Annex XV report

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

Substance Name: Dodecamethylcyclohexasiloxane

EC Number: 208-762-8

CAS Number: 540-97-6

Submitted by: European Chemicals Agency (ECHA) at the request of the European Commission

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ABBREVIATIONS/ACRONYMS

BCF_k	Kinetic bioconcentration factor
BCF_{kg}	Growth corrected, kinetic bioconcentration factor
BCF_{ss}	Steady state bioconcentration factor
BCF_{ssL}	The lipid normalised steady state bioconcentration factor
BSAF	Biota-sediment accumulation factor
BMF	Biomagnification factor
cVMS	Cyclic volatile methylsiloxanes
D4	Octamethylcyclotetrasiloxane
D5	Decamethylcyclopentasiloxane
D6	Dodecamethylcyclohexasiloxane
HLC	Henry's law constant
LOD	limit of detection
LOQ	limit of quantification
MDL	Method detection limit
MSC	Member State Committee
PDMS	Polydimethylsiloxanes
p,p'-DDE	Dichlorodiphenyldichloroethylene
RAC	Committee for Risk Assessment
TMF	Trophic magnification factor
VMSs	volatile methylsiloxanes
w/w	weight by weight

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EC Number: 208-762-8

CAS number: 540-97-6

- It is proposed to identify the substance as very persistent and very bioaccumulative (vPvB) according to Article 57 (e) of Regulation (EC) No 1907/2006 (REACH) due to its intrinsic properties.
- It is proposed to identify the substance as very persistent and very bioaccumulative (vPvB) with ≥ 0.1% w/w octamethylcyclotetrasiloxane (D4) (EC no: 209-136-7) and/or with ≥ 0.1% weight by weight (w/w) decamethylcyclopentasiloxane (D5) (EC no: 208-764-9).
- It is proposed to identify the substance as persistent, bioaccumulative and toxic (PBT) according to Article 57 (d) of Regulation (EC) No 1907/2006 (REACH) with ≥ 0.1% w/w octamethylcyclotetrasiloxane (D4) (EC no: 209-136-7).

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as vPvB based on its intrinsic properties. All available relevant information (such as the results of standard tests, monitoring and modelling, information from the application of the analogue approach (grouping, read-across), benchmarking approach and (Q)SAR results) was considered together in a weight-of-evidence approach.

Persistence

Dodecamethylcyclohexasiloxane (D6) is considered to be not readily biodegradable and so meets the screening persistent (P) and very persistent (vP) criteria. Read-across from D4 and D5 to D6 has been considered appropriate for the assessment of persistence. Based on the comparison of physico-chemical properties of D4, D5 and D6, D6 can be expected to be more persistent than D4 and D5. Data for the analogue substances D4 and D5 provide that the vP criterion is met in sediment (see Annex XV reports of D4 and D5 (2018a and 2018b)).

Bioaccumulation

The available data from laboratory bioaccumulation tests show that D6 meets the vB criterion based on a kinetic BCF of around 6600 – 12 600 l/kg in common carp (*Cyprinus carpio)*. In addition, the available field data provides evidence that biomagnification and trophic magnification occur in certain food webs in the environment. The available information on biomagnification and trophic magnification factors (BMF/TMF) in the field indicating that biodilution occurs in some food chains or in parts of some food chains, does not invalidate the other lines of evidence. Correlation of levels of D6 in some pelagic food webs with levels of known biomagnifying substances (TMFs >1) e.g. PCB-153 and p,p'-

DDE (as part of a benchmarking approach), also tends to demonstrate that D6 can biomagnify. A comparison of the TMF data for D6 with that for D4 and D5 suggests that D6 has a generally similar biomagnification potential to both D4 and D5 in the environment based on the TMF. A similar picture is seen when comparing the D6 BCF values in *Cyprinus carpio* with those for D4 and D5 where the D6 values are similar or higher. However, the BCF for D6 is lower than those for D4 and D5 when comparing the data for *Pimephales promelas*. Taking together all lines of evidence on bioaccumulation potential, it can be concluded that D6 meets the vB criterion.

Toxicity

Several data are available on human health toxicity and ecotoxicity of D6, but these were not assessed for this report.

Relevant constituents, impurities and/or additives

D6 contains octamethylcyclotetrasiloxane (D4) and/or decamethylcyclopentasiloxane (D5) as impurities. D4 fulfils the PBT and vPvB criteria and D5 meets the vPvB criteria (see Annex XV reports of D4 and D5 (2018a and 2018b)). Taking all information into account, including the concentration of D4/D5 and the properties of these substances, D6 thereby fulfils the PBT criteria with impurity D4 in concentration of ≥0.1 % (w/w) and the vPvB criteria with either one or both of the impurities D4 and D5 in concentration of ≥0.1 % (w/w).

Conclusion

Dodecamethylcyclohexasiloxane (D6) meets the criteria for a vPvB substance according to Article 57 (e) of REACH based on its intrinsic properties. Additionally, D6 meets the criteria for a vPvB substance due to its impurity octamethylcyclotetrasiloxane (D4) and/or decamethylcyclopentasiloxane (D5) (concentration ≥ 0.1 % w/w). Furthermore, D6 meets the criteria for a PBT substance. This conclusion is drawn because D6 contains octamethylcyclotetrasiloxane (EC no: 209-136-7; D4) which is typically present as an impurity in relevant concentrations (typically above or equal to 0.1 % w/w).

In conclusion, dodecamethylcyclohexasiloxane (D6) is identified as a PBT/vPvB substance according to Art. 57(d) and (e) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

Registration dossiers submitted for the substance? Yes

PART I

Justification

Octamethylcyclotetrasiloxane (D4, EC 209-136-7) and decamethylcyclopentasiloxane (D5, EC 208-764-9), respectively, are closely related to D6 and data from D4 and D5 have been used in this assessment as a read-across data and for the benchmarking purposes (see Justification on read-across approach in Annex I). The Member State Committee (MSC) provided an opinion on the persistent and bioaccumulative properties of D4 and D5 at the request of the Executive Director of ECHA under Article 77(3)c of REACH (ECHA, 2015) during the process to restrict the use of these two substances. D4 and D5 were subsequently concluded by the Committee for Risk Assessment (RAC) - based on the opinion of the MSC- to fulfil the criteria of Annex XIII of REACH as a vPvB substance (see RAC opinion on the restriction proposal: (ECHA, 2016)). In March 2016, whilst evaluating the UK restriction proposal, the Committee for Risk Assessment (RAC) concluded that D4 meets the REACH Annex XIII criteria for toxicity based both on aquatic and mammalian endpoints (ECHA, 2016). Currently, D4 and D5 are being proposed as SVHCs (see Annex XV reports of D4 and D5 (2018a and 2018b) in parallel to D6.

1 Identity of the substance and physical and chemical properties

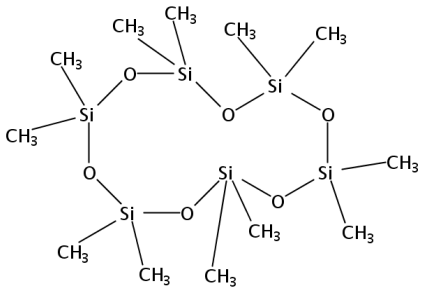
1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	208-762-8
EC name:	Dodecamethylcyclohexasiloxane
CAS number (in the EC inventory):	540-97-6
CAS number: Deleted CAS numbers:	
CAS name:	Cyclohexasiloxane, 2,2,4,4,6,6,8,8,10,10,12,12-dodecamethyl-
IUPAC name:	Dodecamethylcyclohexasiloxane
Index number in Annex VI of the CLP Regulation	Not available
Molecular formula:	$C_{12}H_{36}O_6Si_6$
Molecular weight range (g/mol):	444.92
Synonyms:	D6 Baysilone SF 1217 Silsoft 1217 Cyclomethicone

Note: The abbreviation D6 is used throughout this document to refer to the substance for brevity. Octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane are also referred to in abbreviated form (respectively, D4 (EC no: 209-136-7) and D5 (EC no: 208-764-9)).

Structural formula:



1.2 Composition of the substance

Name: Dodecamethylcyclohexasiloxane

Description:

Substance type: mono-constituent

Table 2: Constituents other than impurities/additives

Constituents	Concentration range
Dodecamethylcyclohexasiloxane *EC 208-762-8*	≥ 80%

Table 3: Impurities

Impurities	Typical concentration	Concentration range
Decamethylcyclopentasiloxane (D5) EC 208-764-9	-	-
Octamethylcyclotetrasiloxane (D4) EC 209-136-7	-	-
Hexamethylcyclotrisiloxane (D3) EC 208-765-4	-	-
Tetradecamethylcycloheptasiloxane (D7) EC 203-496-9	-	-

Table 4: Additives

Additives	Typical concentration	Concentration range	Remarks
Not relevant	Not relevant	Not relevant	Not relevant

1.3 Identity and composition of degradation products/metabolites relevant for the SVHC assessment

Not relevant for this assessment.

1.4 Identity and composition of structurally related substances (used in a grouping or read-across approach)

Octamethylcyclotetrasiloxane (D4, EC 209-136-7) and decamethylcyclopentasiloxane (D5, EC 208-764-9), respectively, are closely related to D6 and data from D4 and D5 has been used in this assessment as a read-across in cases where it is relevant for the assessment of D6 (see Justification on read-across approach in Annex I). Both D4 and D5 have been assessed for their PBT/vPvB properties by the MSC and the RAC (see the “Justification” section).

Table 5: Structurally related substance identity of octamethylcyclotetrasiloxane (D4)

EC number:	209-136-7
EC name:	Octamethylcyclotetrasiloxane
CAS number (in the EC inventory):	556-67-2
CAS number: Deleted CAS numbers:	
CAS name:	Cyclotetrasiloxane, 2,2,4,4,6,6,8,8-octamethyl-
IUPAC name:	Octamethylcyclotetrasiloxane
Index number in Annex VI of the CLP Regulation	014-018-00-1
Molecular formula:	$C_8H_{24}O_4Si_4$
Molecular weight range:	296.62 g/mol
Synonyms:	D4, cyclotetrasiloxane

Substance type: mono-constituent

Structurally related substance formula (D4):

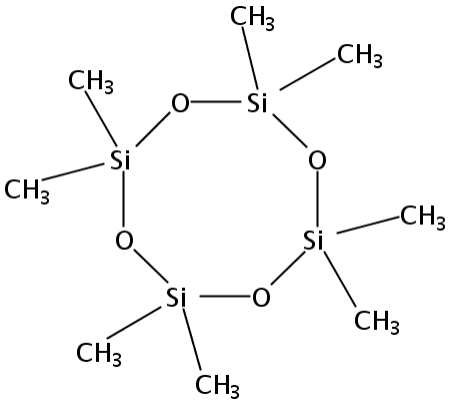


Table 6: Constituents of structurally related substance D4 (other than impurities/additives)

Constituents	Concentration range	Remarks
Octamethylcyclotetrasiloxane (EC no: 209-136-7; D4)	80 – 100 % w/w	

Table 7: Structurally related substance identity of decamethylcyclopentasiloxane (D5)

EC number:	208-764-9
EC name:	Decamethylcyclopentasiloxane
CAS number (in the EC inventory):	541-02-6
CAS number: Deleted CAS numbers:	-
CAS name:	Cyclopentasiloxane, 2,2,4,4,6,6,8,8,10, 10-decamethyl-
IUPAC name:	Decamethylcyclopentasiloxane
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$C_{10}H_{30}O_5Si_5$
Molecular weight range:	370.77 g/mol
Synonyms:	D5, cyclopentasiloxane

Substance type: mono-constituent

Structurally related substance formula (D5):

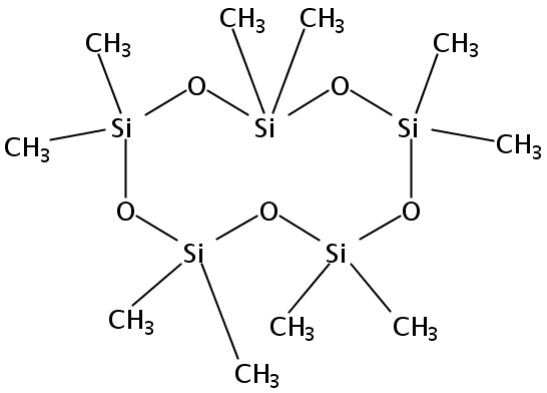


Table 8: Constituents of structurally related substance D5 (other than impurities/additives)

Constituents	Typical concentration	Concentration range
Decamethylcyclopentasiloxane (EC no: 208-764-9; D5)	98.5 % w/w	80 – 100 % w/w

Table 9: Impurities of structurally related substance D5

Impurities	Typical concentration	Concentration range
Octamethylcyclotetrasiloxane (EC no: 209-136-7; D4)	1.5 % w/w	-

1.5 Physicochemical properties

Table 10: Overview of physicochemical properties

	Description of key information	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa	*Liquid*	-	*Merck (1996)*
Melting/freezing point	*-3 °C*	*Reliable Handbook data*	*Kirk-Othmer (1978)*
Boiling point	*245 °C at 1013 hPa*	*Reliable Handbook data*	*Merck (1996)*
Vapour pressure	*4.6 Pa at 25 °C* *(ca. 5 Pa at 20-25 °C)*	*Value determined by extrapolation from a temperature-vapour pressure correlation; reported in REACH registration and used in UK Risk Assessment.* *(REACH registration "weight of evidence" result, based on average of QSAR predicted vapour pressure of 4.7 Pa at 20°C by using appropriate QSAR prediction method (Reconsile 2009) and the selected value)*	*DIPPR (2004)* *Joint submission*
Density	-		
Water solubility	*5.3 µg/l ± 0.48 µg/l at 23 °C* *(5 µg/l)*	*(original reference not available for review and no further information available)*	*Varaprath et al. (1996)* *(reported in secondary literature (Mazzoni, 1997)*
Partition coefficient n-octanol/water (log value)	*8.87 at 24 °C* *(9.06)*	*Kaw, Kow and Koa of ^{14}C-labelled D6 simultaneously determined at room temperature through establishment of octanol/air/water three-phase equilibrium.* *(modelled value based on a linear extrapolation QSAR method used in UK RA)*	*Xu (2009)* *Environment Agency (2009a)*
Dissociation constant	-	*REACH registration: study waived owing to lack of ionisable groups in the molecule.*	*https://echa.europa.eu/substance-information/-/substanceinfo/100.007.967* *(Accessed: 07.02.2017)*

Log K_{oa}	*5.86±0.12 at 24°C* *(5.76 at 24°C)*	*Recent value determined in same study as log Kow.* *(measured value used in UK RA)*	*Xu (2009)* *Environment Agency (2009a)*
Surface tension	-	*REACH registration: Test waived owing to low water solubility (< 1 mg/L), in accordance with column 2 of REACH Annex VII.*	*https://echa.europa.eu/substance-information/-/substanceinfo/100.007.967* *(Accessed: 07.02.2017)*

Note: where more than one value for one endpoint is presented, the value considered most relevant is highlighted in bold.

2 Harmonised classification and labelling

D6 does not have harmonised classification according to the Regulation (EC) No 1272/2008.

3 Environmental fate properties

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

The stability of D6 in water has been assessed by two methods (Kozerski, 2009) from the same study, reported in the REACH registration, as described below.

1) A property-activity relationship was used to predict the second-order rate constants for the hydronium- and hydroxide-catalyzed hydrolysis reactions of D6. A model was developed using reported catalytic constants, k(H+) and k(-OH) for D3, D4 and D5 at 25°C and their reported aqueous solubilities. These rate constants were obtained from reportedly reliable studies under acid and base conditions in dilute aqueous buffer solutions. Linear regression analysis of log k versus log S (where k is k(H+) or k(-OH) and S is the water solubility) was used to establish correlations that were used to estimate hydrolysis rates for D6 by extrapolation.

The results from linear regression analysis of log k on log S for D3, D4 and D5 were as follows:

Hydronium catalysis: slope = 1.114, intercept = 1.033, R^2 = 0.9998

Hydroxide catalysis: slope = 1.544, intercept = 1.103, R^2 = 0.9862

Extrapolating to D6 (log S = 1.06) gave predicted values of k(H+) and k(-OH) of 165 and 554 M^{-1} h^{-1}, respectively. These values were used to calculate a predicted pseudo-first order rate constant at any pH using the equation: k(pred) / h^{-1} = $K(H^+)[H^+]$ + $k(^-OH)[^-OH]$. This assumed that contributions from uncatalyzed or buffer-catalyzed hydrolysis reactions were negligible under the prevailing conditions (which the registrant stated has been shown for other cyclic siloxanes that have been studied). For pH 7 and 25 °C, the calculated rate constant for hydrolysis of D6 was 7.19×10^{-5} h^{-1}, equivalent to a half-life of 401 days.

2) Screening experiments at elevated temperature under basic pH conditions, using methods developed in studies of the hydrolysis of other cyclic methylsiloxanes, were carried out. ^{14}C-enriched D6 was spiked into aqueous boric acid/lithium borate buffer, pH 9 or 10, at a concentration 50% of the aqueous solubility. The solution was transferred to a set of borosilicate glass tubes that were immediately flame-sealed. The tubes were aged in a temperature controlled incubator at 40 or 60 °C (nominal). At various times, individual tubes were removed from the incubator, opened, and their contents analyzed. The distribution of the ^{14}C in aqueous solution, as parent ^{14}C-D6 and hydrolysis intermediates/product, was determined by HPLC separation with fraction collection and subsequent analysis by liquid scintillation counting (LSC). A separate aliquot of the reaction mixture was analyzed directly by LSC for total recovery of ^{14}C activity, relative to that measured in the bulk solution immediately after spiking. Because some of the ^{14}C-D6

partitioned into the small but unavoidable headspace in the sealed tube, non-linear regression analysis (using Berkeley Madonna version 8.0.1 for Windows) was performed to distinguish this process from hydrolysis and obtain a rate constant for the reaction. The conditions of pH and temperature were chosen to target a half-life of ca. 4 h. Basic conditions were chosen because base-catalysis is generally more important in determining the half-lives of cyclic volatile methylsiloxanes. In the pH 9 experiment, an additional set of reaction tubes filled with unspiked buffer was aged alongside the tubes contained ^{14}C-D6. The pH was measured at several intervals through to termination of the kinetic experiment with no observed change in pH.

The two results obtained by method 2 (k(-OH) at 25°C = 9.2 and 15 M^{-1} h^{-1}) are in reasonable agreement with each other but differ significantly from the predicted value of 550 M^{-1} h^{-1} from method 1. The authors of the study could not explain the discrepancy or draw a conclusion as to which is more accurate. However, the data were sufficient for the conclusion to be drawn that the hydrolysis half-life of D6 at pH 7 and 25°C would exceed 1 year. The registrant commented that the actual half-life would be very difficult to measure due to the long disappearance time, very low water solubility and relatively high vapour pressure.

The results of the methods described above fit with the general pattern of hydrolysis half-life reported for this homologous series of cyclic siloxanes (D3, D4, D5, D6): at pH 7 and 25°C D3 has a half-life of 23 minutes, D4 69 hours, and D5 71 days. Further, it has been shown that the presence of dissolved organic carbon (DOC) in natural environments can slow hydrolysis, owing to the adsorptive nature of these substances (Environment Agency 2009b and 2009c).

Overall, it is concluded that hydrolysis is unlikely to be a relevant degradative pathway for D6 in the environment, the half-life being >1 year at pH 7 and 25 °C.

3.1.1.2 Oxidation

No information on oxidation is available for this substance.

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 Phototransformation in air

The rate constant for the reaction of D6 with atmospheric hydroxyl radicals is estimated as 1.80×10^{-12} cm^3/molecule/s using the AopWin (v1.92) program, part of the US-EPA EPI Suite (v4.1) estimation software (based on an OH radical concentration (12 h average) of 1.5×10^6 mol/cm^3). This is equivalent to a half-life of 6.0 days. In addition, according to AopWin, the sorbed fraction may be resistant to atmospheric oxidation.

Safron et al. (2015) studied the reaction of cyclic volatile methyl siloxanes with OH radicals. The authors applied the relative rate technique to study the kinetics of the reaction in a temperature range between 313 and 353 K. The Arrhenius equation was used to extrapolate from these results to a temperature of 298 K, yielding reaction rate constant of $2.8 \cdot 10^{-12}$ cm^3 /molecule/s for D6. This rate constant would result a half-life of 5.7 days assuming an average atmospheric hydroxyl radical concentration of 5×10^5 molecule cm^{-3}. While the study by Safron et al. (2015) appears to be well-conducted, the extrapolation from higher temperatures to 298 K is expected to introduce some uncertainty.

Work by Whelan *et al.* (2004) was discussed in the UK risk assessment of D6 (Environment Agency (2009a), as follows. Whelan *et al.* (2004) assessed the atmospheric fate of VMSs (volatile methylsiloxanes) and their degradation products. The assessment used a simple equilibrium-partitioning model to investigate the relative rates of removal of two

representative volatile methylsiloxanes (VMSs; the linear decamethyltetrasiloxane and D4) and their siloxanol degradation products by reaction and atmospheric deposition. Although the calculations are for only one cyclic VMS, the findings of the paper are equally applicable to other cyclic VMSs such as D6. The modelling is based on the work of Atkinson (1991) and Sommerlade *et al.* (1993) which demonstrates that siloxanes break down in the atmosphere to form hydroxy-substituted siloxanes (or "siloxanols") by reaction with atmospheric hydroxy radicals. As substitution proceeds the siloxanols become increasingly water-soluble and less volatile, and so tend to be washed out of the atmosphere by wet deposition. Oligomeric siloxanols are also assumed to be subject to hydrolysis reactions when dissolved in liquid water droplets. Removal by dry deposition is also accounted for in the approach, but scavenging of particulates from the air by wet deposition is not. The findings from the model indicate that the parent siloxanes and the monohydroxy degradation products occur mainly in the vapour phase, with only relatively small amounts associated with the water and particulate phases (although the small size of the water- and particulate-phase compartments in the atmosphere means that the concentrations in these phases can approach or exceed those in the vapour phase). The degradation products of the hydroxyl substitution are thought to be associated mainly with the dissolved and particulate phases. However, the decreasing concentration of precursor molecules as this degradation proceeds means that the maximum dissolved- and particulate phase concentrations occur for degradation products with two hydroxyl substituents. The concentrations of degradation products with higher levels of hydroxyl substitution are predicted to decrease markedly with increasing substitution. The siloxanediols in the precipitation are predicted to undergo further reaction via hydrolysis to give a mixture of siloxane hydrolysis products (depending on the atmospheric residence time and the pH). Overall it is concluded that >99 per cent of the VMSs are removed from the atmosphere as siloxanols in wet deposition and <1 per cent as siloxanols in dry deposition. The products from the reaction are expected to be siloxanols that are removed from the atmosphere by wet deposition. Buch *et al.* (1984) demonstrated that dimethylsilanediol and other water-soluble dimethylsiloxanols can be degraded further by aqueous photolytic oxidative demethylation reactions. The final products of the degradation of dimethylsiloxanols are expected to be silicic acid and/or silica and carbon dioxide (CO2) (Buch *et al.*, 1984; Chandra, 1997).

D6 contains no chromaphores that would absorb visible or UV radiation, so direct photolysis is not likely to be significant.

3.1.1.3.2 Phototransformation in water

No information on phototransformation in water is available for this substance.

3.1.1.3.3 Phototransformation in soil

No information on phototransformation in soil is available for this substance.

3.1.1.4 Summary on abiotic degradation

Hydrolysis is unlikely to be a relevant degradative pathway for D6 in the environment, the half-life being >1 year at pH 7 and 25 °C.

While D6 is predicted to have a long atmospheric half-life of 6.0 days (AopWin (v1.92)), Whelan *et al.* (2004) demonstrated that siloxanes break down in the atmosphere to form hydroxy-substituted siloxanes (or "siloxanols") by reaction with atmospheric hydroxy radicals. Overall it was concluded that >99 per cent of the VMSs are removed from the atmosphere as siloxanols in wet deposition and <1 per cent as siloxanols in dry deposition.

Direct photolysis is not likely to be significant, as D6 contains no chromaphores that would absorb visible or UV radiation.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

3.1.2.1.1 Estimated data

No estimated data are included as experimental data are available for this substance.

3.1.2.1.2 Screening tests

Springborn Smithers Laboratories (2005) studied the biodegradability of D6 using the OECD 310 methodology [ready biodegradability – CO_2 in sealed vessels (headspace test)]. The D6 tested was 99.6 per cent pure and sodium benzoate was used as a reference substance in the test. The inoculum was derived from activated sludge and sewage from a WWTP that received primarily domestic waste, and soil from a wooded area. The inoculum was added to the test medium at a concentration of 10 mg solids/l, D6 added at a concentration of 10 mg carbon/l and incubated in the dark at 22 ± 2°C. At intervals during the test the amount of CO_2 (measured by total carbon analysis) in the headspace was determined (four replicates were sampled on each occasion). A control (inoculum only), positive control (which contained sodium benzoate at a concentration of 10 mg carbon/l), and toxicity control (which contained sodium benzoate and D6, both at a concentration of 10 mg carbon/l) were also run. The test showed 4.5 per cent degradation of D6 over the 28-day test period. The degradation in the positive control was 106 per cent after 28 days (with >60 per cent degradation within the ten-day window), which indicates that the inoculum used was viable, and the toxicity control showed that D6 was not toxic to the microorganisms present. However, the solubility of D6 is limited (5.3 µg/l), which may have reduced the bioavailability of D6 in this test. In addition, it is possible that a proportion of the D6 could have occupied the headspace [the OECD 310 guideline suggests that this could be significant for substances with a Henry's law constant >50 Pa m^3/mol, and that for D6 is well above this value (2,536,000 Pa m^3/mol)], and so could have contributed to the carbon measured in the headspace.

As part of a study into the fate and behaviour of D6 in a municipal waste water treatment plant in Beijing City, China, Xu *et al.* (2013) carried out an *in vitro* non-guideline study on the anaerobic degradation of D6. The test used a batch system consisting of sealed glass vials containing 40 ml of an activated sludge-liquid mixture obtained from the anaerobic tank of the waste water treatment plant. The sludge mixture had a dry solids content of 10 g/l and a pH of 6.5-6.8. D6 was added to the vial at either 2, 5 or 10 µg/l and then incubated at 30°C with shaking for up to 60 hours under a nitrogen-carbon dioxide headspace (approximately 20 ml). The amount of D6 present in liquid phase and the headspace was determined at intervals (0, 10, 20, 40 and 60 hours). Sterile sludge was used as a control. Degradation of D6 in this test system was found to be minimal, with around 0.5-1.8% degradation after 10 hours and 3-18% degradation after 60 hours. Given the relatively short hydraulic retention times in the anaerobic tanks of waste water treatment plant studies (typically 1.5-2.5 hours) Xu *et al.* (2013) concluded that degradation of D6 during anaerobic waste water treatment would be minimal.

No other biodegradation tests are available for D6.

Overall, based on these results, D6 is not considered to be readily biodegradable.

3.1.2.1.3 Simulation tests (water and sediments)

No information on simulation tests in water and sediments is available for D6.

A study carried out on the analogue substance, D5, is reported below. It can be expected

that D6 behaves similarly upon adsorption to sediments but may be even less degradable in such conditions due to its higher adsorption potential and lower water solubility, which generally decrease the bioavailability.

Xu (2010) investigated the degradation of ^{14}C-labelled D5 in aquatic sediment under both aerobic and anaerobic conditions (incubation under a nitrogen atmosphere). The method used was based on the OECD Test Guideline 308 but with modifications to minimise the headspace volume (to limit loss from volatilisation) and to add the test substance (as a solution in ethylene glycol monomethyl ether) directly to the sediment phase rather than the water phase. The sediment used was natural freshwater sediment collected from Lake Pepin, Minnesota, USA (this lake is known to receive inputs of D5 from urban sources upstream (for more details, see Section 3.4.3.1) and so the sediment was likely to have been pre-exposed to D5). The tests were carried out at 24°C. The sediment had a pH of 7.9 and an organic carbon content of 3.7 per cent.

The incubations were carried out using 250 ml flasks containing 25 g dry weight sediment (approximate depth 2.5 cm) and overlying water. The overlying water was lake water, and this was added to fill the flask leaving only a small headspace of 40 ml. The initial D5 concentration used was ~130-200 µg/kg dry weight. Sterile controls were prepared in a similar way but with the addition of sodium azide.

At occasions during the test, aeration was carried out for the aerobic sediments and nitrogen gas exchange was carried out for the anaerobic experiments. The exchanged gases were collected and analysed for $^{14}CO_2$ and $^{14}CH_4$ and any ^{14}C-containing volatile substances in the exchanged gases were collected in a cooled (-68 to -74°C) glass coil, transferred to an air tight syringe and reintroduced into the headspace of the test vessels. In addition at various time points duplicate sediment samples were sacrificed for analysis of the parent substance and ^{14}C present in the sediment and water phases and the headspace.

The experiments were carried out for up to 245 days under aerobic conditions and up to 201 days under anaerobic conditions. The total number of sampling periods during this time was seven for the aerobic and anaerobic controls, seven for the biotic anaerobic samples and nine for the biotic aerobic samples. Two test vessels were sacrificed for analysis at each time point.

The recovery of radioactivity in the experiment was generally >80 per cent (average 83.0 per cent excluding two samples with a lower recovery) under aerobic conditions, but lower (average 68.3 per cent) under anaerobic conditions. As the recovery rates were generally constant over the entire experimental period this indicated that the low recovery was most likely related to loss during the spiking process or in the early incubation period. Therefore, the kinetics for degradation were determined based on the total amount of radiolabel recovered rather than the total amount of radiolabel added as this would be less sensitive to the low recovery.

The majority of the ^{14}C-D5 in the system (>96 per cent) was found to be associated with the sediment phase. Degradation of D5 was evident under both aerobic and anaerobic conditions (a slow decrease in the amount of D5 occurred while the amounts of the major degradation products, dimethylsilanediol and non-extractable substances presumed by the authors to most likely be other silanols, increased), but the degradation rate was found to be slow. In addition a slow degradation was also evident in the sterile controls indicating that at least part of the degradation was abiotic in nature. The half-lives at 24°C were estimated to be around 1,200 days under the biotic, aerobic conditions, 2,700 days under sterile aerobic conditions, 3,100 days under biotic, anaerobic conditions and 800 days under sterile anaerobic conditions. Minimal amounts of mineralisation products ($^{14}CO_2$ and $^{14}CH_4$) were found to be formed.

The sediment used in these studies was collected on the 22nd May 2008 but the degradation studies themselves were not initiated until 13th January 2009; therefore the sediment was stored for over seven months (the sediment was stored at 4°C in sealed containers and the containers were opened on three occasions to allow air exchange to occur and the sediment for the aerobic experiment was very well mixed at test initiation in order to provide further aeration). The OECD Test Guideline 308 recommends that the sediment is stored at 4°C for a maximum of four weeks and that the sediment used for the aerobic studies should be stored with free access to air. The effect of the prolonged storage used in the current study on the biological viability of the sediment is unknown.

In addition, only one sediment was tested here whereas the OECD 308 Test Guideline recommends that two different sediments are used (one with a high organic carbon content (2.5-7.5 per cent) and fine texture and one with a low organic carbon content (0.5-2.5 per cent) and coarse texture). The organic carbon content of the Lake Pepin sediment was 3.7 per cent (it is not clear if this was determined at the time of collection of the sediment or the time of the test initiation) and the effect of the prolonged storage on the organic carbon content of the sediment (or indeed changes in the organic carbon content over the timescale of the actual degradation experiment) is unknown.

Although these deviations from the OECD Test Guideline are not ideal, the results of the study suggest strongly that degradation of D5 in sediment is predominantly an abiotic process and so the prolonged storage of the sediment prior to test initiation may not be so important in this case. The effect of organic carbon content of the sediment on the degradation rate is currently unknown.

It should be noted that the study above was used for the assessment of persistence of D5 by the MSC (ECHA 2015).

3.1.2.2 Biodegradation in soil

Xu (1999) investigated the degradation of D6 in soil in a study that mainly focused on D4 and D5. The study was designed to analyse the significance of all possible degradation pathways, including ring-opening polymerization reactions (essentially to form polydimethylsiloxanes, PDMS), demethylation reactions, and hydrolysis reactions. The soil used in the study was the Wahiawa Series from the Kunia area, Hawaii, and it was air dried before use. ^{14}C-D6 (radiochemical purity >99 per cent) was dissolved in pentane prior to spiking the soil. The tests were carried out in Teflon® tubes that contained either 1 g or 5 g of soil, with 0.25 ml of the pentane solution of D6 added to the soil, and the tube being flushed with air for two minutes. The initial target D6 concentrations were in the range 40–200 mg/kg dry weight. The spiked soil was then incubated in the closed tubes in the dark at room temperature for between ten minutes and seven days. At the end of the incubation period the soils were solvent extracted, and the D6 that remained and the degradation products were determined. The overall recovery of total ^{14}C in the study was not given for D6, but as the recovery was very high for the more volatile D4 (recovery 98.7 per cent) and D5 (recovery 99 per cent), a similar high recovery is expected for D6 (CES, 2005).

D6 hydrolysed rapidly in the experiments, to form more polar products. For example, around 10 per cent of the D6 disappeared and eight hydrolysis products were evident after 0.5 hours' incubation, and by 24 hours only two main hydrolysis products in addition to D6 remained. The reaction was thought to proceed via ring-opening hydrolysis to form the linear dodecamethylhexasiloxanediol (hexamer diol), with subsequent loss of dimethylsilanediol to form the decamethylpentasiloxanediol (pentamer diol), octamethyltetrasiloxanediol (tetramer diol), hexamethyltrisiloxanediol (trimer diol), tetramethyldisiloxanediol (dimer diol), and eventually dimethylsilanediol. One other unidentified product was found after 0.5 hours' incubation, but this had totally disappeared by 24 hours. Earlier studies, cited in Xu (1999), showed that dimethylsilanediol (the final

degradation product of D6) is lost from soil by volatilisation (Lehmann and Miller, 1996) and biodegradation (Lehmann et al, 1998; Sabourin et al, 1996). The ultimate biodegradation products of dimethylsilanediol are likely to be CO_2 and silica (Chandra, 1997). In addition, any dimethylsilanediol lost from the soil by volatilisation may be photodegraded to CO_2 and silicic acid and/or silica in the atmosphere. This, therefore, provides a complete degradation pathway for D6 in soil.

Xu and Chandra (1999) carried out more experiments on two soils to establish the rate of degradation and volatilisation from soil more satisfactorily. One was a typical temperate soil (coarse-textured alfisol), with a pH of 7.6 and organic matter content of 2.4 per cent, and consisted of 50 per cent sand, 28 per cent silt, and 22 per cent clay. The predominant clay minerals in this soil were illite and chlorite. The other soil was a highly weathered soil (a clay oxisol), with a pH of 4.9 and organic matter content of 2.2 per cent, and consisted of 21.2 per cent sand, 24.0 per cent silt, and 54.8 per cent clay. The predominant clay minerals in this soil were kaolinite, gibbsite, and goethite. Most of the tests were carried out with D4, but some tests were also carried out with D5 and D6. The general conclusions found with the experiments with D4 are also relevant for the other cyclic siloxanes. The substances tested were ^{14}C-labelled (radiochemical purity >99 per cent). The degradation experiments were carried out using sealed systems under different relative humidities (32, 92, and 100 per cent). The soils were prepared by pre-equilibrating samples of 5 g of air-dried soil in 30 ml Teflon® tubes to the required relative humidity atmosphere in a desiccator. After a seven day pre-equilibration period the soil was spiked with the ^{14}C-labelled substance as a solution in pentane (the amount of substance added is equivalent to an initial soil concentration of ~40 mg/kg dry weight) and the tube was immediately capped. After two minutes the cap was removed and the tube flushed with air of the correct humidity for 90–120 seconds to evaporate the solvent. After this the tubes were recapped and incubated at 22°C for between 0 and 21 days. The experiments to investigate the volatilisation loss were prepared in a similar way, but incubated without capping. At various times the amount of ^{14}C substance in the soil was determined. D4 degraded in the test system, and the rate increased as the relative humidity decreased. The rate of degradation was also generally faster in the weathered soil than in the temperate soil. The degradation half-lives are around 0.89 days (21 hours), 0.08 days (1.9 hours), and 0.04 days (58 minutes) in the weathered soil at relative humidities of 100, 92, and 32 per cent, respectively, and 5.25 and 3.54 days in the temperate soil at relative humidities of 92 and 32 per cent, respectively (little or no degradation of D4 occurred in the temperate soil at 100 per cent relative humidity). Results for D5 and D6 were only given for the weathered soil at a relative humidity of 32 per cent. Under these conditions, the half-lives for D5 and D6 are 0.08 days (1.9 hours) and 1.38 days, respectively, compared with 58 minutes for D4 under the same conditions. Overall, it is concluded that the rate of degradation is D4 > D5 >> D6 in these test systems.

Degradation is thought to result from hydrolysis reactions catalysed by the surface activity of soil clays. The increase in moisture of the soil is thought to decrease the surface acidity and thus the hydrolysis rate. The differences in the degradation rates obtained in the weathered soil compared with those in the temperate soil occurred because the weathered soil had a higher clay content, and the clay minerals in this soil were kaolinite (around 50 per cent of the clay minerals) and gibbsite (around 10 per cent of the clay minerals), both of which are highly effective catalysts of PDMS. In contrast, as well as having a lower clay content, the clay minerals in the temperate soil were illite and chlorite (the former is one of the least-effective catalysts for hydrolysis of Si–O–Si linkages). The volatilisation experiments were carried out with D4 only in temperate soils. These show that loss through volatilisation from soil is a significant competing process for D4 in soils in open systems. At a relative humidity of around 50 per cent, volatilisation accounts for around 40 per cent of the total loss of D4, but loss through volatilisation is negligible compared to that through degradation in dry soils (relative humidity 32 per cent). In soils at high relative humidity (~100 per cent) loss through volatilisation is the dominant removal process (e.g. 80 per cent loss through volatilisation over the incubation period compared

with 5 per cent by degradation). These results are relevant for D6 but, given the higher log Kow and lower vapour pressure for D6 compared with D4, the loss of D6 by volatilisation is expected to be lower than for D4.

Based on the outcome of the above studies, it can be concluded that degradation of D6 occurs in dry soils (most probably by an abiotic mechanism). However, moisture significantly reduces the rate of degradation such that when the dried soil is equilibrated to a 100 per cent relative humidity atmosphere essentially no degradation occurs. In terms of the environment, although dry soils may exist in some situations (e.g. drought), most soils contain moisture, and even dry soils are exposed to moisture in the air [as simulated in the studies by Xu (1999) and Xu and Chandra (1999)]. Thus, although it is possible that such degradation in soils could occur under some circumstances in the environment (low relative humidity drought conditions) this is unlikely to be the typical case. Furthermore one of the main soil compartments relevant to the risk assessment is agricultural soil. Here crops are likely to be watered during dry conditions and so the degradation under such situations is likely to be slow.

Further discussion on this was included in the UK risk evaluation (Environment Agency, 2009a), as follows. Another analysis of the soil degradation data for D6 was carried out [Xu, personal communication, as reported by CES (2005) and Xu (2007a)]. The analysis is based on the data of Xu and Chandra (1999) and uses the assumptions:

- the ratio of degradation rates of the various cyclic VMSs relative to D4 are the same at any given moisture level in different soils;
- the rates of degradation of any given cyclic VMS are linearly related to water potential (which is, in turn, linearly related to log {relative humidity} as measured with Londo soil).

The estimated half-lives of D6 in two types of soil using this approach are summarised below [the Xu and Chandra (1999) study was carried out at 22°C]:

Relative humidity (%)	Half-life Temperate soil (days)	Half-life Tropical soil (days)
50	158	1.8
70	179	2.3
90	202	3.0

The half-lives above relate to a dry soil exposed in air of the stated relative humidity. CES (2005) indicate that, for comparison, the water content of Londo soil [as used by Xu and Chandra (1999)] in the 32.5 per cent relative humidity experiment is 2.1 per cent. Using similar assumptions to the above, a half-life of 115 days is estimated for a typical soil in the dry season in France [Xu, personal communication, as reported in CES (2005)]. In France the soil moisture content may regularly decline to between 5 and 10 per cent during the summer months.

New data for the analogue substance D5 available on degradation in sediment show that D5 has a long degradation half-life (in the order of 800-3,100 days at 24°C, expected to be longer at lower temperatures).

3.1.2.3 Summary and discussion on biodegradation

One ready biodegradation test is available for D6 showing very limited degradation in 28 days (4.5%). Information for related substances (D4 and D5), reinforces the conclusion that D6 is not readily biodegradable.

D6 has been measured to degrade fast in dry tropical soil conditions whereas half-lives have been observed to increase with increasing humidity of the soil. Half-lives up to ca.

200 days have been obtained in the same study in temperate conditions. In the available soil degradation study also analogue substances D4 and D5 were measured and the relative half-lives were D4 < D5 < D6.

Due to its high potential of volatilisation, it is expected that part of D6 (6.75%) is removed from aquatic systems and terrestrial systems by volatilisation into the atmosphere. However, its high potential of adsorption to sediment and soil (high Koc value) is expected to limit its potential of volatilisation. It should be noted that D6 has lower water solubility, lower volatility and higher adsorption potential to soil and sediment than D4 and D5. As is the case with the analogue substance D5 (sediment half-lives of 800-1200 days), D6 can be expected to persist in sediments following partitioning in the aquatic environment. Under some conditions (e.g. particularly dry spells; as summarised above) the degradation of D6 in soil could become more rapid (and become the dominant removal process from the soil). However, this would not represent a realistic worst-case situation.

3.1.3 Field data

No field data are available for D6.

3.1.4 Summary and discussion on degradation

Degradation of D6 occurs in the atmosphere by reaction with atmospheric hydroxyl radicals. Hydrolysis of D6 has been shown to be negligible in water (half-lives >1 year).

One ready biodegradation test is available for D6 showing very limited degradation in 28 days (4.5%). Information for related substances (D4 and D5), reinforces the conclusion that D6 is not readily biodegradable.

Due to its volatility, it is expected that part (6.75%) of D6 is removed from aquatic systems and terrestrial systems by volatilisation into the atmosphere. However, its high potential of adsorption to sediment and soil (high Koc value) is expected to limit its potential of volatilisation (see details in section 3.1.5). D6 can be expected to persist in sediments following partitioning in the aquatic environment based on the screening data on biodegradation on D6 and on degradation simulation data on the analogue substance D5 (half-lives 800-1200 days). Under some conditions (e.g. particularly dry spells) the degradation of D6 in soil could become more rapid (and become the dominant removal process from the soil). However this would not represent a realistic worst-case situation, as explained above.

In its opinion on the persistency and bioaccumulation of D4 and D5 (ECHA, 2015), the Member State Committee states the following:

> *MSC has evaluated non-degradation processes and concluded that these do not have a large impact on the sediment removal half-life, and thus cannot be used to refute the relevance of the sediment compartment in the assessment of persistence.*
>
> [...]
>
> *Based on OECD TG 308 sediment simulation studies (Xu, 2010), D5 has a degradation half-life in freshwater sediment of the order of 800-3,100 days at 24°C. MSC concludes that D5 meets the Annex XIII criteria for a very persistent (vP) substance in sediment according to Regulation (EC) No 1907/2006.*

Among the new studies published after the MSC and RAC opinion making processes, three studieswere identified to be relevant for the degradation assessment of D5. These studies

were evaluated and taken into account for the overall weight-of-evidence determination. These studies support the conclusion that the substance (D5) is very persistent in sediment.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

A value for K_{oc} of 8.7×10^5 l/kg (log K_{oc} 5.9) with the MCI method and K_{oc} of 5×10^7 l/kg (log K_{oc} 7.7) with the Kow method can be estimated for D6 from its chemical structure using KOCWIN (v2) from the US-EPA EPI Suite (v4.1) estimation software, although such derived values must be viewed with caution as it is not clear if the model's training set contained cyclic siloxanes. Chandra (1997) used four different correlation equations (which relate K_{oc} to water solubility or log K_{ow}) to estimate K_{oc} for D6. The mean value for the Koc estimated was 120,230 l/kg (log K_{oc} 5.08).

The partition coefficients below are estimated using a log Kow value of 8.87 and the methods outlined in the EU TGD.

- Organic carbon–water partition coefficient (Koc): 1.93×10^7 l/kg
- Solids–water partition coefficient in soil (Ksoil): 3.86×10^5 l/kg
- Solids–water partition coefficient in sediment (Ksed): 9.64×10^5 l/kg
- Solids–water partition coefficient in suspended matter (Ksusp): 1.93×10^6 l/kg
- Soil–water partition coefficient (Ksoil–water): 5.78×10^5 m^3/m^3
- Suspended matter–water partition coefficient (Ksusp–water): 4.82×10^5 m^3/m^3
- Sediment–water partition coefficient (Ksed–water): 4.82×10^5 m^3/m^3

Again, such derived values must be viewed with caution as it is not clear if the models' training sets contained cyclic siloxanes.

The partitioning of cyclic siloxanes to organic carbon from different sources has been reported in a poster presentation by van Egmond *et al.* (2010). The sources of organic carbon included river sediment, activated sludge, digester sludge and waste water treatment plant influent and effluent, peat and humic acid, however D6 was only studied with activated sludge. The experiments were carried out by equilibrating the organic carbon source with pure water for 24 hours and then determining the concentration of D6 in the water phase (via a headspace technique) and the total sediment phase. The activated sludge samples contained sufficient native D6 to carry out the investigation (i.e. no further D6 was added to the samples). The mean log K_{oc} value determined (±standard deviation) was 6.18±0.12. This corresponds to a Koc value of 1.5×10^6 l/kg.

No further experimental values for the Koc of D6 are available. Studies with D4 and D5 determined the Koc to be 1.7×10^4 and 1.5×10^5, respectively, using three different soils with organic carbon contents between 2 and 5.5 per cent by weight (Environment Agency, 2009b and 2009c). The Koc values obtained in these studies are similar in all three soils which implies that the majority of the adsorption measured was associated with the organic phase of the soil, and so the log Kow value should be a good measure of the relative adsorption of these substances. Using the log Kow values for D4 (6.49), D5 (8.03), and D6 (8.87) it is possible to estimate, by linear extrapolation, that the actual Koc value for D6 should be around 2.23×10^5 l/kg (log Koc=5.35).

The REACH registration selected a value of log Koc of 6.03 based on the average of four values: 1) similarly generated by linear extrapolation from D4 and D5 data (but using an

estimated Kow for D6 rather than the measured value of 8.87); 2) linear solvation energy relationship (LSER) model using measured solvation descriptors; 3) the US-EPA EPI Suite estimation; and 4) correlation with measured/estimated log K_{ow} using models for hydrophobics, non-hydrophobics. Few other details are available. The log Koc of 6.03 (equivalent to a Koc of 1.1×10^6 l/kg) is consistent with the value determined by van Egmond *et al.* (2010).

The high Koc for D6 (2.2×10^5 to 1.5×10^6 l/kg) means that D6 adsorbs strongly to sediment and soil. Given that it is also of very low water solubility and highly volatile, leaching from soil is not expected to be a significant process in the environment.

3.2.2 Volatilisation

Several values for Henry's law constant (HLC) and the air-water partitioning coefficient (K_{aw}), modelled and measured, are available for D6. The UK risk assessment (Environment Agency, 2009a) selected an HLC value of 4,943,000 Pa m^3/mol at 25°C based on a K_{aw} of 1995, from modelling work by Xu *et al.* (2007) using four different methods to estimate log K_{aw} for D6, as follows:

- a bond-contribution method based on the directly measured log Kaw for D4 and D5 (2.69 and 3.13, respectively);
- calculated from the measured octanol–air partition coefficient (log Koa) for D6 (5.76) and the estimated log *K*ow for D6 (9.45, obtained using the HPLC method);
- estimated from the measured log *K*oa for D6 (5.76) and the log Kow for D6 (9.06, estimated by linear extrapolation from the measured log Kow of D4 and D5 based on the number of –(CH2)–Si–O– units); and
- calculated based on linear free energy relationships using measured solute descriptors for D6.

The resulting log K_{aw} values estimated at 25°C were:

- 3.57 by the bond contribution method;
- 3.69 estimated from log Koa and log Kow;
- 3.30 by the linear extrapolation method;
- 3.08 by the linear free energy relationship method.

Xu *et al*. (2007) recommend a log Kaw value of 3.30 (Kaw = 1995; as obtained from the linear extrapolation method, equivalent to 4,943,000 Pa m^3/mol) for D6 as this value is close to the average log Kaw from the four methods (the average log Kaw was 3.41).
The Henry's law constant can be estimated as 0.165 atm m3/mol (16,700 Pa m^3/mol; bond method) using HENRYWIN (v3.20) from the US-EPA EPI Suite (v4.1) estimation software. The value is estimated from the chemical structure using the bond contribution method.

The lead REACH registration selects a Kaw value of 1,023 at 23.6 °C (equivalent to 2,536,000 Pa m^3/mol). This was derived in the same study as the log Kow (Xu, 2009). The Kaw, Kow and Koa of ^{14}C-labelled D6 were simultaneously determined at room temperature. A custom-made glass apparatus was used for the test, which allowed for the establishment of an octanol/air/water three-phase equilibrium. Concentrations of the test substance in the three phases were analysed by LSC and HPLC/RAM.

An earlier study by Kochetkov et al (2001) determined dimensionless Henry's law constant values of 2.7 ± 0.2 (headspace method) and 5.9 ± 2.9 (vapour entry loop method). The REACH registration considered the analytical method used not suitable for the substance and so discounted these results (equivalent to a Henry's law constant of 6,712 Pa m^3/mol and 14,667 Pa m^3/mol, respectively).

Using a water solubility of 0.0053 mg/l at 23°C and a vapour pressure of 4.6 Pa at 25°C, the Henry's law constant can be estimated as 386,140 Pa m^3/mol. From the available data it is apparent that the Kochetkov *et al*. (2001) measured values are significantly lower than predicted on the basis of water solubility and vapour pressure alone, whereas the estimates from Xu *et al*. (2007) and measured data by Xu (2009) are slightly higher than this predicted value. The prediction of Henry's law constant from water solubility and vapour pressure is dependent on the substance showing ideal behaviour in solution; from the available data it is possible that this is not the case for D6.

The measured Henry's law constant of 2,536,000 Pa m^3/mol at 23.6 °C (based on a measured Kaw of 1,023; Xu (2009)) is selected in this report as it is consistent with the measured data available for both D4 and D5 (K_{aw}s equivalent to HLCs of 1,215,000 and 3,344,000 Pa m^3/mol, respectively), is consistent with other partition coefficients estimated for D6 and is from a reliable study.

3.2.3 Distribution modelling

Level III fugacity modelling for D6 (from US-EPA EPI Suite (v4.1)) using equal and continuous loading rates for air, soil, and water of 1000 kg/h and the inputs given in Table 10 and selected HLC shows environmental distributions of 6.75% in air, 30.1% in water, 9.93% in soil, and 53.3% in sediment. Due to its low water solubility, higher volatility and partitioning properties, D6 released into soil is expected to remain in soil (75.4%) and to volatilise (24.6%), D6 released into air is expected to remain in air (99.6%), while D6 released into water is expected to partition largely to the sediment (63.8%) with 36% remaining in the water.

A series of modelling studies has been carried out looking at the behaviour of D6 in various aquatic systems using local and regional modelling approaches. The studies are summarised in Table 11: Predicted persistence of D6 in water in various aquatic systems. They were carried out using the best measured data for the physico-chemical properties of D6 available at the time of the study, taking into account their known (or predicted) temperature dependence (for log K_{ow}, the air-water partition coefficient and the octanol-air partition coefficient). The variation of the predicted behaviour with temperature/season was investigated in some of the studies. The models were parameterised to reflect as closely as possible the particular environment being modelled, though the resulting predictions are subject to uncertainties resulting from the underlying assumptions and simplifications in the models.

The release rate of D6 into the water compartment of the model was generally based on a per capita release rate to waste water (taken from EA, 2009a; this essentially assumed that 10 per cent of the use in personal care products is released to waste water and 90 per cent of the use is released to air) and took into account the size of the population releasing into the environment being modelled, and the removal during waste water treatment.

With one exception no sensitivity analysis was carried out in the studies other than investigating the effect of temperature, and no predictions were made for known substances of concern. For the Whelan (2009d) study, a limited sensitivity analysis was carried out in relation to the predictions. This found that several key model outputs (for example the concentrations and persistence in sediment) were very sensitive to the organic carbon-water partition coefficient and the sedimentation velocity assumed in the model in particular.

Table 11: Predicted persistence of D6 in water in various aquatic systems

System	Model used	Main assumptions[1]	Main findings	Reference
Lake Pepin	Quantitative Water Air Sediment Interaction (QWASI Model). This is a steady-state non-equilibrium Level III fugacity model. The model was parameterised to reflect the properties of Lake Pepin.	Total D6 flux to lake 41-123 kg/year via waste water after waste water treatment (removal during waste water treatment assumed to be between 94 per cent and 98 per cent). The estimate was based on a population of 4,200,000 discharging into the river feeding the lake. Concentration of D6 in air was assumed to be constant at 10 ng/m^3. Degradation in water takes place by hydrolysis at pH 8 and 14°C (the mean annual water temperature in the lake) in the dissolved phase only. This results in a degradation half-life in water of 170 days and a degradation half-life in sediment of 96 years (the sediment half-lives were estimated at a temperature of 8°C which was considered to be more appropriate for sediment than the mean annual water temperature). log K_{oc} = 6.03 (at 25°C). log K_{ow} = 9.06 (at 25°C) or 8.83 (at 14°C). log K_{aw} = 2.68 (at 14°C). log K_{oa} = 6.15 (at 14°C).	The predicted total concentration in water and sediment are 0.82-2.5 ng/l and 41-123 µg/kg dry weight respectively. The fraction of the total steady-state mass in the lake is estimated to be distributed 6 per cent in the water phase and 94 per cent in the sediment phase. The persistence[2] in the model system was estimated by investigating the effect of the cessation of emissions after a certain time period. The persistence in sediment was estimated to be 126 days (approximate half-life of 87 days). The main driving force in this persistence estimate was sediment burial and re-suspension (a sediment burial flux of 14 g/m^2/day was assumed in the model to reflect the very high sediment accumulation rates in Lake Pepin). However, it should be noted that the recent sediment core data from Lake Pepin suggest a much longer half-life for D6 in sediment, although this may be because the QWASI model is relevant for surface sediments, not core strata. The persistence in the water column was found to be 8.92 days (approximate half-life 6.2 days) reflecting loss via advective outflow and volatilisation, along with hydrolysis to a lesser extent, and the overall persistence was estimated to be 71.7 days (approximate half-life 49.7 days).	Whelan (2009a)

Inner Oslofjord	Coastal Zone Model for Persistent Organic Pollutants (CoZMo-POP) and the Oslofjord POP model. Both models are multimedia fate and transport models The models were parameterised to reflect the properties of Oslofjord.	Total D6 flux via waste water 15.7 kg/year after waste water treatment (removal during waste water treatment was assumed to be 98 per cent for D6). This estimate was based on a population of 1,600,000 discharging into the catchment. Degradation in water takes place by hydrolysis in the dissolved phase only. The resulting degradation half-lives in water at 25°C were assumed to be 401 days at pH 7 and 40 days at pH 8. The equivalent values for sediment (at 25°C) were 522 years at pH 7 and 63 years at pH 8. log K_{oc} = 6.03 (at 25°C). log K_{ow} = 9.06 (at 25°C). Vapour pressure 2.2 Pa at 25°C. Although the above properties refer to 25°C the actual modelling was carried out using the known seasonal temperature variation in the water of Oslofjord. Three water compartments were assumed, freshwater/estuarine (temperature varied between ~0°C and ~16°C), open/coastal seawater (temperature varied between ~3°C and ~17°C) and deep seawater (at a constant temperature of approximately 7°C) (all temperatures are approximate here as they are read from a graph in the report).	The concentrations predicted were found to vary seasonally with water temperature reflecting the temperature dependence of hydrolysis and volatilisation (concentrations generally highest in the winter time and lowest in the late summer). The total concentrations in the water column were estimated to be below the levels that would be detectable analytically with current methods (<10 ng/l). The predicted concentrations of D6 in sediment were between around 1 and 8 µg/kg dry weight with the Oslofjord POP model and a maximum of 5 µg/kg dry weight with the CoZMo-POP model. The persistence of D6 was also investigated by modelling the decline in concentrations following cessation of emissions. The concentrations were found to decline rapidly in all compartments using the Oslofjord POP model. The CoZMo-POP model also predicted a rapid decline in the concentrations in water and estimated the dissipation half-life in sediment to be around 405 days, mainly as a result of sediment burial. Volatilisation was found to be the most important loss process from the water column, accounting for >50 per cent of the emissions.	Whelan (2009b)

Lake Ontario	QWASI Model adapted to Lake Ontario.	Total D6 flux to lake 250 kg/year via waste water after waste water treatment (removal during waste water treatment was assumed to be 94 per cent for D6). This estimate was based on a population of 7,135,800 discharging into the catchment. Concentration of D6 in air was assumed to be constant at 10 ng/m^3. Degradation in water takes place by hydrolysis at pH 8 and 9°C in the dissolved phase only. This results in a degradation half-life in water of 340 days and a degradation half-life in sediment of 96 years. log K_{oc} = 6.03 (at 25°C). log K_{ow} = 9.06 (at 25°C). Temperature correction was applied to partition coefficients.	The predicted concentrations in water and sediment were 0.07 ng/l and 7.9 µg/kg dry weight respectively. The fraction of the total steady-state mass in the lake is estimated to be distributed 30.8 per cent in the water phase and 69.2 per cent in the sediment phase. These data refer to 9°C. When the simulation was run at 2°C the predicted concentrations in water and sediment were 0.10 ng/l and 6.8 µg/kg dry weight respectively, and the percentage steady-state mass was distributed 41 per cent in the water phase and 59 per cent in the sediment phase. At 20°C the predicted concentrations were 0.032 ng/l in water and 6.2 µg/kg dry weight in the sediment, with 19.8 per cent of the steady-state mass in the water phase and 80.2 per cent in the sediment. The persistence in the model system was estimated by investigating the effect of the cessation of emissions after a certain time period. The persistence in sediment was estimated to be 2,983 days equivalent to a half-life of around 2,068 days) at all three temperatures. The main driving force in this persistence estimate was sediment burial and re-suspension. The persistence in the water column was found to range between 85 days at 20°C (summer) to 266 days at 2°C (winter) (equivalent to half-lives of 59 days (summer) and 184 days (winter). The overall persistence ranged between 385 days (summer) and 575 days (winter), equivalent to half-lives of 267 days (summer) and 399 days (winter).	Whelan (2009c)

Regional scale model system representing a freshwater – estuarine –coastal – open marine continuum	CoZMo-POP. The model was set up with environmental parameters consistent with the Baltic Proper.	Emissions to the environment were estimated on a per capita basis taking into account the population surrounding (and hence discharging to) the Baltic Proper. For this simulation it was assumed that the total emissions of D6 were the same as estimated for D5 (1,991.7 tonnes/year to air and 7 tonnes/year to water after waste water treatment) to allow the modelling results for D6 to be compared directly with those for D5. Degradation in water takes place by hydrolysis in the dissolved phase only. This results in degradation half-lives in water (at 25°C) of 401 days for freshwater (at pH 7) and 40 days for marine waters (at pH 8). A temperature correction was applied to the half-lives in the models. The half-lives in sediment were estimated to be 964 years for freshwater and 5 years for marine water. log K_{ow} = 9.06 (at 25°C). log K_{aw} = 3.3 (at 25°C). log K_{oa} = 5.76 (at 25°C). Temperature correction was applied to partition coefficients and the modelling was carried out using seasonal temperature profiles appropriate to the Baltic Proper.	Only limited modelling was carried out for D6 (the focus of the study was D5). It was estimated that the concentrations in sediment would decline rapidly after cessation of emissions, with the peak sediment concentrations being reduced to around 5 per cent of their steady-state concentrations within 2-3 years for coastal and open water sediments (but longer in deep water sediments).	Whelan (2009d)

Note: 1) K_{oc} = organic carbon-water partition coefficient.
K_{ow} = octanol-water partition coefficient.
K_{aw} = air-water partition coefficient.
K_{oa} = octanol-air partition coefficient.
2) Persistence is defined as the time taken for the concentration to fall to 1/e of its starting value, i.e. the environmental half-life ≈ 0.69×persistence.

3.2.4 Field data

No field data is available for the environmental distribution of this substance.

3.2.5 Summary and discussion of environmental distribution

D6 adsorbs strongly to sediment and soil (high Koc value 2.2×10^5 to 1.5×10^6 l/kg). Given that it is also of very low water solubility (5.3 µg/l at 23°C) and highly volatile (Henry's law constant of 2,536,000 Pa m^3/mol at 23.6 °C), leaching from soil is not expected to be a significant process in the environment.

Based on the Level III fugacity modelling, D6 equally released to air, soil and water is expected to partition largely to the sediment (53.3%) and the water (30.1%). Due to its low water solubility, higher volatility and partitioning properties, D6 released into soil is expected to remain in soil (75.4%) and to volatilise (24.6%); D6 released into air is expected to remain in air (99.6%); while D6 released into water is expected to partition largely to the sediment (63.8%) with 36% remaining in the water.

3.3 Data indicating potential for long-range transport

Information on potential for long-range transport of D6 is available, but the latter information was not assessed for this report.

3.4 Bioaccumulation

Several studies to investigate the accumulation of D6 are available. ^{14}C-D6 was used in some of the accumulation studies. In these experiments measurements of body burdens (and hence accumulation factors) based on total ^{14}C measurements may overestimate the actual accumulation of D6 (as such measurements may include contributions from metabolites) when compared with measurements based on parent substance analysis.

D6 has a log K_{ow} of 8.87-9.06. Therefore the substance meets the screening criteria for B and vB.

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

Several studies are available:

1) Annelin and Frye (1989) studied the uptake of D6 from water by fish. The D6 used in this test was not radiolabelled and was from a commercial source (no other information is available on the purity of the substance used). The study used a resaturation method (whereby the exposure solution was continuously passed through a column that contained sand coated with D6) to maintain a reasonably constant exposure concentration. The bioconcentration experiments were carried out with rainbow trout (*Oncorhynchus mykiss*) of approximately 0.7–2.3 g in size. The water used in the test had a hardness of 104 mg/l as $CaCO_3$, pH of 7.6, and a dissolved oxygen concentration of 8.0 mg/l. The exposure tank had a total volume of 120 l and the recirculation rate through the resaturation column was 10 l/hour. Exposure was for 35 days at 12 °C. Both the water phase and the fish were analysed for D6 using a gas–liquid chromatography (GLC) method. After 35 days' exposure, the concentration of D6 in the fish reached 1–2 mg/kg. The concentration of D6 in the water phase was below the limit of detection (<1 µg/l). Based on these data it is possible to estimate a BCF for D6 as >1 000 to >2 000 l/kg based on parent substance measurements.

Given the paucity of information available for the study and lack of quantification in the water, the results of the study cannot be used reliably.

2) The bioconcentration of D6 was also investigated using fathead minnows (*Pimephales promelas*) (Drottar, 2005). The study was carried out at 22 °C according to OECD Guideline 305 using a flow-through system with a 49-day exposure period and a 98-day depuration period. The substance tested was ^{14}C-D6 with a radiochemical purity of 99.57 per cent. Two concentrations of D6 were tested [mean measured concentrations (± standard deviation) of 0.41 ± 0.029 µg/l and 4.4 ± 0.23 µg/l] and no treatment-related signs of toxicity occurred throughout the test. Stock solutions of the test substance were prepared in dimethylformamide and delivered to sealed mixing chambers (at a flow rate of 0.060 ml/minute), in which they were mixed with dilution water [dechlorinated tap water (hardness ~120 mg/l as $CaCO_3$, pH 7.6–8.6) at a flow rate of 600 ml/minute). The concentration of DMF in the test vessel was 0.1 ml/l and a solvent control was run at this concentration. Two replicates were carried out for each treatment level. At various times during the test, four fish per treatment group (or two for the control) were analysed for D6 by total radioactivity measurements. The tissue concentrations of D6 (measured as total radioactivity) reached steady state after 35 days of exposure (no statistically significant difference ($p = 0.05$) was found in the tissue concentrations measured on days 35, 42, and 49) and the steady-state BCF (based on total ^{14}C measurements) was 1160 l/kg for the 0.41 **µg/l** treatment and 240 l/kg for the 4.4 **µg/l** treatment, based on the mean tissue concentrations measured between day 35 and day 49. Around 79 per cent of the body burden was found to be present as the parent substance, which implies that the BCF based on parent substance may be lower than that based on total ^{14}C measurements. However, for this study the concentration in water was also based on total ^{14}C measurements, which may overestimate the concentration of the parent substance in the water phase if excreted metabolites are in the water (no information is available on the fraction of the radioactivity in the water phase that was parent substance). Thus, taking into account that 79 per cent of the radioactivity in the fish was parent substance, it is estimated that the BCF based on parent substance alone is **≥916** l/kg for the 0.41 **µg/l** treatment and **≥190** l/kg for the 4.4 **µg/l** treatment. Depuration of the accumulated radioactivity was slow [the first-order rate constants for depuration ranged between 0.0233/day (0.41 µg/l treatment group) and 0.0260 day (4.4 µg/l treatment group)]. These are equivalent to depuration half-lives of 27–30 days. The corresponding first-order rate constants for the uptake phase of the study were 38.8 day^{-1} (for the 0.41 µg/l treatment) and 8.29 day^{-1} (for the 4.4 µg/l treatment). Based on the kinetic data, a BCF (based on total ^{14}C measurements) of 1 660 l/kg (for the 0.41 **µg/l** treatment) and 319 l/kg (for the 4.4 **µg/l** treatment) is estimated (the equivalent BCFs corrected for the fraction of the total radioactivity in the fish that was parent substance are **≥1** 311 l/kg and **≥252** l/kg, respectively). These kinetic data support the BCFs above based on the steady-state measured body burdens. The fish used in this test had a mean lipid content [based on the analysis of a subset of six individuals (two each from the controls, and low- and high treatment groups)] of 4.5 per cent (range 3.12–5.27 per cent) at day 0, 2.9 per cent (range 1.76–5.47 per cent) at the end of the uptake phase (day 49), and 4.5 per cent (range 3.05–6.17 per cent) at the end of the depuration phase (day 147). The higher concentration tested in this study (4.4 µg/l) is very close to the water solubility of the test substance (5.3 µg/l). Although the test concentration was adequately maintained at this level, the analytical methodology used involved collection of the water samples from mid-depth using a pipette and analysing the water samples directly by scintillation counting. Thus, the measured levels represent total levels of D6 and not necessarily dissolved concentrations only. It is therefore possible that, at the higher concentration tested, some of the D6 may not have been present in the dissolved phase (which may explain why a generally lower level of accumulation was found at the higher test concentration compared with the lower test concentration). For this reason, the result obtained at

steady state for the 0.41 µg/l treatment (i.e. a BCF of 1 160 l/kg based on total ^{14}C measurements) is considered to be the most representative value for the BCF of D6 from this study. This study and result is selected as the key data in the REACH lead registration.

3) CERI (2010) measured the BCF of D6 in common carp (*Cyprinus carpio*). The original study report is in Japanese but the report was translated into English. The study was conducted according to OECD TG 305 with an uptake period of 60 days and a depuration period of 32 days. The fish were between 6.5 and 11.9 cm in length at the start of the test. The test was carried out at 24-25 °C. The dissolved oxygen concentration was between 5.6 and 7.8 mg/l and the pH was between 7.6 and 8.0. Fish were sampled in duplicate from each treatment group during the uptake and depuration period of the test.

Two test concentrations were used, nominally 0.1 and 1 µg/l. A 1 000 mg/l stock solution of the test substance was firstly prepared in 2-propanol containing a dispersant (HCO-40 at a concentration 10 times that of the test substance) and this was then diluted in 2-propanol to give stock solutions of 50 mg/l test substance (containing 500 mg/l HCO-40) for the higher exposure concentration and 5 mg/l test substance (containing 50 mg/l HCO-40) for the lower exposure concentration. The test was carried out using 70 litre glass aquaria and the flow rate used in the test was 2,000 ml/min (~2,800 l/day) dilution water and 0.04 ml/min of the relevant stock solution. The actual exposure concentration was verified analytically at intervals during the uptake period and the concentrations measured are summarised in Table 12: Bioconcentration of D6 by *Cyprinus carpio*. The mean measured concentrations were 0.086 µg/l (86% of nominal) and 0.91 µg/l (91% of nominal) for the low and high treatment groups respectively. The data show that the exposure concentrations were adequately maintained throughout the uptake phase of the experiment. A solvent control was also run.

The reported BCF values are also summarised in Table 12: Bioconcentration of D6 by *Cyprinus carpio*; it should be noted that these were determined using the mean water concentration for the time period corresponding to the exposure day (i.e. the average concentration in water between the sampling time and the previous sampling time) rather than the overall mean water concentration over the entire study. However, as can be seen from Table 12: Bioconcentration of D6 by *Cyprinus carpio*, the water concentration was reasonably constant over the entire exposure period and so essentially the same values would be obtained using the overall mean water concentration. The data also show that steady-state was reached after around 34 days' exposure (the individual mean values at days 34, 47 and 60 were within 1 – 12 % of the overall mean value for days 34-60). The mean steady-state BCF values based on the values determined after 34 days were 4 042 l/kg at the lower exposure concentration and 2 344 l/kg at the higher exposure concentration. The main test report did not contain the raw fish concentration data themselves but these have very recently been received from the Japanese authorities (personal communication, 2017a).

Table 12: Bioconcentration of D6 by *Cyprinus carpio*

Exposure time (days)	Measured concentration in water **(µg/l)**		BCF (l/kg)	
	A	B	A	B
7	0.0855	0.878		
11	0.0806	0.827	2 400 2 900	1 400 1 500
20	0.0883	0.896	3 100 3 300	1 800 1 700
34	0.0872	1.02	3 900 3 400	2 200 2 000
47	0.0868	0.882	4 700 4 400	2 600 2 500
60	0.0896	0.945	4 100 3 800	2 500 2 300
Overall mean (±standard deviation)	0.0863 (±0.003)	0.908 (±0.067)		
Mean day 34 to 60 (±standard deviation)	0.0879 (±0.002)	0.949 (±0.069)	4 042 (±453)	2 344 (±213)

Notes: A) Nominal 0.1 µg/l treatment group.
B) Nominal 1 µg/l treatment group

Depuration of the substance from the fish was studied following the 60-day uptake period. This is reported as a separate part of the study, and commenced on day-62. The report indicates that the depuration half-life was 25 days for each exposure concentration (notably greater than those for D4 and slightly greater than D5 measured in similar studies). The main report provides depuration data as a percentage of the steady-state concentration remaining in the fish after 1, 5, 15 and 32 days depuration, and these are summarised in Table 13: Retention rate in elimination test (% of steady-state concentration remaining).

Table 13: Retention rate in elimination test (% of steady-state concentration remaining)

Nominal concentration (mg/l)	After 1 day	After 5 days	After 15 days	After 32 days
0.1	112	78	75	42
	116	86	73	47
1.0	89	84	61	49
	99	117	57	37

Fish concentration data was used to calculate the depuration rate constants as shown in Figure 1 and Figure 2. The overall depuration rate constants have been determined as 0.0273 day^{-1} at the lower exposure concentration and 0.0279 day^{-1} at the higher concentration. These rate constants are equivalent to an overall depuration half-life of 24.8 to 25.4 days (which are in agreement with the quoted elimination half-lives in the test report).

Figure 1: Depuration plot for low concentration (no growth correction)

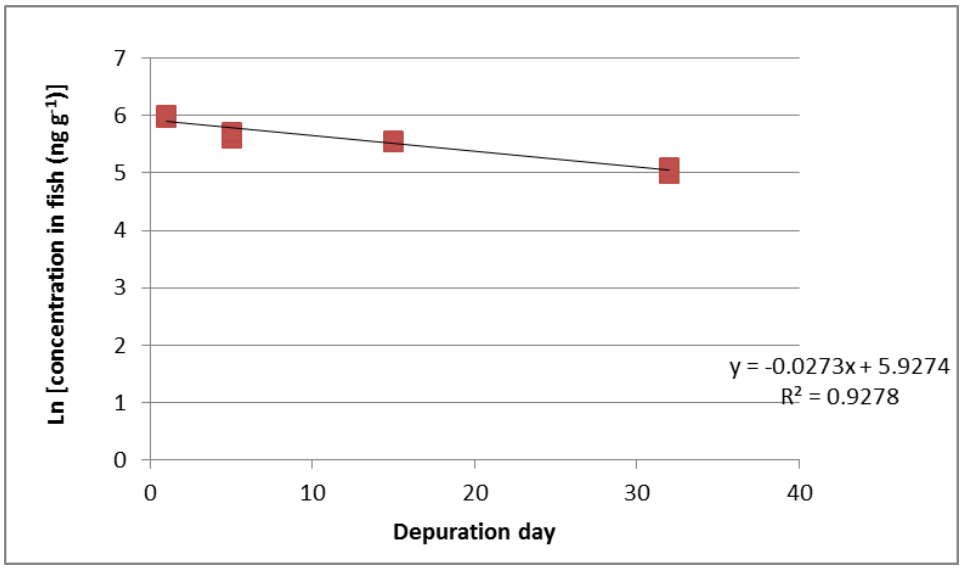
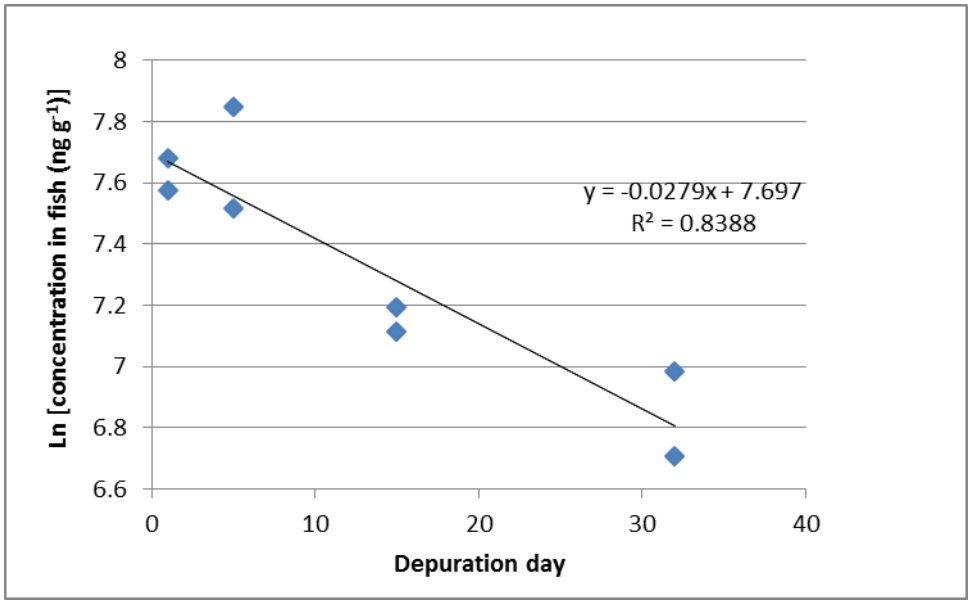


Figure 2: Depuration plot for high concentration (no growth correction)



Obtaining a kinetic BCF value

Kinetic fitting of the data has been performed using sequential and simultaneous fitting. Sequential fitting used linear regression to obtain the k2 values as described above, with the k_1 value then determined sequentially in the usual way. The results of this are provided in Table 14: Results of the sequential fitting of the data without growth or lipid correction with the plots of the sequential fit provided in Figure 3 and Figure 4.

Table 14: Results of the sequential fitting of the data without growth or lipid correction

Nominal concentration (mg/l)	k_1	k_2	BCF_k (l/kg)
0.1	164	0.0273	6010
1.0	96.2	0.0279	3451

Figure 3: Low concentration (0.1 µg/l nominal) sequential fit

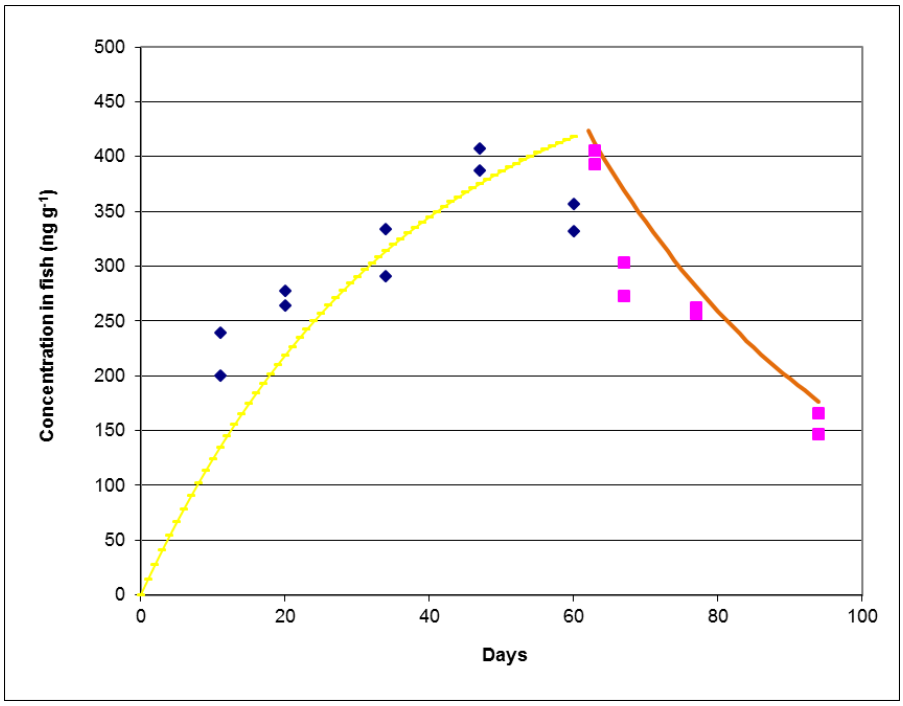
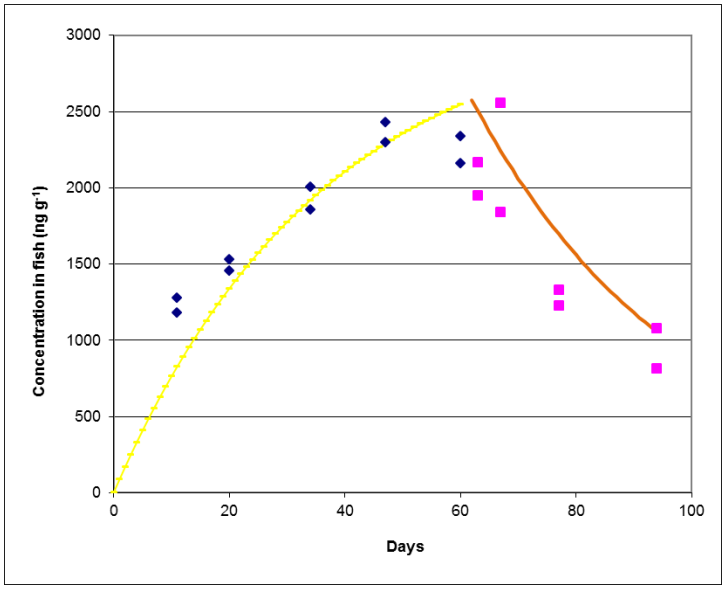


Figure 4: High concentration (1 µg/l nominal) sequential fit



Simultaneous fitting of the kinetic BCF was performed using the bcmfr package[1] (v0.3-2) in the R programme. The package offers three options for data transformation: untransformed, log, and Box-Cox. For details, see outputs in Annex IV. For both concentrations the outputs of these options were reviewed:

For the lower concentration, the Box-Cox transformation is preferred due to better diagnostics and main fit than the untransformed or log-transformed data. It does have a slightly wider standard error.

[1] Available as part of the OECD TG 305 fish bioaccumulation guidance (OECD, 2017)

For the high concentration the untransformed data is preferred as neither of the transformations were better, although the Box-Cox transformation performs similarly. It is noted that the actual results are little different from the three options despite for example the log-transformation fit and residual plots suggesting that this should not be preferred.

The results of the chosen fits are shown in Table 15 with Figure 5 and Figure 6 providing the simultaneous fits, together with 95th percentile confidence intervals.

Table 15: Results of simultaneous fitting of the data without growth or lipid correction

Nominal concentration (mg/l)	k_1	k_2	BCF_k (l/kg)
0.1 95 % CI	232 177 - 287	0.049 0.035 – 0.064	4692 4334 - 5051
1.0 95 % CI	116 94 - 138	0.041 0.031 – 0.05	2860 2615 - 3105

Figure 5: Simultaneous fit for the low concentration (0.1 µg/l nominal)

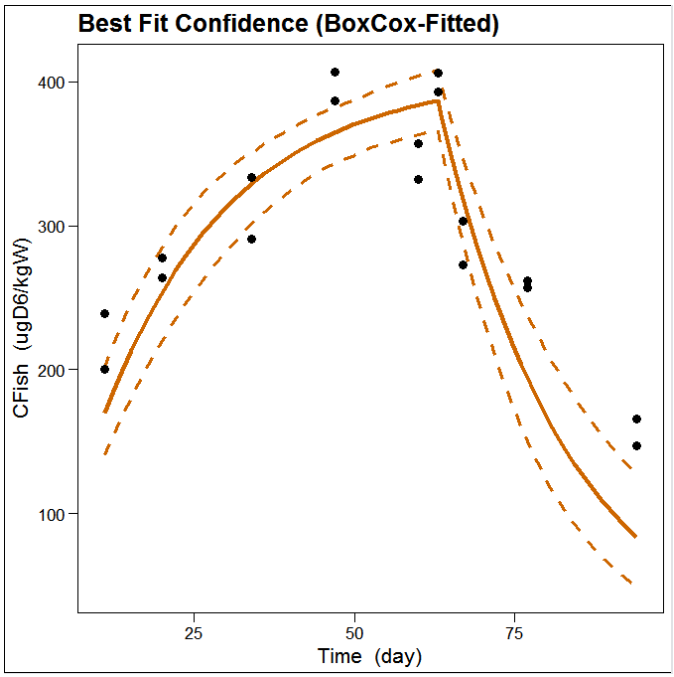
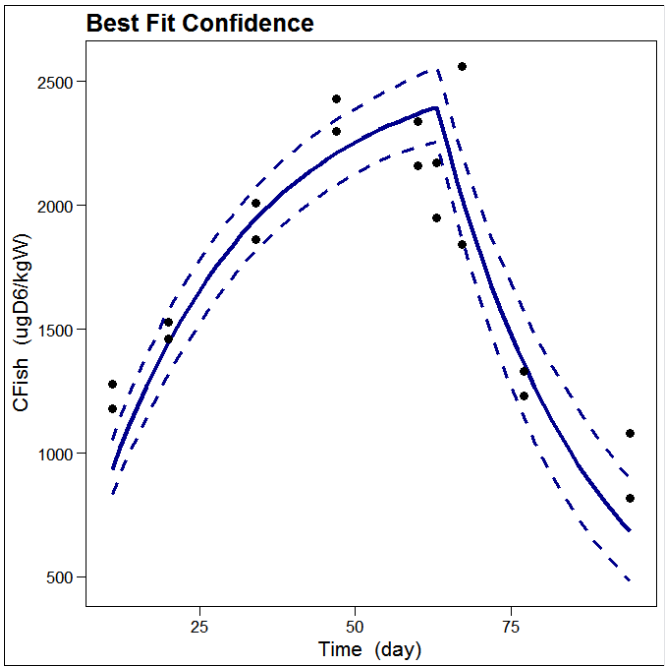


Figure 6: Simultaneous fit for the high concentration (1 µg/l nominal)

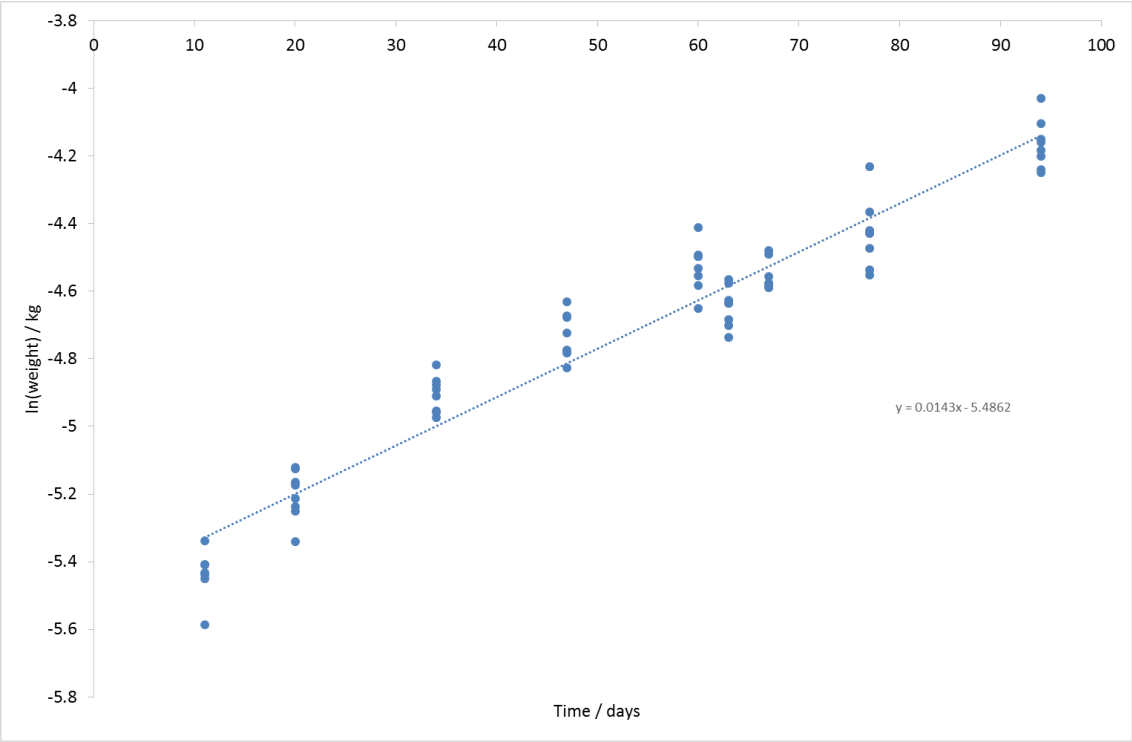


When deriving the results from BCF tests it is also relevant to consider the effects of lipid normalisation (i.e. normalising the results to a standardised lipid content of 5 %) and correcting for growth dilution. In the CERI (2010) study the lipid content of the fish was 4.85 % at the start of the test and 5.78 % at the end of the depuration period. Lipid normalisation can therefore be neglected in this case because these values are so close to the "standard" lipid content (the mean lipid content over the two sampling points is 5.3 %; normalising to 5 % would lead to BCF values that are 94 % of the reported values).

Of greater importance is growth correction of the data. On request, the Japanese authorities have provided the fish weight data (personal communication, 2017b) for days 11, 20, 34, 47 and 60 during uptake, and days 1, 5, 15 and 32 of depuration (no weight information is available from the start of the test). This has been used to determine the growth rate constant for the study using a linear plot of ln(fish weight) versus time (). Individual plots of uptake and depuration growth rates indicated little difference (the latter is marginally smaller)[2]. Similarly there was little difference between the growth rates for the two BCF concentrations. On this basis the growth rate (k_g) used to correct k_2 is calculated from all fish weight data, and is 0.0148 d^{-1} (95th percentile CI: 0.0140 – 0.0157).

[2] This is different to the D4 study where little growth occurred during depuration for Carp study.

Figure 7: Plot of fish growth (based on mass) during the test



Growth correction of the sequential fit gave k_2 values of 0.0130 d^{-1} and 0.0136 d^{-1}. These are equivalent to depuration half-lives of 53 and 51 days, respectively, which are more than double the uncorrected values. For comparison the growth-corrected simultaneously fitted depuration values are 0.035 and 0.026 d^{-1} for the lower and upper concentrations, which equate to half-lives of 20 and 26 days respectively. Therefore the results from the two kinetic fitting methods have quite different uptake rate constants and depuration rate constants

Table 16: Summary of BCF values derived from different fits provides a summary of the final BCF values that have been derived from the original study report. There is currently no agreed method to growth-correct steady-state BCF values. As growth appears to account for at least half of the elimination, the steady-state BCF values derived in the study report under-estimate the bioaccumulation potential of D6. This means the steady-state values should be given a low weight in the bioaccumulation assessment.

Table 16: Summary of BCF values derived from different fits

Nominal concentration (mg/l)	BCF_k sequential fitting (growth corrected) (l/kg)	BCF_k simultaneous fitting (growth corrected) (l/kg)	BCFss (not growth corrected) (l/kg)
0.1 95 % CI	12632	6605 5411 - 7799	4 042
1.0 95 % CI	7071	4419 3571 - 5266	2 344

The difference in the rate constants between the simultaneous and sequential fits is also reflected in the different BCF values. Nevertheless, the growth-corrected 'low concentration' BCF_k values for both simultaneous and sequential fitting significantly exceed 5 000 L/kg. At the high concentration, the sequentially fitted BCF_k significantly exceeds 5 000 L/kg, but the simultaneously fitted BCF_k does not, although it is close to the threshold.

The OECD guidance indicates that generally the simultaneous kinetic fit is preferred to the sequential fit (OECD, 2017). However in this instance it is noted that:

Uptake of the substance in fish drops off at the final measurement during uptake. There is a delay between the final uptake measurement (and exposure continues) and the start of depuration in clean water.

Neither aspect affects the determination of the depuration value in the sequential fitting, but will affect the depuration value derived in the simultaneous fit, (where both uptake and depuration constants are derived at the same time). This can be seen in the simultaneous fits where the final fish concentrations during depuration are above the fitted line.

Regardless of whether sequential or simultaneous fitting is preferred, Table 9 and Table 15: Results of simultaneous fitting of the data without growth or lipid correction show that while the depuration rates are broadly consistent at the low and high concentrations for a particular kinetic fit, uptake at the high concentration is slower than at the low concentration. One reason could be that at the higher concentration the substance was close to saturation in the test medium, reducing the availability of the test substance. However, the higher concentration is only 17 % of the solubility in pure water[3]. A solubiliser (HCO-40) was used in the study for both concentrations, but it is not possible to determine how this affected bioavailability. A further possible explanation could be some form of adverse effect at the higher concentration that reduced uptake in the fish, although no such observation is mentioned in the report.

Overall, the study appears to be well carried out and is considered to be valid. The kinetic BCF values of > 5 000 L/kg is considered to be reliable, with preference given to the sequential BCF values as the fitting appears to be less affected by the delay in depuration commencing and drop in fish concentration towards the end of uptake. In any case, using the sequential or the simultaneous fitting method will not change the overall B/vB conclusion for D6 as the kinetic BCF values are above 5000 L/Kg.

Opperhuizen et al. (1987) studied the uptake and elimination of D6 in guppies (*Poecilia reticulata*) and goldfish (*Carassius auratus*) through exposure via water or food. The exposures were to a mixture of cyclic siloxane oligomers (ranging from D3 to D9) and linear oligomers (ranging from hexamethyldisiloxane to hexadecylmethylheptasiloxane). The substances tested were from commercial sources and were not radiolabelled (no other information is available on the purity of the substances used). The analytical method used involved analysis of the parent substances by gas chromatography equipped with a flame ionization detector or a mass spectrometer. The spiked food was prepared by adding a solution of the test substances in pentane to the food and evaporation of the solvent. The concentrations in food that resulted were stated to be in the range 306 – 425 mg/kg for the cyclic oligomers in the goldfish experiments and 1 008 – 1 044 mg/kg in the guppy experiments, but when displayed graphically in the paper these concentrations appear to be around 1 mg/kg. No information is given in the paper on whether freshly spiked food was prepared at regular intervals during the experiment or how stable the concentrations were on storage of the food. For the water-exposure experiments a saturated solution of the test substances was prepared using a continuous-flow saturation system. However, a film of test substance was always present on the surface of the water when solutions were prepared in this manner. The saturated solution was continuously circulated through the exposure vessels during the experiment. The actual concentrations in the test vessels are not reported. The water exposure experiments were only carried out with guppies. The guppies used in the test had an average weight and lipid content of 0.17 g and 6.5 per

[3] The temperature of the water solubility test was 23 °C compared to 24-25 °C for the bioconcentration study, so this is unlikely to have made a significant difference.

cent, respectively (for the goldfish these were 1.8 g and 2.3 per cent, respectively). The tests were carried out at 22 °C using a mixture of 50 per cent tap water and 50 per cent demineralised water. The water was continuously aerated during the dietary exposure experiments and in the water-exposure experiments it was aerated with pure oxygen added via a capillary tube. In the feeding experiments, the feeding rate used was 25 mg/g each day and the exposure period was for up to 12 weeks. In the water-exposure experiments the fish were exposed for 20 days. In all cases the exposed fish were placed on a clean diet and in clean water after the exposure period to monitor the depuration of the accumulated chemicals.

4) Uptake of the cyclic oligomers occurred in both the water-exposure experiments and the dietary exposure experiments. For D6 the steady-state BCF was 1 200 l/kg and the steady-state biomagnification factor (BMF) from the food experiment was 0.06 for guppies (similar results were stated for goldfish). These values are based on parent-substance analysis. The depuration half-life was around 4.3 days. Given the uncertainties over the exposure concentrations discussed above, these values should be treated with caution. Opperhuizen et al. (1987) also carried out a similar experiment in which fish were exposing to either a single linear oligomer (hexadecylmethylheptasiloxane) or a single cyclic oligomer (D7). Some of these experiments provide evidence that cVMS (ranging from D5 to D9) form in fish, but it cannot be established whether this was the result of impurities in the materials, or whether such materials were formed by transformation in the water phase followed by subsequent uptake, or by metabolic processes in the fish.

5) Bruggeman et al. (1984) attempted to determine the dietary uptake of D6 by guppies (*Po. reticulata*). The substance tested was not radiolabelled and was from a commercial source (no other information on the purity of the substance tested is given). The analytical method used in the study was gas chromatography with flame ionisation detection or mass spectrometric detection (parent-substance analysis). A standard mixture of linear and cyclic siloxane oligomers (including D6) together with several chlorinated benzenes and chlorinated biphenyls (as reference substances) was prepared in toluene (concentration of each component was 250 µg/ml). The spiked food (commercial dry fish food; lipid content 10 per cent of dry weight) was prepared by adding 3 ml of the toluene solution to food and evaporating the solvent. This led to an initial measured concentration of D6 in the food of between 20 and 50 mg/kg food (the more volatile siloxanes tested completely evaporated from the food during sample preparation). The dietary exposure tests were carried out using 110 male guppies (lipid content 1.7 per cent of wet weight). These were fed the spiked food at a rate of ~20 mg dry food/g wet weight of fish each day for up to ten weeks. Samples of six fish were collected for analysis each week (after a period of two days without feeding). The detection limit for D6 in the fish was around 0.3 mg/kg wet weight. D6 was not detected in the fish during the course of this study and a magnification factor [defined as the concentration in fish (on a lipid basis) divided by the concentration in food (on a lipid basis)] was <0.03 based on parent-substance analysis.

There appear to be several shortcomings in this experiment, not least that preparation of the spiked food would have allowed loss via evaporation of D6 and, although the concentrations initially in the spiked food appear to be verified analytically, no details are given on the frequency of the preparation of the spiked food, the repeatability of the spiking of the food, or whether the concentration of D6 in the spiked food was maintained during any storage, etc. Therefore, there is a large amount of uncertainty over the actual exposure concentration of D6 during the course of this experiment.

6) The bioconcentration of D6 by invertebrates (*Daphnia magna*) was also investigated (Dow Corning, 1985). Few details of the test are available, but it was carried out over 32 days using a saturated solution of radiolabelled D6 (concentration given as 0.004 ppm, measured, in the REACH registration) prepared by a re-saturation system. The organisms used were reportedly a mixed-age culture and no control culture appears

to have been used. The steady-state BCF for D6 was ~2 400 l/kg. It appears this value is based on total ^{14}C analysis or parent-substance analysis, and so may include a contribution from formed metabolites or radiolabel impurities present in the test material.

7) A study in a sediment dwelling invertebrate (*Lumbriculus variegatus*) is available in the REACH registration (Wildlife International, 2008). This was conducted according to ASTM E 1706 with 28 days exposure with a semi-static exposure regime and 30 days depuration. The test substance used was as described in section 1.2 (no other details are available). Nominal concentrations of 100 and 1000 mg active ingredient/kg were spiked to artificial sediment (mean measured concentrations were 28.1 and 484 mg a.i./kg based on dry weight of sediment). Table 17: Accumulation of D6 by *Lumbriculus variegatus* below gives details of measured concentrations during the uptake phase in the sediment and organisms' tissue, the derived steady-state BAF and the kinetic BAF for both of the concentration groups.

Table 17: Accumulation of D6 by *Lumbriculus variegatus*

Treatment group	Parameter	Duration of exposure			
		0 days	14 days	28 days	Depuration 14 days
Negative control	Concentration in the sediment (mg a.i./kg; two samples)	<10.5; <9.16	<8.34; <8.36	<9.91; <10.0	
	Concentration in tissue samples (mg a.i./kg)	<0.423	<0.515	<0.820	<0.457
Nominal concentration 100 mg a.i./kg	Concentration in the sediment (mg a.i./kg); three samples)	31.7; 28.2; 39.6	27.1; 33.2; 41.3	17.8; 19.6; 14.5	
	Concentration in tissue samples (mg a.i./kg)		17.8	18.5	2.85
	Bioaccumulation factor			0.66	
	Kinetic bioaccumulation factor				0.67 (k1 = 0.090; k2 = 0.134)
Nominal concentration 1000 mg a.i./kg	Concentration in the sediment (mg a.i./kg); three samples)	668; 558; 645	412; 592; 702	314; 274; 187	
	Concentration in tissue samples (mg a.i./kg)		66.2	33.7	3.13
	Bioaccumulation factor			0.070	
	Kinetic bioaccumulation factor				0.070 (k1 = 0.012; k2 = 0.170)

The half-life for the substance in the test organism was calculated as 5.2 and 4.1 days for the low and high treatments, respectively. The time to achieve 90% of steady state was calculated as 17.2 and 13.6 days for the low and high treatments, respectively. All replicates appeared normal during the test, with a few observations of abnormal behaviour in the treatments and control group but these were concluded to be non-treatment related. The number of worms increased during the test indicating that reproduction had occurred. Few other details are available in the registration on this study.

Benchmarking fish bioaccumulation data
Kinetic BCF values for D6 in common carp are comparable to those for D5, but greater than D4. However, the depuration half-life of D6 is longer than either D4 or D5. Fish growth was found to be significant in the D4 study, and suspected to be significant in the D5 study, although no data were available. Growth was also significant in the D6 study. The D5 results were lipid normalised, but. D4 and D6 results were not as the lipid content was around 5%.

In the tests using Fathead minnow, only the steady-state BCF values were considered fully reliable for all three chemicals. When those results are compared, the values for D4 and D5 significantly exceed those for D6. The depuration rate constant for D6 is similar to D5, which might suggest that D6 was less bioavailable in the test (i.e. uptake was lower). However, there is some uncertainty associated with these kinetic values. None of these studies included growth correction. It was noted to be significant in the D5 study, but due to the limitation with the kinetics a growth correction could not be applied. None of the results are lipid normalised.

Table 18 provides a comparison of the key fish bioaccumulation data for the main cVMS.
Kinetic BCF values for D6 in common carp are comparable to those for D5, but greater than D4. However, the depuration half-life of D6 is longer than either D4 or D5. Fish growth was found to be significant in the D4 study, and suspected to be significant in the D5 study, although no data were available. Growth was also significant in the D6 study. The D5 results were lipid normalised, but. D4 and D6 results were not as the lipid content was around 5%.

In the tests using Fathead minnow, only the steady-state BCF values were considered fully reliable for all three chemicals. When those results are compared, the values for D4 and D5 significantly exceed those for D6. The depuration rate constant for D6 is similar to D5, which might suggest that D6 was less bioavailable in the test (i.e. uptake was lower). However, there is some uncertainty associated with these kinetic values. None of these studies included growth correction. It was noted to be significant in the D5 study, but due to the limitation with the kinetics a growth correction could not be applied. None of the results are lipid normalised.

Table 18: Comparison of key fish bioaccumulation data for D4, D5 and D6

	D4		D5		D6	
	BCF (l/kg)	$t_{1/2}$ (days)	BCF (l/kg)	$t_{1/2}$ (days)	BCF (l/kg)	$t_{1/2}$ (days)
Common carp	SS: 3 000 – 4 000 K: 4 100 – 5 500 (gc)	6.5-8.8	SS: 10 550 – 11 048 K: 12 566 - 14 009 (not gc)	19-22	SS: 4 042 & 2 344 Kseq: 12632 & 7071 (gc) Ksim: 6605 & 4419 (gc)	Seq. 51-53 (gc) Sim. 20-26 (gc)
	BMF study	30 (gc)	BMF study	30 (gc)	-	
Fathead minnow*	SS: ≥11 495 K: 14 900 (not gc)	3.8 (not gc)	SS: 5 860 (K: 11 039 & 4 358, not gc)	(24-39) (not gc)	SS: 1 160 (K: 1 330, not gc)	(30, not gc)
Rainbow trout	BMF study	105 (gc)	BMF study	74 (gc)	-	

SS = steady-state; K = kinetic (assumed to be sequential unless noted otherwise); (gc) = growth corrected;

Data in parentheses indicate uncertainty noted in previous assessments;
* total radioactivity adjusted for parent was used to calculate the BCF and $t_{1/2}$.

A benchmarking overview with a broader set of PBT/vPvB substances has been included in Annex III, Table 45. As can be seen from the Table 45, the concentration range for D6 is within the range of whole fish concentrations generally achieved for substances with vB properties.

3.4.2 Bioaccumulation in terrestrial organisms (soil dwelling organisms, vertebrates)

No data available.

3.4.3 Field data

Several field studies investigating the bioaccumulation of D6 have been carried out. It should be noted that there is a lack of agreed guidelines and methodologies for carrying out such studies, and interpretation of such studies encompasses several uncertainties (see section.11.4.1.2.6 of ECHA Guidance on PBT/vPvB assessment[4]) It should also be noted that although the ECHA Guidance document indicates that the results from such field studies should be considered as part of the overall evaluation of the data, Chapter R.11.4.1.2 of the Guidance indicates that the absence of a biomagnification potential cannot be used on its own to conclude that the B or vB criteria are not fulfilled. The new data are summarised below.

Trophic magnification

Eight food chains (Lake Pepin, Lake Opeongo, Oslofjord, Lake Mjøso (two studies), Lake Erie, Tokyo Bay, Dalian Bay and Lake Champlain) have been investigated in some detail.

- The bioaccumulation of D6 has been studied in a natural freshwater aquatic food chain in Lake Pepin, Upper Mississippi River, Minnesota, USA (44°29'N 92°18'W) (Powell *et al.*, 2009a). The lake has a surface area of 102.7 km^2, a length of 33.5 km and a mean depth of 5.4 m. The hydraulic residence time of the lake ranges from around 6 days (high flow) to 47 days (low flow). The lake is around 80 km downstream of the cities of Minneapolis and Saint Paul (estimated population of 3.2 million in 2006). The lake acts as a sink for sediment-associated contaminants from the inflowing river and sediment accumulation rates range from 20-30 kg/m^2/year in the upstream end of the lake to 3-5 kg/m^2/year in the downstream end of the lake.

  The food chain considered included surface sediment, benthic macroinvertebrates (two genera, two families) and 15 fish species (14 genera, 9 families). The fish were collected on the 4th and 5th September 2007 and the surface sediments and benthic macroinvertebrates were collected between the 20th and 22nd May 2008 (the influence of temporal differences in exposure conditions is unknown). The fish were collected in near-shore areas of the lake (apparently over most of the length of the lake; since fish move the sampling location does not necessarily reflect where they are exposed), and sediment and benthic macroinvertebrates were collected from 25 locations along five shore-to-shore transects positioned perpendicular to the flow axis of the lake. Small

[4] https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

fish and macroinvertebrates were pooled into composite samples for each species whereas large fish were analysed as individuals. A rigorous quality control procedure was implemented during the sampling and analysis to minimise contamination of the samples. This included field blanks and field spiked samples for sediment and laboratory blanks for sediment and fish. The measured concentrations were corrected for background levels found in laboratory blanks.

Trophic level (TL) of the organisms was determined by means of $\delta^{15}N$ measurements[5] and ranged from TL ~2.0 (benthic detrivores such as *Chironomus* sp. and *Hexagenia* sp.) to TL ~3.7 (pelagic piscivores such as largemouth bass and walleye). The trophic levels, and concentrations found, are summarised in Table 19: Accumulation of D6 in the Lake Pepin food chain. The following points should be noted in relation to the concentrations found and the limit of detection (LOD), method detection limit (MDL) and limit of quantification (LOQ)[6].

- The concentrations of D6 in the sediment were all greater than the MDL but were less than the LOQ in all 25 samples.
- The concentrations of D6 in the benthic invertebrates were all greater than the MDL but were less than the LOQ for mayfly.
- The concentrations of D6 in fish were less than the MDL in 6 out of 16 species and less than the LOQ in 11 out of 16 species.

A plot of the natural logarithm (ln) of the mean measured concentrations (on a lipid weight basis) against the trophic level is shown in Figure 8. The antilog of the slope of the regression line gives the Trophic Magnification Factor (TMF). The TMF for D6 in this food web can therefore be estimated to be around 0.28 based on the mean measured lipid normalised concentrations. The TMF value quoted in Powell *et al.* (2009a) is slightly smaller than this value (TMF 0.18) and this value was derived based on a regression using all 52 individual observations rather than the mean values per species. As the value derived by Powell *et al.* (2009a) is based on each individual data point it is preferred over the TMF derived from the mean concentration for each species in Figure 8 as it minimises errors associated with unbalanced sampling (for example different numbers of organisms were collected for each species)[7]. Powell *et al.* (2009a) estimate a further TMF of 0.11 using trophic guilds (here the data were assigned to one of six trophic guilds[8] and the mean value per trophic guild used in the regression). Based on these analyses, the TMF for D6 is clearly less than 1 in this food web, and lies in the approximate range 0.1-0.2.

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[5] $\delta^{15}N = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$ where R_{sample} is the $^{15}N/^{14}N$ abundance (in parts per thousand) in the sample and $R_{standard}$ is the $^{15}N/^{14}N$ abundance in a standard (atmospheric nitrogen gas). The trophic level of a consumer is defined as follows, assuming the trophic level of midge larvae is 2:

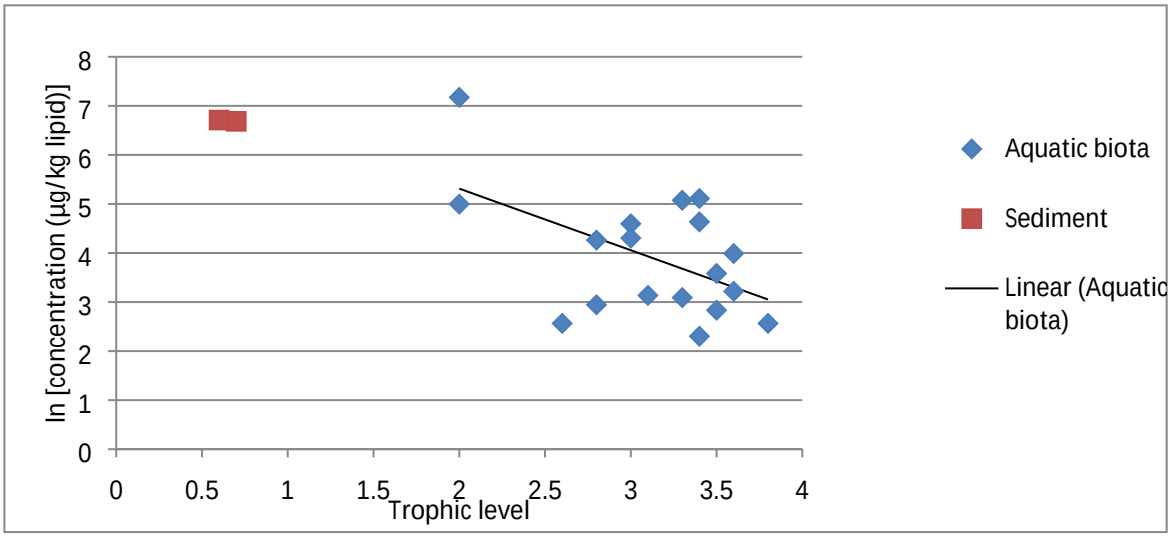
$$TL = 2 + \frac{\left(\delta^{15}N_{consumer} - \delta^{15}N_{midge}\right)}{3.4}.$$

[6] Limit of detection (LOD) is based on the ability of the analytical method to distinguish between signal and noise. The method detection limit (MDL) is a measure of the analytical method's ability to quantify an analyte in a sample matrix. The limit of quantification (LOQ) is the minimum level of a substance in a sample that can be detected and accurately quantified (this was defined as three times the MDL in the current study).

[7] The test report does not give the individual concentrations for each data point (rather they are shown graphically). Therefore the mean data reported by Powell *et al.* (2009a) have had to be used here to construct Figure 8 in order to illustrate the findings. Given the different numbers of samples for each species it would have been preferable to reconstruct Figure 8 here using the individual data points for this evaluation report but this was not possible.

[8] The six trophic guilds considered were detrivores, planktivores, omnivores, invertivores, carnivores and piscivores.

Figure 8: Plot of ln [mean concentration] (on a lipid weight basis[9]) against trophic level for the Lake Pepin food chain



9 The sediment concentration is on a ng/g organic carbon basis.

Table 19: Accumulation of D6 in the Lake Pepin food chain

Sample	Number of samples analysed	Trophic level	Mean measured D6 concentration (±standard deviation)	
			µg/kg wet weight	µg/kg lipid
Surface sediment - samples taken from whole lake	25	0.7	6.3±0.6	800±121[1]
Surface sediments - samples taken from where benthic macroinvertebrates were collected	5	0.6	6.6±0.7	821±142[1]
Midge (*Chironomous sp.*)	5 composites	2.0	10.5±3.7	1,305±550
Burrowing mayfly (*Hexagenia sp.*)	2 composites	2.0	3.7±0.3	148±28
White sucker (*Catostomus commersoni*)	1	2.6	(0.3)[2]	(13)[2]
Common carp (*Cyprinus carpio*)	3	2.8	9.4±7.1	71±52
Gizzard shad (*Dorosoma cepedianum*)	4	2.8	1.7±2.5	19±26
Gizzard shad (young of year) (*Dorosoma cepedianum*)	3 composites	3.0	3.7±4.0	99±111
Silver redhorse (*Moxostoma anisurum*)	3	3.0	5.8±4.9	74±49
Bluegill sunfish (*Lepomis macrochirus*)	3	3.1	(1.2±0.8)[2]	(23±14)[2]
River carpsucker (*Carpiodes carpio*)	1	3.3	26.9	160
Shorthead redhorse (*Moxostoma macrolepidotum*)	3	3.3	(1.4±1.3)[2]	(22±19)[2]
Freshwater drum (*Aplodinotus grunniens*)	3	3.4	4.9±4.0	103±84
Emerald shiner (*Nitropis atherinoides*)	4 composites	3.4	5.1±4.3	166±130
Black crappie (*Pomoxis nigromaculatus*)	3	3.4	(0.7±0.0)[2]	(10±0)[2]
White bass (*Morone chrysops*)	3	3.5	(1.1±1.1)[2]	(17±20)[2]
Smallmouth bass (*Micropterus dolomieu*)	3	3.5	2.0±2.5	36±47
Quillback carpsucker (*Carpiodes cyrinus*)	2	3.6	6.9±4.1	54±26
Walleye (*Stizistedion vitruem*)	3	3.6	1.8±1.8	25±21
Largemouth bass (*Micropterus salmoides*)	3	3.8	(0.5±0.1)[2]	(13±4)[2]

Note: 1) Sediment concentrations are expressed on a total organic carbon basis rather than a lipid basis.
2) Concentrations <MDL but >LOD; reported as the actual measured concentration.

The paper also estimated the biomagnification factor (BMF) for various organisms, taking into account the composition of the diet of each organism[10], and biota-sediment accumulation factors (BSAF). A correction was also applied to the BMF to take account of the trophic level increase (this was designated BMF_{TL}) in the Powell *et al.* (2009a) report. However it was later found out that the correction originally applied was incorrect (CES, 2010a) and so the BMF_{TL} values are not considered here. The BSAF and BMF values are summarised in Table 20:.

As can be seen from Table 20:, the BMF is only above 1 for midge larvae (a benthic macroinvertebrate species at the bottom of the food chain). The remaining BMFs are in the range <0.1 to 0.3. This generally confirms the results of the TMF analysis that trophic dilution of D6 appears to be occurring in this food chain.

The BSAFs obtained are all less than 1 for the fish species and above 1 for midge larvae. Overall, despite the small sample sizes and large variation in tissue concentrations for some individual species, the results of this study suggest that the concentrations of D6 were generally highest in the benthic microinvertebrates and decreased with increasing trophic level within the food chain. Powell *et al.* (2009a) considered that the fact that the concentrations and various accumulation factors were highest in the organisms having a close association with the sediment compartment indicated that the main source of D6 in the food chain was sediment rather than water, and that most uptake in the food chain occurred from dietary exposure rather than water-phase exposure. Based on this Powell *et al.* (2009a) concluded that bioconcentration was not an important process in this food chain but the uptake was rather controlled by dietary uptake and associated mitigation processes such as metabolism, growth dilution and low uptake and assimilation efficiencies.

10 The BMF was calculated by dividing the mean lipid normalised concentration in the predator by the mean lipid normalised concentration in the diet of the predatory. The concentrations in diet were calculated as the mean diet-weighted concentration taking into account the fraction of each prey item that constituted the diet. The assumed feeding relationships were complex and took into account the known (or assumed) composition of the diet for each species – it was not a simple single predator- single prey relationship.

Table 20: BMF and BSAF values derived for D6 for the Lake Pepin food chain

Sample	Trophic level	BSAF	BMF[2]
Midge	2.0	1.6	1.6[1]
Burrowing mayfly	2.0	0.2	0.2[1]
White sucker	2.6	<0.1	<0.1
Common carp	2.8	0.1	0.1
Gizzard shad	2.8	<0.1	<0.1
Gizzard shad (young of year)	2.9	0.1	0.1
Silver redhorse	3.0	0.1	0.2
Bluegill sunfish	3.1	<0.1	0.1
River carpsucker	3.3	0.2	0.3
Shorthead redhorse	3.3	<0.1	<0.1
Freshwater drum	3.4	0.1	0.1
Emerald shiner	3.4	0.2	0.3
Black crappie	3.4	<0.1	<0.1
White bass	3.5	<0.1	0.1
Smallmouth bass	3.5	<0.1	0.1
Quillback carpsucker	3.6	0.1	0.1
Walleye	3.6	<0.1	0.2
Largemouth bass	3.8	<0.1	0.1

Note: 1) For the benthic macroinvertebrates the diet was considered to consist mainly of sediment detritus (75-80 per cent) and plankton (20-25 per cent). No concentration data were available for sediment detritus or plankton and so it was assumed that the concentrations were the same as the organic carbon normalised concentration in sediment. Therefore the BMF is numerically equivalent to the BSAF.

2) In order to carry out these estimates the diets of the species were simplified and in many cases included a component from sediment detritus, plankton, fish eggs and terrestrial insects along with the other species included in the study. As no concentrations were measured for some of these assumed dietary components, the concentrations were estimated and this introduces some uncertainty into the resulting BMF values.

Although the data show that D6 does not have trophic magnification in this food chain (as demonstrated by the low TMF and declining BMFs with increasing trophic level), the results are not so conclusive as to whether or not uptake via bioconcentration was significant compared with dietary exposure. The reason for this is that there are no data available on the levels of D6 in the water phase and so the contribution from the water phase cannot be fully assessed. Although the concentrations are clearly higher in the organisms associated with the sediment, and so accumulation through sediment and diet appears to be the most likely explanation, it cannot totally be ruled out that the concentration found in these organisms is contributed to by exposure via sediment pore water or overlying water (i.e. bioconcentration processes). It should also be noted that many of the same mitigation processes suggested by Powell *et al.* (2009a) in relation to dietary exposure would also be relevant if significant uptake also occurred via the water phase, for example increasing metabolic capacity (or other elimination mechanisms) with increasing trophic level would equally explain the decreasing concentrations with increasing trophic level if the exposure was mainly via the water phase or via diet. In practical terms, it is not so

important to determine the exact route of exposure as the BMF, TMF and BSAF will reflect the combined exposure via both water and food in this food chain.

When considering these data one final point is important. The sediment and benthic macroinvertebrates were collected at a different point in time than the fish (May 2008 versus September 2008). This introduces some uncertainties when comparing the concentrations found in fish to those found in sediment and benthic macroinvertebrates as the concentration of D6 in the sediment (and overlying water) may have been different on the two sampling occasions (for example the hydraulic residence time of the lake has been shown to vary between around 6 days (high flow) and 47 days (low flow)), and the modelling work carried out by Whelan (2009b), admittedly on a different aquatic system, indicates that some seasonality in the concentration in water may occur owing to the temperature dependence of hydrolysis and volatilisation (resulting in higher concentrations in winter time and lower concentrations in late summer). However, as the fish were all sampled at the same time this finding would not affect the conclusions that can be drawn regarding the trends in concentration with trophic level in the fish samples.

As a follow-on to the Lake Pepin field study a number of mink (*Mustela vison*) from the same area have been analysed for the presence of D6 (Woodburn and Durham, 2009). The samples (three males and one female) were collected from the tributaries of Lake Pepin between the 5th and 12th November 2008. Samples of fat, liver and muscle from each individual were analysed. The stomach contents of the mink indicated that the dietary composition of the mink ranged from predominantly aquatic organisms (one of the mink) to virtually exclusively terrestrial species (two of the mink). The concentrations of D6 found in the mink ranged between not detected and 1.8 µg/kg lipid in muscle (detectable in two out of four samples), 1.8 and 14 µg/kg lipid (mean 4.7 µg/kg lipid; detectable in all four samples) in fat and not detected and 0.7 µg/kg lipid in liver (detectable in two out of four samples). Comparing these concentrations with the concentrations measured in fish in Lake Pepin (Table 19: Accumulation of D6 in the Lake Pepin food chain) it can be seen that the lipid normalised concentrations in mink are generally lower than found in the fish, providing further evidence that although D6 can be transferred through this food chain all the way up to top predators, biomagnification does not appear to be occurring (at least for the aquatic food web; it should also be recognised that only a limited number of samples was included that may not be fully representative of all possible top predatory diets and species).

- A second field study investigating the bioaccumulation of D6 has been carried out in Lake Opeongo, Algonquin Park, Canada (Powell *et al.*, 2009b and 2010a). Lake Opeongo is around 250 km north of Toronto (45°42'N 78°24'W) and is considered to be relatively remote from major population centres. The lake is oligotrophic and has a surface area of 58.6 km^2, a maximum depth of 49.4 m and a mean depth of 14.6 m. The lake is free from potential sources of D6 resulting from sewage and runoff, although there is recreational camping and canoeing in the area. Samples of surface sediment, sediment cores and zooplankton were collected on the 2nd and 3rd October 2007 and samples of yellow perch (*Perca flavescens*), cisco (*Coreogonus artedi*) and lake trout (*Salvelinus namaycush*) were collected on the 26th to 31st October 2007. The sediment and zooplankton were collected at representative locations throughout the lake, whereas the fish were sampled from the southern arm of the lake only (the exact locations were not given). Zooplankton were known to represent a significant fraction of the diet for the forage fish (e.g. small yellow perch and cisco) and these fish were thought to be a significant fraction of the diet for lake trout (Martin and Fry (1972), Vander Zanden and Rasmussen (1996) and Vander Zanden *et al.* (1999 and 2000)).

  With the exception of the fish, the sampling procedure included field quality control samples which enabled contamination during collection, handling and subsequent analysis to be assessed. However it was not possible to include field quality control samples for the fish samples and, although precautions were taken to avoid

contamination (for example the personnel carrying out the sampling were instructed to refrain from using personal care products), it was not possible to assess the extent of contamination of the fish samples that may have occurred in the field and subsequent handling. In particular, CES (2010a) notes that the predatory species (lake trout) and the forage species (yellow perch and cisco) were collected on two separate days by two separate field crews. Furthermore the lake trout were subject to greater handling in the field (as they were measured for length and weight) compared with the forage species.

The concentrations of D6 measured in the samples are summarised in Table 21: Accumulation of D6 in the Lake Opeongo food chain. A variable instrumental blank response was seen (presumably originating from the laboratory reagents used in the analytical procedure) in all analyses which made detection and accurate quantification in the samples difficult. All of the concentrations reported were corrected for this background contamination but the variability in the background contamination introduced some uncertainty into the data. The method detection limit in all samples ranged from 0.15 to 0.74 µg/kg wet weight. The following points should be noted in relation to the concentrations found and the limit of detection (LOD), method detection limit (MDL) and limit of quantification (LOQ):

- For sediment and zooplankton the levels of D6 were all less than the LOD. The concentration present was assumed to be equal to the LOD divided by the sample mass that was analysed.
- For yellow perch, the concentration of D6 was less than the LOD in three out of seven fish. The mean background corrected concentration of D6 in yellow perch was above the MDL.

Table 21: Accumulation of D6 in the Lake Opeongo food chain

Sample	Number of samples analysed	Trophic level	Mean measured D6 concentration (±standard error)	
			µg/kg wet weight	µg/kg lipid
Surface sediment	9 (2 sediment cores and 7 surface sediments)		[0.48±0.03][3]	[44.0±3.0][1, 3]
Zooplankton	3 pooled samples	2.0[2]	[0.77±0.07][3]	[19.1±1.9][3]
Cisco	7 composite samples and individuals	3.0	0.52±0.02	10.8±0.4
Yellow perch	7 composite samples and individuals	3.1	0.49±0.06	11.9±1.4
Lake trout	5 individuals	3.7	1.23±0.27	16.1±4.3

Note: 1) Sediment concentrations are expressed on a total organic carbon basis rather than a lipid basis.
2) No $\delta^{15}N$ data were available. Zooplankton was assumed to be in trophic level 2.
3) Values in square brackets are where the measured concentrations were below the limit of detection (LOD). Here the concentration was estimated to be equal to the limit of detection divided by the sample mass that was analysed.

The trophic level of each species was not determined using $\delta^{15}N$ values. In this case the trophic level was determined relative to the $\delta^{15}N$ value for cisco, which was assumed to be in trophic level 3. No $\delta^{15}N$ data were available for zooplankton, which was assumed to be in trophic level 2. The trophic level data are summarised in Table 21: Accumulation of

D6 in the Lake Opeongo food chain.

Based on the lipid normalised data, Powell *et al.* (2010a) estimated predator-prey BMF values[11] for lake trout-perch and lake trout-cisco by bootstrap analysis using Monte-Carlo simulation. The mean BMFs estimated were 1.4 (95 per cent confidence interval 0.9 to 2.1) for the lake trout-perch relationship and 1.5 (95 per cent confidence interval 0.9 to 2.2) for the lake trout-cisco relationship. The bootstrap analysis indicated that there was a high probability (95 per cent or more) that the BMF values were above 1.

The source of D6 in Lake Opeongo is unknown. Powell *et al.* (2010a) considered it likely that the main source was from personal care products of people using the lake for recreational purposes, although atmospheric transport could not be ruled out. Powell *et al.* (2010a) considered that such recreational use would lead to D6 entering the water column and that accumulation in the food chain would be driven by bioconcentration processes combined with dietary exposures. Thus the pattern of accumulation seen in Lake Opeongo appears to differ from that seen in Lake Pepin, with uptake in the latter appearing to be driven by accumulation from sediment and the food chain according to the authors.

Overall the data for Lake Opeongo suggest that uptake via water exposure is important in this food chain, and that the BMFs for a top predator are greater than 1, implying biomagnification is occurring. However it should be recognised that there are some significant uncertainties with the Lake Opeongo study. These are summarised below.

- The levels found in some parts of the food chain were less than the analytical detection limit.
- There was a relatively high (and variable) analytical background contamination.
- The quality control program for the fish sampling did not allow the extent of contamination during sampling and handling to be assessed. As noted earlier, lake trout were subject to greater handling in the field than both yellow perch and cisco, so there is a possibility that the statistically significantly higher ($p<0.01$) concentrations in this species were caused to some extent by contamination.

To address these uncertainties, Powell *et al.* (2010a) indicated that it was intended that further fish would be sampled (using an appropriate quality control program) and analysed under laboratory conditions that have recently been optimized to minimise and better control the laboratory background contamination. However CES (2010b) indicates that this is now not possible owing to analytical sensitivity issues associated with samples from this system coupled with the increased difficulty in transporting samples from Canada into the United States. As a result of this, CES (2010b) reported that other lakes were being evaluated as a substitute for Lake Opeongo. The criteria being used for selection of a suitable lake include that the lake must receive some waste water effluent and the food web in the lake must be comparable to that in Lake Opeongo (i.e. a pelagic food chain consisting of zooplankton, cisco and lake trout). However, no further studies have been performed yet.

- A further field study investigating the bioaccumulation potential of D6 has been carried out for the aquatic marine food chain of inner and outer Oslofjord, Norway (Powell *et al.*, 2009c and 2010b). The samples analysed included surface sediment, zooplankton, benthic macroinvertebrates (three species, three genera, three families), shellfish (four species, three genera, two families) and finfish (14 species, 13 genera, seven families). The samples were all collected between the 12th and 14th November 2008 and the

[11] These were defined as the concentration in predator (on a lipid normalised basis)/concentration in prey (on a lipid normalised basis) and assume that the diet of predator (in this case lake trout) consisted solely of the single prey species.

trophic level of each species was determined based on $\delta^{15}N$ measurements relative to that of zooplankton (assuming that the trophic level of zooplankton was 2).

The study included a quality control program that investigated the possible contamination of the samples during sampling and analysis. This included field quality control samples for fish (but not sediments, zooplankton and macroinvertebrates) and a rigorous laboratory quality control program. The field crew refrained from using any personal care products during the collection of the samples.

Atlantic cod (*Gadus morhua*) were found to occupy the highest trophic level (TL ~4) and investigation of the gut contents indicated that they were feeding exclusively on shrimp at the time of collection (the gut contents of the other fish species were not evaluated). Analysis of carbon flows (based on ^{13}C-measurements) in the food chain suggested that the trophic dynamics in Oslofjord were best described as representing a compressed food web that was dominated by a benthipelagic food chain. The dominant species in this food chain were identified and the analysis of the data concentrated on these dominant species.

The lipid-normalised concentrations of D6 were found to be highly variable across species and the levels found were generally higher in samples from the inner Oslofjord than the outer Oslofjord. Fish can presumably move between the two locations, although the extent to which this occurs in the sampled species' populations is unknown. The concentrations found are summarised in Table 22: Concentrations of D6 measured in Oslofjord.

It was found that the concentrations of total cVMS (i.e. D4, D5 and D6) were typically greatest in the lowest trophic levels species (such as benthic macroinvertebrates and zooplankton) and decreased with increasing trophic level, with the lowest concentrations being found in the highest trophic level (e.g. Atlantic cod).

^{13}C-measurements in the various organisms were used to determine the food web dynamics operating in both the inner and outer Oslofjord. Based on similarities in the 13C-signatures the various species were assigned to one of four food chains12. The dominant food chain13 was found to include 14 of the 22 species in the study and the trophic magnification factors (TMFs) for this dominant food chain were derived using the lipid normalised concentration data. The TMFs derived for D6 are summarised in Table 23: Trophic magnification factors (TMF) and biomagnification factors (BMFs) for D6 in Oslofjord.

[12] Based on a significant difference in the signature compared with that for Atlantic cod, northern shrimp and Atlantic herring.

[13] The dominant food chain consisted of worms, sea urchin, mussel (species A and B), jellyfish, northern shrimp, European whiting, haddock, European plaice, long rough dab, common sole, Vahl's eelpout, poor cod and Atlantic cod.

Table 22: Concentrations of D6 measured in Oslofjord

Species	Inner Oslofjord				Outer Oslofjord			
	Number of samples	Trophic level	Concentration (±standard error)		Number of samples	Trophic level	Concentration (±standard error)	
			µg/kg wet weight	µg/kg lipd[1]			µg/kg wet weight	µg/kg lipd[1]
Sediment (0-1 cm depth)	7		29.3±3.4	3,423±256	5		3.5±0.8	492±91
Sediment (1-2 cm depth)	8		25.7±3.1	2,744±365	6		3.4±0.3	450±40
Blue mussel (*Mytilus edulis*)	5	1.5	1.5±0.7	120±25				
Sea Urchin (*Brissopsis lyrifera*)					3	2.1	10.1±2.2	3,156±675
Worms	1	1.7	20.1	6,266	1	2.1	1.3	405
Jellyfish	1	2.0	0.4	71	1	2.2	0.1	13
Plankton	1	2.0	2.9	397	1	2.2	0.6	55
Mussels (species A)	2	2.6	7.7±1.0	1,118±282	3	3.1	4.2±0.2	306±13
Mussels (species B)	2	2.8	1.9±0.5	213±16	3	3.0	1.2±0.2	118±5
Atlantic herring (*Clupea harengus*)	6	3.0	18.7±3.3	241±30				
Northern shrimp (*Pandalus borealis*)	6	3.0	3.2±0.5	104±5	6	3.0	1.0±0.2	29±1
European plaice (*Pleuronectes platessa*)	6	3.1	26.6±3.6	543±79	5	3.4	7.5±1.3	261±45
Coalfish (*Pollachius virens*)	6	3.3	18.6±1.5	809±52	6	3.6	2.8±0.4	53±7
Common sole (*Solea vulgaris*)					3	3.4	2.9±0.8	54±10

Norway pout (*Trisopterus esmarkii*)	6	3.3	15.4±1.5	181±6	10	3.5	2.5±0.2	37±3
European hake (*Merluccius merluccius*)	4	3.4	12.9±6.3	428±163				
Starry skate (*Amblyraja radiate*)					3	3.5	1.2±0.4	46±12
Haddock (*Melanogrammus aeglefinus*)	4	3.8	22.5±3.4	413±36	12	3.7	4.5±0.7	132±15
European whiting (*Merlangius merlangus*)	6	3.8	2.3±0.5	185±22				
Long rough dab (*Hippoglossoides platessoides*)	6	3.8	13.5±2.9	794±247	6	3.6	1.9±0.6	58±13
Vahl's eelpout (*Lycodes vahlii*)	6	3.8	3.1±0.9	362±132				
North Atlantic Pollock (*Pollachius pollachius*)	6	3.8	24.0±6.3	576±124				
Poor cod (*Trisopterus minutus*)	6	3.8	3.9±1.0	96±12				
Atlantic cod (*Gadus morhua*)	6	4.0	4.2±1.3	137±15	6	4.1	1.1±0.3	41±5

Note: 1) The concentrations in sediment are µg/kg organic carbon.

The TMF was below 1 for both the inner and outer Oslofjord (the TMF values determined for D6 were 0.4 for the inner Oslofjord and 0.2 for the outer Oslofjord; in both cases the probability that the TMF was >1 was 0.0 per cent). Powell *et al.* (2010b) indicated that future work will include better identification and characterisation of the Oslofjord food web so that TMFs can be calculated for all appropriate food chains.

In addition to the TMFs, Powell *et al.* (2010b) also determined biomagnification factors (BMFs) for various predator-prey interactions. The BMF values determined for D6 were 1.7-1.8 for Atlantic cod-shrimp (probability of a BMF >1 was 80-81 per cent) and 0.9 for Atlantic cod-herring (probability of a BMF >1 was 29 per cent). The data are also summarised in Table 23: Trophic magnification factors (TMF) and biomagnification factors (BMFs) for D6 in Oslofjord.

It should be noted that the BMFs were not corrected for differences in trophic level in this case as both predator-prey relationships were separated by a single trophic level step.

Powell *et al.* (2010b) concluded that the data show that biomagnification of D6 was not occurring in this food chain based on the TMF <1. It is noted that the number of samples was small.

Table 23: Trophic magnification factors (TMF) and biomagnification factors (BMFs) for D6 in Oslofjord

Food web grouping	Location	Derived accumulation factor[3]
Dominant food chain[2] trophic magnification factor	Inner Oslofjord	Mean TMF = 0.4[1] (95% confidence interval 0.2 to 0.6; probability TMF >1 0.0%; mean fit of regression model (r^2) 36%)
	Outer Oslofjord	Mean TMF = 0.2 (95% confidence interval 0.2 to 0.4; probability TMF >1 0.0%: mean fit of regression model (r^2) 46%)
Atlantic cod-shrimp biomagnification factor	Inner Oslofjord	Mean BMF = 1.7 (95% confidence interval 0.6 to 3.6; probability BMF>1 80%)
	Outer Oslofjord	Mean BMF = 1.8 (95% confidence interval 0.6 to 4.9; probability BMF>1 81%)
Atlantic cod-herring biomagnification factor	Inner Oslofjord	Mean BMF = 0.9 (95% confidence interval 0.4 to 2.0; probability BMF>1 29%)
	Outer Oslofjord	No estimate possible

Note: 1) The TMF were calculated based on regression analysis of the log transformed lipid normalised concentration against trophic level.
2) The dominant species present in the food chain were identified based on ^{13}C flows.
3) Variability associated with the TMF and BMF was evaluated by bootstrap analysis using Monte Carlo simulation.

- Borgå (2012) reports the results of a further study investigating the TMF for D6. This study was carried out on a pelagic food web in Lake Mjøsa in Norway (60°53'N, 10°41E). The lake is 117 km long, 14 km wide with an average and maximum depth of 153 m and 453 m, respectively. The lake is situated in an agricultural area and there

is also some industrial activity. The top predator in the food chain is brown trout (*Salmo trutta*) and the food chain has been studied previously for other contaminants.

The samples included in the study were zooplankton from the epilimnion (predominantly *Daphnia galeata*) and hypolimnion (predominantly copepods *Limnocalanus macrurus*), *Mysis relicta* from the hypolimnion and the following fish species, vendace (*Corogonus albula*), smelt (*Osmerus eperlanus*) and brown trout (*Salmo trutta*). The zooplankton samples along with *Mysis relicta* samples were collected mid-lake near to Skreia on either the 22nd September 2010 or 27th September 2010 and the fish samples were collected either in the northern part of the lake (smelt) or near to Skreia (vendace and trout) between 11th September and 19th October 2010. As all three fish species are pelagic, Borgå (2012) considered that the influence of sampling location on contaminant exposure would be negligible.

Precautions were taken during the sampling and subsequent analysis of the samples to avoid inadvertent contamination of the samples. The measures taken included avoidance of use of personal care products 24 hours prior to sampling, collection of field blanks during sampling and analysis of procedural blanks, field blanks and an internal matrix control sample (herring homogenate) with each set of eight samples along with duplicate analysis of three brown trout and two vendace samples. The limit of quantification was set to the mean plus ten times the standard deviation of the procedural blanks. The results were not blank corrected (samples that contained less than five times the corresponding field blank were considered to be below the limit of quantification). The trophic level of the samples was assigned based on $\delta^{15}N$ measurements and $\delta^{13}C$ measurements were used to identify whether the carbon source to the food web was predominantly pelagic or benthic in origin. A number of chlorinated and brominated substances[14] were also analysed in the samples as benchmark substances.

The concentration of D6 was found to be above the limit of quantification in all of the fish samples and one zooplankton sample; the concentration was below the limit of quantification in the remaining zooplankton samples and the *Mysis relicta* samples. The amount of D6 in field blanks was generally low compared with the concentrations in the samples. The results are summarised in Table 24: Accumulation of D6 in the Lake Mjøsa food chain.

The $\delta^{13}C$ measurements demonstrated that the food web was predominantly pelagic and the trophic level assignments were consistent with known feeding relationships in the food web. Borgå (2012) considered that trout feed predominantly on smelt and some vendace. Smelt were thought to feed predominantly on *Mysis* and zooplankton with an increasing degree of cannibalism when the fish are larger than 10 cm (the fish sampled in this study were 20.5-23.7 cm in length). Vendace were thought to feed mainly on zooplankton. For the invertebrates, *L. macrurus* is omnivorous and feeds on algae and zooplankton, *D. galeata* feeds predominantly on algae and *Mysis relicta* feeds predominantly on water fleas.

[14] PCB-153 (2,2',4,4'5,5'-hexachlorobiphenyl); PCB-180 (2,2',3,4,4',5,5'-heptachlorobiphenyl); p,p'-DDE (p,p'-dichlorodiphenyldichloroethylene); BDE-47 (2,2',4,4'-tetrabromodiphenyl ether); BDE-99 (2,2',4,4',5-pentabromodiphenyl ether).

Table 24: Accumulation of D6 in the Lake Mjøsa food chain

Sample	Number of samples analysed	Trophic level	Mean Measured D6 concentration	
			Range of wet weight concentrations (µg/kg wet weight)	Mean lipid normalised (µg/kg lipid) (±standard deviation)[1]
Zooplankton (predominantly *Daphnia galeata*) - epilimnion	4 pooled samples	2.0	<1.8 to 4.4	<870
Zooplankton (predominantly *Limnocalanus macrurus*) - hypolimnion	4 pooled samples	2.7	<2.1 to <2.8	<230
Mysis relicta - hypolimnion	4 pooled samples	2.6	<0.77 to <1.2	<51
Vendace	5 muscle samples	3.6	1.1 to 6.7	100 (±18)
Smelt	5 muscle samples	4.1	2.8 to 7.2	640 (±160)
Brown trout	5 muscle samples	4.2	0.82 to 5.7	130 (±20)

Note: 1) Standard deviations were not reported for the zooplankton or *Mysis relicta* samples.

No TMF for the whole food chain was estimated by Borgå (2012) for D6 owing to the lack of detection of the substance at the lower trophic levels. The TMFs for the benchmark substances for the whole food web were 4.9 for PCB-153, 6.01 for PCB-180, 3.90 for p,p'-DDE, 5.82 for BDE-47 and 2.43 for BDE-99.

It is important to note that the number of samples analysed in this study is relatively small (four to five per species). Further the fish samples analysed were muscle samples rather than whole fish. For these samples/species it is not known how the concentrations measured in muscle relate to the likely whole fish concentration. However, in another study from Japan (SIAJ, 2011; see below) the wet weight concentration in whole fish samples (pale chub, common carp, yellowfin goby, flathead mullet and Japanese seabass) tended to be higher than in the edible part of the same fish. These factors introduce some further uncertainty into the results from this study.

- Börga *et al.* (2013a and 2013b) carried out a further (repeat) study of the pelagic food web in Lake Mjøsa and extended the study to include a similar lake in the same area (Lake Randsfjorden) and a lake thought to be remote from any known sources of emission (Lake Femunden). All three lakes are deep and contain well-defined pelagic food webs including zooplankton, planktivorous fish and brown trout as a top predator.

  Although Lake Femunden was considered a remote lake and there was no waste water treatment plant discharging into the lake, the map given in the paper shows a small village close by and so point sources of emission cannot be totally ruled out.

  Lake Mjøsa has a pelagic food web with brown trout (*Salmo trutta*) as the top predator, smelt (*Osmerus eperlanus*) and vendace (*Coregonus albula*) as primary planktivorous prey, and an invertebrate community consisting of cladocerans, copepods and *Mysis relicta*. Lake Randsfjorden has some similarities to Lake Mjøsa and has a well-defined

pelagic food web with brown trout and arctic char (*Salvelinus alpinus*) as top predators, and whitefish (*Coregonus lavaretus*) and smelt as planktivorous prey. Lake Femunden has a pelagic fish community of brown trout, arctic char and whitefish.

The main food web difference between the lakes is that Lake Mjøsa includes *Mysis relicta* in the invertebrate community, vendace among the planktivorous fish, and excludes Arctic char as top predator. Whitefish is assumed to be a benthic feeding species in Lake Mjøsa but assumed to replace vendace in the pelagic food web of Randsfjorden and Femunden.

The samples were collected between July and September 2012. Fish and invertebrates were sampled from the pelagic zone in all three lakes. In addition benthic fish (whitefish, perch (*Perca fluviatilis*) and burbot (*Lota lota*)) were sampled from Lake Mjøsa. As well as biota samples, samples of surface sediments were also collected from all three lakes along with surface water and effluent samples from Lake Mjøsa and Lake Randsfjorden.

The majority of biota samples in Lake Mjøsa (zooplankton, *Mysis reticta*, vendace and smelt) were collected mid-lake in an area south of the town of Helgøya. Brown trout were collected from close to the town of Gjøvik but it was noted that as trout use the entire lake in search of food it was thought that these samples were representative of a larger geographical area. In Lake Randsfjorden the biota samples were all collected mid-lake from an area south of Brandu and in Lake Femunden the biota samples were collected from the southern basin.

The fish samples consisted of skinless fillets from one individual except for small smelt where five or six skinless fillets were pooled for each sample. For burbot, both fillets and liver were sampled. Pre-cleaned field blanks were handled in the same way as the biotic samples. Sediment samples were taken from the surface layer (0-1 cm depth) in areas close to the discharge from waste water treatment plants where this was possible. Each sample consisted of a pool of three cores from each sampling area. Deeper sediments (typically from 30 cm or deeper) were also collected to act as reference samples. Water samples from Lake Mjøsa were collected from a depth of 15 m[15]. Grab samples of effluent were collected from the outlets of three waste water treatment plants in each of Lake Mjøsa and Lake Randsfjorden. Precautions were taken during sampling to avoid inadvertent contamination of the samples (for example all personnel avoided the use of personal care products).

As in previous studies the trophic level of each species was assigned based on $\delta^{15}N$ measurements and the carbon source for the organism was determined based on $\delta^{13}C$ measurements. The zooplankton from the epilimnion was defined as the baseline consumer and assigned a trophic level of 2. The other trophic levels were assigned relative to this using an enrichment factor (ΔN) of 3.4 ‰ TL^{-1}.The number of samples collected and trophic level assigned are summarised in Table 25: Summary of levels of D6 in samples collected from Lakes Mjøsa, Randsfjorden and Femunden.

The samples were analysed for the presence of cVMS (D4, D5 and D6). In addition known bioaccumulative substances (polychlorinated biphenyls (PCB-153 and PCB-180) and dichlorodiphenyldichloroethylene (p,p'-DDE) in Lake Mjøsa and Lake Randsfjorden, and polybrominated diphenyl ethers (PBDE-47 and PBDE-99) in Lake Mjøsa) were analysed in the sample to act as reference substances. Procedural blanks, field blanks and an internal matrix control (homogenate of herring from the Baltic sea for biota samples and a sediment sample from Lake Mjøsa for abiotic samples) were also analysed at intervals along with the samples. The limit of quantification (LOQ) for

[15] For the surface water samples the particulate phase was analysed for cVMS and the dissolved phase was analysed for the reference substances.

biota was set to the mean plus 10×standard deviation of the procedural blanks and the LOQ for sediment was set at 3×maximum quantity measured in the reference sediments. The levels found are summarised in Table 25: Summary of levels of D6 in samples collected from Lakes Mjøsa, Randsfjorden and Femunden. The levels were not blank-corrected[16].

Table 25: Summary of levels of D6 in samples collected from Lakes Mjøsa, Randsfjorden and Femunden

Lake	Sample	Food web	Number of samples analysed	Mean trophic level (±standard error)	Mean concentration of D6 (ng/g lipid) (±standard error)
Lake Mjøsa	Zooplankton (epilimnion)	Pelagic	3	2.0±0.0	<48
	Zooplankton (hypolimnion)	Pelagic	4	2.6±0.2	48
	Mysis relicta	Pelagic	4	2.8±0.1	59±13
	Vendace (*Coregonus albula*)	Pelagic	7	3.9±0.0	786±117
	Smelt, small (*Osmerus eperlanus*)	Pelagic	5	3.8±0.1	184±21
	Smelt, large (*Osmerus eperlanus*)	Pelagic	5	4.4±0.0	325±55
	Brown trout (*Salmo trutta*)	Pelagic	5	4.4±0.0	285±45
	Whitefish (*Coregonus lavaretus*)	Benthic	5	3.6±0.1	<122
	Perch (*Perca fluviatilis*)	Benthic	6	4.0±0.1	<66
	Burbot, liver (*Lota lota*)	Benthic	6		260±73
	Burbot, muscle (*Lota lota*)	Benthic	6	4.4±0.1	174±21
Lake Randsfjorden	Zooplankton (epilimnion)	Pelagic	4	2.0±0.0	<37
	Zooplankton (hypolimnion)	Pelagic	3	3.0±0.3	48±10
	Whitefish (*Coregonus lavaretus*)	Bentho-pelagic	9	3.2±0.1	<30
	Smelt (*Osmerus eperlanus*)	Pelagic	5	3.5±0.1	58±9

[16] The total content of D5 and D6 in the field blanks from Lake Mjøsa was in all cases low compared to the total amount extracted from the samples above LOQ (ratio >4.4 up to 3,499). For D4 the difference between field blanks and samples was lower (total range 3-94). For Randsfjorden, although more samples were close to or below the LOQ for D4 and D6, the biota sample to field blank ratio for D5 was greater than 5 for all but 6 samples. In Femunden only D5 was quantified above the LOQ in trout, with values 15-23 times higher than the field blank.

Lake	Sample	Food web	Number of samples analysed	Mean trophic level (±standard error)	Mean concentration of D6 (ng/g lipid) (±standard error)
	Brown trout (*Salmo trutta*)	Pelagic	5	3.8±0.1	132±31
Lake Femunden	Arctic char (*Salvelinus alpinus*)	Pelagic			<40
	Brown trout (*Salmo trutta*)	Pelagic			<80

The levels of D6 found in Lakes Mjøsa and Randsfjorden were higher than found in Lake Femunden, reflecting the local sources of release into the lakes. The amount of D6 was above the LOQ in 58% of the biota samples (a total of 91 samples were analysed) and 73% of the sediment samples (a total of 18 samples were analysed). In Lake Femunden, all cVMS were below LOQ in all samples analysed[17] except for a few trout in which D5 was above the LOQ.

All of the effluent water samples contained all cVMS above the LOQ, with the exception of D6 in a sample from Lillehammer, Mjøsa. For the particulate samples of surface water, an error in the field resulted in no field blank being available. Since it could therefore not be excluded that these samples were contaminated, the measured concentrations were designated "<" values.

The sediment samples showed a high spatial variation in the concentration of cVCMs in Lake Mjøsa and Lake Randsfjorden, with the highest concentrations near to the towns of Brandbu and Grjøvik respectively, reflecting the local sources of input (i.e. waste water treatment plants) in these areas.

The $\delta^{13}C$ measurements showed a clear separation of the pelagic feeding fish from the benthic feeding fish in Lake Mjøsa. In Lake Ransfjorden, a relatively high variation in the $\delta^{13}C$ value was found in whitefish, suggesting that there was some variation in the diet of this species. Earlier investigations of stomach contents of whitefish from this lake had shown both purely pelagic feeding fish and fish feeding on benthic and terrestrial invertebrates. Therefore the TMFs for Lake Ransfjorden were calculated both including and excluding whitefish.

The TMF was estimated from the slope of a plot of the natural logarithm of lipid normalised concentration in biota versus trophic level. The benthic fish (from Lake Mjøsa) and sediment samples were not included in the analysis. Where the concentration of D6 was <LOQ but >LOD (limit of detection) the actual estimated concentration was used in the analysis (rather than replacing the <LOQ value with a fixed or random value). For Lake Randsfjorden, one hypolimnion zooplankton sample was identified as a multivariate outlier and so was excluded from the analysis. A plot showing the mean concentrations against the trophic level for Lake Mjøsa is shown in Figure 9. The TMFs derived from the data are summarised in Table 26 (these values were derived in the actual publications from plots of the individual data points rather than the mean data points).

The TMF for D6 was found to be similar between Lakes Mjøsa and Randsfjorden when the whitefish was omitted (when whitefish was included the TMF was statistically significantly

[17] As low levels in this lake were foreseen, sediments and samples of the top predators brown trout and arctic char were analysed first. As only low levels were found, the remaining samples collected in Lake Femunden (zooplankton, whitefish, arctic char) were not analysed.

different between the two lakes ($p<0.05$)). The statistical significance of the TMF being above 1 was also reduced for Lake Randsfjorden when whitefish were included compared with the situation when whitefish were omitted (for example see the 95% confidence intervals and the p-values in Table 26: Summary of TMFs derived by Börga *et al.* (2013a and b), although the actual magnitude of the TMF was similar in both cases. The lower significance of the TMF in Randsfjorden when whitefish were included resulted from the fact that the D6 concentrations in whitefish in this lake were lower compared with other species at the same trophic level which suggests that the source of D6 in whitefish may have been different from the other, purely pelagic species considered, for example as a result of feeding in the littoral zone on terrestrial and benthic prey.

In Lake Mjøsa benthic feeding fish (perch, whitefish and burbot) generally had lower levels of D6 than pelagic fish of a similar trophic level (the benthic species were not included in the TMF derivation for the pelagic food web).

Figure 9: Plot of ln [mean concentration in biota (ng/g lipid)] versus trophic level for Lake Mjøsa

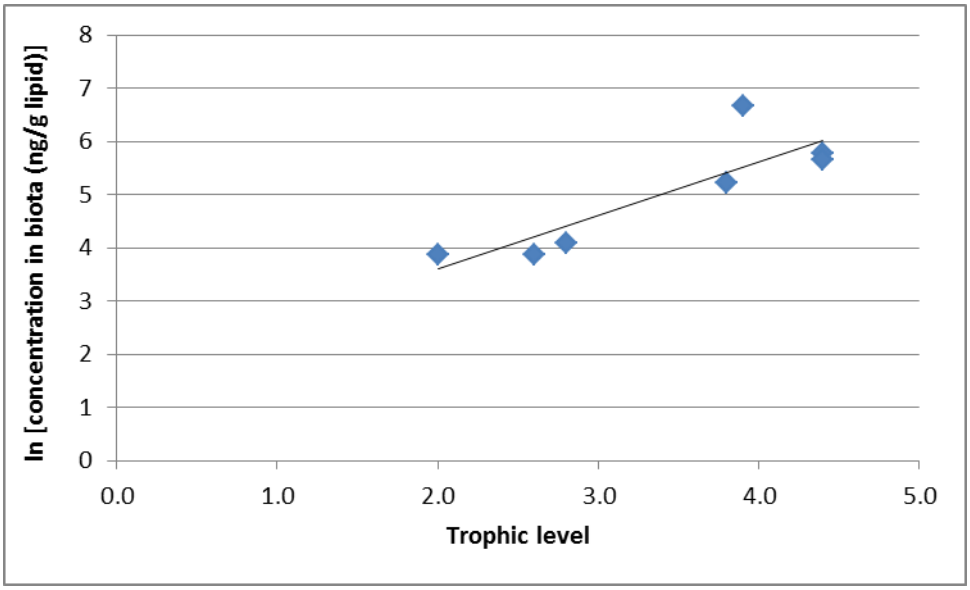


Table 26: Summary of TMFs derived by Börga *et al.* (2013a and b)

Lake	Number of data points	TMF	95% confidence interval	p-value[a]	R^2 of regression	Comment
Lake Mjøsa	33	2.72	1.96-3.77	<0.0001	0.55	Not including whitefish
Lake Randsfjorden	17	1.60	1.09-2.34	0.0189	0.32	Not including whitefish; over 50% of the data were below the limit of quantification
	26	1.46	0.90-2.36	0.117	0.10	Including whitefish; over 50% of the data were below the limit of quantification
Combined Lake Mjøsa and Lake Randsfjorden	51	2.30	1.76-3.02	<0.0001	0.59	Not including whitefish for Lake Ransfjorden.

Note: a) The p-value indicates the statistical significance of the regression. Statistically significant difference is usually taken as a value of $p \leq 0.05$.

The levels of D6 in the pelagic food webs were also found to correlate with the levels of known biomagnifying substances, for example PCB-153 and p,p'-DDE. The TMFs for these reference substances were higher in Lake Mjøsa than Lake Randsfjorden but were above 1 in both lakes.

As is the case with the other available study there are a number of uncertainties associated with these results, including the following:

- The brown trout in Lake Mjøsa were sampled from a different area of the lake than the other biota samples. The rainbow trout were sampled near to Grjøvik and the sediment samples suggested that this area may have been more heavily contaminated than other parts of the lake. However, it was noted that brown trout use the entire lake for feeding and so the levels found in this species are probably more reflective of the levels in the whole lake rather than the specific area sampled. In addition, a similar level of trophic magnification was evident in the food webs of both Lake Mjøsa and Lake Randsfjorden.

- The fish samples analysed were skinless fillets (with the exception of burbot livers). The levels found may not necessarily reflect the levels present in whole fish. The burbot data show that the levels of D6 (and the halogenated reference substances) were generally higher in liver than in fillets, however the liver will contribute only a relatively small fraction of the total weight of the fish (although this would likely vary from fish to fish). The concentration (or amount) of D6 present in other, non-fillet, portions of the fish are not known.

- A number of species included in the regressions had levels of D6 below the limit of quantification (particularly for Lake Randsfjorden). In addition the number of samples analysed for each species was relatively limited (varied between 3 and 9).

Overall this study shows evidence for biomagnification of D6 in pelagic food webs of both Lake Mjøsa and Lake Randsfjorden. The TMF determined in both lakes was similar and the overall TMF from both lakes combined was determined to be 2.3 with a 95% confidence interval of 1.8-3.0. In addition the levels of D6 were found to correlate in the pelagic food chain with the reference substances which are known to biomagnify. Thus although there are, as always with this type of study, some uncertainties resulting from, for example, the limited number of samples, different sampling areas for some species, the use of fish fillets versus whole fish, this does provide some support that D6 is biomagnifying in the pelagic food chains of Lake Mjøsa and Lake Randsfjorden. Borgå *et al*. (2013a and b) also considered the available data from the other field studies and concluded that the available field studies suggest that the TMF of D6 is sensitive to the food web composition and that a possible explanation of the differences found between studies may come from differences in ecosystem characteristics that affect both the trophic transfer and retention of contaminants, and hence the degree of biomagnification. These could include, for example pelagic versus benthic/benthopelagic habitats, water temperature, residence time of the water in the system, water depth, species composition and salinity.

- A further field study investigating the biomagnification of D6 has recently been published (McGoldrick *et al.,* 2014a). This study was carried out in the western basin of Lake Erie, Canada. The biota used in the study were collected in the summer/autumn of 2009[18] in the vicinity of Middle Sister Island and included zooplankton, mayflies (*Hexagenia* sp.), common shiner (*Luxilus cornutus*), yellow perch (*Perca flavescens*), emerald shiner (*Notropis atherinoides*), trout perch (*Percopsis omiscomaycus*), white perch (*Morone americana*), freshwater drum (*Aplodinotus grunniens*) and walleye

[18] The samples were frozen immediately in the field and then stored at either -80°C (zooplankton and benthos) or -20°C (fish) in the laboratory until processing. The length of storage of the samples prior to processing and analysis is not given

(*Sander vitreus*). The fish were analysed as whole fish samples (walleye and freshwater drum were analysed as individual fish, the other species were analysed as composite samples of between 2 and 60 individuals with each composite being divided into 5 subsamples). Precautions were taken during sampling and analysis to avoid inadvertent contamination of the samples.

As with the other studies, the trophic level of each species was determined based on $\delta^{15}N$ measurements and $\delta^{13}C$ measurements were used to establish the carbon source. The relative contribution of pelagic- and benthic-based carbon to the diet of each species was estimated using a single isotope-two source mixing model. This analysis showed that the fish in the study were predominantly feeding on benthic-based carbon sources but that two of the species, emerald shiner and trout perch, were feeding on benthic- and pelagic-based carbon sources.

The concentration of D6 measured in each species, along with the assigned trophic levels and lipid contents are summarised in Table 27: Summary of levels of D6 in samples collected from Lake Erie. The TMFs were estimated from the data using the lipid equivalent concentrations and various assumptions over the food web composition. The TMF for D6 was determined to be 0.71 (95% confidence interval 0.47-1.0; probability of TMF >1 4.6%) when all species were included, 0.71 (95% confidence interval 0.47-1.0; probability of TMF >1 5.3%) when the zooplankton were excluded and 0.97 (95% confidence interval 0.62-1.4; probability of TMF >1 40%) when both zooplankton and walleye were excluded[19].

Table 27: Summary of levels of D6 in samples collected from Lake Erie

Sample	Estimated diet composition	Number of samples analysed	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D6 (ng/g wet weight) (±standard deviation)
Zooplankton		1	2.0±0.32	0.3	Not detected
Mayfly (*Hexagenia* sp.)		1	2.2±0.08	1.3	5.7
Common shiner (*Luxilus cornutus*)	13% pelagic – 87% benthic	2	3.1±0.08	3.5	6.9±9.7
Yellow perch (*Perca flavescens*)	15% pelagic – 85% benthic	5	3.4±0.1	1.6	11±7.7
Emerald shiner (*Notropis atherinoides*)	40% pelagic – 60% benthic	5	3.6±0.07	2.1	13±2.2
Trout perch (*Percopsis omiscomaycus*)	49% pelagic – 51% benthic	5	3.6±0.08	0.7	13±4.4
White perch (*Morone Americana*)	3% pelagic – 97% benthic	4	3.7±0.05	5.3	8.2±8.2
Freshwater drum (*Aplodinotus grunniens*)	28% pelagic – 72% benthic	5	4.0±0.12	3.4	9.9±5.6
Walleye	20% pelagic	15	4.2±0.12	13	14±7.2

[19] The sensitivity of the TMF to food web structure was evaluated by excluding organisms from the lowest and highest trophic levels. D6 was not detectable in zooplankton leading to essentially the same TMF as derived for the whole dataset when the zooplankton were excluded.

Sample	Estimated diet composition	Number of samples analysed	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D6 (ng/g wet weight) (±standard deviation)
(*Sander vitreus*)	– 80% benthic				

The study also included analysis of PCB180 as a reference substance that is known to bioaccumulate. The TMF derived for this substance was 1.2 when all species were included, 1.7 when mayfly were excluded, 0.55 when zooplankton were excluded, 2.1 when both mayfly and walleye were excluded and 0.58 when both zooplankton and walleye were excluded. This suggests that the TMF is dependent on the food web structure.

Again, as with the other studies there are some uncertainties with this study resulting, for example, from the relative small sample sizes and, in this case, the inclusion of species with a relatively high contribution from pelagic carbon sources in what is essentially a benthic food web. It is also relevant to note that the recoveries of the ^{13}C-D6 used as analytical standard range from 19% to 104%, were highest for the zooplankton samples and generally decreased as the lipid content of the fish increased. This may have introduced some bias into the results as the fish at the higher trophic levels generally had higher lipid contents than the fish at lower trophic levels (e.g. the lipid contents for the fish in trophic levels between 3.7 and 4.2 were in the range 3.4 to 13% compared to lipid contents between 0.7% of 3.5% for fish at lower trophic levels). This could potentially lead to an underestimation of the concentrations in fish at the higher trophic levels compared with lower trophic levels.

Overall the results of this study suggest that biomagnification of D6 was not occurring in this predominantly benthic food chain, in line with the findings from other benthic food chains.

- A further study of the bioaccumulation of D6 is available (Powell *et al.*, 2017). A pre-publication draft of the study was made available for the assessment. The study was of a pelagic marine food web in Tokyo Bay. The samples for the study included sediment and fish (see Table 28: Summary of levels of D6 in samples collected from Tokyo Bay) collected between 4th and 15th November 2011 from a defined 500 km^2 area covering approximately 55% of inner Tokyo Bay. The area was defined using a two-dimensional probability design based on 25 km^2 square grids extending seaward from the head of the bay to the narrows between Cape Kannon and Cape Futtsu. Sediments were collected from 20 locations by systematically sampling each 25 km^2 grid and fish were collected within the northern part of the study area. Precautions were taken during sampling, storage and analysis to avoid unintentional contamination of the samples and loss from evaporation and degradation. As well as D6, the study included PCB-180 as a benchmark chemical and PCB-153 as a reference chemical.

  The trophic positions of the organisms were determined based on δ^{15}N measurements and δ^{13}C measurements were used to assess the sources and flow of dietary carbon in the food web. The trophic levels assigned to the organism (using a Δ^{15}N of 3.4 ‰ TL^{-1}) are shown in Table 28: Summary of levels of D6 in samples collected from Tokyo Bay along with the concentrations of D6 measured. In all cases the concentration of D6 was below the method detection limit[20] but the actual uncensored values measured were used in the subsequent analysis.

[20] The method detection limit (MDL) was the level in a sample matrix that could be measured and reported with >99% certainty as being greater than zero. The limit of quantification was defined as 3 times the MDL. The actual non-censored values were reported.

The concentration of D6 (and also PCB-153 and PCB-180) in sediment varied spatially across the area, generally decreasing with distance from the inner part of the estuary (close to the mouths of the Arakawa River and the Edogawa River). The $\delta^{15}N$ and $\delta^{13}C$ measurements in sediment also appeared to be related to the proximity of the rivers entering the bay but no significant trends were apparent. As a result of the existence of this concentration gradient in the levels in sediment the study area was stratified and mean concentrations in sediments were calculated using appropriate methods for a stratified experimental design.

Table 28: Summary of levels of D6 in samples collected from Tokyo Bay

Sample	Number of samples analysed	Trophic level (based on a **Δ15N** value of 3.4 ‰ TL^{-1})	Mean lipid content (%)	Mean concentration of D6 (ng/g lipid) (±standard deviation)[a]
Dotted gizzard shad (juvenile) (*Konosirus punctatus*)	3 composites (each of 11 individuals)	3.0	8.0	39.1±5.7
Silver croaker (*Pennahia argentata*)	3 composites (each of 13 individuals)	3.1	5.9	198±41.2
Japanese sardinella (*Sardinella zunasi*)	3 composites (each of 48 individuals)	3.1	4.5	95.5±7.6
Japanese anchovy (*Engraulis japonicas*)	3 composites (each of 55 individuals)	3.5	3.9	106±13.7
Dotted gizzard shad (adult) (*Konosirus punctatus*)	1 composite (of 5 individuals)	3.8	17.0	70.6±(14.1)
Chub mackerel (*Scomber japonicas*)	1 composite (of 4 individuals)	4.1	20.0	34.2±(6.8)
Red barracuda (*Sphyraena pinguis*)	1 composite (of 5 individuals)	4.1	11.0	78.7±(15.7)
Japanese sea bass (*Lateolabrax japonicas*)	6 individuals	4.4	6.3	74.9±31.5

Notes: a) The concentrations of D6 were below the method detection limit (MDL). This was the level in a sample matrix that could be measured and reported with >99% certainty as being greater than zero. The limit of quantification was defined as 3 times the MDL. The actual non-censored values were reported. For the species where only one sample was analysed the standard deviation (given in brackets) was estimated using sampling variances from other studies conducted on cVMS.

For the fish samples the $\delta^{13}C$ measurements indicated that all species were feeding on a similar carbon source, and that this carbon source was different to that in the sediment. The $\delta^{15}N$ measurements suggested that the food web covered around 1.4 trophic steps with planktivorous forage species at the base of the food web (e.g. juvenile dotted gizzard shad (*Konosirus punctatus*), silver croaker (*Pennahia argentata*) and Japanese sardinella

(*Sardinella zunasi*)) and piscivorous predatory species at the top of the food web (e.g. red barracuda (*Sphyraena pinguis*), chub mackerel (*Scomber japonicus*) and Japanese sea bass (*Lateolabrax japonicas*)). Examination of the gut contents indicated that the Japanese sea bass were feeding exclusively on Japanese anchovy (*Engraulis japonicas*) and Japanese sardinella at the time of sampling. With the exception of Japanese sea bass the species sampled were thought to actively migrate throughout the estuary (Japanese sea bass were not thought to migrate as actively as other species).

Trophic magnification factors were firstly estimated from the fish data from the slope of a plot of ln [concentration in fish (ng/g lipid)] versus trophic level. The TMF for D6 was 0.8 with a 95% confidence interval of 0.5 to 1.4 and was not statistically different from 1 (p=0.46). The TMFs derived for PCB-153 and PCB-180 were 2.7 and 2.8 respectively.

The TMFs were also estimated from the same data using a probabilistic method (in order to take into account bias resulting from experimental design). This resulted in a median TMF for D6 of 0.7 (95% confidence interval 0.5-1.0, probability of TMF >1 2.6%). The median TMFs derived for PCB-153 and PCB-180 were both 2.2 using this method.

Next the data were analysed using a benchmarking approach combined with the probabilistic method, using PCB-180 as the benchmarking chemical. For this approach the TMF for PCB-180 was assumed to be 4.0 (the median value of published data for this substance) and this was used to calibrate the food web, resulting in a benchmarked $\Delta^{15}N$ value of 5.9 ‰ TL^{-1}. This value was then used to derive the TMF for D6 and PCB-153. Using this approach the median TMF for D6 was 0.5 (95% confidence interval 0.3-1.0, probability of TMF >1 2.6%). The median TMF for PCB-153 was 3.9. Although this approach resulted in a TMF value for PCB-153 that was in line with the expected value for this substance the $\Delta^{15}N$ value derived was outside the accepted range for aquatic food webs (generally taken to be between 3.0 ‰ TL^{-1} and 5.0 ‰ TL^{-1}). Powell *et al.* (207) suggested that this was indicative of variable exposure in the current food web.

As the sediment data also indicated the existence of concentration gradients within the sampled area, and hence the possibility of variable exposure of the fish sampled, an analysis was undertaken to correct for this based on estimated migration patterns for each species (based on their known ecology) and the concentrations in sediment (used as an indicator of exposure based on the assumption that the concentrations in water and sediment were in equilibrium over the long-term). This was carried out by estimating BSAF values for each species based on the mean concentration in each species (ng/g lipid) by the relative exposure concentration in sediment (ng/g total organic carbon) for that species. The BSAFs derived are summarised in Table 29: Summary of bioaccumulation parameters derived for Tokyo Bay. The BSAF for D6 was in all cases <1 and it was found to generally decrease with increasing trophic level, which was in contrast to the BSAFs calculated for PCB-153 and PCB-180.

The BSAFs for PCB-180 were used to apply an exposure correction to the food web. Using this approach an exposure-corrected $\Delta^{15}N$ value of 3.9 ‰ TL^{-1} was calculated using the benchmarking approach outlined above. This was then used to estimate the TMF for D6 and PCB-153 using the probabilistic approach. The exposure-corrected median TMF for D6 was 0.6 (95% confidence interval 0.4-0.8, probability of TMF >1 0.3%). The median TMF for PCB-153 was estimated to be 3.6. This method was considered by Powell *et al*. (2017) to provide the best estimates of the TMFs for this food chain. The various TMFs estimated in this study are summarised in Table 29: Summary of bioaccumulation parameters derived for Tokyo Bay.

Table 29: Summary of bioaccumulation parameters derived for Tokyo Bay

Parameter		D6	PCB-153	PCB-180
Biota-sediment accumulation factor (BSAF)	Dotted gizzard shad (juvenile)	0.045	1.0	0.44
	Silver croaker	0.20	0.87	0.57
	Japanese sardinella	0.088	1.3	0.65
	Japanese anchovy	0.10	1.4	0.94
	Dotted gizzard shad (adult)	0.085	2.6	1.5
	Chub mackerel	0.038	3.3	1.8
	Red barracuda	0.066	2.5	1.6
	Japanese sea bass	0.052	5.4	3.3
TMF using the standard method; $\Delta^{15}N$ = 3.4 ‰ TL^{-1}	TMF	0.8	2.7	2.8
	95% Confidence Interval	0.5-1.4	1.4-5.3	1.4-5.6
	TMF statistically significantly different from 1	No (p=0.46)[a]	Yes (p=0.01)[a]	Yes (p=0.01)[a]
Probabilistic TMF; $\Delta^{15}N$ = 3.4 ‰ TL^{-1}	Median TMF	0.7	2.2	2.2
	95% Confidence Interval	0.5-1.0	1.7-2.9	1.7-3.0
	Probability TMF >1	2.6%	>99.9%	>99.9%
Benchmark TMF; $\Delta^{15}N$ = 5.9 ‰ TL^{-1}	Median TMF	0.5	3.9	4.0
	95% Confidence Interval	0.3-1.0	2.4-6.3	2.4-6.9
	Probability TMF >1	2.6%	>99.9%	>99.9%
Corrected benchmark TMF; $\Delta^{15}N$ = 3.9 ‰ TL^{-1}	Median TMF	0.6	3.6	4.0
	95% Confidence Interval	0.4-0.8	2.6-4.9	2.9-5.7
	Probability TMF >1	0.3%	>99.9%	>99.9%

Note: a) The p-value indicates the statistical significance of the regression. Statistically significant difference is usually taken as a value of p≤0.05.

Overall the study is well carried out and the analysis of the data is comprehensive. As with the other studies there are some uncertainties associated with the study (including small sample size, possibility of variable exposure) but the analysis carried out has attempted to minimise these. However it is relevant to note the following points.

- The concentration of D6 in all biota samples was below the method detection limit (and also the limit of quantification). This introduces some further uncertainty on the actual concentrations of D6 present in the samples, and hence the TMF derived.
- The species sampled covered 1.4 trophic levels, which is smaller than in some of the other studies available. However in the current study only fish were sampled and when this is compared with other studies the range of trophic levels covered is more similar to that in other studies (for example the Lake Erie study in Table 27: Summary of levels of D6 in samples collected from Lake Erie considered fish samples between trophic level 3.1 and 4.2, compared with fish samples between trophic level 3.0 and 4.4 in the Tokyo Bay study).
- The exposure correction applied was based on the data for PCB-180. It is possible that the distribution of D6 throughout the estuary may have been different to that for PCB-180. No detailed analysis of this was given in the paper but, from visual inspection of the sediment data, it would appear that the concentrations of D6 followed a similar pattern to that of PCB-180.

- The $\Delta^{15}N$ value assumed, while important for determining the magnitude of the TMF, does not affect whether or not the TMF is above or below 1. This is because the $\Delta^{15}N$ value affects the size of the slope of the ln [concentration] versus trophic level plot and not whether the slope is positive (TMF >1) or negative (TMF <1).

Overall, the results of this study suggest that the TMF for D6 in this marine pelagic food web was <1.

- A further TMF study has been published for D6 (Jia *et al*., 2015). The food web studied was from Dalian Bay, Northern China. Dalian Bay is in the northern region of the Yellow Sea and has an area of 40 km^2 and an average depth of 15 m (maximum depth 35 m). Biota samples were collected in September 2013 from four main locations within the Bay and included five fish species (Pacific herring (*Clupea pallasii*), mackerel (*Pneumatophorus japonicas*), greenling (*Hexagrammos otakii*), schlegel's black rockfish (*Sebastes schlegelii*), sea catfish (*Synechogobius hasta*)), mud crab (*Scylla serrata*), Surf clam (*Mactra veneriformis*), short-necked clam (*Raditapes philippinarum*), mussel (*Mytilus galloprovincialis*), black fovea snail (*Omphalus rustica*), clamworm (*Perinereis aibuhitensis*), arthritic Neptune (*Neptunea coming*) and sea lettuce (Latin name not given). The numbers of samples and sampling locations for each species are summarised in Table 30: Summary of levels of D6 in samples collected from Dalian Bay. Precautions were taken to avoid contamination of the samples during collection and analysis, including the use of field blanks.

Table 30: Summary of levels of D6 in samples collected from Dalian Bay

Species	Sampling location	Number of samples analysed	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D6 (ng/g wet weight) (±standard deviation)	Mean concentration of D6 (ng/g lipid) (±standard deviation) – as reported
Pacific Herring (*Clupea pallasii*)	S2	26 individuals	3.15±0.11	9.23 ±2.77	20.2±9.29	124±84.2
Mackerel (*Pneumatophorus japonicas*)	S2	15 individuals	2.22±0.10	5.45 ±1.65	15.6±6.96	153±73.4
Greenling (*Hexagrammos otakii*)	S2	7 individuals	3.58±0.20	3.60 ±1.25	26.9±24.8	314±295
Schlegel's black rockfish (*Sebastes schlegelii*)	S2	6 individuals	3.40±0.18	1.98 ±0.79	8.9±5.67	255±213
Sea catfish (*Synechogobius hasta*)	S2	7 individuals	3.79±0.22	3.18 ±0.81	22.8±12.1	365±156
Mactra quadrangularis (*Mactra veneriformis*)	S2 & S3	7 composites (21 individuals in total)	1.46±0.04	2.44 ±0.30	20.6±10.8	415±178
Short-necked clam (*Ruditapes philippinarum*)	S2 & S3	10 composites (30 individuals in total)	2.00±0.07	3.73 ±0.36	13.0±3.64	175±46.3

Species	Sampling location	Number of samples analysed	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D6 (ng/g wet weight) (±standard deviation)	Mean concentration of D6 (ng/g lipid) (±standard deviation) – as reported
Mussel (*Mytilus galloprovincialis*)	S2 & S3	10 composites (30 individuals in total)	1.58±0.11	3.44 ±0.90	8.33±6.62	127±107
Arthritic neptune (*Neptunea cumingi*)*	S1 & S2	3 composites (9 individuals in total)	2.69±0.08	2.12 ±0.52	23.5±13.7	534±191
Black fovea snail (*Omphalus rustica*)	S2 & S3	3 composites (71 individuals in total)	2.06±0.02	3.84 ±0.53	14.7±3.24	187±39.4
Mud crab (*Scylla serrata*)	S2	5 composites (15 individuals in total)	2.83±0.24	4.15 ±0.72	18.4±6.06	224±66.7
Clamworm (*Perinereis aibuhitensis*)*	S1 & S2	6 composites (60 individuals in total)	1.61±0.07	2.79 ±0.92	9.29±5.77	160±87.3
Sea lettuce (*Ulva pertusa*)	S1, S2 & S3	8 samples	1.91±0.06	1.48 ±0.39	10.5±11.7	320±286

Note: The Sampling sites were designated by letter only but shown on map of Dalian Bay. Site S1 was closest to the shore. Site S2 was approximately in the middle of the bay and site S3 was close to the mouth of the bay.
*Species not included in the TMF calculation (see text).

The organisms were assigned to trophic levels on the basis of $\delta^{15}N$ measurements using a $\Delta^{15}N$ enrichment factor of 3.4 ‰. Short-necked clams (*Ruditapes philippinarum*) were assumed to represent trophic level 2 and the trophic levels of the other organisms were determined relative to this. The tropic levels assigned are summarised in Table 30: Summary of levels of D6 in samples collected from Dalian Bay.

The fish were analysed as individuals but only the concentration of D6 in muscle samples were determined rather than the concentration in whole fish. The other species were analysed as composite samples of several individuals (see Table 30: Summary of levels of D6 in samples collected from Dalian Bay).

Low levels of D6 were found to be present in field and procedural blanks and the limit of detection (LOD) and limit of quantification (LOQ) for D6 were set to 1.68 ng/g ww and 3.39 ng/g ww respectively. D6 was found at concentrations above the LOQ in 94 % of the samples analysed. It was below the LOQ in one sample of greenling, one sample of Schlegel's black rockfish, three samples of mussel, one sample of clamworm and one sample of sea lettuce. The concentrations determined are summarised in Table 30: Summary of levels of D6 in samples collected from Dalian Bay, along with the determined lipid contents of the samples. For the subsequent calculation of the TMFs, Jia *et al.* (2015) set values below the LOQ to two thirds of the LOQ (i.e. 2.26 ng/g ww).

It is important to note that the concentrations on a lipid weight basis reported in Table 30: Summary of levels of D6 in samples collected from Dalian Bay are as they are given in the Jia *et al.* (2015) paper. On closer inspection it is not clear how these values have been determined from the reported wet weight concentration and the reported lipid contents. For example, the mean concentration of D6 in Pacific herring is 20.2 ng/g ww and the mean lipid content is given as 9.23 %. Based on these data the mean lipid normalised concentration for that species would be expected to be around 220 ng/g lw weight (i.e. lipid normalised concentration = wet weight concentration×100/% lipid) but the value given in the paper is 124 ng/g lw. A similar apparent discrepancy in the data is evident for all of the other species (using both the reported mean concentrations in Table 30: Summary of levels of D6 in samples collected from Dalian Bay and the individual data reported in the supporting information for the Jia *et al.* (2015) paper). This, therefore, casts some uncertainty over the lipid normalised concentrations given in the paper.

To try and better understand how the lipid normalised data have been determined the authors of the Jia et al. (2015) have been contacted. At the time of writing, no further information on this has been received. Therefore the following paragraphs consider the TMF values as derived by Jia *et al*. (2015) using the data as reported. In addition a further analysis of the raw data has been undertaken in order to investigate other ways of calculating the lipid normalised concentrations from the given lipid contents and wet weight concentrations.

TMF analysis of data as reported by Jia *et al*. (2015)

Jia *et al*. (2015) used stable carbon isotope measurements to identify species feeding on common carbon sources. The stable carbon isotope ratios were in the range (mean±standard deviation) from -22.7±0.5 ‰ to -25.7±0.5 ‰ for fish species, from -23.3±0.5 ‰ to -26.2±0.2 ‰ for invertebrates (with the exception of arthritic Neptune and clamworm) and -22.7±0.8 ‰ for sea lettuce. The stable carbon isotope ratios for arthritic Neptune and clamworm were -19.4±0.4 ‰ and -20.1±0.5 ‰ respectively, indicating that they were feeding on different carbon sources than the other species included in the study. These two species were therefore not included in the TMF calculations.

The TMF for D6 was estimated from the slope of the plot of the logarithm of the lipid normalised concentration against trophic level. In addition bootstrapping methods were used to estimate the TMF for various configurations of the marine food web.

No statistically significant correlations were found between the lipid normalised concentration and trophic level for the data in this study. The TMF for D6 was estimated to be 1.01 (95 % confidence interval: 0.84-1.22; 66.9 % probability of observing a TMF >1).

A polybrominated diphenyl ether (BDE-99) was also included in the study as a benchmark chemical, and the TMF for this substance was determined to be 3.27 (95 % confidence interval: 2.49-4.30; 99.7 % probability of observing a TMF >1).

TMF analysis of data

For this analysis the lipid normalised concentrations of each species has been recalculated from the raw wet weight data and the raw lipid content data by using lipid normalised concentration = wet weight concentration×100/% lipid content. The concentrations have been estimated from the individual data reported in the supporting information to the Jia et al. (2015) paper, and the mean values have been estimated both excluding concentrations that were below the LOQ and using a value of two thirds of the LOQ for concentrations below the LOQ (as done in the

original paper). The data obtained using this approach are summarised in Table 31: Recalculated lipid normalised concentrations of D6 in samples collected from Dalian Bay.

Table 31: Recalculated lipid normalised concentrations of D6 in samples collected from Dalian Bay

Species	Sampling location	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D6 (ng/g wet weight) (±standard deviation)	Recalculated mean concentration of D6 (ng/g lipid) (±standard deviation) – omitting samples <LOQ	Recalculated mean concentration of D6 (ng/g lipid) (±standard deviation) – assuming <LOQ = 2/3 LOQ
Pacific Herring (*Clupea pallasii*)	S2	3.15±0.11	9.23±2.77	20.2±9.29	248±168	248±168
Mackerel (*Pneumatophorus japonicas*)	S2	2.22±0.10	5.45±1.65	15.6±6.96	308±148	308±148
Greenling (*Hexagrammos otakii*)	S2	3.58±0.20	3.60±1.25	26.9±24.8	846±563	733±595
Schlegel's black rockfish (*Sebastes schlegelii*)	S2	3.40±0.18	1.98±0.79	8.9±5.67	599±432	515±437
Sea catfish (*Synechogobius hasta*)	S2	3.79±0.22	3.18±0.81	22.8±12.1	728±311	728±311
Mactra quadrangularis (*Mactra veneriformis*)	S2 & S3	1.46±0.04	2.44±0.30	20.6±10.8	830±364	830±364
Short-necked clam (*Ruditapes philippinarum*)	S2 & S3	2.00±0.07	3.73±0.36	13.0±3.64	349±91.6	349±91.6
Mussel (*Mytilus galloprovincialis*)	S2 & S3	1.58±0.11	3.44±0.90	8.33±6.62	336±212	255±217
Arthritic neptune (*Neptunea cumingi*)*	S1 & S2	2.69±0.08	2.12±0.52	23.5±13.7	1,075±368	1,075±368
Black fovea snail (*Omphalus rustica*)	S2 & S3	2.06±0.02	3.84±0.53	14.7±3.24	386±79.6	386±79.6
Mud crab (*Scylla serrata*)	S2	2.83±0.24	4.15±0.72	18.4±6.06	448±134	448±134
Clamworm (*Perinereis aibuhitensis*)*	S1 & S2	1.61±0.07	2.79±0.92	9.29±5.77	365±156	321±176
Sea lettuce (*Ulva pertusa*)	S1, S2 & S3	1.91±0.06	1.48±0.39	10.5±11.7	705±601	641±586

Note: The sampling sites were designated by letter only but were shown on a map of Dalian Bay. Site S1 was closest to the shore. Site S2 was approximately in the middle of the bay and site S3 was close to the mouth of the bay.
*Species not included in the TMF calculation (see text).

Using the recalculated lipid concentration data the TMF of D6 can be estimated as 1.02 (95 % confidence interval: 0.84-1.23; value not statistically significantly different from 1 (p=0.85)) assuming that concentrations below the LOQ are two thirds of the LOQ (see

Figure 10). In addition the TMF has also been estimated omitting the data points below the LOQ. In this case the TMF is estimated to be 0.98 (95 % confidence interval: 0.82-1.18; value not statistically significantly different from 1 (p=0.86); Error! Reference source not found.). These values are similar to the TMF values reported by Jia *et al*. (2015) and suggest that the TMF for D6 is around 1 for this food chain.

Figure 10: Plot of ln [concentration] (on a lipid weight basis) against trophic level for the Dalian Bay food chain assuming concentrations <LOQ are two thirds of the LOQ

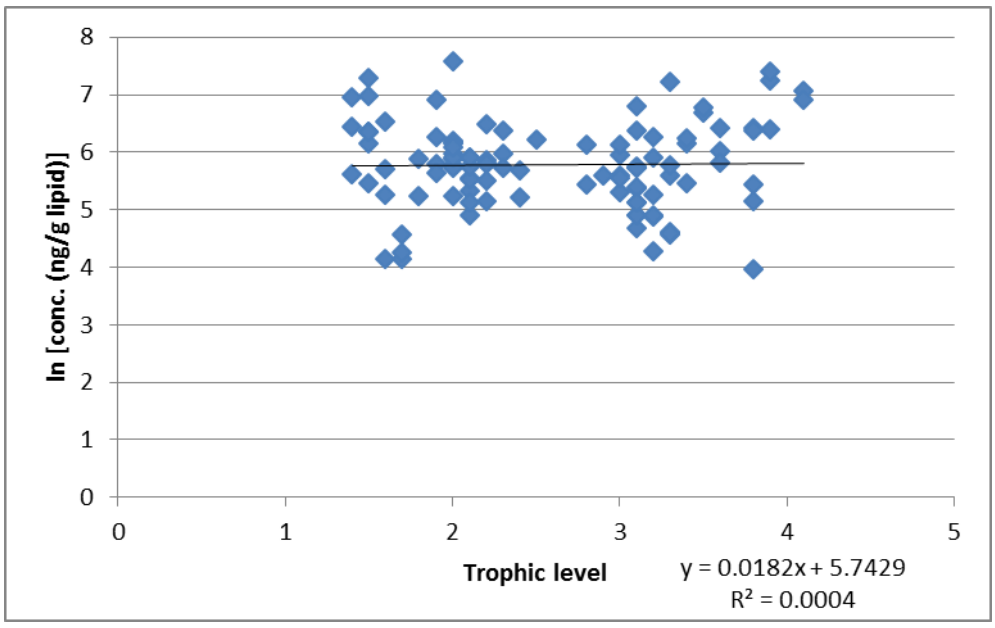
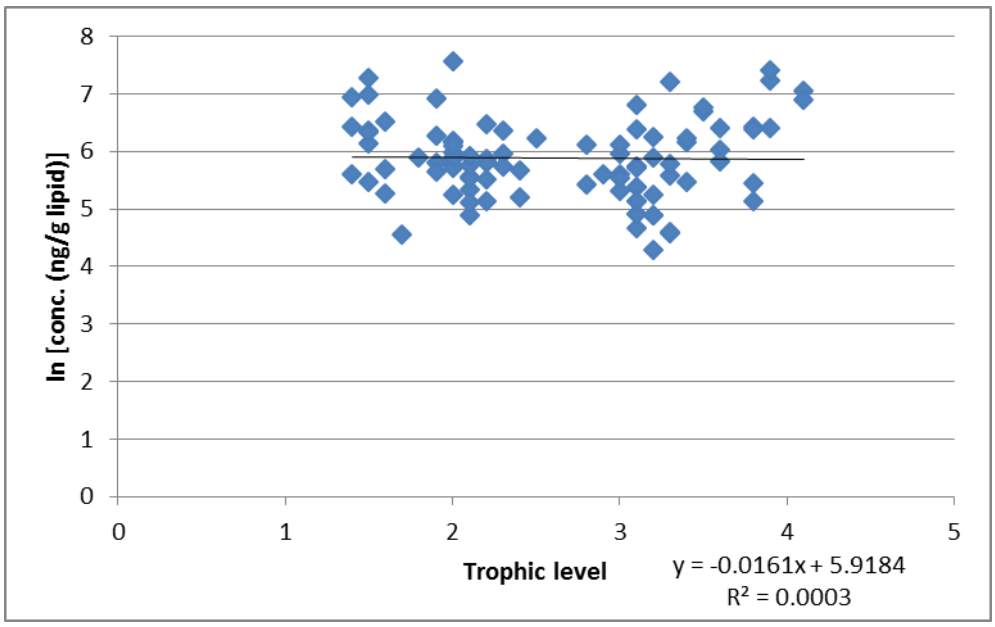


Figure 11: Plot of ln [concentration] (on a lipid weight basis) against trophic level for the Dalian Bay food chain omitting concentrations <LOQ



It is also relevant to note (as has been indicated in a critique of the study by Powell *et al*. (2015)) that there is no information in this study on possible concentration gradients in the water or sediment in the study area. However, the biota samples were

collected from three main areas and, importantly, the fish and mud crab were all collected at the same time and location (designated areas S2 in the paper; see Table 31: Recalculated lipid normalised concentrations of D6 in samples collected from Dalian Bay). When the fish and mud crab alone are considered, the TMF can be estimated as 1.45 (95 % confidence interval: 1.06-1.99; value statistically significantly different from 1 (p=0.022)) when the concentration below the LOQ is taken to be two thirds of the LOQ (see Figure 12) and 1.58 (95 % confidence interval: 1.18-2.13; value statistically different from 1 (p=0.0028)) when the concentrations below the LOQ are omitted (see Figure 13. These data suggest that concentrations gradients could have potentially led to an underestimate of the TMF for D6 in this study.

Although concentration gradients can of course complicate the interpretation of such studies, particularly where species that migrate widely are included, the available data from Jia *et al*. (2015) are suggestive of a TMF of around 1 or above for D6. However, it is not possible to investigate this further with the current data set and, given the uncertainties about the reported lipid concentrations, the results cannot be considered conclusive.

Figure 12: Plot of ln [concentration] (on a lipid weight basis) against trophic level for the fish and mud crab samples from site S2 in Dalian Bay food chain assuming concentrations <LOQ are two thirds of the LOQ

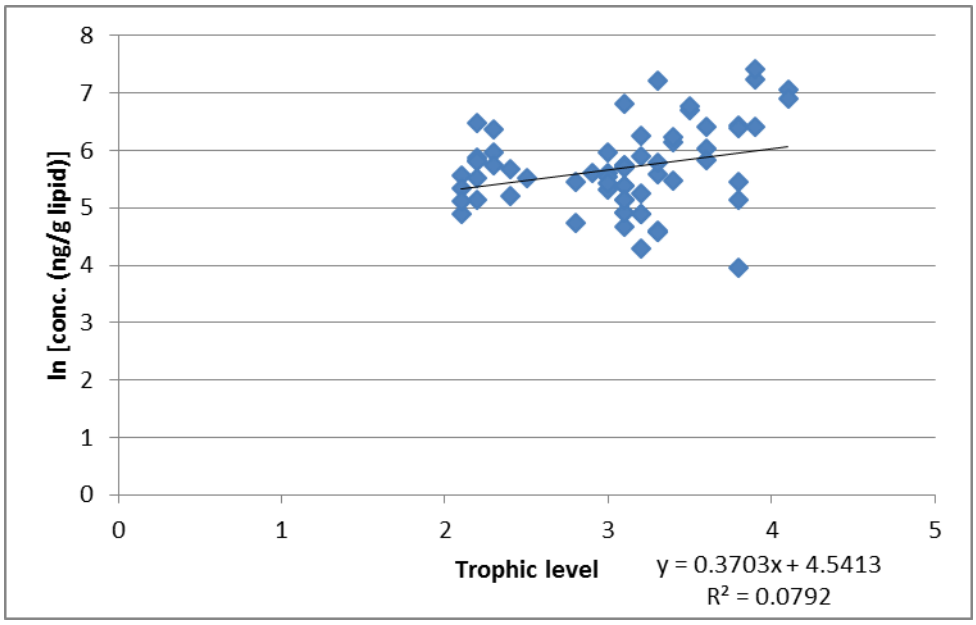
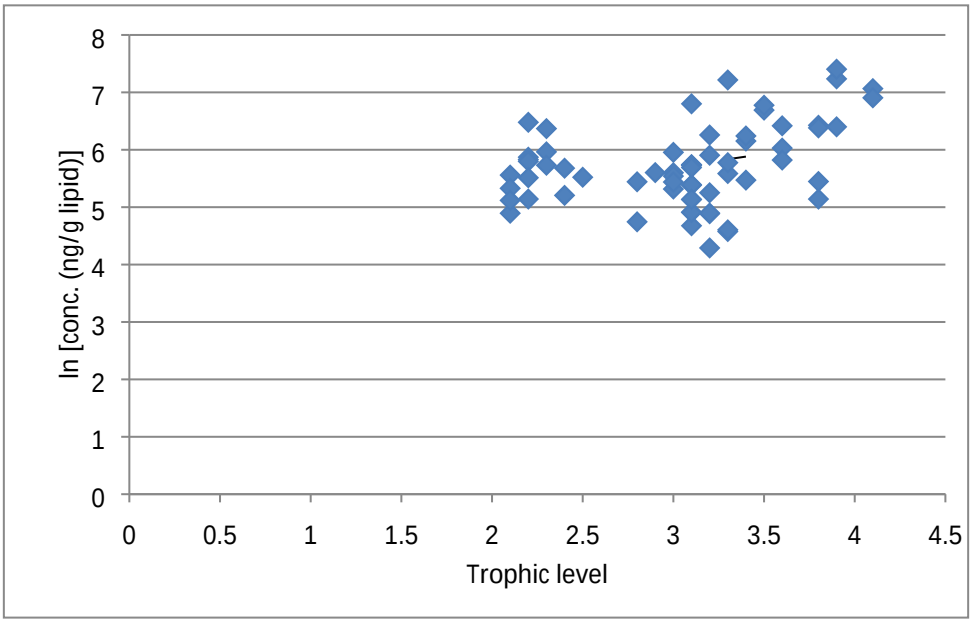


Figure 13: Plot of ln [concentration] (on a lipid weight basis) against trophic level for the fish and mud crab samples from site S2 in Dalian Bay food chain omitting concentrations <LOQ



- An interim report is also available for another TMF study with D6 (Powell et al, 2014b). The study was carried out in Lake Champlain, United States. Lake Champlain is 200 km long, 19 km wide (at its widest point) and has an average depth of 19.5 m (maximum depth 122 m). The sampling was carried out in the main lake basin. The main lake basin is the deepest part of the lake and is mesotrophic to oligotrophic in nature. The sampling was carried out over an area of 800 km^2 using a two-dimensional *a priori* probability design based on one minute latitude by one minute longitude grid resolution. Samples were collected between the 22 and 29 October 2012. A total of 59 surface sediment samples were collected from 59 locations within the study area in order to evaluate spatial variability of the D6 concentration across the study area. Biota samples were collected from 13 locations across six sites in the defined study area, with a total of 5 to 11 samples (either pooled or individual) being collected for each species. The samples included zooplankton, mysid shrimp (*Mysis relicta*) and ten species of fish (white perch *Morone americana*, alewife *Alosa pseudoharengus*, lake cisco *Coregonus artedi*, slimy sculpin *Cottus cognatus*, trout perch *Percopsis omiscomaycus*, lake whitefish *Coregonus clupeoformis*, rainbow smelt *Osmerus mordax*, yellow perch *Perca flavescens*, brown trout *Salmo trutta* and lake trout *Salvelinus namaycush*. Lake trout represented the highest trophic level of the species sampled.

  The food web structure was evaluated using $\delta^{15}N$ and $\delta^{13}C$ measurements. The relative trophic levels of the species were determined using both a value of $\Delta^{15}N$ of 3.4 ‰ and a value of $\Delta^{15}N$ derived from the sampled food web (see below). The $\delta^{13}C$ data for zooplankton ($\delta^{13}C$ = -28.6 ‰) were lower than those of the other species sampled ($\delta^{13}C$ = -27.7 ‰ to -23.4 ‰) which suggested that the zooplankton may have been feeding on a different source of carbon to the other species. However, Powell et al. (2014b) considered this possibility unlikely. The $\delta^{15}N$ data for zooplankton ($\delta^{15}N$ = 18.2 ‰) were also anomalous with those for the other species sampled ($\delta^{15}N$ in the range 12.7 to 18.0 ‰ , which would indicate that zooplankton occupied the highest trophic level position in this food chain). The authors considered that the stable isotope data for zooplankton may have been biased as the samples were collected in autumn and it was likely that large quantities of seston and detritus may have been collected

with the zooplankton. Therefore the zooplankton data were not used in the TMF determination.

Precautions were taken during sampling and analysis to avoid inadvertent contamination of the samples with D6. The concentrations of a benchmark chemical, PCB-180 (TMF assumed to be 4), and a reference substance, PCB-153 (TMF assumed to be >3.5), were also determined in the samples. The individual concentrations of D6 determined in the samples are only shown graphically in the report. The concentrations of D6 in sediment were found to vary spatially across the study areas and generally decreased with increasing distance from areas of point source discharges (Burlington, Vermont and Plattsburgh). The organic carbon normalised concentrations of D6 were generally greatest on the eastern side of the sampled basin, whereas the concentrations of PCBs were generally greatest in the middle sections and west side of the sampled area. Similarly the concentrations of D6 and PCBs in biota samples were variable across the lake and generally appeared to be related to sampling location.

Powell et al. (2014b) estimated the TMF for D6 using a number of different methods. The derived TMF values are summarised in Table 32: Summary of TMF values determined for D6 from Lake Champlain (Powell et al., 2014b). The standard TMF was estimated using linear regression to determine the slope of a plot of ln [concentration (ng/g lipid)] versus trophic level. This was done using a $\Delta^{15}N$ value of 3.4 ‰, which is the typical value used in TMF studies, and a $\Delta^{15}N$ value of 4.38 ‰, which is the value obtained by benchmarking the data using PCB-180 as the benchmark chemical (TMF assumed to be 4). The TMF for D6 using these two approaches was 1.7 (95 % confidence interval: 1.2-2.5) and 2.0 (95 % confidence interval: 1.2-3.3) respectively. The slopes of the regression plots were statistically significantly different from 0 ($p<0.05$).

Table 32: Summary of TMF values determined for D6 from Lake Champlain (Powell et al., 2014b)

Method	Parameter	D6	PCB-180	PCB-153
Linear regression using $\Delta^{15}N$ = 3.4 ‰	Median TMF	1.7	2.9	2.6
	95% Confidence interval	1.2-2.5	2.1-4.2	1.9-3.5
	R^2	8.6%	31.7%	31.2%
	p-value	0.008	<0.001	<0.001
Benchmarked linear regression using $\Delta^{15}N$ = 4.38 ‰	Median TMF	2.0	4.0	3.4
	95% Confidence interval	1.2-3.3	2.5-6.3	2.3-5.0
	R^2	8.6%	31.7%	31.2%
	p-value	0.008	<0.001	<0.001
Bootstrap regression using $\Delta^{15}N$ = 3.4 ‰	Median TMF	1.9	2.9	2.4
	95% Confidence interval	0.9-4.1	1.3-6.5	1.2-5.0
	R^2	11.5%	25.1%	23.3%
	p-value	0.256	0.081	0.094
	Probability of TMF >1	96.2%	99.6%	99.3%
Benchmarked bootstrap regression using $\Delta^{15}N$ = 4.47 ‰	Median TMF	2.4	4.0	3.2
	95% Confidence interval	0.9-6.2	1.4-11.8	1.3-8.5
	R^2	11.7%	25.1%	23.4%

Method	Parameter	D6	PCB-180	PCB-153
	p-value	0.254	0.082	0.094
	Probability of TMF >1	96.3%	99.5%	99.1%
Adjusted for exposure - linear regression using $\Delta^{15}N$ = 3.4 ‰	Median TMF	0.5-2.8	1.9-13	1.2-8.3
	R^2	0.1%-58.4%	13.6%-87.3%	0.9%-91.3%

The next method used was a probabilistic/bootstrapping approach similar to that used on other studies (e.g. Powell et al., 2017). This was carried out using a $\Delta^{15}N$ value of 3.4 ‰ and a benchmarked $\Delta^{15}N$ value of 4.47 ‰ derived from the data for PCB-180. Using these methods the median TMF was determined as 1.9 and 2.4, respectively, with the probability of the TMF being >1 estimated to be 96.2-96.3 %, respectively. However, the slopes of the regression plots were not statistically significantly different from 0 ($p>0.05$).

Powell et al. (2014b) noted that the r^2-values of the regressions for D6 were relatively low (8.6-11.7 %) indicating that trophic position alone was a relatively weak descriptor for the lipid normalised concentrations across the food web, and considered that this was evidence for complications from exposure gradients across the study area. In addition, Powell et al. (2014b) noted that the $\Delta^{15}N$ value obtained by benchmarking (4.4-4.5 ‰) was outside the range of values normally expected (typically 3.0-4.4 ‰) and that this was also indicative of food webs that are confounded by variable exposures.

To try to correct for variable exposure across the foodchain, Powell et al. (2014b) used an iterative process using the concentrations in sediment as a marker of exposure and assumptions over the home range of the organisms. This resulted in variable and uncertain estimates of the TMF that depended on the starting point of the iteration process. The exposure-adjusted TMF values for D6 were in the range 0.5-2.8 using this approach.

The potential effect of exposure gradients on the TMF of D6 was investigated further using a hypothetical food chain model. This indicated that the TMF is a function of the log K_{ow} of a substance and the rate of biotransformation, and the modelling estimated that a TMF of <1 would be obtained regardless of the log K_{ow} value so long as the metabolic rate constant was >0.05 d^{-1} (or half-life <20 days.

Overall, it is likely that the results of this TMF study are confounded by the presence of concentration gradients in the study area. However it is difficult to determine the actual significance of this based on the information reported as it is not clear which sample was collected at each sampling station/location and the raw concentration data are not available. It is, however, relevant to note that all methods used to estimate the TMF of D6 have resulted in at least some TMF values >1.

In addition to the above field studies, some preliminary results have been provided on the levels of D6 in pike (*Esox lucius*) and roach (*Rutilus rutilus*) obtained from the River Cam in the UK (R van Egmond, personal communication). The fish were obtained from a section of the river that receives effluent from the city of Cambridge. Two individual pike (one 30 cm in length and one 50 cm in length) and a composite sample of eight roach were analysed. The lipid content of the two pike was 0.44 per cent and 0.49 per cent and the lipid content of the roach composite sample was 0.62 per cent. The concentration of D6 in the roach sample was 2.47 mg/kg lipid (mean of duplicate analyses of the sample). The concentration of D6 in the pike was lower at 0.46 mg/kg lipid in one sample (mean of duplicate analyses of the sample) and 1.09 mg/kg lipid (single analysis). Thus these results show a decrease in concentration between roach and pike. The significance of this finding is unclear given the small sample size.

When considering the available field studies that have investigated trophic magnification the limitations of the studies should be taken into account. As noted earlier, no agreed methodology currently exists for carrying out such studies, or interpretation of the results of such studies, although it is recognised that work is now underway to address this. For the available studies for D6 there are limitations in terms of the sampling (in general only a small number of samples were obtained for each species and in some cases single samples were collected) which introduces some uncertainty over how representative the data are for each species in the areas sampled, particularly when samples are taken at different time points or locations within the water body.

CES (2010b) and Powell (2010b) summarise the developing thinking in terms of analysis of data from such studies based on the HESI/SETAC/USEPA Expert Workshop on 'Lab to Field Bioaccumulation' that was held on the 18-19th November 2009 (now published in two publications, Borgå *et al.* (2011) and Conder *et al.* (2011). CES (2010b) recommends that the level of uncertainty associated with the TMF value is best investigated using Monte-Carlo simulation with bootstrapping (as was done with the Oslofjord data) as this allows the probability of a TMF>1 to be estimated. In addition it was recommended that the TMF should be derived based on regression analysis across all individual samples, rather than by using the mean concentration per species as this reduces bias introduced by unequal sample sizes for each species. It is understood that in some of the available studies, although only the mean concentrations per species were generally reported in the study report, the TMF values reported were derived using the individual data points rather than the species means (for example in Lake Pepin). For the Lake Mjøsa study, the influence of each species was weighted dependent on the number of samples.

CES (2010b) and Powell (2010b) also suggest that the use of Monte-Carlo simulation with bootstrap analysis can be used to reduce the uncertainty associated with seasonal variability. However this would imply that the distribution of concentrations is known (or could be estimated) for all species at different times of the year. This may not necessarily be the case with Lake Pepin for example, as the macroinvertebrates were sampled in May and the fish were sampled in September and so the distribution of concentrations found for each species will not contain a seasonal element.

CES (2010b) and Powell (2010b) outline a number of other possible areas of uncertainty where further work may be needed in order to better understand the derivation and interpretation of TMF values. These are briefly summarised below.

- Improved knowledge of the ecology of food webs, including guidance on the use of $\delta^{15}N$ and $\delta^{14}C$ in trophic level assignment.
- Uncertainty in field measurements resulting from potential spatial and temporal inhomogeneity in exposure and sample collection, including:
  - Unbalanced test designs (over/under representation of certain species).
  - Sample collection bias.
  - Lack of statistical power.
  - Seasonal variability of short-lived species.
  - Age variation of long-lived species.
- Different food chains (benthic versus pelagic)[21], which may give rise to:

[21] These may be relevant considerations when comparing the data from Lake Mjøsa (and Lake Opeongo) with those from Lake Pepin and Oslofjord.

  - Differences in chemical accumulation dynamics between benthic and pelagic food webs.
  - Disproportionate/different exposure levels for contaminants across benthic versus pelagic food chains.
- Multiple sources of contamination in food webs (exposure via food, water and sediment).
- Use of reference materials with known bioaccumulation properties.

The available TMF data for siloxanes up to 2009 have been considered at an expert panel workshop organized by the Global Silicones Council (Global Silicones Council, 2009). This workshop identified the following as sources of uncertainty and challenges associated with the interpretation of TMF values.

- Different energy requirements and biotransformation abilities between poikilotherms and homeotherms.
- Opportunistic feeders rather than specialist feeders may confound the results.
- Variations with size of a given species, particularly invertebrates.

The workshop recommended that, where possible, TMF is the most relevant parameter for evaluating bioaccumulation.

Powell *et al.* (2017)[22] carried out a comparison of the TMFs derived for D6, along with D4 and D5, from the various studies available up until 2014. This included, where necessary, recalculation of the TMF for the food chain using the probabilistic approach with a $\Delta^{15}N$ of 3.4 ‰ TL^{-1} and species-specific probability density functions for $\delta^{15}N$ and the lipid normalised concentrations defined by the means and standard deviations reported in each study. The probabilistic approach was considered by Powell *et al*. (2017) the most appropriate method of analysing the data to minimise bias resulting from experimental design. The results of this analysis are summarised in Table 33: Summary of TMFs derived for cVMS in field studies (based on Powell et al. (2017)). The analysis did not consider the data from Lake Opeongo, nor the recent studies in Dalian Bay and Lake Champlain.

Table 33: Summary of TMFs derived for cVMS in field studies (based on Powell et al. (2017))

Location	Food web	Range of trophic levels covered by the food chain	Median TMF (95 % confidence interval given in brackets)		
			D4	D5	D6
Tokyo Bay	Pelagic – marine	3.0-4.4	0.6 (0.5-0.8)[a]	0.6 (0.4-0.8)[a]	0.7 (0.5-1.0)
Inner Oslofjord	Benthic – marine	1.5-4.0	0.6 (0.5-0.8)	0.2 (0.2-0.3)	0.3 (0.2-0.5)
	Pelagic – marine		0.7 (0.4-1.0)	0.3 (0.2-0.6)	0.8 (0.6-1.2)
Outer Oslofjord	Benthic – marine	2.1-4.1	0.7 (0.5-1.0)	0.4 (0.3-0.6)	0.3 (0.2-0.4)
	Pelagic – marine		1.0 (0.6-1.4)	0.5 (0.3-0.9)	0.9 (0.7-1.1)
Lake Pepin	Benthic - freshwater	2.0-3.8	0.5 (0.3-0.7)	0.3 (0.2-0.6)	0.4 (0.2-0.6)
Lake Mjøsa	Pelagic – freshwater	2.0-4.2	1.3 (0.8-2.1)	2.5 (1.6-4.0)	0.8 (0.6-1.2)
	Pelagic – freshwater	2.0-4.4	0.8 (0.5-1.1)	3.1 (2.3-4.3)	2.7 (2.0-3.8)
Lake Ransfjorden	Pelagic – freshwater	2.0-3.8	0.6 (0.3-1.1)	2.2 (0.9-4.7)	1.6 (0.9-2.9)

[22] This study was included in the previous version of this fact sheet referenced as Powell et al. (2014a). The reference has simply been updated to the final report.

Location	Food web	Range of trophic levels covered by the food chain	Median TMF (95 % confidence interval given in brackets)		
			D4	D5	D6
Lake Erie	Benthic and pelagic - freshwater	2.0-4.2	0.6 (0.4-0.9)	0.8 (0.5-1.1)	0.7 (0.4-1.3)

Note: a) An earlier unpublished preliminary study of Tokyo Bay suggested a TMF of 0.4-0.6 for D4 and 0.5 for D5.

Based on this analysis the only studies that result in TMFs >1 for cVMS are the two studies in Lake Mjøsa (although a TMF of 1 is indicated for D4 in Outer Oslofjord) and the study in Lake Randsfjorden (for D5 and D6), although since this analysis was done, TMFs close to or above 1 have been reported for Dalian Bay and Lake Champlain. For Lake Mjøsa, the TMF for D5 was similar in both studies but for D4 and D6 one study gave a TMF <1 and one study gave a TMF >1. For both these substances a significant number of the data points had concentrations below the limit of quantification which may have introduced some uncertainty into the analysis.

The results from the Lake Mjøsa and Lake Randsfjorden study were considered further by Powell *et al*. (2017). The probabilistic TMFs determined for these two lakes for D5 and D6 were statistically significantly higher than for the other study areas. For the other study areas no significant difference was evident between the values obtained in benthic food webs compared with pelagic food webs for D4 and D5 but the TMF in pelagic food webs for D6 (TMF 0.7-0.9) was significantly greater than from benthic food webs (TMF 0.3-0.4). Powell et al. (2017) considered that the differences between the TMFs derived in Lakes Mjøsa and Randsfjorden compared with the other systems may be related to variable exposure resulting from non-uniform migration patterns of some species and food web dynamics. Powell et al. (2017) noted that the range of $\delta^{13}C$ across the food web was larger in both Lake Mjøsa and Lake Randsfjorden than in other study areas suggesting that omnivorous feeding by consumers may have occurred or that samples were inadvertently collected from trophically distinct food webs. In addition Powell *et al.* (2017) considered that variable exposure resulting from concentration gradients may be a confounding factor in these studies (as is potentially a case with most studies). When considering these concerns it is also relevant to note that the analysis carried out by Powell *et al.* (2017) was attempting to investigate if there were any scientific explanations for the difference between the TMF found in Lake Mjøsa and Lake Randsfjorden and those found in the other studies and so concentrated on the potential uncertainties with the Lakes Mjøsa and Randsfjorden study. However, as noted above there are potential uncertainties with all of the other field studies and these were not discussed in the same level of detail as those for Lakes Mjøsa and Randsfjorden in the paper by Powell *et al.* (2017). Overall, although the concerns raised by Powell *et al.* (2017) are real it is not currently possible to assess the significance of the various uncertainties on the TMFs derived in Lake Mjøsa and Lake Randsfjorden.

A further independent review of the McGoldrick et al. (2014a) TMF study in Lake Erie and the Powell et al. (2017) TMF study in Tokyo bay has been made available (Borgå and Starrfelt (2015)[23]. The focus of the comments made by Borgå and Starrfelt (2015) on the Powell et al. (207) study relate to the benchmarking of the TMFs against PCB-180 and to the probabilistic method used to estimate the TMF. It was noted that the scaling used in the benchmark approach increases (or decreases) the absolute value of the TMF but does not affect the statistical tests for the TMF (i.e. the statistical tests for the TMF being >1 or <1 remain unaltered). Thus the effect of such benchmarking is to scale the extent that the TMF deviates from 1 without altering the probability that the TMF is >1.

For the probabilistic method used by Powell et al. (2017), Borgå and Starrfelt (2015) noted

[23] The authors received a pre-publication version of Powell et al, 2017.

that the approach may give a false impression of the precision of the TMF estimate. This is because the probabilistic method, as applied by Powell et al. (2017) to a large extent does not take into account the variability in the individual estimates of the TMF values used in the method. This is because the method essentially estimates the confidence intervals around the TMF based on the 2.5 and 97.5 percentiles of 10 000 estimates of the TMF obtained using random sampling of probability distributions within the species. However, Borgå and Starrfelt (2015) noted that each of the individual TMF estimates will have its own confidence bounds associated with it and when these are taken into account different conclusions can be reached on the probability of the TMF being <1. For example Powell et al. (2017) concluded that the TMF for D6 in this study was <1 with over 90% probability but Borgå and Starrfelt (2015) showed that each of the 10,000 TMF estimates used in the analysis included a TMF of 1 within their respective individual confidence intervals. Borgå and Starrfelt (2015) concluded that the probabilistic method used by Powell et al. (2017) may give a false impression of the precision of the TMF estimate and should be interpreted with care. The same conclusions would also apply to other studies that have used a similar probabilistic method to estimate the TMF.

For the McGoldrick et al. (2014a) study Borgå and Starrfelt (2015) noted that a similar probabilistic method to the Powell et al. (2017) method had been used, and so the above comments would also apply here. In addition, Borgå and Starrfelt (2015) noted a number of other potential issues with the study including the following:

- Low sample size for organisms at the base of the food web (i.e. a single composite sample for zooplankton and benthic invertebrates). It was assumed that the variance in concentration in these samples would be similar to that in organisms higher in the food web. It is unclear how this affects the estimated TMFs.
- The TMF obtained is sensitive to which species are included in the regression. The study only considers shortening the food web and does not investigate the sensitivity to including/excluding fish that feed on benthopelagic organisms and not only benthic organisms.
- The trophic levels in the study were assigned using zooplankton as the baseline although most species included in the study feed on benthic organisms and not purely pelagically. Borgå and Starrfelt (2015) considered that this might introduce some uncertainty into the trophic levels assigned as the pelagic and benthic food web baselines differ. However it is considered that this is likely to be only a minor issue with the study as all the trophic levels are assigned relatively to the baseline species, and this only moves the plot of the ln [concentration] versus trophic level along the x-axis and does not affect the slope (TMF).

McGoldrick et al. (2014a) suggested that variability in the environmental distribution of the contaminants is a possible explanation for the variability of TMFs, and considered a general assumption that home range is allometrically scaled, so that larger fish have a larger home range than smaller fish. However, Borgå and Starrfelt (2015) considered that home range was very much species- rather than size-dependent.

A study by Warner *et al.* (2014) has investigated whether various allometric parameters (particularly length, weight and age) of fish may play a role in the bioaccumulation of cVMS, including D6, and whether this may contribute to variations observed in the bioaccumulation potential. For the study, samples of Atlantic cod (*Gadus morhua*) liver and sediment were collected in November 2010 and April 2011 from two locations near to Tromsø, Norway. The sampling locations were Tromsøysund, the harbour next to Tromsø, and Nipøya, a small island approximately 30 km northeast of Tromsø. Precautions were taken during sampling and analysis to avoid accidental contamination of the samples. D6 was found to be present in all samples of cod liver, and no statistically significant differences were found between the concentrations present in samples in 2010 compared with 2011 at both locations, and so the data for the two years were combined for further analysis. The mean (± standard deviation) concentration of D6 found at Tromsøysund was

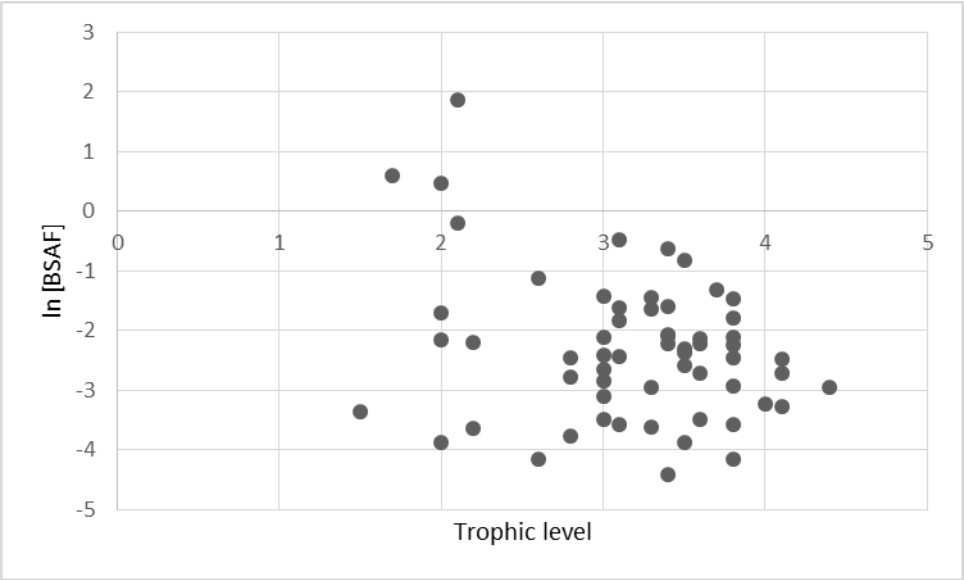
77.2±28.8 µg/kg lipid compared with 38.2±47.5 µg/kg lipid from Nipøya. The concentrations at Nipøya were statistically significantly lower than at Tromsøysund ($p<0.05$) and this was explained in terms of the higher anthropogenic activity at Tromsøysund compared with Nipøya. The stomach contents of the fish indicated that fish from both areas were consuming similar diets and so the difference in concentration was unlikely to be a result of differences in feeding. The difference between locations was confirmed by the sediment data, where D6 was only detectable in sediment samples from Tromsøysund.

The concentration of D6 in cod livers from Tromsøysund was found to be negatively correlated with both fish length and weight using linear regression and Spearman correlation ($p<0.01$). However for the samples from Nipøya, the D6 concentration was found to be negatively correlated to fish length ($p<0.05$, Spearman correlation) but not weight. This discrepancy was thought to result from the smaller number of fish containing detectable concentrations at Nipøya compared with Tromsøysund. No signification correlations were found at either site between D6 concentration and liver weight or fish age. The latter finding was considered to be surprising by Warner *et al.* (2014) as fish length is closely associated with age; however, it was noted that the fish sampled covered a rather limited age range (3-6 years). It was postulated by Warner *et al.* (2014) that the negative correlation with fish size is indicative of greater capacity within the fish for elimination of D6 with increasing size but it is not possible to say from these results if this is driven by increasing metabolism or other elimination processes (such as growth dilution) with increasing fish size.

When considered as a whole the biomagnification behaviour of D6 is broadly similar to that of both D4 and D5, with median TMFs in the range 0.5-1.3 being derived for D4, 0.2-3.1 being derived for D5 and 0.3-2.7 being derived for D6 within the same food webs (see Table 19 and also the Jia et al. (2015) study in Dalian Bay and the Powell et al. (2014b) study in Lake Champlain). The uncertainties with the studies are essentially the same for D4, D5 and D6 with the exception that, for D5, the levels present in the organisms were generally higher than for D4 and D6 (as would be expected based on the higher tonnage of D5 used compared with D4 and D6) which means complications from the presence of non-detectable concentrations amongst the data set were generally less for D5 than for D4 and D6 (see the Annex XV reports for D4 and D5 (2018a and 2018b) for more details of the concentrations measured for these substances).

As well as TMF values, it is also theoretically possible to estimate other bioaccumulation parameters from the information in field studies. For example, if the water or sediment concentrations are known reliably in the areas sampled then it is possible to estimate either bioaccumulation factors (BAFs) relating the concentration in the organism to the concentration in water, or biota-sediment accumulation factors (BSAFs), relating the lipid normalised concentration in an organism to the organic carbon normalised concentration in sediment. For the available studies, reliable information on the concentrations in water in the areas sampled is not available and so it is not possible to estimate BAF values. However, for some studies, information on the sediment concentration is available and, where this is representative of the areas sampled, this can be used to estimate the BSAF values. Such BSAF values have already been derived from some of the studies (see the reviews of the studies earlier in this Section) but this information, and other studies where a BSAF has been estimated has been consolidated in Annex II. This analysis showed that the BSAF is only above 1 for organisms at trophic level 2 or below; all derived BSAF for trophic levels higher than this are <1. Furthermore, there is a tendency for the BSAF to decrease with increasing trophic level, as can be seen from plot of ln BSAF versus trophic level in Figure 14. Such a trend would be expected for a substance that undergoes biodilution in a food chain rather than biomagnification.

Figure 14: Plot of log BSAF against trophic level



Other measures of accumulation

Field studies comparing the uptake of D6 with certain benchmark chemicals have also been carried out. The results from some of these studies are currently available as poster presentations only. The available details are summarised below.

- The accumulation of D6 in the Humber Estuary, UK, has been studied by Kierkegaard (2011). Six intertidal sites in the lower estuary were sampled between 24th September and 15th October 2009. The samples of surface sediment (1-2 cm depth; 9 samples per site, three samples collected within 1 m of each of the three ragworm sampling locations at the site), ragworm (50 individuals from each of three locations at each site) and flounder (1-3 samples per location, although no flounder were obtained at one of the sites) were collected from the six locations in the estuary and were analysed for both D6 and the benchmarking chemical, 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB-180). All personnel involved in the sampling and analysis avoided use of personal care products in order to minimise the potential for inadvertent contamination of the samples. The ragworm samples were depurated for 24 hours prior to analysis and pooled samples of 5-10 individuals were analysed. For the flounder skin-free dorsal fillets from individuals were analysed. Field blanks were incorporated into the sampling scheme in order to check for possible inadvertent contamination of the samples during collection and processing and procedural blanks and control samples were routinely analysed along with the samples. The D6 concentrations found ranged between around 30 and 95 µg/kg dry weight (1,300-3,200 µg/kg organic carbon) in sediment and 2.5 and 27 µg/kg fresh weight in ragworm. D6 was also detectable at up to 4.7 µg/kg fresh weight concentration in two of the 34 flounder fillet samples. The highest concentrations were generally found at the sampling site in the inner estuary and the concentrations were found to decrease down the estuary. D6 was found to be present in field blank samples for the worm sampling but the reported concentrations in ragworm were not corrected for this (and so the reported concentrations in ragworm may be biased high). The lipid levels in biota were not measurable in many of the samples and so a "benchmarking" ratio approach, based on the ratio of the multi-media bioaccumulation factor (mmBAFs) for D6 to that of PCB-180 was used to investigate the bioaccumulation potential of D6. The mmBAF represents the fraction of the chemical present in an environment that has accumulated in an organism and is estimated as the ratio of the amount of chemical in an organism to the amount of

chemical in the environment. For the current study the mmBAF ratio of D6:PCB-180 approximates to the ratio of the sediment-biota bioaccumulation factors (BSAF) for D6 to that for PCB-180 in the same sample. A total of 19 ratios for ragworms and 2 ratios for flounder were calculated from the measured data. The mean ratio was around 0.19 for ragworm (i.e. the mean mmBAF for D6 was around five times lower than that for PCB-180). The ratio for flounder was 0.1 or lower. These results indicate that D6 was bioaccumulating to a lesser extent than PCB-180 in these organisms.

It should be noted that for the flounder samples fillets were analysed. For these samples it is not known how the concentrations measured in muscle relate to the likely whole fish concentration and, importantly, if this relationship is the same for both D6 and PCB-180. In another study from Japan (SIAJ, 2011; see below) the wet weight concentration in whole fish samples (pale chub, common carp, yellowfin goby, flathead mullet and Japanese seabass) tended to be higher than in the edible part of the same fish. It should also be noted that the extraction efficiency of the analytical method used was not 100% (for example the extraction efficiency was reported to be 56% for D6 in flounder) and hence the concentrations of D6 in some of the biota samples may have been underestimated. These factors introduce some further uncertainty into the flounder results from this study.

- A further study of cVMS has been carried out in fish from the Baltic Sea (Kierkegaard *et al.*, 2010 and 2013). The samples analysed were taken from the sample bank of the Swedish Museum of Natural History and included herring (collected in 2007) and blue mussel, European flounder, perch, smelt, white fish, eelpout, turbot, cod, cod liver and grey seal (all collected in 2008). D6 was found to be present in the herring, and it was reported that the D6 concentrations in herring were around 6 times lower than those of D5 and the D5 levels were reported to be in the range 15-720 µg/kg lipid in herring. Therefore it is likely that the D6 levels were present at levels up to 120 µg/kg lipid (shown graphically in the Kierkegaard *et al*., 2013 paper). The herring samples allowed the spatial trend in the concentration of D6 to be investigated. The highest levels were generally found in samples from the Baltic Proper (consistent with a wastewater source) and markedly lower levels being found in samples collected from the Swedish west coast.

- The levels of D6 in the seal blubber samples from 2008 was in the range 4.4-9.5 µg/kg wet weight however the biomagnification potential of D6 could not be assessed fully as the concentration of D6 in samples of herring from 2008 was below the limit of quantification (this was thought to be a result of the low lipid content of the herring from 2008 (<0.1-0.43%) compared with those from 2007 (1.9-6.7%) however a comparison of the levels in seal blubber from 2008 with herring samples from the same area from 2007 revealed that the concentrations in seal were around 6 time lower than in the herring, suggesting that D6 did not biomagnify in seals (Kierkegaard *et al*., 2013). There is clearly some uncertainty this conclusion as the seals and herring were from different years and the temporal variability in the concentrations of D6 in these species is unknown.

- McGoldrick *et al.* (2014b) determined the concentration of D6 in predatory freshwater fish from various lakes in Canada. The fish were collected from 16 water bodies including lakes, rivers and reservoirs and included sites remote from emission sources and sites in heavily populated/industrial areas. The fish samples were collected in 2009 or 2010. The main species sampled were lake trout (*Salvelinus namaycush*) but at some locations where lake trout were not present the species sampled was walleye (*Sander vitreus*). Lake trout and walleye were thought to occupy the uppermost trophic levels of the various water bodies sampled. Between 3 and 10 individuals were collected at each site. Precautions were taken during sample collection and analysis to avoid contamination of the samples with D6. Whole body homogenates of the fish were analysed and the limit of quantification of the analytical method used was 0.37 µg/kg wet weight for D6. D6 was detectable in all 87 fish samples analysed. The concentration

of D6 was generally similar to that of D4 in fish from the same location but was around 10 times lower than that of D5. The highest D6 concentrations were found in fish from the Laurentian Great Lakes, particularly in lake trout from Lake Ontario and the eastern basin of Lake Erie, and were in the range 4.7-16 µg/kg wet weight in lake trout collected from the sampling point closest to Niagara on Lake Ontario. The levels of the cVMS in the fish generally declined in a north-west geographic gradient, but evidence for a possible difference between the environmental behaviour of D6 compared with D4 and D5 was also found in the data. For example, the concentration of D6 in the fish appeared to increase slightly in samples from the more northerly sites (a slight increase in concentration from Lake Huron to Lake Superior and Lake Athabasca to Lake Kusawa was seen for D6 but not D4 or D5). The concentrations of the cVMS in walleye were also generally slightly lower than in rainbow trout, possibly indicating that the potential for accumulation in walleye was lower than in rainbow trout. However, McGoldrick *et al.* (2014b) indicated that these conclusions should be considered speculative at present and that further work would be needed to investigate the spatial distribution and interspecies differences in the body burden before definitive conclusions can be drawn.

- A programme of long-term monitoring of the levels of D6 in sediments and biota samples from Lake Pepin and Lake Ontario is being carried out (Seston et al., 2014a, 2014b, 2015a and 2015b). The overall objective of the work is to investigate any temporal trends in the concentrations in these two areas. The study has been designed to detect a statistically significant (α=0.05) annual rate of change of ±6% in the concentration over a five year period with 80% power (β=0.20). Results of the study are available for 2011 and 2012 and these are discussed below. The temporal analysis has not yet been conducted and will be completed in the final study report for the project.

The results for Lake Pepin (Seston et al., 2015a and 2015b) are summarised in Table 34: Summary of monitoring data for Lake Pepin for the temporal trends study (Seston et al., 2015a and 2015b). For the 2011 samples the mean concentration in zooplankton was below the method detection limit whereas the mean concentration in mayfly was 69.7 ng/g lipid and the mean concentration in fish ranged from 30.7 to 38.1 ng/g lipid. For 2012 the only species where the mean concentration was above the method detection limit was mayfly larvae, which has a mean concentration of 207 ng/g lipid.

Table 34: Summary of monitoring data for Lake Pepin for the temporal trends study (Seston et al., 2015a and 2015b)

Species/ sample	2011				2012			
	Date	No of samples	D6 concentration (mean± standard deviation)		Date	No of samples	D6 concentration (mean± standard deviation)	
			ng/g ww	ng/g lipid			ng/g ww	ng/g lipid
Sediment (0-1 cm stratum)	June	7	3.07±0.33	397±72[c]	May	5	3.26±0.32	416±67[c]
Sediment (0-5 cm stratum)	June	7	4.03±0.70	440±120[c]	May	13	4.05±0.53	418±64[c]
Sediment (0-1 cm stratum)	October	5	3.21±0.32	453±57[c]	Not collected			
Sediment (0-5 cm stratum)	October	5	4.03±0.17	475±38[c]	Not collected			
Zooplankton	Jume	4 pooled	(1.53±0.48)[a]	(102±18)[a]	Not collecte			

Species/ sample	2011				2012			
	Date	No of samples	D6 concentration (mean± standard deviation)		Date	No of samples	D6 concentration (mean± standard deviation)	
			ng/g ww	ng/g lipid			ng/g ww	ng/g lipid
					d			
Mayfly larvae (*Hexagenia* sp.)	June	5 pooled	1.55±0.09[b]	69.8±11.8[b]	May	5 pooled	1.77±0.53	207±127
Gizzard shad (*Dorosoma cepedianum*) (young of year)	October	11 pooled	0.95±0.17[b]	38.1±6.9[b]	October	11 pooled	(1.09±0.54)[b]	(12.7±6.27)[b]
Walleye (*Sander virteus*)	Not collected				October	20 individuals	(0.52±0.28)[b]	(8.38±4.17)[b]
Suager (*Sander canadensis*)	October	20 individuals	0.84±0.33[b]	30.7±17.3[b]	October	21 individual	(0.43±0.34)[b]	(6.57±4.99)[b]

Notes: a) Value is below the method detection limit (3.0 ng/g ww for biota for the samples in 2011 and 1.1 ng/g ww for the samples in 2012).
b) Samples were reanalysed owing to relatively high background levels of D6 in procedural blanks in the original analysis. The method detection limit for the reanalysis was 0.07 ng/g ww.
c) Sediment concentrations are reported on a ng/g organic carbon basis.

The results for Lake Ontario (Seston et al., 2014a and 2014b) are summarised in Table 35: Summary of monitoring data for Lake Ontario for the temporal trends study (Seston et al., 2014a and 2014b). For the 2011 samples the mean concentration in mysid shrimp was 24.1 ng/g lipid and the mean concentration in fish ranged from 39.4 to 182 ng/g lipid. For 2012 the mean concentration in mysid shrimp was 14.4 ng/g lipid and the mean concentration in fish ranged from 26.0 to 289 ng/g lipid.

Table 35: Summary of monitoring data for Lake Ontario for the temporal trends study (Seston et al., 2014a and 2014b)

Species/ sample	2011				2012			
	Date	No of samples	D6 concentration (mean± standard deviation)		Date	No of samples	D6 concentration (mean± standard deviation)	
			ng/g ww	ng/g lipid			ng/g ww	ng/g lipid
Sediment - Lake (0-1 cm stratum)	August	2	4.39 (±2.52-6.26)[a]	497 (±267-727)[a, b]	August	2	4.18 (±1.97-6.38)[a]	485 (±217-754)[a, b]
Sediment – Lake (0-5 cm stratum)	August	2	3.57 (0.76-6.38)[a]	397 (83.3-711)[a, b]	August	2	4.24 (1.43-7.05)[a]	453 (177-730)[a, b]
Sediment – Hamilton harbour (0-1 cm stratum)	Decem-ber	3	23.7±9.4	2,288 ±695[b]	Decem-ber	3	28.4±8.4	2,680 ±659[b]
Sediment – Hamilton harbour (0-5 cm stratum)	Decem-ber	3	22.2±5.3	1,990 ±242[b]	Decem-ber	3	30.3±8.1	2,580 ±630[b]
Mysid shrimp (*Mysis*	August	4 pooled	1.89±0.91	24.1 ±10.4	August	5 pooled	1.23±0.36	14.4±2.5

Species/ sample	2011				2012			
	Date	No of samples	D6 concentration (mean± standard deviation)		Date	No of samples	D6 concentration (mean± standard deviation)	
			ng/g ww	ng/g lipid			ng/g ww	ng/g lipid
relicta)								
Round goby (*Neogobius meanostomus*) - small	August	6 pooled	2.52±0.24	136 ±9	August	6 pooled	2.64±0.25	139±37
Round goby (*Neogobius meanostomus*) - moderate	August	6 pooled	5.09±1.30	18 2±63	August	6 pooled	5.92±1.58	189±23
Rainbow smelt (*Osmerus mordax*)	August	9 pooled	2.29±1.20	39.4 ±12.4	August	3 pooled	1.56 ±0.14	26.0±6.5
Alewife (*Alosa pseudohar-engus*)	August	5 pooled	3.07±0.46	56.3 ±14.0	August	7 pooled	1.91 ±0.38	27.3±13.7
Lake trout (*Salvelinus namaycush*)	August	19 individ-uals	10.7±3.8	58.5 ±19.2	August	20 individ-uals	11.9±4.4	68.2±26.1

Notes: a) Where only two samples were available the values in parentheses represent the range rather than the standard deviation.
b) Sediment concentrations are reported on a ng/g organic carbon basis.

As data are only available for two years it is not possible to draw any tentative conclusions on temporal trends as yet. It is important to note that the study design used is designed to detect temporal trends in concentration. Seston et al. (2014a, 2014b, 2015a and 2015b) indicate that the data should not be used for biomagnification potential as the studies do not evaluate or control confounding factors such as variable exposure, concentration gradients and shifts in dietary preference. However, a preliminary investigation of the 2011 data for Lake Ontario has been undertaken (CES Personal Communication). The evaluated food web consisted of mysid shrimp (TL=3.0), alewife (TL=3.1), small goby (TL=3.5), large goby (TL=3.7), rainbow smelt (TL=3.9), and lake trout (TL=4.6). The results of the preliminary assessment estimated the TMF for D6 to be 0.9 using the standard plot of ln [concentration] versus trophic level and a $\Delta^{15}N$ value of 3.4 ‰ and 0.5 when benchmarked to PCB-180 and corrected for exposure concentration gradients. Probabilistic/bootstrapped estimates for the TMF were 1.1 for D6 where exposure gradients were not taken into account and 0.6 for D6 when corrected for exposure. These are in the same range of values determined in other studies.

When considering these data comparing the apparent accumulation of D6 with that of a reference substance, it is important to note that a similarity between the pattern seen for D6 and the reference substance does not necessarily mean that D6 accumulates by the same processes as the reference substance. This is discussed further below.

For the comparison using the biota-sediment accumulation it is important to also consider the underlying properties and behaviour of the substances in the environment. In this respect there are a number of important differences between D6 and the reference substance PCB-180. These are summarised below (based on arguments put forward for two related cVMS (D4 and D5) by Fisk and Wilmot (2010)).

- The releases of PCB-180 to the environment should have decreased (or more or less ceased) over recent years and so any substance detected probably has been

in the environment for many years. This contrasts with D6 where the presence in the samples reflects current (and on-going) emissions. Furthermore, PCBs in general are known to bind to two broad types of site on sediment particles (reversible and almost irreversible), and therefore a high degree of irreversible adsorption may be expected for PCB-180, particularly in aged sediments (see Fisk and Wilmot (2010) for further discussion). In contrast to this, the adsorption of D6 is thought to be reversible with little or no effect of ageing. This results in an important distinction between PCB-180 and D6 as it would be expected that PCB-180 may be associated with deeper sediments but can be found in surface sediments as a result of disturbance, etc., whereas D6 would be expected to occur in newly deposited sediments and interstitial water.

- The consequence of this is that the exposure of organisms through sediment is likely to be predominantly via ingestion for PCB-180 as little or no substance would be expected to be present in interstitial water, whereas the exposure to D6 would be expected to be via both ingestion and interstitial water.

- As the actual BSAF value is a combination of all routes of exposure it is not possible to infer from the data how the accumulation potential of D6 through any one route (e.g. diet) compares to that for PBC-180. However it can be inferred that the net result of all routes of exposure would lead to lower concentrations in the organism for D6 than for PCB-180 for a given concentration in sediment.

Overall, the available “benchmarking” studies appear to show that the accumulation potential for D6 is lower than for PCB-180. However, there are considerable uncertainties in interpreting these data.

A field study from Japan has recently investigated the sediment biota accumulation factor (BSAF) for D6 in fish (SIAJ, 2011). The samples of sediment and biota were collected from the Tama River, Arakaw River and Tone River, which are representative of the rivers in the Kanto Region. Both the Tama River and Arakawa River flow into Tokyo Bay. The samples were collected at various locations along the river lengths during 2010 (some sampling on the Tama River was also carried out in 2009). The sediment samples consisted of the surface layer (top 3 cm) from areas on the river where sediment was likely to accumulate. Fish were caught by net or rod in the same area (fish were generally collected within a two to three week period for each species at a site, but a month or so apart for different species at some sites). The samples collected were analysed for the presence of D6 (both whole fish and edible parts were analysed).

It should be noted that the method used for extraction of D6 from sediment involved solvent extraction in hexane and then concentrating the hexane extracts to a total volume of 1 ml by evaporation at 25°C under a stream of nitrogen. It is not clear whether this step in the extraction process would have resulted in loss of D6 and hence underestimation of the concentration present in sediment (no similar evaporation step was included in the extraction of biota). However, the quality control procedures used included recovery tests (carried out in 2009) and these showed a recovery of 101 per cent with a standard deviation of 3.8 per cent (total of seven recovery samples) for D6 indicating that loss of D6 during sample extraction was limited (no recovery tests appear to have been carried out for the 2010 sampling).

It should also be noted that no information is given in the report on measures that were taken to avoid inadvertent contamination of the samples during collection (e.g. avoidance of the use of personal care products containing D6).

The results are summarised in Table 36: Summary of BSAFs derived from rivers in Japan.

Table 36: Summary of BSAFs derived from rivers in Japan

River	Location[1]	Sample[8]	N[7]	Concentration[2]		Derived biota-sediment accumulation factor[3]
				µg/kg wet wt.	µg/kg organic carbon or µg/kg lipid	
Tama River	Mid-stream	Sediment	3	6.6±1.2	4,400,±810	
		Pale chub	3	{6.7±0.91}[5]	{150±20}[5]	{0.0}
		Common carp	3	[42±1.1][4]	[1,100±28][4]	[0.3]
	Down-stream	Sediment	6	23±13	7,500±810	
		Yellowfin goby	3	[14±.17][4]	[510±6.0][4]	[0.1]
		Flathead mullet	3	52±5.8	1,000±110	0.1
		Japanese seabass	3	{8.4±0.91}[5]	{360±40}[5]	{0.0}
Arakawa River	Mid-stream	Sediment	6	17±16	6,900±7,000	
		Pale chub	3	{12±0.18}[5]	{170±2.6}[5]	{0.0}
		Common carp	3	[37±7.1][4]	[1,500±280][4]	[0.2]
	Down-stream	Sediment	6	99±13	8,000±1,400	
		Yellowfin goby	3	[20±2.4][4]	[940±110][4]	[0.1]
		Flathead mullet	3	44±1.6	840±30	0.1
		Japanese seabass	3	{7.4±0.47}[5]	{260±16}[5]	{0.0}
Tone River	Mid-stream	Sediment	6	19±6	1,600±350	
		Pale chub	3	{9.2±0.92}[5]	{130±13}[5]	{0.1}
		Common carp	3	{8.0±0.72}[5]	{330±30}[5]	{0.2}
	Down-stream	Sediment	6	18±3.0	2,300±700	
		Yellowfin goby	3	(3.6±0.15)[6]	(180±7.7)[6]	(0.1)
		Flathead mullet	3	[16±0.46][4]	[270±7.7][4]	[0.1]
		Japanese seabass	3	(2.7±0.58)[6]	(32±6.7)[6]	(0.0)

Note: 1) These terms are used in the SIAJ (2011b) report, and relate to the distance downstream from the origin of the river. Midstream relates to sampling at approximately mid-length of the river. Downstream relates to sampling at the river mouth.

2) Mean ± standard deviation. The concentrations in fish represent whole fish concentrations. The concentrations in the edible portions were determined separately and were found to be generally lower than the whole fish concentrations.

3) The BSAFs were calculated using the lipid-normalised concentration in biota/organic carbon-normalised concentration in sediment.

4) The concentration was above the method detection limit but below the limit of quantification. The method detection limit was determined by repetitive analysis of samples. The limit of quantification was defined as three times the method detection limit.

5) The concentration was above the limit of detection but below the method detection limit. The limit of detection was determined by repetitive analysis of reagent blanks.
6) The concentration was below the limit of detection.
7) Number of samples: with the exception of the midstream sample from Tama River, three sediment samples were collected from each of two locations.
8) Latin names: Pale chub – *Zacco platypus*, common carp – *Cyprinus carpio*, yellowfin goby – *Acanthogobius flavimanus*, flathead mullet – *Mugil cephalus* and Japanese seabass – *Lateolabrax japonicas*.

The sampling sites were generally influenced by local sources (e.g. waste water treatment plants (WWTP) and densely populated urban areas; WWTP discharge contributes up to about 50-70% of the river flow in some locations). The BSAF values derived (based on the lipid-normalised concentration in fish/organic carbon-normalised concentration in sediment) were well below 1 for all samples. The highest (detectable) concentrations were generally found in flathead mullet. This species was reported to feed on sediment, ingesting detritus, algae and polychaetes present in the sediment and this was thought to result in a higher intake of D6 than the other species analysed. It should be noted that the number of samples was very small so their representivity is unknown. The fish samples were generally collected in October or November, so seasonal variation is also unknown.

$\delta^{13}C$-analysis was carried out on both the sediment and biota samples in this study in order to determine the likely origin of the carbon in the food chain (land origin or marine origin). The sediment from midstream and downstream locations generally showed the sediment to be deposition of land origin (the midstream sample from the Arakawa River gave a $\delta^{13}C$ value midway between land and marine origin). The carp samples from midstream had $\delta^{13}C$ values typical of land origin but the pale chub from midstream showed a wider range of $\delta^{13}C$ values, with the pale chub from the Arakawa River having a value more consistent with marine origin (possibly reflecting the findings for sediment) than land origin. The $\delta^{13}C$ values from the downstream biota samples reflected differences in habitat and food web between the species. Yellowfin goby is a demersal fish that lives over sediments of land origin. Flathead mullet feeds mainly on detritus accumulated on the river bottom (and attached algae) but also takes up sand and mud along with these items. Therefore food of flathead mullet is likely to be highly influenced by the local concentrations of D6 in the sediment. Both the yellowfin goby and flathead mullet had $\delta^{13}C$ values close to those expected for a food chain of land origin. In contrast, Japanese seabass are thought to travel long distances between the river mouth area and the ocean and the $\delta^{13}C$ values for this species were found to be intermediate between land and marine origin. The probable movement of Japanese seabass in and out of the sampling area means that the actual exposure of this species via sediment is uncertain.

In addition to these data, samples of fish were also collected from Tokyo Bay. These showed generally lower concentrations of D6 (≤220 µg/kg lipid). SIAJ (2011) used the carbon and nitrogen stable isotope ratio determined in the various samples to try to assign each species to a trophic level. However, clear predator-prey relationships were not established and so trophic levels could not be calculated.

3.4.3.1 Measured concentrations in biota

The available monitoring data for D6 in general up to around 2008 are summarised in Environment Agency (2009a). Of most relevance to the PBT and vPvB assessment are data on the occurrence of D6 in biota from marine areas and from remote regions. The available relevant data are briefly summarised below.

- D6 was not detectable (<5 µg/kg wet weight) in 19 samples of fish muscle from various locations (including background sites and sites near to potential point sources) in and around Sweden. The fish species included Baltic herring, herring, eelpout, salmon, flounder and perch (Kaj *et al.*, 2005).

- EVONIK Industries (2007) carried out a survey of the levels of D6 in freshwater and marine fish from Europe. The analytical detection limit was 15 µg/kg wet

weight. For the marine samples D6 was not detected in samples of 11 species from the North East Atlantic, six species from the Baltic Sea close to the mouth of the Odra River, and one species from the Baltic Sea. For the freshwater fish, D6 was not detected in three species from Lake Nipgård, Denmark, and three species from Lake Constance. In contrast to these data, D6 was present at up to 0.1 mg/kg wet weight in one sample of eel from the River Rhine, Germany (close to the Dutch Border), but it was not detectable in two other species of freshwater fish from the same area.

- TemaNord (2005) reports levels of D6 of <5 to 74 µg/kg fresh weight in biota from Nordic countries. The concentrations were generally elevated in urban areas and in areas close to sewage treatment plants, and only few background samples showed detectable levels. The samples included marine and freshwater fish, marine mammals and seabird eggs. The highest level found was 74 µg/kg fresh weight for cod liver from the Inner Oslofjord in Norway but detectable levels of D6 were also found in samples of flounder from Denmark and seal blubber from Denmark.

- Schlabach *et al.* (2007) investigated the levels of D6 in biota from the Inner Oslofjord. The samples included common mussels, flounder fillet, flounder liver, cod liver and cod stomach contents (mainly krill, shrimp and small crabs). D6 was detectable in all of the samples. The highest levels found were in cod liver (~109-152 µg/kg wet weight or 328-829 µg/kg lipid), which were comparable with the levels found in cod liver in the same area in the TemaNord (2005) study.

- A preliminary screening study of the levels of D6 in mussels from the Southern North Sea was carried out by Boehmer *et al.* (2007). Around 30-50 blue mussels were collected from the intertidal areas from sites at Rømø and Hu Bugt (Denmark), Norderney (Germany), Ameland (the Netherlands), and Ambleteuse and Cap Gris Nez (France). In all a total of 23 composite samples (each of two to six individuals) were analysed. The levels of D6 found were below the method detection limit (<6.6 µg/kg) in 22 samples and between the method detection limit and the method limit of quantification in one sample (the estimated concentration was 5.0 µg/kg).

A number of further studies of the levels of D6 in biota have become available since the Environment Agency (2009a) evaluation was completed. The available new information, including biota samples from remote regions, is summarised in Table 37: Measured concentrations of D6 in biota. The sampling and analysis protocols in the majority of these studies have generally attempted to minimise the potential problems from inadvertent/background contamination of the samples. Where this is not necessarily the case this is noted in the table.

Of most relevance to the PBT and vPvB assessment are the studies by Campbell (2010; very brief details of this study are given in an interim report by Campbell (2009) and some of the results appear to be given in a poster presentation by Warner *et al.* (2010a) and a paper by Warner *et al.* (2010b)) and by Evenset *et al.* (2009) of the levels of D6 in biota from remote regions (around Svalbard).

For the Campbell (2010) study, the samples were collected on two expeditions, one carried out in July and August 2008 and one in July and August 2009. Three laboratories were involved in analysing the 2009 samples in order to allow inter-laboratory comparisons of the results to be made (these laboratories also analysed the 2008 samples but in some cases the analysis for a particular species was carried out by one laboratory only). Precautions were taken during sampling and analysis to avoid contamination and the samples were collected by appropriately trained experts/personnel. The sampling locations and samples collected are summarised below.

- Kongsfjorden in 2008. Benthic organisms, zooplankton, kittiwakes and black guillemot.
- Liefdefjorden in 2008. Benthic organisms.
- Bjørnøya in 2008. Glaucous gull.
- Sweden in 2008. Herring, sprat and herring gull.
- Adventfjorden in 2009. Sediment, juvenile Atlantic cod and sculpin.
- Kongsfjorden in 2009. Sediment, bearded seals, Atlantic cod and zooplankton.
- Liefdefjorden in 2009. Sculpin and zooplankton.
- Nordkappsundet in 2009. Zooplankton

The 2008 sampling was carried out in Kongsfjorden (~78°55'N 11°54'E) and Liefdefjorden (~79°34'N 12°44'E) within the Svalbard archipelago, Bjørnøya (Svalbard) and off the west coast of Sweden. The 2009 samples were collected mainly from Adventfjorden (~78°13'N 15°40'E), Kongsfjorden and Liefdefjorden within the Svalbard archipelago, with some additional zooplankton samples collected from Nordkappsundet (~81°N, 21°E). Liefdefjorden is accessible only from the north and has no settlements on its shores but has frequent visits from cruise ships during the summer months. Liefdefjorden was considered by Cambell (2010) to be the most remote of the locations sampled on Svalbard in 2009. Kongsfjorden is located on the west coast of Svalbard and has a permanent research station in the area (at Ny Alesund) with up to 150 personnel in the summer. Cruise ships also make periodic stops at Ny Alesund during spring and summer. Adventfjorden was considered to be the least remote of the 2009 sampling sites as Longyearbyen (the capital of Svalbard with around 2,500 inhabitants) is located in the area.

The results are summarised in Error! Reference source not found. (where D6 was not detected in one or more samples the method detection limit is given; the limit of quantification was generally set as three times the method detection limit[24]).

D6 was detectable in some samples of Atlantic cod (*Gadus morhua*) liver, bivalves (*Chlamys islandies*), glaucous gull (*Larus hyperboreus*) liver and muscle, herring gull (*Larus argentatus*) muscle and liver, sculpin liver[25] and whole body minus liver, sea urchin[25], seal blubber[25], shrimp[25], and zooplankton.

Where detectable, the concentration of D6 was generally close to the method detection limit. However, it is noteworthy that levels up to 15.6 µg/kg wet weight (Atlantic cod liver) were found in samples from Kongsfjord (which may reflect a local source). D6 was also still detectable in some of the samples from the more remote locations.

It is interesting to note that in this study some of the higher concentrations are found in fish such as Atlantic cod rather than invertebrates (in contrast with some of the field bioaccumulation studies reported in Section Field data). The lack of information on predatory-prey relationships and lipid contents, and the limited numbers of samples, etc., precludes a detailed evaluation of the bioaccumulation potential for D6 in this food chain.

The Evenset *et al.* (2009) study showed that D6 was detected in a number of samples of

[24] In many of the samples, although D6 was detectable, the concentration present was below the limit of quantification. Here the actual concentration reported has been given regardless of whether it is above or below the limit of quantification. There is therefore some uncertainty in the accurate quantification of concentrations close to the limit of detection.
[25] Species name not given.

polar cod (*Boreogadus saida*; whole fish and liver) but was not detectable in the liver of Atlantic cod (*Gadus morhua*). D6 was also not detectable in samples of seabird liver or in sediment samples collected on the west coast of Spitsbergen. The source of D6 exposure is not known.

Table 37: Measured concentrations of D6 in biota

Species	Location	Measured concentration[1]	Reference
Atlantic cod (*Gadus morhua*) - liver	Samples from remote region around Svalbard (Kongsfjorden)[2]	Not detectable (<9.7 µg/kg wet weight or <31.7 µg/kg lipid) (5 samples)	Evenset *et al.* (2009)
	Samples from remote region around Svalbard (Kongsfjorden)[4]	1.4-15.6 µg/kg wet weight (detectable in 19 out of 19 samples[5]).	Campbell (2010)
	Samples from remote region around Svalbard (Adventfjorden)[4]	2.4-4.3 µg/kg wet weight (detectable in 11 out of 11 samples[5] in 2009).	Campbell (2010)
	Samples from remote region (the exact location is unclear but was probably either Kongsfjorden or Liefdefjorden)	7.3-9.5 µg/kg wet weight (detected in 3 out of 3 samples from 2008).	Campbell (2010)
Bivalve (*Astarte borealis*)	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Net detectable in a single sample from 2008 (method detection limit 0.61 µg/kg wet weight).	Campbell (2010)
Bivalve (*Chlamys islandies*)	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	0.53-0.64 µg/kg wet weight (detectable in 3 out of 3 samples from 2008).	Campbell (2010)
	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 3 samples in 2008 (method detection limit 1.07-1.38 µg/kg wet weight).	Campbell (2010)
	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 4 samples in 2008 (method detection limit 1.07-1.33 µg/kg wet weight).	Campbell (2010)
Bivalve (*Mya truncate*)	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 4 samples in 2008 (method detection limit 1.06-1.52 µg/kg wet weight).	Campbell (2010)
	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 2 samples in 2008 (method detection limit 1.27-1.44 µg/kg wet weight).	Campbell (2010)

Species	Location	Measured concentration[1]	Reference
Bivalve (*Serripes groenlandica*)	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 2 samples in 2008 (method detection limit 1.08-1.61 µg/kg wet weight).	Campbell (2010)
	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 2 samples in 2008 (method detection limit 1.15-1.24 µg/kg wet weight).	Campbell (2010)
Black guillemot (*Cepphus grille*) - liver	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 2 samples in 2008 (method detection limit 1.7 µg/kg wet weight).	Campbell (2010)
Black guillemot (*Cepphus grille*) - muscle	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 2 samples in 2008 (method detection limit 1.7 µg/kg wet weight).	Campbell (2010)
Black guillemot (*Cepphus grille*) - plasma	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 10 samples in 2008 (method detection limit 5.16-5.31 µg/kg wet weight).	Campbell (2010)
Black guillemot (*Cepphus grille*) – blood cells	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 10 samples in 2008 (method detection limit 3.92-11.1 µg/kg wet weight).	Campbell (2010)
Common eider (*Somateria mollissima*) - liver	Samples from remote region around Svalbard (Kongsfjorden)[2]	Not detectable (<2.6 µg/kg wet weight or <156 µg/kg lipid) (5 samples)	Evenset *et al.* (2009)
Glaucous gull (*Larus hyperboreus*) - liver	Samples from remote region - Bjørnøya	1.8-20.5 µg/kg wet weight (detectable in 8 out of 8 samples[5] in 2008).	Campbell (2010)
Glaucous gull (*Larus hyperboreus*) - muscle	Samples from remote region - Bjørnøya	3.25-9.75 µg/kg wet weight (detectable in 2 out of 5 samples in 2008; method detection limit 1.7 µg/kg wet weight).	Campbell (2010)
Herring[3]	Samples from west coast of Sweden (Skagerrak)	Not detectable in 6 samples from 2008 (method detection limit 0.59-0.94 µg/kg wet weight).	Campbell (2010)

Species	Location	Measured concentration[1]	Reference
Herring gull (*Larus argentatus*) - liver	Samples from remote region around the west coast of Sweden	1.35-1.58 µg/kg wet weight (detectable in 2 out of 12 samples[5] in 2008; method detection limit was between 0.79 and 1.7 µg/kg wet weight).	Campbell (2010)
Herring gull (*Larus argentatus*) - muscle	Samples from remote region around the west coast of Sweden	1.67-4.38 µg/kg wet weight (detectable in 3 out of 9 samples[5] in 2008; method detection limit was between 0.84 and 1.7 µg/kg wet weight where reported).	Campbell (2010)
Kittiwake (*Rissa tridactyla*) - liver	Samples from remote regions around Svalbard (Kongsfjorden and Liefdefjorden)[2]	Not detectable (<2.5 µg/kg wet weight or <96.8 µg/kg lipid) (9 samples).	Evenset *et al.* (2009)
Kittiwake (*Rissa tridactyla*) - blood	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 13 samples in 2008 (method detection limit in the range 2.00-6.14 µg/kg wet weight).	Campbell (2010)
Polar cod (*Boreogadus saida*) – liver and whole fish	Samples from remote regions around Svalbard (Liefdefjorden, Billefjorden and close to Moffen)[2]	<8.1-10.7 µg/kg wet weight or <20.8-30.6 µg/kg lipid (detected in 2 out of 6 liver samples; the two detectable samples were from Liefderfjorden). 2.2-3.8 µg/kg wet weight or 50.6-94.4 µg/kg lipid (detected in 5 out of 5 whole fish samples from Moffen).	Evenset *et al.* (2009)
Sculpin[3] - liver	Samples from remote region around Svalbard (Liefdefjorden)	0.98-3.61 µg/kg wet weight (detectable in 5 out 18 samples[5] in 2009; method detection limit was 0.92 to 1.06 µg/kg wet weight).	Campbell (2010)
	Samples from remote region around Svalbard (Adventfjorden)[4]	1.0-4.9 µg/kg wet weight (detectable in 15 out of 16 samples[5] in 2009).	Campbell (2010)
	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Not detectable in 5 samples from 2008; method detection limit 0.83-1.44 µg/kg wet weight).	Campbell (2010)
Sculpin[3] – whole body	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Not detectable in 5 samples from 2008 (method detection limit 1.7 µg/kg wet weight).	Campbell (2010)
Sculpin[3] – whole body minus liver	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	0.70-2.78 µg/kg wet weight (detected in 5 out of 5 samples in 2008).	Campbell (2010)

Species	Location	Measured concentration[1]	Reference
Sea urchin[3]	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	0.30-0.48 µg/kg wet weight (detected in 3 out of 3 samples in 2008).	Campbell (2010)
Seal[3] blubber	Samples from remote region around Svalbard (Kongsfjorden)[4]	1.27-2.01 (detected in 2 out of 10 samples from 2009; method detection limit 1.36 to 2.60 µg/kg wet weight).	Campbell (2010)
Shrimp (*Pandulus borealis*)	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 3 samples (method detection limit 0.92-1.06 µg/kg wet weight).	Campbell (2010)
Shrimp[3]	Samples from remote region around Svalbard (Liefdefjorden)	1.36 µg/kg wet weight (detected in one out of two samples[5] from 2008; method detection limit for second sample 1.7 µg/kg wet weight).	Campbell (2010)
Shrimp[3] – composite samples	Samples from remote region around Svalbard (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Not detectable in 2 composite samples from 2008 (method detection limit 0.64-0.83 µg/kg wet weight).	Campbell (2010)
Sprat[3]	Samples from west coast of Sweden (Skagerrak)	Not detectable in 4 samples from 2008 (method detection limit 0.78-1.00 µg/kg wet weight).	Campbell (2010)
Zooplankton	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 9 samples[5] in 2009 (method detection limit was in the range 0.92-1.06 µg/kg wet weight).	Campbell (2010)
	Samples from remote region around Svalbard (Kongsfjorden)[4]	Not detectable in 9 samples[5] in 2009 (method detection limit was in the range 0.92-1.06 µg/kg wet weight).	Campbell (2010)
	Samples from remote region around Svalbard (Kongsfjorden)	0.76 µg/kg wet weight (detected in 1 out of 3 samples from 2008; method detection limit 0.56-0.61 µg/kg wet weight).	Campbell (2010)
	Samples from remote region (Nordkappsundet)	Not detectable in 4 samples from 2009 (method detection limit was in the range 0.92-0.93 to 2.60 µg/kg wet weight).	Campbell (2010)

Note: 1) Precautions were taken during sampling and analysis to avoid contamination with cVMS in all of these studies. “Not detectable” means below the stated limit of detection.
2) Marine sediment samples were also collected in Kongsfjorden and Liefdefjorden. D6 was not detected in any of the sediment samples (concentration typically <17 µg/kg dry weight).
3) The species scientific name was not given in the paper.
4) Marine sediment samples were also collected in Kongsfjorden and Adventfjorden in 2009. D6 was not detected in 15 sediment samples from Kongsfjorden or 15 sediment samples from Adventfjorden (method detection limit 1.96 to 6.14 µg/kg wet weight).
5) The total number of samples here refers to the total number of samples analysed across each laboratory. As three laboratories were involved, and generally two or three of the laboratories each analysed a sub-sample from each organism, the total number of organisms collected would be smaller than indicated by the sampling numbers.

Overall the Campbell (2010) and Evenset *et al.* (2009) studies confirm that D6 is present in some biota samples from remote regions, generally at very low concentrations (close to the limit of detection). It is interesting to note that D6 was not detectable in sediment samples from some of the same areas where D6 was found in biota. However, the results for other cVMS (e.g. D5) indicate that local sources may exist even in remote locations (and may lead to locally elevated concentrations). Although it is not clear if local sources can explain all such findings, the possibility of local sources even in remote locations means that the interpretation of the data in terms of long-range transport potential for D6 is difficult.

An interlaboratory comparison of the levels of D6 in cod liver from the inner Oslofjord has been carried out by Durham *et al.* (2009). Seventeen fish were collected in December 2007 and were sent to three laboratories for dissection (each laboratory received five or six fish) and the liver samples were then analysed by all three laboratories. Overall agreement between the three laboratories was generally good and D6 was found in all samples at concentrations between around 1.6 and 396 µg/kg wet weight. The levels found were in agreement with those of previous studies in the area (e.g. TemaNord (2005) and Schlabach *et al.* (2007)) and confirm that elevated concentrations of D6 occur in biota taken from areas close to sources of release.

3.4.4 Summary and discussion of bioaccumulation

The available bioaccumulation data are summarised in Table 38: Summary of available bioaccumulation data for D6. Of these, the most reliable report steady-state BCFs of 1 160 l/kg in Fathead minnow *Pimephales promelas* (Drottar, 2005) and kinetic BCF values of 4 419 – 12 632 l/kg in common carp *Cyprinus carpio* (CERI, 2010). Uptake of D6 by fish from food was also demonstrated, but these feeding studies are not sufficiently accurate to allow a reliable accumulation factor to be determined. High BCF values (up to ~2 400 l/kg) are also found in aquatic invertebrates.

The kinetic BCF values in the carp study are corrected for fish growth, which was significant. This means that the apparent steady-state BCF values (4 042 and 2 344 l/kg) reported in that study underestimate bioaccumulation. There are differences between the results from the two statistical methods of kinetic fitting. Preference is given to the sequential results of 12 632 and 7071 l/kg as the fitting appears to be less affected by the delay in depuration commencing and drop in fish concentration towards the end of uptake. In any case, using the sequential or the simultaneous fitting method will not change the overall B/vB conclusion for D6 as the kinetic BCF values are above 5000 L/Kg. The depuration half-lives for carp are also rather long (around 25 days before growth correction for the sequential fit).

A number of field studies have investigated the biomagnification of D6 in aquatic food webs. Most of these studies suggest that the TMF for D6 is <1. However a recent study in Lake Mjøsa and Lake Randsfjorden reported a higher TMF for D6 of up to 2.7 which suggested that biomagnification may be occurring in those particular food webs. Similarly, a TMF of around 1 or slightly above has been found in a study in Dalian Bay and a TMF in the range 0.5-2.8 (with most estimates above 1) has been reported in a study in Lake Champlain, although there are issues with interpretation for both these studies. In addition although many of the field studies suggest a TMF for D6 of <1, the BMFs for some individual predator-prey interactions within some of the food webs are close to or above 1 in some of these studies (for example a BMF of 1.6 for midge larvae in Lake Pepin, a BMF of 1.7-1.8 for Atlantic cod-shrimp in Oslofjord, a BMF of 0.9 for Atlantic cod-herring in Oslofjord, a BMF of 1.4 for Lake trout-perch in Lake Opeongo and a BMF of 1.5 for Lake trout-cisco in Lake Opeongo)[26]. The experimental measurement of TMF is still at a relatively early stage of development and the lack of agreed guidelines for carrying out such field studies and analyzing the results means that, although there are possible explanations for the differences found between the various studies, it is not currently possible to disregard these higher values. In addition, correlation of levels of D6 in some pelagic food webs with levels

[26] The TMF effectively represents the average BMF per trophic level step within a food chain. Therefore it is possible for individual BMFs for some predator-prey interactions to be >1 and the overall TMF to still be <1.

of known biomagnifying substances (TMFs >1) e.g. PCB-153 and p,p'-DDE (as part of a benchmarking approach), also tends to demonstrate that D6 can biomagnify. A comparison of the TMF data for D6 with that for D4 and D5 suggests that D6 has a generally similar biomagnification potential to both D4 and D5 in the environment based on the TMF. A similar picture is seen when comparing the D6 BCF values in *Cyprinus carpio* with those for D4 and D5 where the D6 values are similar or higher. However, the BCF for D6 is lower than those for D4 and D5 when comparing the data for *Pimephales promelas*.

Table 38: Summary of available bioaccumulation data for D6

Species	Exposure concentration	Value	Validity	Reference
Carassius auratus	306-425 mg/kg food (mixture of oligomers)	Value not given but reported to be similar to that for *P reticulata* (BMF ~ 0.06)	Invalid – exposure concentration not well defined – based on parent substance.	Opperhuizen *et al.*, 1987
	Saturated solution	Value not given but reported to be similar to that for *P reticulata* (BCF ~ 1 200)		
Daphnia magna	Saturated solution	BCF = 2 400 l/kg	Use with care – full experimental details not available – basis for measurement (total ^{14}C or parent substance) is not clear (most probably total ^{14}C).	Dow Corning, 1985
Oncorhynchus mykiss	<1 µg/l	BCF >1 000->2 000 l/kg	Invalid – exposure concentration not well defined - based on parent substance.	Annelin and Frye, 1989
Pimephales promelas	0.41 µg/l	BCF = 1 160 l/kg	Valid – steady-state value based on total ^{14}C analysis – the estimated value based on parent substance is ≥916 l/kg.	Drottar, 2005
		BCF = 1 660 l/kg	Use with care – kinetic value based on total ^{14}C analysis – supportive of steady-state value – the estimated value based on parent substance is ≥1,311 l/kg.	
	4.4 µg/l	BCF = 240 l/kg	Use with care – steady-state value based on total ^{14}C analysis – the concentration tested was very close to the water solubility – the estimated value based on parent substance is ≥190 l/kg.	Drottar, 2005

Species	Exposure concentration	Value	Validity	Reference
		BCF = 319 l/kg	Use with care – kinetic value based on total ^{14}C analysis – the concentration tested was very close to the water solubility – the estimated value based on parent substance is ≥252 l/kg.	
Cyprinus carpio	0.1 µg/l 1 µg/l	BCF = 12 632 l/kg BCF = 7 071 l/kg	Valid. Fish growth significant in study. Values are corrected for growth.	CERI, 2010
Poecilia reticulata	1,008-1,044 mg/kg food (mixture of oligomers)	BMF = 0.06	Invalid – exposure concentration not well defined – based on parent substance.	Opperhuizen *et al.*, 1987
	Saturated solution	BCF = 1 200 l/kg		
	20-50 mg/kg food	BMF <0.03	Invalid – exposure concentration not well defined – based on parent substance.	Bruggeman *et al.*, 1984
Lumbriculus variegatus	28.1 mg/kg 484 mg/kg	BAF_{ss} 0.66; BAF_k 0.67 BAF_{ss} 0.07; BAF_k 0.07	Use with care – full experimental details not available – basis for measurement not discussed.	Wildlife International, 2008

4 Human health hazard assessment

Information on human health hazard of D6 is not reported in this section. Toxicity data of the impurity D4 has been addressed by RAC (ECHA 2016). See also Annex XV report on D4 (ECHA 2018a).

5 Environmental hazard assessment

Information on ecotoxicity of D6 is not reported in this section. Ecotoxicity data of the impurity D4 has been addressed by RAC (ECHA 2016). See also Annex XV report on D4 (ECHA 2018a).

6 Conclusions on the SVHC Properties

6.1 PBT and vPvB assessment

6.1.1 Assessment of PBT/vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as PBT/vPvB. All available information (such as the results of standard tests, monitoring and modelling, information from the application of the category and analogue approach (grouping, read-across), benchmarking approach and (Q)SAR results) was considered together in a weight-of-evidence approach.

6.1.1.1 Persistence

Degradation of D6 occurs in the atmosphere by reaction with atmospheric hydroxyl radicals. Hydrolysis of D6 has been shown to be negligible in water (half-lives >1 year).

One ready biodegradation test is available for D6 showing very limited degradation in 28 days (4.5%). Information for related substances (D4 and D5), reinforces the conclusion that D6 is not readily biodegradable.

Due to its volatility, it is expected that part (6.75%) of D6 is removed from aquatic systems and terrestrial systems by volatilisation into the atmosphere. However, its high potential of adsorption to sediment and soil (high Koc value) is expected to limit its potential of volatilisation (see details in section 3.1.5). D6 can be expected to persist in sediments following partitioning in the aquatic environment based on the screening data on biodegradation on D6 and on degradation simulation data on the analogue substance D5 (half-lives 800-1200 days). Under some conditions (e.g. particularly dry spells) the degradation of D6 in soil could become more rapid (and become the dominant removal process from the soil). However this would not represent a realistic worst-case situation.

In its opinion on the persistency and bioaccumulation of D4 and D5 (ECHA, 2015), the Member State Committee assessed the degradation data on the analogues D4 and D5 and also the question on overall persistence due to the anticipated removal via air. MSC evaluated that non-degradation processes do not have a large impact on the sediment removal half-life, and thus cannot be used to refute the relevance of the sediment compartment in the assessment of persistence. The Member State Committee concluded both D4 and D5 to fulfil the criteria of very persistent in sediment.

New studies which became available on D4 and D5 after the MSC opinion have been evaluated in the Annex XV reports on D4 and D5 (2018a and 2018b), respectively. They have been considered to support the conclusion that these substances are very persistent in sediment.

Overall, D6 meets the screening criteria for P or vP based on the results of an OECD TG 310 study, enforced by similar data on analogues D4 and D5; the substance is not readily biodegradable. Sediment studies with the analogues D4 and D5 showed these substances to meet the Annex XIII vP criterion in sediment. This conclusion can be read-across to D6 which might be even more persistent than D4 and D5 because it is more adsorptive, more hydrophobic and less volatile than these analogues. Finally, it is concluded that D6 meets the criteria of vP of Annex XIII to REACH for sediment.

6.1.1.2 Bioaccumulation

Several studies investigating bioaccumulation of D6 in aquatic organisms are available.

The key data come from an aqueous bioconcentration study in common carp *Cyprinus carpio* (CERI, 2010). The reported steady-state BCF values (2 344 – 4 042 l/kg) are unreliable because the fish were growing significantly. The kinetic BCF values are considered reliable with preference given to the sequential fitting as this appears to be less affected by the delay in depuration commencing and drop in fish concentration towards the end of uptake. This yields a kinetic BCF of around 7 000 – 12 600 l/kg when growth correction is taken into account. A growth-corrected kinetic BCF of around 6 600 l/kg is also obtained for one of the test concentrations if the simultaneous fitting method is used (the other test concentration yields a kinetic BCF of around 4 400 l/kg). D6 therefore meets the Annex XIII vB criterion (BCF > 5 000 l/kg). The depuration half-lives for carp are also rather long (around 25 days before growth correction for the sequential fit), which is consistent with other substances (such as D4 and D5) that are agreed to meet the vB criteria.

Uptake of D6 by fish from food has been demonstrated, but the available feeding studies are not sufficiently accurate to allow a reliable accumulation factor to be determined. High BCF values (up to ~2 400 l/kg) are also found in aquatic invertebrates.

A number of field studies have investigated the biomagnification of D6 in aquatic food webs. Confidence intervals for TMF estimates are typically rather wide, but out of ten food webs investigated, a median TMF above 1 was obtained in Lake Mjøsa and Lake Randsfjorden, Norway. A TMF above 1 is also not excluded for Inner and Outer Oslofjord and Lake Erie, and studies in Dalian Bay and Lake Champlain also suggest a TMF for D6 of around 1 or above (up to 2.8). As with most field stuies, there are interpretational issues, particularly concerning sample numbers and representivity and potential concentration gradients. Nevertheless, even for field studies that suggest a median TMF for D6 below 1, BMFs for some individual predator-prey interactions within some of the food webs are above 1 (for example a BMF of 1.6 for midge larvae in Lake Pepin, a BMF of 1.7-1.8 for Atlantic cod-shrimp in Oslofjord, a BMF of 1.4 for Lake trout-perch in Lake Opeongo and a BMF of 1.5 for Lake trout-cisco in Lake Opeongo). The lack of agreed guidelines for carrying out field studies and analysing the results means that although there are possible explanations for the differences found between the various studies on D6, it is not currently possible to disregard the higher values. On that basis, it seems reasonable to conclude that D6 can biomagnify in some food webs or feeding relationships, particularly those in the pelagic zone. A comparison of the TMF data for D6 with that for D4 and D5 suggests that D6 has a generally similar biomagnification potential to both analogues (based on the TMF). A similar picture is seen when comparing the BCF values in *Cyprinus carpio* with those for D4 and D5 where the D6 values are similar or higher. In addition, correlation of levels of D6 in some pelagic food webs with levels of known biomagnifying substances (TMFs >1) e.g. PCB-153 and p,p'-DDE (as part of a benchmarking approach), also tends to demonstrate that D6 can biomagnify. The Annex XIII criteria do not contain specific cut-off values for TMF or BMF values in relation to the B and vB criteria but a TMF or BMF >1 could be taken as strong evidence that the substance at least meets the Annex XIII criterion for B.[27]

The highest steady-state BCF value measured in Fathead Minnow *Pimephales promelas* (≥916 l/kg) does not meet the Annex XIII B or vB criteria. The BCF for D6 is lower than those for both D4 and D5 with this species, which is not consistent with the trend from the carp or field data. Difference in bioaccumulation observed between Fathead Minnow and common carp can be

[27] For example Inoue *et al*. (2012) carried out a comparison between lipid normalised BMF from dietary studies and lipid normalised (to 5% lipid) BCF for several poorly water soluble substances in carp (Cyprinus carpio). This analysis found that a lipid normalised BMF of 0.31 (95% confidence interval 0.11-0.87) from an OECD TG 305 dietary study corresponded to a lipid normalised BCF of 5 000 l/kg. For D6 the available data are TMF and BMF values from field studies and so a direct comparison is not possible here (a TMF and BMF from a field study integrates exposure from all possible sources, not just diet, and the TMF effectively represents the average BMF for one trophic level step across the entire food web).

explained by the fact that Fathead minnow is able to metabolise D6 while common carp does not seem to metabolise it. Overall, it is concluded that D6 fulfils the vB criterion of Annex XIII to REACH.

6.1.1.3 Toxicity

Human health toxicity and ecotoxicity properties of D6 were not assessed in this report.

6.1.1.4 Assessment based on relevant constituents, impurities and/or additives

According to the introductory section of Annex XIII to REACH,

[…] The identification shall also take account of the PBT/vPvB properties of relevant constituents of a substance and relevant transformation and/or degradation products. […]

As recommended in ECHA's Guidance on PBT/vPvB assessment[28], if a constituent, impurity or additive of a substance fulfils the PBT/vPvB properties (based on the assessment of the registrant or of ECHA), a ≥0.1 % (w/w) threshold applies for concluding the substance as fulfilling the same PBT or vPvB criteria. For substances containing PBT/vPvB constituents, impurities or additives in individual amounts <0.1 % (w/w) of the substance, the same conclusion need not normally be drawn. This limit of 0.1% (w/w) is set based on a well-established practice recognised in European Union legislation to use this limit as a generic limit[29]. This is also in line with the threshold used for considering PBT and vPvB substances in mixtures (Article 14(2)(f) of REACH).

D6 contains D4 and D5 as impurities. D4 has been assessed by MSC (ECHA 2015) and RAC (ECHA 2016) as PBT and vPvB (see also Annex XV report on D4 (ECHA 2018a)). RAC (ECHA 2016) concluded that *D4 meets the REACH Annex XIII criteria for toxicity based on both aquatic and mammalian end points*, whereas conclusion on mammalian endpoints was based on harmonised classification (reproductive toxicity category 2). For ecotoxicity, a harmonised classification proposal is currently under opinion making process in RAC. D5 has been assessed by MSC (ECHA 2015) and RAC (ECHA 2016) as vPvB. The properties of the two impurities, their concentration and taking into account all information available on these substances, render the substance D6 also to fulfil PBT and vPvB properties as release of substance D6 would unavoidably cause release and exposure of D4 and D5 (when present in D6 as impurities), which are PBT/vPvB and vPvB, respectively.

Consequently, as provided above, D6 fulfils PBT criteria with impurity D4 in concentration of ≥0.1 % (w/w) and vPvB criteria with either one or both of the impurities D4 and D5 in concentration of ≥0.1 % (w/w).

[28] https://echa.europa.eu/documents/10162/13632/information_requirements_r11_en.pdf/a8cce23f-a65a-46d2-ac68-92fee1f9e54f

[29] The limit of 0.1% (w/w) is indicated in the European Union legislation, where there is no specific reason (e.g., based on toxicity) to establish a concentration limit specific to the case. Examples of this generic concentration limit are, i.a., another category of substances of very high concern according to Article 57 of REACH, where the default concentration of Carcinogenic/Mutagenic (category 1A/1B) ingredients in a mixture requiring a Carcinogen/Mutagen (1A/1B) classification of the mixture under Regulation (EC) No 1272/2008 is 0.1% (w/w). Furthermore, Articles 14(2)(f), 31(3)(b) and 56(6)(a) of REACH apply a similar principle and the same concentration limit for PBT/vPvB substances in mixtures regarding some obligations under REACH. Additionally, the Judgments of the General Court (Seventh Chamber, extended composition) of 7 March 2013 in cases T-93/10, T-94/10, T-95/10 and T-96/10 (see in particular paragraphs 117 to 121) confirmed the validity of this approach for PBT/vPvB constituents of a substance.

6.1.2 Summary and overall conclusions on the PBT and vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as vPvB based on its intrinsic properties. All available relevant information (such as the results of standard tests, monitoring and modelling, information from the application of the analogue approach (grouping, read-across), benchmarking approach and (Q)SAR results) was considered together in a weight-of-evidence approach.

Persistence

Dodecamethylcyclohexasiloxane (D6) is considered to be not readily biodegradable and so meets the screening persistent (P) and very persistent (vP) criteria. Read-across from D4 and D5 to D6 has been considered appropriate for the assessment of persistence. Based on the comparison of physico-chemical properties of D4, D5 and D6, D6 can be expected to be more persistent than D4 and D5. Data for the analogue substances D4 and D5 provide that the vP criterion is met in sediment (see Annex XV reports of D4 and D5 (2018a and 2018b)).

Bioaccumulation

The available data from laboratory bioaccumulation tests show that D6 meets the vB criterion based on a kinetic BCF of around 6600 – 12 600 l/kg in common carp (*Cyprinus carpio)*. In addition, the available field data provides evidence that biomagnification and trophic magnification occur in certain food webs in the environment. The available information on biomagnification and trophic magnification factors (BMF/TMF) in the field indicating that biodilution occurs in some food chains or in parts of some food chains, does not invalidate the other lines of evidence. Correlation of levels of D6 in some pelagic food webs with levels of known biomagnifying substances (TMFs >1) e.g. PCB-153 and p,p'-DDE (as part of a benchmarking approach), also tends to demonstrate that D6 can biomagnify. A comparison of the TMF data for D6 with that for D4 and D5 suggests that D6 has a generally similar biomagnification potential to both D4 and D5 in the environment based on the TMF. A similar picture is seen when comparing the D6 BCF values in *Cyprinus carpio* with those for D4 and D5 where the D6 values are similar or higher. However, the BCF for D6 is lower than those for D4 and D5 when comparing the data for *Pimephales promelas*. Taking together all lines of evidence on bioaccumulation potential, it can be concluded that D6 meets the vB criterion.

Toxicity

Several data are available on human health toxicity and ecotoxicity of D6, but these were not assessed for this report.

Relevant constituents, impurities and/or additives

D6 contains octamethylcyclotetrasiloxane (D4) and/or decamethylcyclopentasiloxane (D5) as impurities. D4 fulfils the PBT and vPvB criteria and D5 meets the vPvB criteria (see Annex XV reports of D4 and D5 (2018a and 2018b)). Taking all information into account, including the concentration of D4/D5 and the properties of these substances, D6 thereby fulfils the PBT criteria with impurity D4 in concentration of ≥0.1 % (w/w) and the vPvB criteria with either one or both of the impurities D4 and D5 in concentration of ≥0.1 % (w/w).

Conclusion

Dodecamethylcyclohexasiloxane (D6) meets the criteria for a vPvB substance according to Article 57 (e) of REACH based on its intrinsic properties. Additionally, D6 meets the criteria for a vPvB substance due to its impurity octamethylcyclotetrasiloxane (D4) and/or decamethylcyclopentasiloxane (D5) (concentration ≥ 0.1 % w/w). Furthermore, D6 meets the criteria for a PBT substance. This conclusion is drawn because D6 contains octamethylcyclotetrasiloxane (EC no: 209-136-7; D4) which is typically present as an impurity in relevant concentrations (typically above or equal to 0.1 % w/w).

In conclusion, dodecamethylcyclohexasiloxane (D6) is identified as a PBT/vPvB substance according to Art. 57(d) and (e) of REACH by comparing all relevant and available information

listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

6.2 Assessment under Article 57(f)

Not relevant for this assessment.

Part II

7 Registration and C&L notification status

7.1 Registration status

Table 39: Registration status

From the ECHA dissemination site[30]	
Registrations	☒ Full registration(s) (Art. 10) ☐ Intermediate registration(s) (Art. 17 and/or 18)

7.2 CLP notification status

Table 40: CLP notifications

	CLP Notifications[31]
Number of aggregated notifications	6
Total number of notifiers	263

8 Total tonnage of the substance

Table 41: Tonnage status

Total tonnage band for the registered substance (excluding the volume registered under Art 17 or Art 18)[32]	10 000 – 100 000 t/pa

[30] https://echa.europa.eu/substance-information/-/substanceinfo/100.007.967 *(accessed 07 Feb 2018)*
[31] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 13 Feb 2018)
[32] https://echa.europa.eu/substance-information/-/substanceinfo/100.007.967 *(accessed 07 Feb 2018)*

9 Information on uses of the substance

Table 42: Uses

	Use(s)	Registered use *(If not, specify the source of the information)*	Use[33] in the scope of Authorisation
Uses as intermediate	• Use as a monomer in the production of polysiloxane polymers and resins – at industrial sites D6 can be present as an impurity >0.1% w/w in polysiloxane polymers and resins dependent on the specific production process and polymer type. In some cases residuals can be removed via a 'devolatilisation' process Polysiloxane polymers and resins have diverse applications across construction, automotive, aerospace, oil/gas and textile industries e.g. as sealants, adhesives, lubricants/greases, anti-foam	Yes	No
Formulation or repacking	• Formulation of cosmetic products (both 'rinse-off' and 'leave on' types) (deodorants, anti-perspirants, skin creams and lotions). Products may be aimed for consumer (general public) or professional use (e.g. in salons) • Formulation of household care products • Formulation of pharmaceuticals and medical devices	Yes Yes Yes	Yes
Uses at industrial sites	Manufacture • Manufacturing of the substance. Uses at industrial sites (non intermediate) • Industrial use of cleaning and maintenance products (e.g. surfactants, defoamer, lubricants) • Use as a laboratory chemical	Yes Yes Yes	No (manufacture) Yes (other)
Uses by professional workers	• Professional use of cosmetic products (leave-on and rinse off) • Professional use of household products: washing and cleaning, polishes and waxes	Yes Yes	Yes
Consumer uses	• Consumer use of cosmetic products • Consumer use of household products: washing and cleaning, polishes and waxes	Yes	Yes

[33] https://echa.europa.eu/documents/10162/13640/generic_exemptions_authorisation_en.pdf

		Yes	
Article service life	• Articles produced from polysiloxane polymers and resins may contain low concentrations of D6 as and impurity (unreacted monomer) or via the degradation of the polymer matrix during the article service life. These materials are widely used widely in construction, aerospace and automotive sectors.	No (ECHA call for evidence for restriction proposal on the use of D4/D5 on consumer / professional products, by analogy)	No

There are several uses of D6 (taken from non-confidential lead REACH Registrant CSR).

- Use as a monomer in the production of silicone polymers (intermediate).
- Use as an intermediate in the production of other organosilicon substances.
- Use in personal care products (comprising formulation, professional use, consumer use).
- Use in household care products (comprising formulation, professional use, consumer use).
- Use as a laboratory reagent in research and development activities.
- Use in pharmaceuticals (small quantities).

The main uses of D6 are as an intermediate (monomer) in the production of silicone polymers and direct uses in cosmetic products (e.g. deodorants, anti-perspirants, skin- and hair-care products). D6 is also registered for use in household care products, such as in washing and cleaning products, polishes and waxes.

Use as an intermediate to make silicone polymers effectively consumes the D6, although trace amounts are still present in the final products can be subsequently released to the environment. Intentional use of D6 in a cosmetic products is likely to occur at concentrations greater than 0.1% w/w and is likely to result in wide-dispersive exposure to the environment. D6 can also occur unintentionally as an impurity in cosmetics and household care products as a result of the use of silicone polymers as ingredients, which is not uncommon.

Although D6 has been registered for use for the formulation of pharmaceuticals, D6 is not currently reported to be present in any authorised human medicinal product in the EU[34]. However, D6 may be present in various authorised human medical products in the EU as a constituent of 'polydimethly cyclosiloxane', which is present as a constituent in various authorised human medical products and which can feasibly contain D6 (as well as other cyclic siloxanes, such as D4 and D5), depending on its specific formulation. By analogy, D6 may also be present in certain types of medical devices (particularly those used on the skin), although no specific information on this use is available in registration dossiers. D6 may also be present as an impurity in silicone polymers used in pharmaceuticals and medical devices.

Aggregated non-confidential tonnage information across the various uses / registrants is not currently available. Environment Agency (2009) report non-confidential tonnage information for use of D6 in Europe in cosmetics of 1858 and 1989 tonnes per annum for 2003 and 2004, respectively. In the same report the authors note an increasing trend for both intermediate and non-intermediate uses based on confidential information from an industry survey.

Environment Agency (2009) also note that other uses of D6 have been reported in the Nordic Substances in Products in the Nordic Countries (SPIN) database include surface treatment,

[34] Personal Communication with the European Medicines Agency (2017). Based on medicinal products containing D6 as part of their composition – i.e. as an active ingredient or as an excipient.

fillers, paints, lacquers, and varnishes, although it is noteworthy that these uses are not registered in the EU. Environment Canada (2008) indicates that in Canada there may be some use of D6 in waxes and polishes (D6 content between 3 and 15 per cent) and in surfactants and defoamers (D6 content between 0.2 and 35 per cent).

It is possible that uses of D6 reported in the literature are actually uses of silicone polymers made from D6 rather than a direct use of D6.

10 Additional information

10.1 Alternatives

Octamethylcyclotetrasiloxane (EC 209-136-7, D4), decamethylcyclopentasiloxane (EC 208-764-9, D5) and D6 may be used as substitutes for each other in some applications, dependent on the properties required. Therefore, risk management should cover all three substances together. Furthermore, D6 usually contains impurities of D4 and D5 and vice versa.

The availability of alternatives for the use of D6 in cosmetics and household care products is implied by the availability of products on the EU market within the same product categories that do not contain D6.

Further possible alternatives for the use as an intermediate in silicone production might be the respective linear siloxanes octamethyltrisiloxane (EC 203-497-4, L3), decamethyltetrasiloxane (EC 205-491-7, L4) and dodecamethylpentasiloxane (EC 205-492-2, L5). However, these substances are listed on the CoRAP as potential PBT/vPvB substances.

Furthermore, silicone production from linear siloxanes would in any case lead to the formation of D4, D5 and D6 as residuals in the polymer.

10.2 Existing EU legislation

None relevant.

10.3 Previous assessments by other authorities

A UK national review of uses of D6 (Environment Agency, 2009), based on the information available at the time and read-across from similar substances, concluded that although D6 had the potential to meet the screening criteria for a persistent (P) or very persistent (vP) substance it did not meet the criteria for either a PBT or vPvB substance. In terms of quantitative risk assessment, risk in soil and predators (secondary poisoning) were considered to be low. However, a lack of suitable ecotoxicity data meant that it was not possible to assess risks in the sediment compartment.

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Annex I - Justification on read-across approach

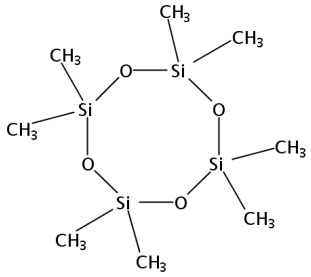
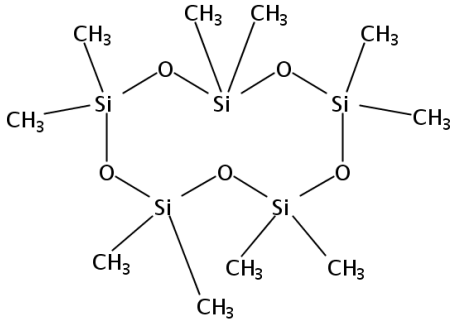
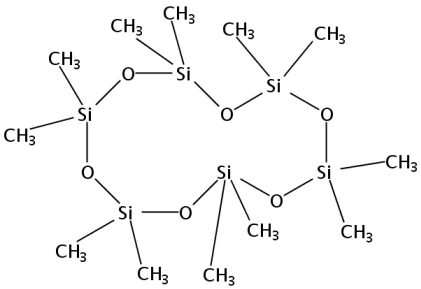
In general, the read-across approach can be applied for substances of which physico-chemical and/or toxicological and/or ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. Those substances may be considered as a group or a category of substances, as indicated in Annex XI Section 1.5 of REACH. According to ECHA`s practical guide 6 “How to report read-across and categories” similarities may be due to a common functional group, common precursor or breakdown products, constant pattern in changing potency or common constituents or chemical class.

Due to their similar physico-chemical properties and common functional groups a read-across approach is considered to be relevant between D6 and D4, D5.

D6 is more hydrophobic and less volatile than the analogues D4 and D5. D6 is more adsorptive to sediments, soils and to lipids than D4 and D5. These properties generally indicate even lower susceptibility to degradation in sediment and soil simulation degradation tests as well as higher bioaccumulation potential than D4 and D5.

A matrix with the relevant information on physico-chemical and environmental fate properties is presented here:

Table 43: Matrix of physical chemical properties and environmental fate properties for D4, D5 and D6 relevant to justify the read-across approach

Substance name:	Octamethylcyclotetrasiloxane (D4)	Decamethylcyclopentasiloxane (D5)	Dodecamethylcyclohexasiloxane (D6)
EC number:	209-136-7	208-764-9	208-762-8
CAS number (in the EC inventory):	556-67-2	541-02-6	540-97-6
Index number in Annex VI of the CLP Regulation	014-018-00-1	-	-
Molecular formula:	$C_8H_{24}O_4Si_4$	$C_{10}H_{30}O_5Si_5$	$C_{12}H_{36}O_6Si_6$
Structural formula			
Molecular weight range (g/mol):	296.62	370.77	444.92
Physico-chemical properties			
Physical state at 20°C and 101.3 kPa	liquid	liquid	Liquid
Melting/freezing point (°C)	17.7	-38	-3

Substance name:	Octamethylcyclotetrasiloxane (D4)	Decamethylcyclopentasiloxane (D5)	Dodecamethylcyclohexasiloxane (D6)
Boiling point (°C)	175	210	245 °C at 1013 hPa
Vapour pressure	132 Pa at 25 °C	33.2 Pa at 25 °C	*4.6 Pa at 25 °C* (ca. 5 Pa at 20-25 °C)
Density	0.95 g/cm^3 at 25°C	0.96 g/cm^3 at 20°C	-
Water solubility	0.0562 mg/L at 23 °C and pH ca. 7	17.03 µg/L at 23 °C and pH ca. 7	*5.3 µg/l ± 0.48 µg/l at 23 °C* (5 µg/l)
Partition coefficient n-octanol/water (log value)	6.488 at 25.1 °C	8.023 at 25.3 °C	*8.87 at 24 °C* (9.06)
Adsorption/desorption (Koc value in L/Kg)	1.7 × 10^4	1.5×10^5	2.2×10^5 to 1.5×10^6
Persistence assessment (P/vP)			
Hydrolysis	Half-life = 16.7 days at pH7 and 12°C	Half-life = 365 days at pH7 and 12°C (freshwater) Half-life = 64 days at pH8 and 9°C (marine water)	Half-life > 1 year in water.
Ready biodegradability screening test	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable (4.5% degradation in 28 days)
Simulation test	DegT50 = 242 days in aerobic sediments DegT50 = 365 days in anaerobic sediments (OECD TG 308; Xu, 2009a & 2009b)	DegT50 = 800-3,100 days in freshwater sediments at 24°C (OECD TG 308; Xu 2010)	Data for the analogue substances D4 and D5 is used in a read-across approach in order to conclude that D6 fulfils the vP criterion in sediment.

Field data	Evidence of persistence from sediment core data from Lake Pepin, USA (Powell, 2009 & 2010)	Evidence of persistence from sediment core data from Lake Pepin, USA (Powell, 2009 & 2010)	No data available.
Bioaccumulation assessment (B/vB)			
BCF (aquatic)	BCF_{ss} = 12,400 L/Kg for Fathead Minnow *Pimephales promelas* (Fackler *et al.*, 1995) BCF_{ss} = 3,000 – 4,000 L/kg (based on parent compound analysis) and BCF_k = 4,100 - 5,500 L/kg (without growth correction; it is higher if growth is taken into account) for Common Carp *Cyprinus carpio* (CERI, 2007 and 2010a).	BCF_{ss} = 7,060 L/Kg for Fathead Minnow *Pimephales promelas* (Drottar, 2005) BCF_{ss} = 12,049 – 12,617 L/kg (based on parent compound analysis) or BCF_{ssL} = 10,550 – 11,048 L/kg for Common Carp *Cyprinus carpio* (CERI, 2010b).	BCF_{ss} = 1160 L/Kg and > 961 L/Kg (parent compound analysis) for Fathead minnows (*Pimephales promelas*) (Drottar, 2005) BCF_{kg} of around 6600 – 12 600 l/kg in common carp *Cyprinus carpio* (CERI, 2010).
Whole body concentrations	D4 can achieve whole fish concentrations similar to a range of substances that are widely accepted as being very bioaccumulative (e.g. UV-328 and UV-320, long chain perfluorocarboxylic acids, musk xylene, hexaBDE and HBCDD).	D5 can achieve whole fish concentrations similar to a range of substances that are widely accepted as being very bioaccumulative (e.g. UV-328 and UV-320, long chain perfluorocarboxylic acids, musk xylene, hexaBDE and HBCDD).	-

Substance name:	Octamethylcyclotetrasiloxane (D4)	Decamethylcyclopentasiloxane (D5)	Dodecamethylcyclohexasiloxane (D6)
Fish dietary bioaccumulation (BMF)	A dietary BMF between 0.47- 4.6 was measured in Rainbow Trout *Oncorhynchus mykiss* (Dow Corning, 2007). The growth-corrected depuration rate constant calculated from this study was 0.00659 day-1. A growth-corrected and lipid-normalised BMF of 0.51 and 0.7 has been measured in *C. carpio* (CERI, 2011). The growth-corrected depuration rate constant calculated from this study was ~0.058 day-1.	A dietary BMF between 0.63 – 3.9 was measured in Rainbow Trout *Oncorhynchus mykiss* (Dow Corning, 2006). The growth-corrected depuration rate constant calculated from this study was 0.00939 day-1. A dietary BMF of 0.96-1.21 (growth-corrected and lipid-normalised) has been measured in *C. carpio* (CERI, 2011). The growth-corrected depuration rate constant calculated from this study was ~0.023 day-1.	Several indications of biomagnification from field studies (BMFs > 1).
Field studies	BSAF values above one have been measured for benthic invertebrates and fish in both laboratory and field studies, and BMFs above one have been measured for some fish feeding relationships in field studies. D4 is present in biota in remote regions.	It is considered that trophic magnification may occur in some food webs whereas trophic dilution occurs in others. D5 is also found in fish, birds and marine mammals sampled from remote regions.	Trophic magnification (TMFs > 1) may occur in some food webs whereas trophic dilution occurs in others. D6 is present in some biota samples from remote regions, generally at low concentrations. Elevated concentrations of D6 occur in biota taken from areas close to sources of release.
Overall PBT/vPvB assessment based on intrinsic properties	PBT/vPvB	vPvB	vPvB

Source of information for D4 and D5: MSC opinion (ECHA, 2015) and RAC opinion (ECHA, 2016); as well as Annex XV reports on D4 and D5 (Annex XV reports for D4 and D5 , 2018a and 2018b)

Annex II - Biota-Sediment Accumulation Factors (BSAFs)

Table 44 below contains BSAF values for D6 that have been reported in the various field studies, or have been estimated using data in the available field studies. The BSAF values are all reported on a fish lipid/sediment organic carbon basis. It is important to note that the derivation of such factors requires knowledge of the sediment concentration in the same area as the organisms were exposed. For some studies the sediment concentration data are variable, showing possible concentration gradients in the areas sampled, and these data have only been included where the authors of the original studies have taken this into account. Nevertheless it is important to note that even where this has been taken into account, there are still uncertainties associated with the derived BSAF.

The BSAF values derived generally show the highest values with the organisms towards the bottom of the food chain. This can be seen from the plot of ln [BSAF] against trophic level (where available) shown in Figure 13. This shows that BSAF values above 1 only occur for organisms around trophic level 2 or below, and there is a general trend to decreasing BSAF with increasing trophic level.

Figure 15: Plot of log BSAF against trophic level

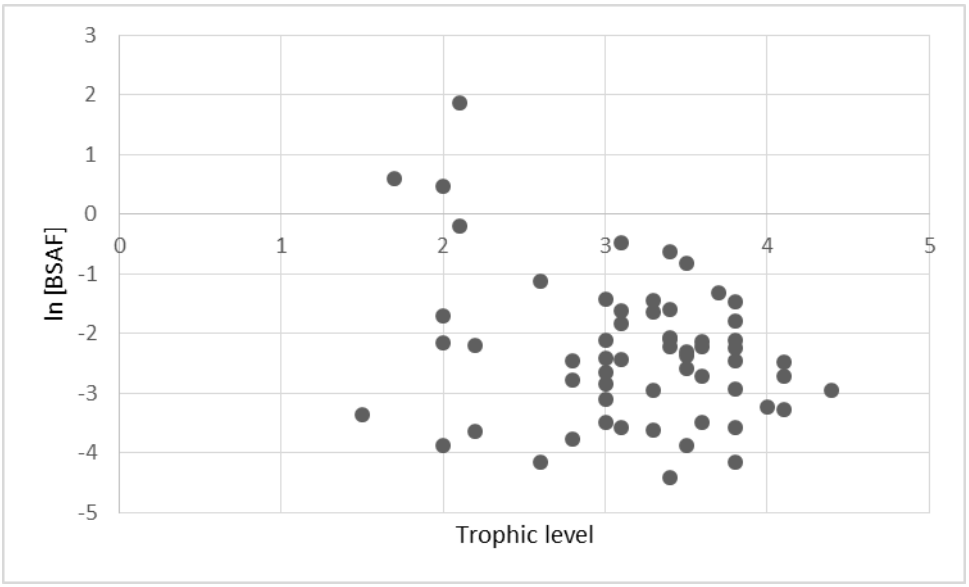


Table 44 Summary of BSAF derived for D6

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Midge (*Chironomous sp.*)	2.0	821	1,305	1.6	Powell et al., 2009a
Burrowing mayfly (*Hexagenia sp.*)	2.0	821	148	0.2	Powell et al., 2009a
White sucker (*Catostomus commersoni*)	2.6	821	(13)	0.02	Powell et al., 2009a
Common carp (*Cyprinus carpio*)	2.8	821	71	0.09	Powell et al., 2009a
Gizzard shad (*Dorosoma cepedianum*)	2.8	821	19	0.02	Powell et al., 2009a
Gizzard shad (young of year) (*Dorosoma cepedianum*)	3.0	821	99	0.1	Powell et al., 2009a
Silver redhorse (*Moxostoma anisurum*)	3.0	821	74	0.09	Powell et al., 2009a
Bluegill sunfish (*Lepomis macrochirus*)	3.1	821	(23)	0.03	Powell et al., 2009a

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
River carpsucker (*Carpiodes carpio*)	3.3	821	160	0.2	Powell et al., 2009a
Shorthead redhorse (*Moxostoma macrolepidotum*)	3.3	821	(22)	0.03	Powell et al., 2009a
Freshwater drum (*Aplodinotus grunniens*)	3.4	821	103	0.01	Powell et al., 2009a
Emerald shiner (*Nitropis atherinoides*)	3.4	821	166	0.2	Powell et al., 2009a
Black crappie (*Pomoxis nigromaculatus*)	3.4	821	(10)	0.01	Powell et al., 2009a
White bass (*Morone chrysops*)	3.5	821	(17)	0.02	Powell et al., 2009a
Smallmouth bass (*Micropterus dolomieu*)	3.5	821	360	0.04	Powell et al., 2009a
Quillback carpsucker (*Carpiodes cyrinus*)	3.6	821	54	0.07	Powell et al., 2009a
Walleye (*Stizistedion vitruem*)	3.6	821	25	0.03	Powell et al., 2009a
Largemouth bass (*Micropterus salmoides*)	3.8	821	(13)	0.01	Powell et al., 2009a

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Blue mussel (*Mytilus edulis*)	1.5	3,423	120	0.04	Powell et al., 2009c and 2010b
Worms	1.7	3,423	6,266	1.8	Powell et al., 2009c and 2010b
Jellyfish	2.0	3,423	71	0.02	Powell et al., 2009c and 2010b
Plankton	2.0	3,423	397	0.1	Powell et al., 2009c and 2010b
Mussels (species A)	2.6	3,423	1,118	0.3	Powell et al., 2009c and 2010b
Mussels (species B)	2.8	3,423	213	0.06	Powell et al., 2009c and 2010b
Atlantic herring (*Clupea harengus*)	3.0	3,423	241	0.07	Powell et al., 2009c and 2010b

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Northern shrimp (*Pandalus borealis*)	3.0	3,423	104	0.03	Powell et al., 2009c and 2010b
European plaice (*Pleuronectes platessa*)	3.1	3,423	543	0.2	Powell et al., 2009c and 2010b
Coalfish (*Pollachius virens*)	3.3	3,423	809	0.2	Powell et al., 2009c and 2010b
Norway pout (*Trisopterus esmarkii*)	3.3	3,423	181	0.05	Powell et al., 2009c and 2010b
European hake (*Merluccius merluccius*)	3.4	3,423	428	0.1	Powell et al., 2009c and 2010b
Haddock (*Melanogrammus aeglefinus*)	3.8	3,423	413	0.1	Powell et al., 2009c and 2010b
European whiting (*Merlangius merlangus*)	3.8	3,423	185	0.05	Powell et al., 2009c and 2010b
Long rough dab	3.8	3,423	794	0.2	Powell et

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
(*Hippoglossoides platessoides*)					al., 2009c and 2010b
Vahl's eelpout (*Lycodes vahlii*)	3.8	3,423	362	0.1	Powell et al., 2009c and 2010b
North Atlantic Pollock (*Pollachius pollachius*)	3.8	3,423	576	0.2	Powell et al., 2009c and 2010b
Poor cod (*Trisopterus minutus*)	3.8	3,423	96	0.03	Powell et al., 2009c and 2010b
Atlantic cod (*Gadus morhua*)	4.0	3,423	137	0.04	Powell et al., 2009c and 2010b
Sea Urchin (*Brissopsis lyrifera*)	2.1	492	3,156	6.4	Powell et al., 2009c and 2010b
Worms	2.1	492	405	0.8	Powell et al., 2009c and 2010b
Jellyfish	2.2	492	13	0.03	Powell et al., 2009c

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
					and 2010b
Plankton	2.2	492	55	0.1	Powell et al., 2009c and 2010b
Mussels (species A)	3.1	492	306	0.6	Powell et al., 2009c and 2010b
Mussels (species B)	3.0	492	118	0.2	Powell et al., 2009c and 2010b
Northern shrimp (*Pandalus borealis*)	3.0	492	29	0.06	Powell et al., 2009c and 2010b
European plaice (*Pleuronectes platessa*)	3.4	492	261	0.5	Powell et al., 2009c and 2010b
Coalfish (*Pollachius virens*)	3.6	492	53	0.1	Powell et al., 2009c and 2010b
Common sole (*Solea vulgaris*)	3.4	492	54	0.1	Powell et al., 2009c and 2010b

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Norway pout (*Trisopterus esmarkii*)	3.5	492	37	0.08	Powell et al., 2009c and 2010b
Starry skate (*Amblyraja radiate*)	3.5	492	46	0.09	Powell et al., 2009c and 2010b
Haddock (*Melanogrammus aeglefinus*)	3.7	492	132	0.3	Powell et al., 2009c and 2010b
Long rough dab (*Hippoglossoides platessoides*)	3.6	492	58	0.1	Powell et al., 2009c and 2010b
Atlantic cod (*Gadus morhua*)	4.1	492	41	0.08	Powell et al., 2009c and 2010b
Dotted gizzard shad (juvenile) (*Konosirus punctatus*)	3.0			0.045	Taken from Powell et al., 2014a)
Silver croaker (*Pennahia argentata*)	3.1			0.20	Taken from Powell et al., 2014a)

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Japanese sardinella (*Sardinella zunasi*)	3.1			0.088	Taken from Powell et al., 2014a)
Japanese anchovy (*Engraulis japonicas*)	3.5			0.10	Taken from Powell et al., 2014a)
Dotted gizzard shad (adult) (*Konosirus punctatus*)	3.8			0.085	Taken from Powell et al., 2014a)
Chub mackerel (*Scomber japonicas*)	4.1			0.038	Taken from Powell et al., 2014a)
Red barracuda (*Sphyraena pinguis*)	4.1			0.066	Taken from Powell et al., 2014a)

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Japanese sea bass (*Lateolabrax japonicas*)	4.4			0.052	Taken from Powell et al., 2014a)
Zooplankton		397	(102)	0.3	Seston et al., 2015a
Mayfly larvae (*Hexagenia* sp.)		397	69.8	0.2	Seston et al., 2015a
Gizzard shad (*Dorosoma cepedianum*) (young of year)		397	38.1	0.1	Seston et al., 2015a
Suager (*Sander canadensis*)		397	30.7	0.08	Seston et al., 2015a
Mayfly larvae (*Hexagenia* sp.)		416	207	0.5	Seston et al., 2015b
Gizzard shad (*Dorosoma cepedianum*) (young of year)		416	(12.7)	0.03	Seston et al., 2015b
Walleye (*Sander virteus*)		416	(8.38)	0.02	Seston et al., 2015b
Suager (*Sander*		416	(6.57)	0.02	Seston et

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
canadensis)					al., 2015b
Mysid shrimp (*Mysis relicta*)		497	24.1	0.05	Seston et al., 2014a
Round goby (*Neogobius meanostomus*) - small		497	136	0.3	Seston et al., 2014a
Round goby (*Neogobius meanostomus*) - moderate		497	182	0.4	Seston et al., 2014a
Rainbow smelt (*Osmerus mordax*)		497	39.4	0.08	Seston et al., 2014a
Alewife (*Alosa pseudoharengus*)		497	56.3	0.1	Seston et al., 2014a
Lake trout (*Salvelinus namaycush*)		497	58.5	0.1	Seston et al., 2014a
Mysid shrimp (*Mysis relicta*)		485	14.4	0.03	Seston et al., 2014b
Round goby (*Neogobius meanostomus*) - small		485	139	0.3	Seston et al., 2014b
Round goby (*Neogobius meanostomus*) - moderate		485	189	0.4	Seston et al., 2014b

	Trophic level	Sediment concentration assumed (µg/kg organic carbon)[1]	Biota concentration (µg/kg lipid)[1]	BSAF	Reference
Rainbow smelt (*Osmerus mordax*)		485	26.0	0.05	Seston et al., 2014b
Alewife (*Alosa pseudoharengus*)		485	27.3	0.06	Seston et al., 2014b
Lake trout (*Salvelinus namaycush*)		485	68.2	0.1	Seston et al., 2014b

Note: 1) Concentrations in ( ) are <MDL but >LOD; reported as the actual measured concentration.

ANNEX III - Comparison of laboratory bioconcentration data between substances

The dossier submitter has also compared the available fish laboratory bioconcentration data for substances that are considered to meet the vB criterion. Comparisons of concentrations actually measured in wildlife have not been included because of the size of the task and variability of use patterns and quantities leading to very different exposures. The data in the following table were collated from agreed (or soon-to-be agreed) regulatory reports produced under REACH. Wet weight whole fish concentrations have been estimated from the cited BCF and water solubilities unless otherwise stated, and do not take account of lipid content. Polyaromatic hydrocarbons other than anthracene have not been considered for the purpose of this exercise (though could in future).

Table 45 Laboratory bioconcentration data of various PBT/vPvB substances

Substance	CAS No.	BCF, L/kg	Maximum fish concentration in BCF test, mg/kg ww		Comment	Reference
			mg/kg ww	mmol/kg ww		
Anthracene	120-12-7	>6,000	-	-	Exposure concentrations are not stated so whole fish concentrations cannot be derived. Molecular weight 178.2 g/mole.	EC (2008)
Alkanes, C_{10-13}, chloro (short chain chlorinated paraffins)	85535-84-8	ca. 7,273	ca. 240	ca. 0.65	Data are for a C_{10-12} 58% wt Cl substance based on parent compound analysis. Fish lipid content not stated. Molecular weight assumed 371 g/mole ($C_{11}H_{26}Cl_6$).	ECHA (2008b)
2-(2H-Benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol (UV-328)	25973-55-1	4,590	0.37	0.0011	Based on average BCF at study end. Fish lipid content 4.2%. Molecular weight 351.5 g/mole.	ECHA (2014a)
2-Benzotriazol-2-yl-4,6-di-*tert*-butylphenol (UV-320)	3846-71-7	9,265	0.93	0.0029	Fish lipid content 3.6%. Molecular weight 323.4 g/mol.	ECHA (2014b)

Substance	CAS No.	BCF, L/kg	Maximum fish concentration in BCF test, mg/kg ww		Comment	Reference
			mg/kg ww	mmol/kg ww		
5-*tert*-Butyl-2,4,6-trinitro-m-xylene (musk xylene)	81-15-2	3,730 and 10,500	9.89 and 32.7 (estimated)	0.033 and 0.11	Steady state not reached – plateau fish concentrations were estimated using a one-compartment model. Fish lipid content 3.4%. Another study resulted in slightly lower fish concentrations (but still >1 mg/kg). Molecular weight 297.3 g/mol.	ECHA (2008c)
Hexabromocyclododecane (HBCDD)	25637-99-4	18,100 and 13,085	112 and 4.45	0.17 and 0.0069	Fish lipid content not specified. Molecular weight 641.7 g/mol.	ECHA (2008a)
Henicosafluoroundecanoic acid	2058-94-8	ca. 2,700 and 3,700	ca. 1.30 and 0.37	ca. 0.0023 and ca. 0.00066	BCF in first study based on carcass only. Lipid normalisation not appropriate. Molecular weight 564.09 g/mol.	ECHA (2012b)
Pentacosafluorotridecanoic acid	72629-94-8	ca. 18,000 and ca. 13,000	ca. 3.60 and ca. 1.30	ca. 0.0054 and ca. 0.0020	BCF in first study based on carcass only; second study cited as a BCF range so estimated fish concentration is based on the average. Lipid normalisation not appropriate. Molecular weight 664.11 g/mol.	ECHA (2012c)
Heptacosafluorotetradecanoic acid	376-06-7	ca. 23,000 and ca. 16,500	ca. 0.32 and ca. 1.65	ca. 0.000045 and ca. 0.0023	BCF in first study based on carcass only; second study cited as a BCF range so estimated fish concentration is based on the average. Lipid normalisation not appropriate. Molecular weight 714.11 g/mol.	ECHA (2012d)
Octamethylcyclotetrasiloxane (D4)	556-67-2	≥11,495	≥2.64	≥0.0089	Fish lipid content 6.4%. Molecular weight 296.62 g/mol.	EA (2009a)

Substance	CAS No.	BCF, L/kg	Maximum fish concentration in BCF test, mg/kg ww		Comment	Reference
			mg/kg ww	mmol/kg ww		
Decamethylcyclopentasiloxane (D5)	541-02-6	≥5,860 and ca. 12,600	≥24.3 and ca. 13.0	≥0.066 and ca. 0.035	In the first study, fish lipid content varied from 2.9 to 4.1% during the uptake phase. In the second study, the variation was less and the mean lipid content was 5.71%. Molecular weight 370.77 g/mol.	EA (2014)
Dodecamethylcyclopenthexasiloxane (D6)	540-97-6	Up to 12632	2.43 and 0.407	0.0055 and 0.00091	Lipid content was between 4.85 and 5.78%. BCF was corrected for growth. Molecular weight 444.92 g/mol. Maximum measured fish concentrations	This report.
Pentabromodiphenyl ether (pentaBDE)	32534-81-9	PentaBDE: ca. 17,700 HexaBDE: ca. 5,640	PentaBDE: ca. 42 HexaBDE: ca. 1.37	PentaBDE: ca. 0.074 HexaBDE: ca. 0.0021	The analysis is complicated because several congeners were tested at the same time, and some corrections have to be made to the data. The cited data are for one pentaBDE and one hexaBDE constituent, respectively. Fish lipid content was 4.8%. Molecular weight 564.7 g/mol (PentaBDE) and 643.6 g/mol (HexaBDE).	EC (2001)

Whole fish concentrations associated with a high BCF depend on the water solubility achieved in the experiment as well as (usually) the size and lipid content of the test organisms, species-specific factors (such as metabolism, which may change with life stage), and growth dilution, etc. Comparisons between studies using the same substance can therefore be complicated, and comparisons between substances should be treated with caution. Nevertheless, from the table, it can be seen that substances with vB properties can generally achieve whole fish concentrations in the range of 0.9 – ca. 50 mg/kg ww, with three substances outside this range[35]. A benchmark of 1 mg/kg ww might therefore be suitable as an indicator of high bioaccumulation potential. The equivalent range on a molar basis is around 0.002 to

[35] In terms of the PBT concept, bioaccumulation concerns are linked to the potential for a substance to reach a toxic threshold in species that have not been tested in the laboratory. It is perhaps open to question whether substances achieving concentrations at the lower end of this range should be considered to be as hazardous as those at the upper end (two orders of magnitude higher), but this will also depend on factors such as molecular weight (i.e. the number of molecules present in the fish) and mode of any toxic action.

0.2mmol/kg.

The maximum fish concentrations measured for Dechlorane Plus in laboratory studies were in the range 0.385 – 8.72 mg/kg ww for *Lepomis macrochirus* and 0.327 mg/kg ww for *Cyprinus carpio* in bioconcentration tests. Some of these concentrations might include substance adsorbed to the skin and they might also partially result from particulate uptake, but conversely, steady-state concentrations could be higher. These values are comparable to substances such as UV-328 and -320, long chain perfluorocarboxylic acids, musk xylene, D4, hexaBDE and HBCDD. Molar concentration is inversely proportional to the molecular weight (MW). The MW of Dechlorane Plus (654 g/mole) is higher than some of thes substances (e.g. henicosafluoroundecanoic acid, 564 g/mole) and similar to others (e.g. HBCDD, 642 g/mole), so there will be more or a similar number of Dechlorane Plus molecules present in the fish compared to these substances when concentrations are the same. As can be seen from the Table 45, the concentration range for D6 is within the range of whole fish concentrations generally achieved for substances with vB properties.

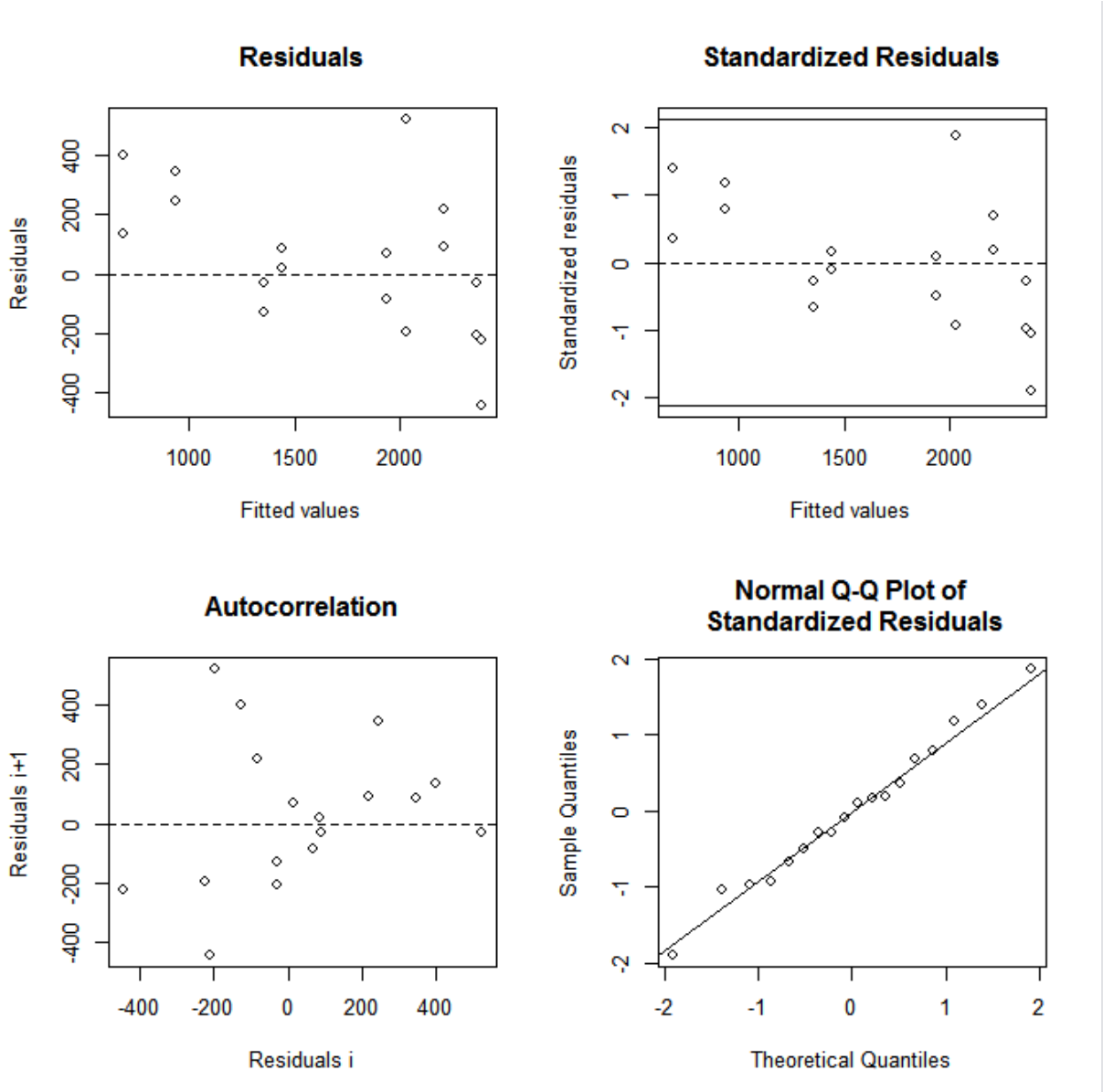
ANNEX IV: OUTPUT FROM R STATISTICAL PACKAGE

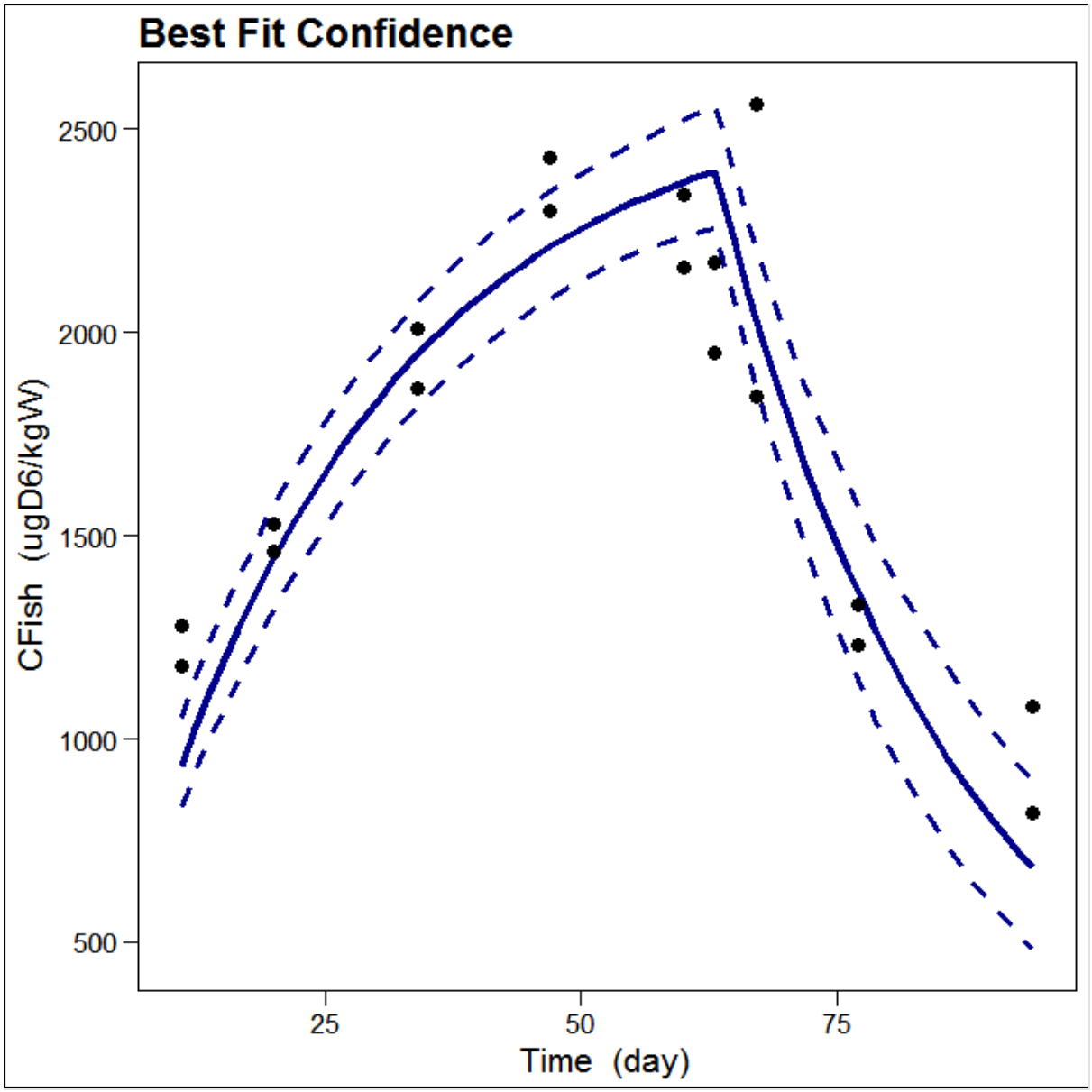
BCMFR PACKAGE, V0.3-2: FOR SIMULTANEOUS FITTING OF KINETIC BCF FOR CERI, 2010
TEST_AQUEOUS_D6_HIGH_FINAL

It should be noted that the model equation passes through 0,0 –point (by default).

```
            value    unit
cwater     0.9080  ugD6/L
tstart     0.0000     day
tdepur    63.0000     day
tend      94.0000     day
kgrowth    0.0143   1/day
lipidfish  5.3000 percent
```

Untransformed fit

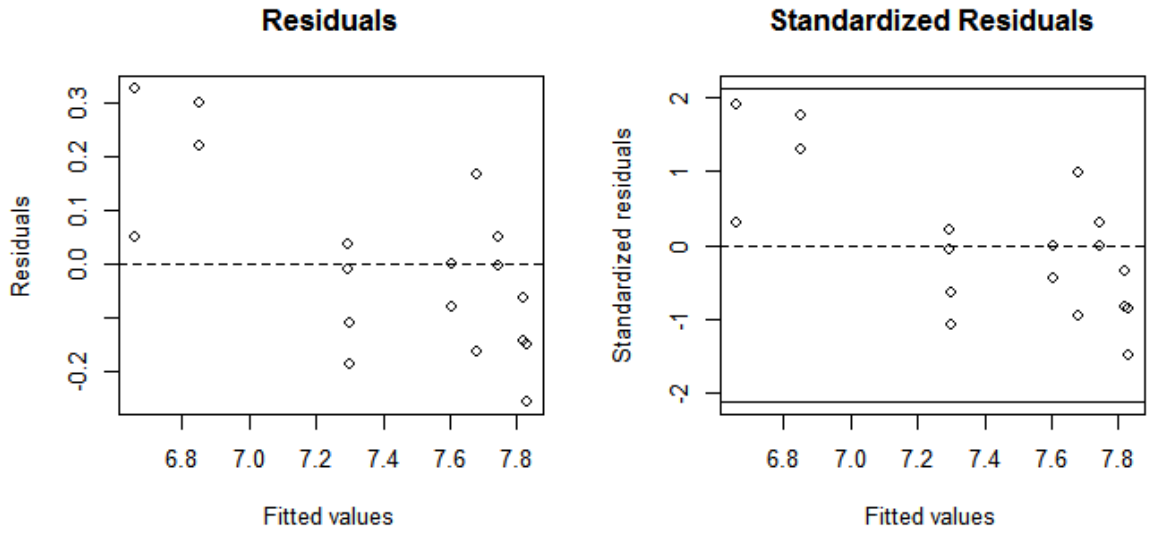


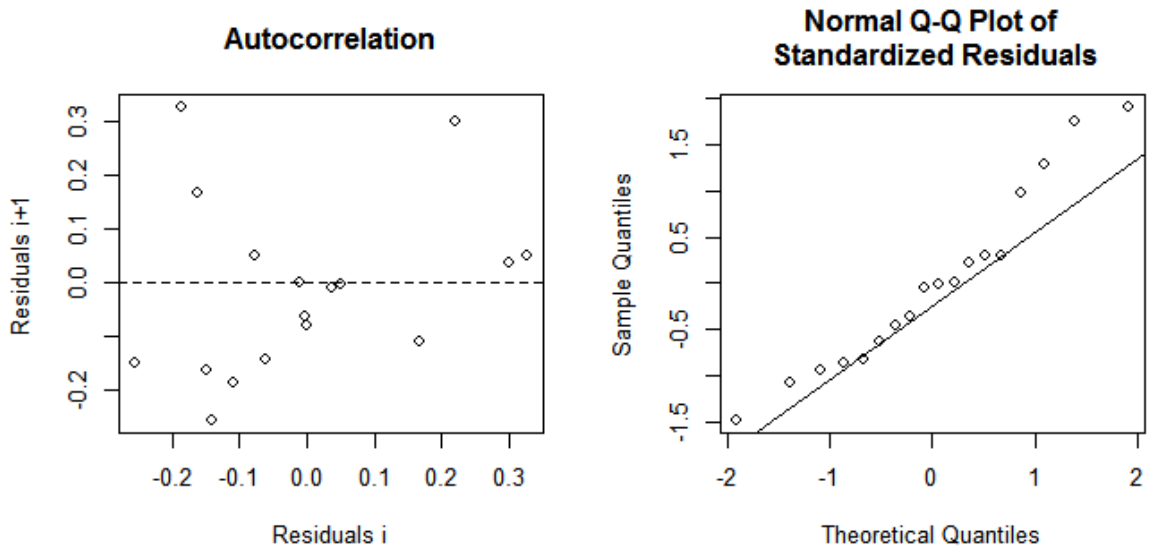


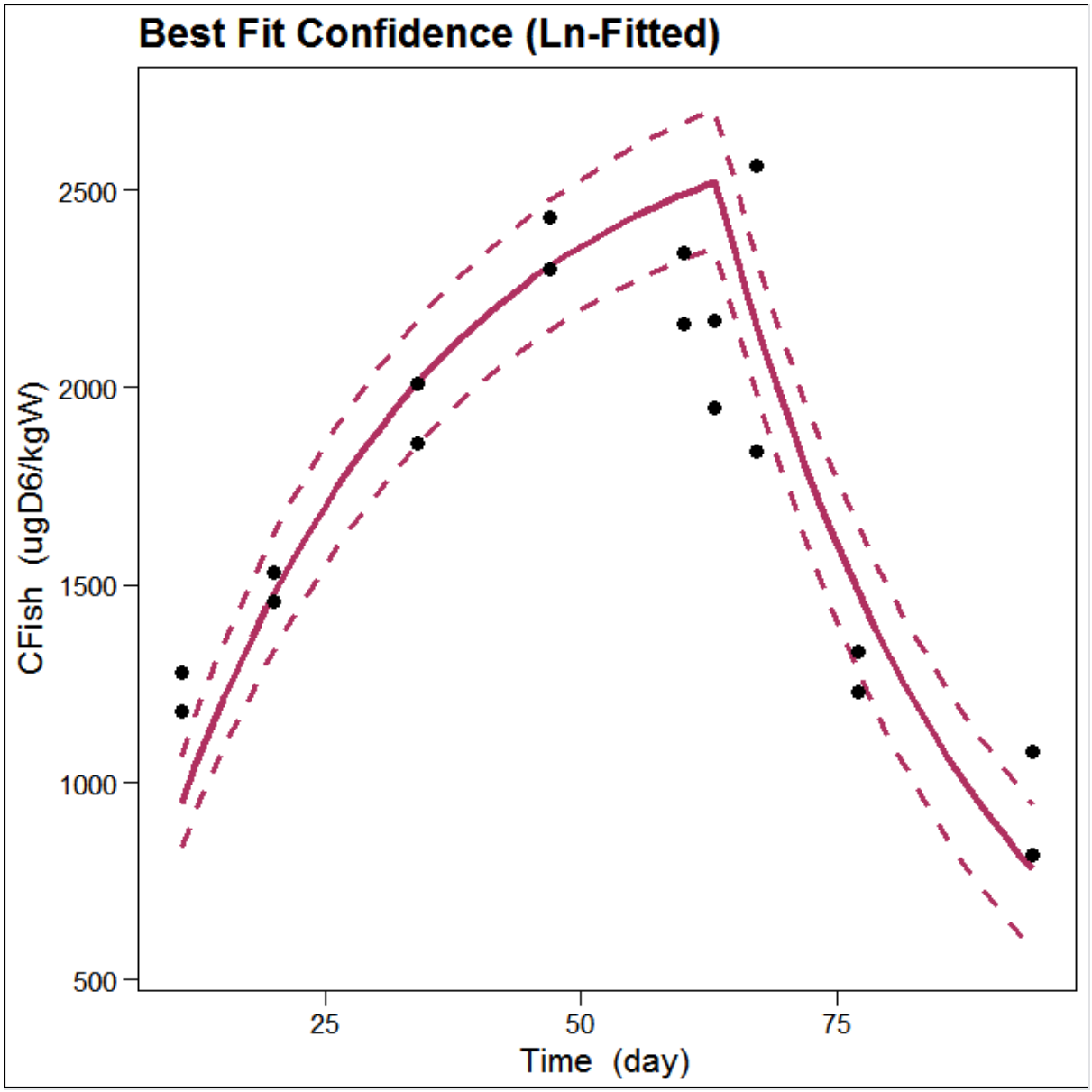
Summary

	Estimate	Std.Error	2.5%	97.5%
k1	115.98	11.028	94.366	137.6
k2	0.041	0.0047	0.031	0.05
k2g	0.026	0.0047	0.017	0.036
BCFK	2860.4	125.01	2615.3	3105.4
BCFKg	4418.7	432.13	3571.8	5265.7
Thalfg	26.403	4.7757	17.042	35.763
BCFKgL	4168.6	407.67	3369.6	4967.7

Natural Log Transformed fit



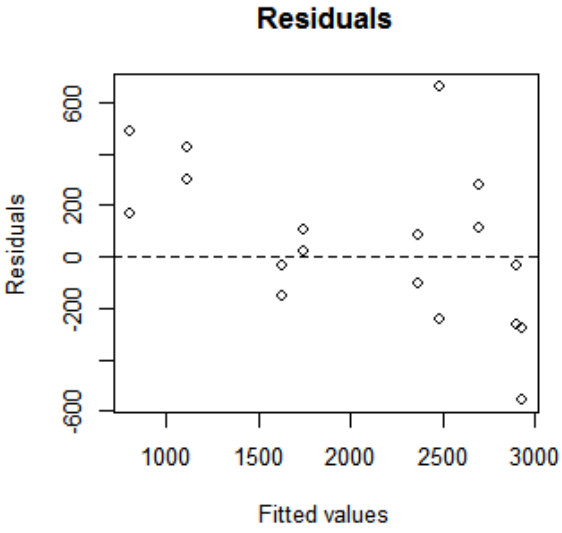


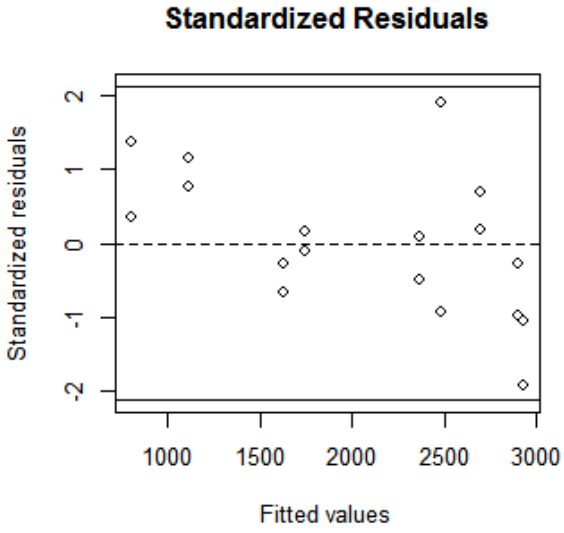


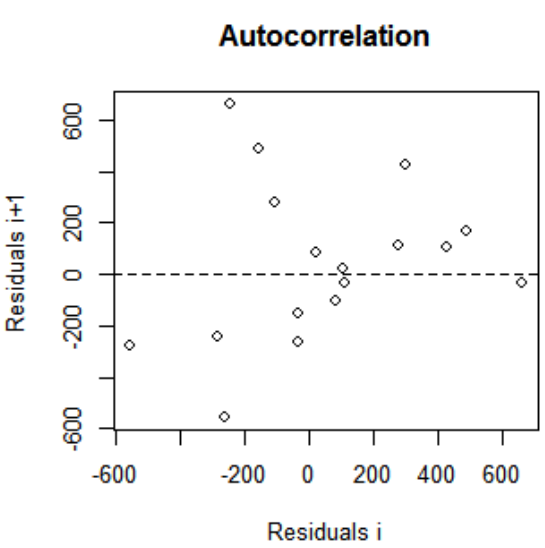
Summary

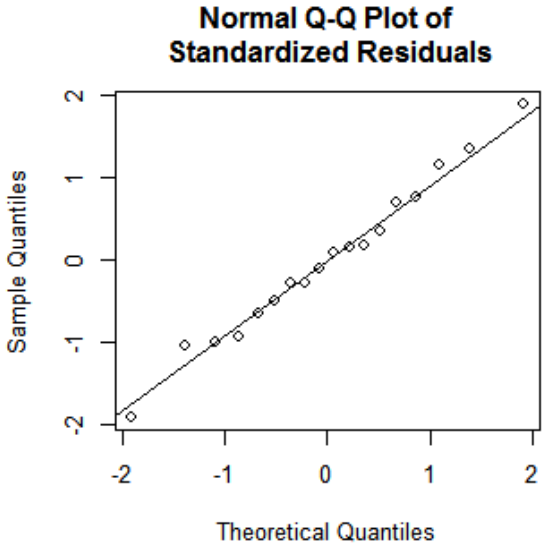
	Estimate	Std.Error	2.5%	97.5%
k1	115.85	8.9746	98.263	133.44
k2	0.038	0.0031	0.032	0.044
k2g	0.024	0.0031	0.018	0.03
BCFK	3055.5	132.41	2795.9	3315
BCFKg	4905.6	373.81	4172.9	5638.2
thalfg	29.344	3.8301	21.837	36.851
BCFKgL	4627.9	352.65	3936.7	5319.1

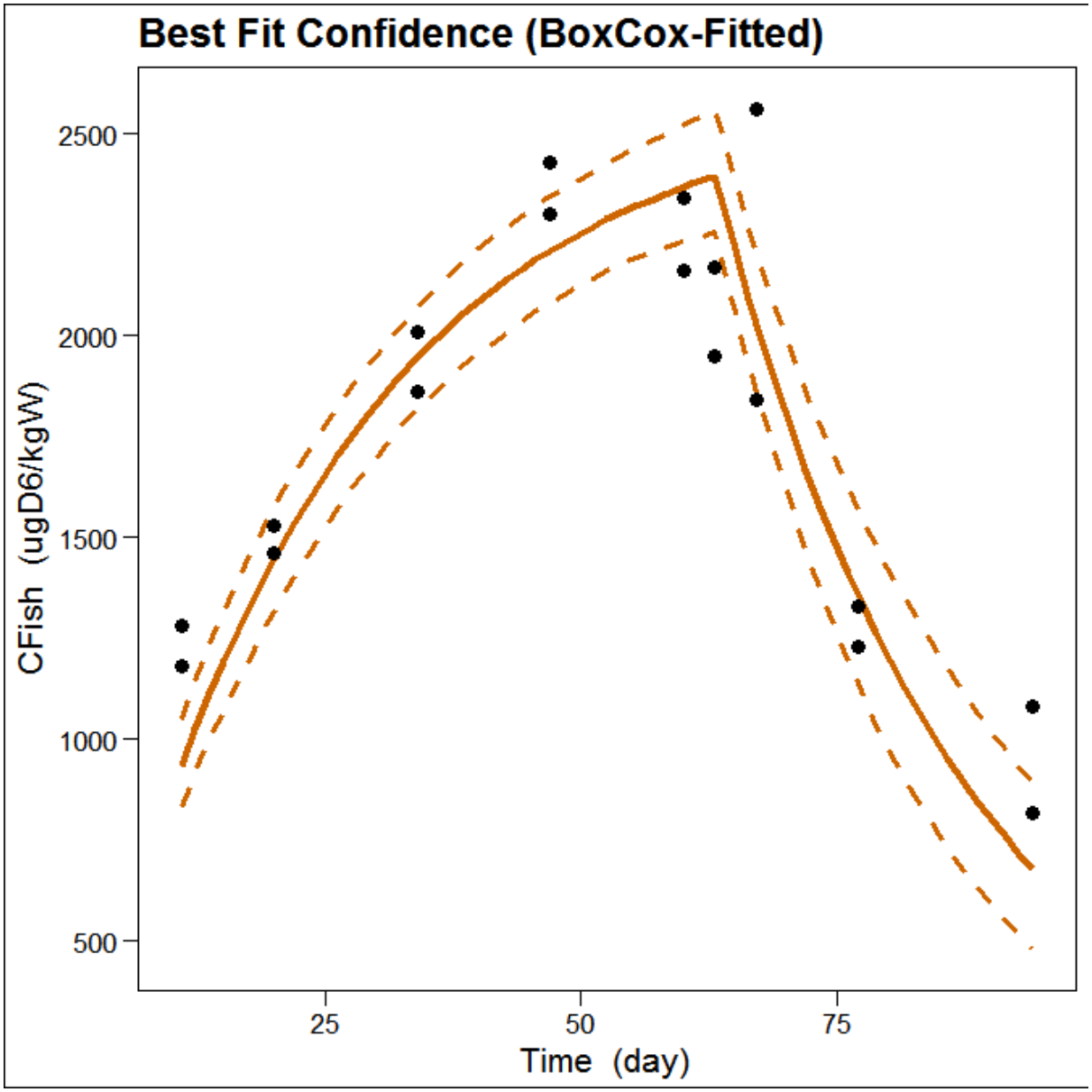
Box-Cox Transformed fit











Summary

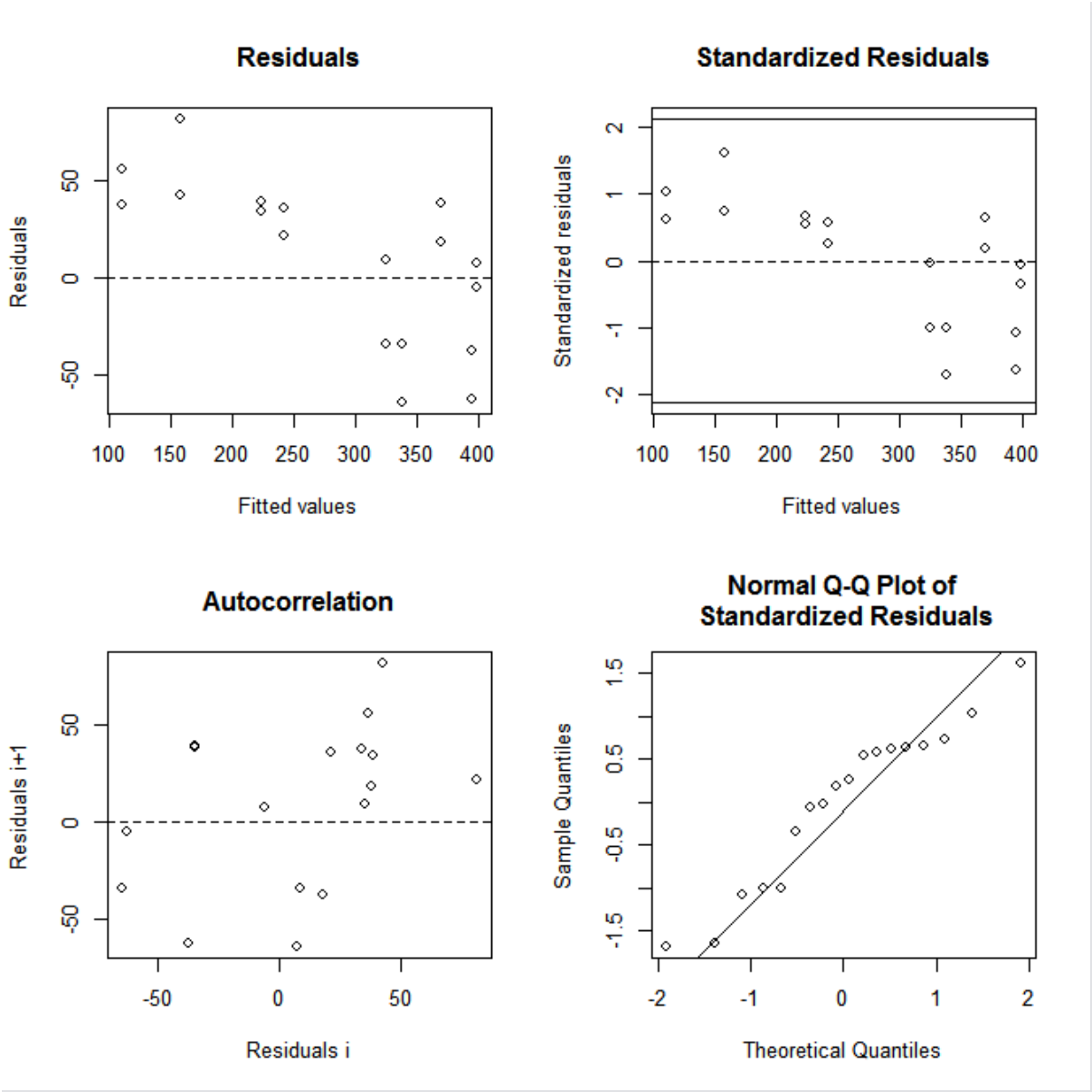
	Estimate	Std.Error	2.5%	97.5%
k1	116.09	11.148	94.24	137.94
k2	0.041	0.0048	0.031	0.05
k2g	0.026	0.0048	0.017	0.036
BCFK	2856.4	125.15	2611.1	3101.7
BCFKg	4407	434.43	3555.5	5258.5
thalfg	26.308	4.8098	16.88	35.735
BCFKgL	4157.6	409.84	3354.3	4960.8

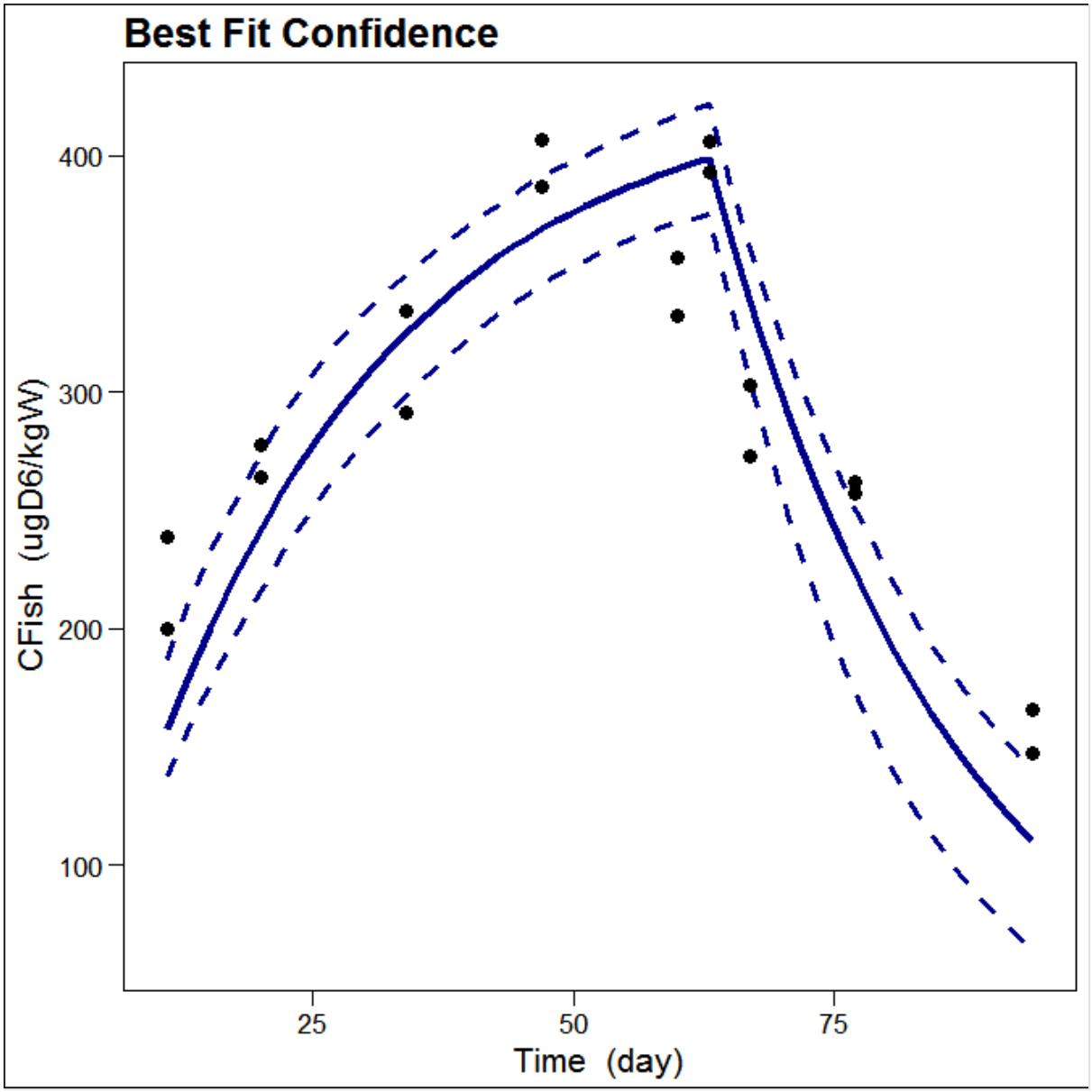
TEST_AQUEOUS_D6_LOW_FINAL

	value	unit
cwater	0.0863	ugD6/L
tstart	0.0000	day
tdepur	63.0000	day
tend	94.0000	day
kgrowth	0.0143	1/day

```
lipidfish  5.3000 percent
```

Untransformed fit

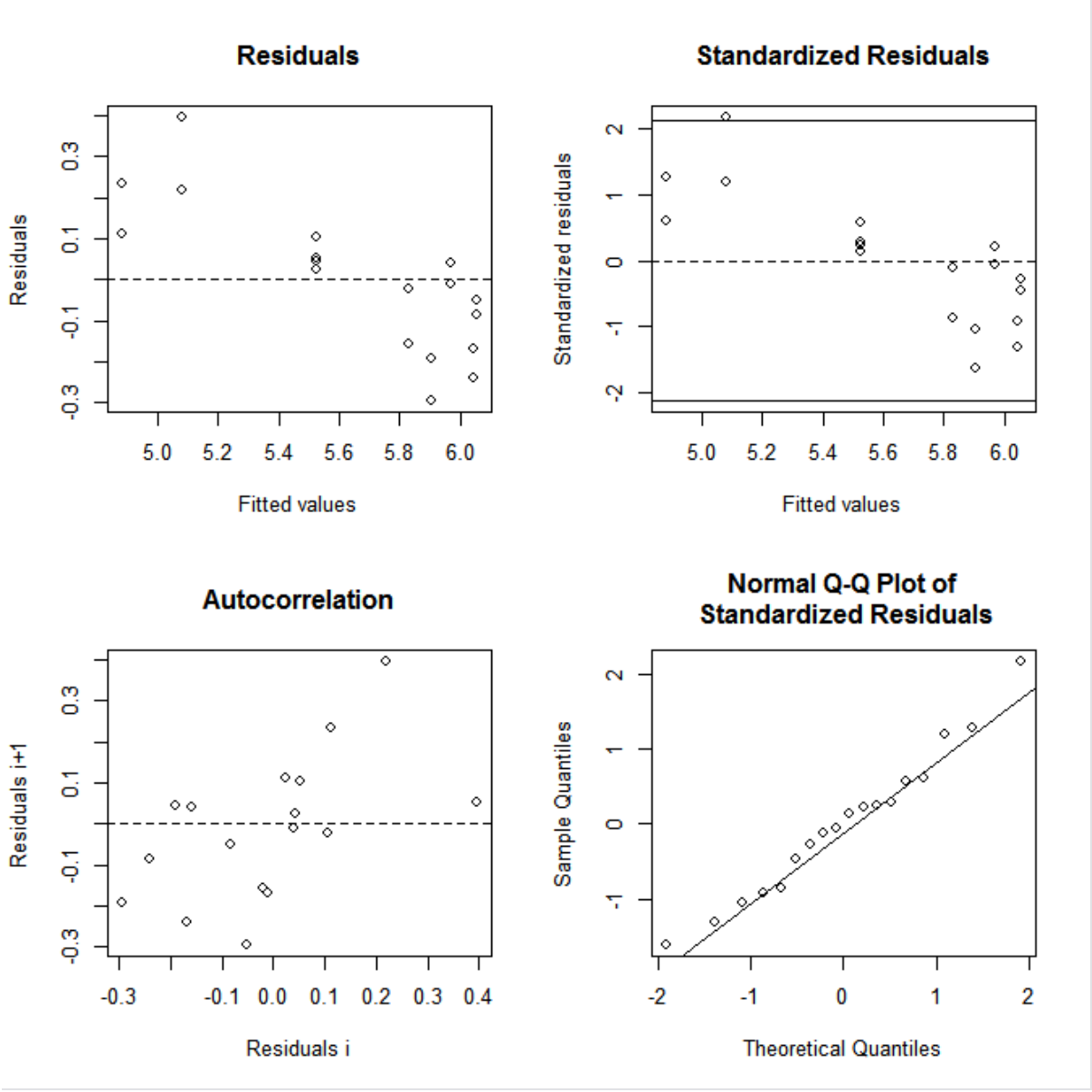


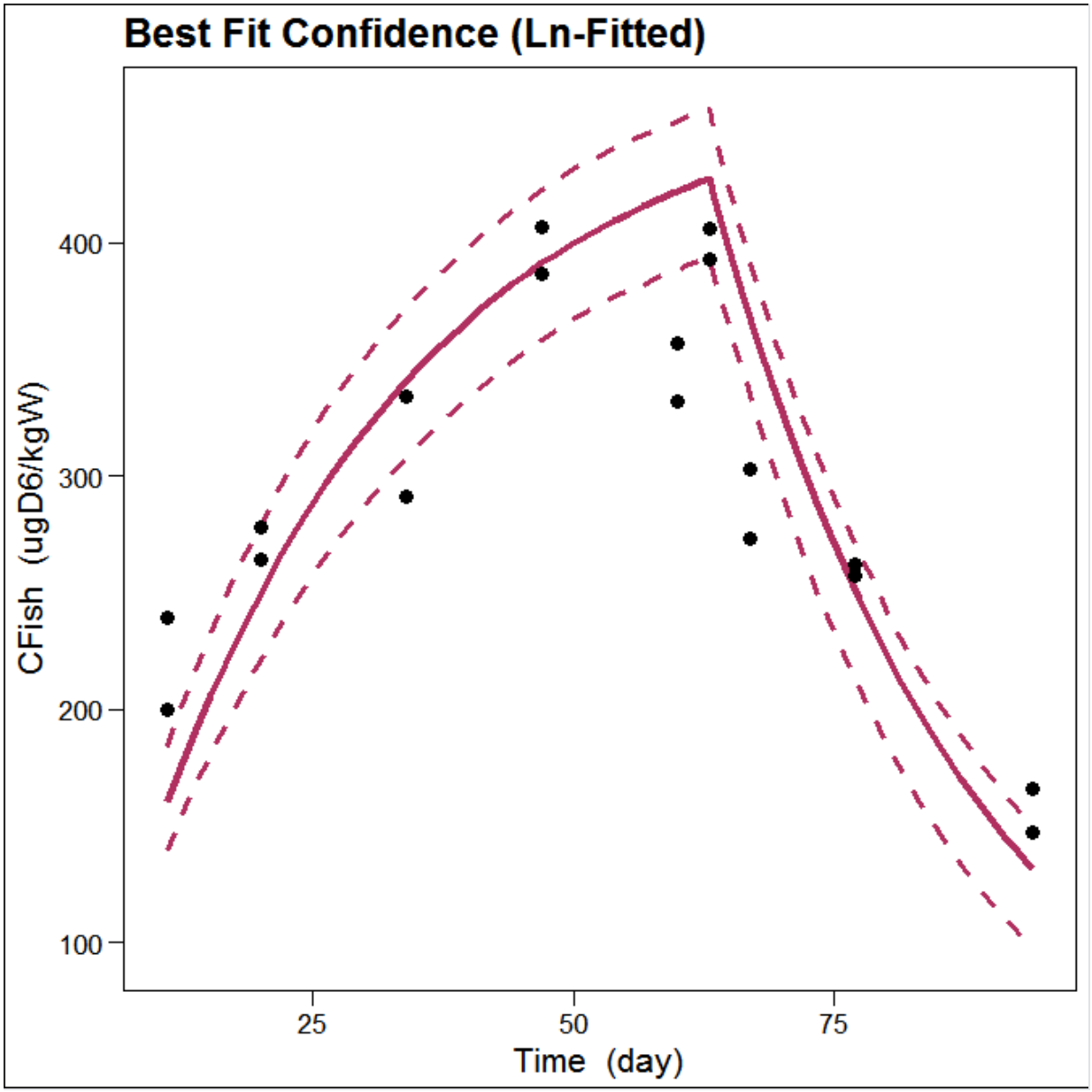


Summary

	Estimate	Std.Error	2.5%	97.5%
k1	206.77	20.322	166.94	246.6
k2	0.041	0.005	0.032	0.051
k2g	0.027	0.005	0.017	0.037
BCFK	4984.4	221.5	4550.2	5418.5
BCFKg	7606.3	741.29	6153.4	9059.2
thalfg	25.493	4.6695	16.34	34.645
BCFKgL	7175.8	699.33	5805.1	8546.5

Natural Log Transformed fit

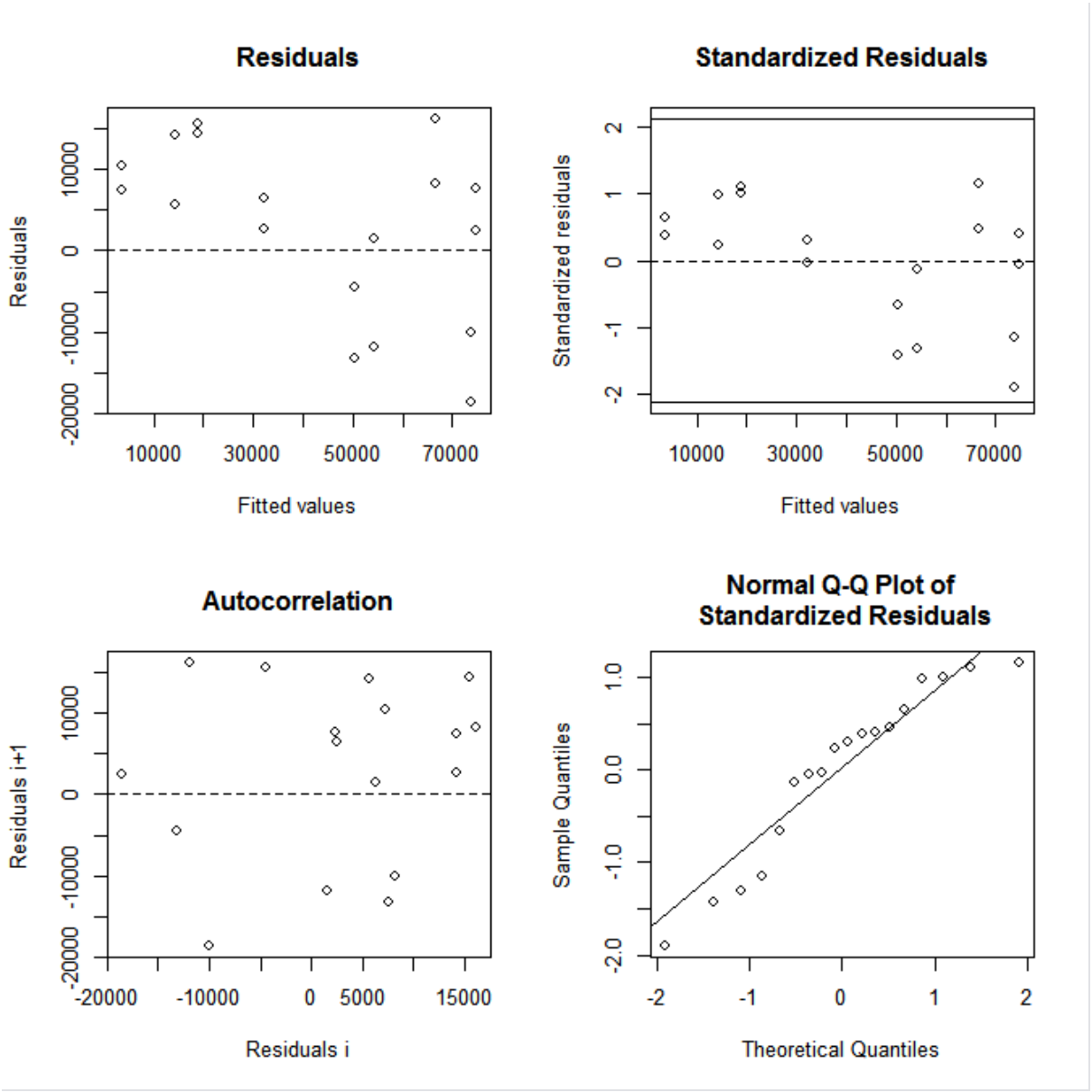




Summary

	Estimate	Std.Error	2.5%	97.5%
k1	207.11	17.059	173.68	240.55
k2	0.038	0.0033	0.032	0.044
k2g	0.024	0.0033	0.017	0.03
BCFK	5449.1	250.84	4957.5	5940.8
BCFKg	8735.9	704.62	7354.9	10117
thalfg	29.231	4.0439	21.305	37.157
BCFKgL	8241.4	664.73	6938.5	9544.3

Box-Cox Transformed fit


Residuals
Residuals
10000
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-10000
-20000
10000
30000
50000
70000
Fitted values
Standardized Residuals
Standardized residuals
2
1
0
-1
-2
10000
30000
50000
70000
Fitted values
Autocorrelation
Residuals i+1
10000
0
-10000
-20000
-20000
-10000
0
5000
15000
Residuals i
Normal Q-Q Plot of Standardized Residuals
Sample Quantiles
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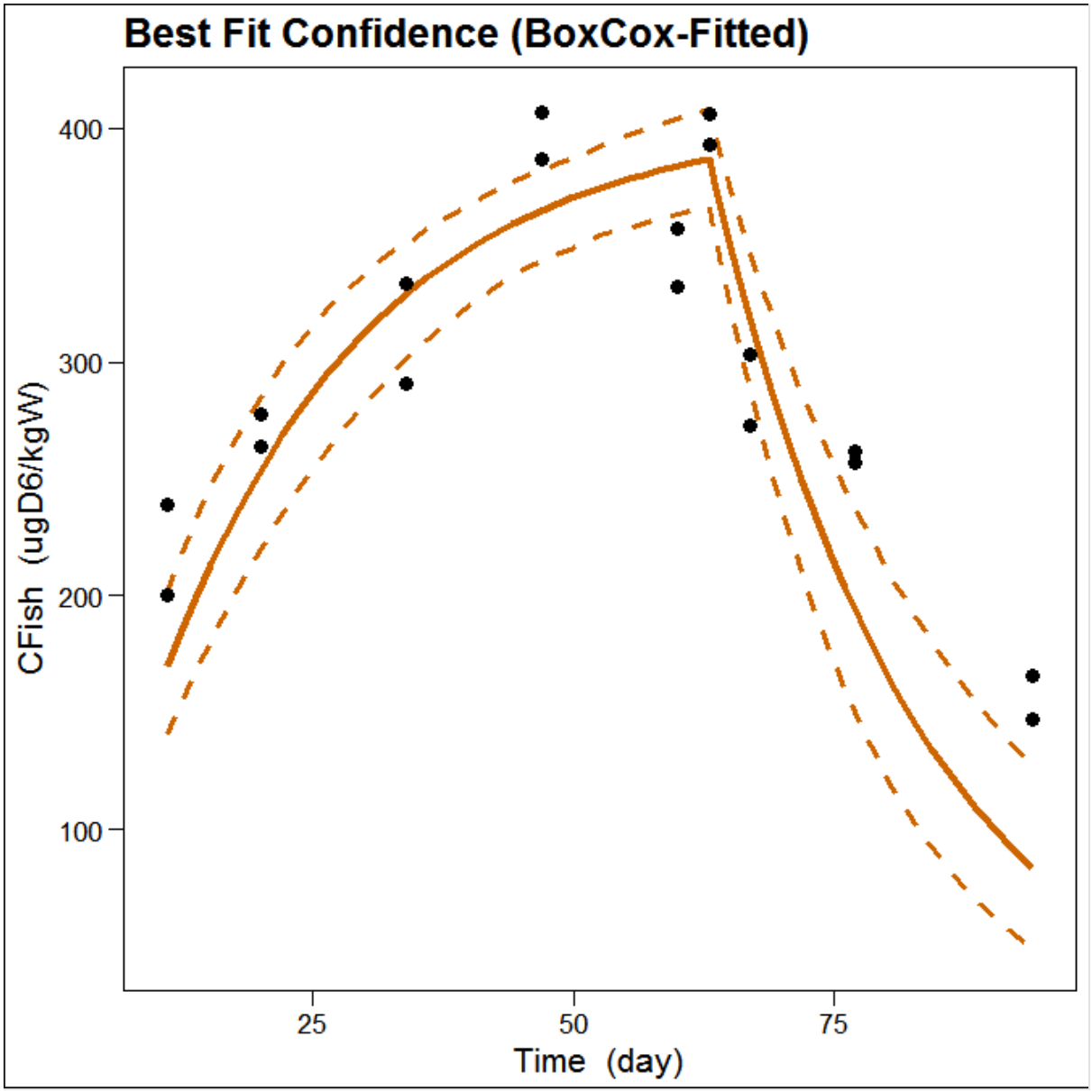
1
2
Theoretical Quantiles



Summary

	Estimate	Std.Error	2.5%	97.5%
k1	231.71	28.132	176.57	286.85
k2	0.049	0.0073	0.035	0.064
k2g	0.035	0.0073	0.021	0.049
BCFK	4692	182.93	4333.5	5050.6
BCFKg	6604.6	609.19	5410.5	7798.6
thalfg	19.753	4.0838	11.749	27.757
BCFKgL	6230.7	574.7	5104.3	7357.1