

IDENTIFICATION OF PBT AND vPvB SUBSTANCE

RESULTS OF EVALUATION OF PBT / vPvB PROPERTIES

This dossier covers the substance manufactured and supplied as detailed below.

Substance name: Octamethylcyclotetrasiloxane

EINECS number: 209-136-7

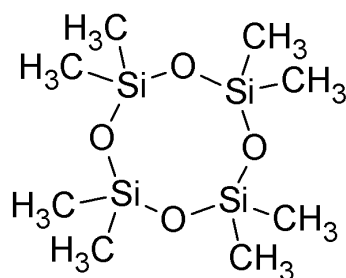
EINECS name: Octamethylcyclotetrasiloxane

CAS number: 556-67-2

Registration number(s): [Link to ECHA dissemination site for D4](#)

Molecular formula: C₈H₂₄O₄Si₄

Structural formula:



Composition: The purity of octamethylcyclotetrasiloxane (D4) is between >96 per cent and >99 per cent. The major impurity is decamethylcyclopentasiloxane¹ (D5; CAS no.: 541-02-6). No additives are present in the commercial substance (EA, 2009).

Summary of how the substance meets the CMR (Cat 1 or 2), PBT or vPvB criteria, or is considered to be a substance of an equivalent level of concern

Octamethylcyclotetrasiloxane (D4) was discussed by the former EU PBT Working Group on a number of occasions. As a result of these discussions the substance was included in Regulation (EC) No. 465/2008 of 28th May 2008, which required industry to conduct an environmental monitoring programme and submit the results by November 2009. In addition, Industry has voluntarily carried out a large number of other studies relevant to the PBT and vPvB assessment for this substance. Following review of this information, the Rapporteur submitted an evaluation report to the European Chemicals Agency (ECHA) in October 2010. Since then, several more studies have been carried out in Japan and submitted to the Rapporteur by the registrants, and some further academic studies have been published. For

¹ The actual amount of D5 present has not been reported but based on the stated purity of D4 it is likely to range from 1 per cent or less to up to approximately 4 per cent.

completeness a literature search was carried out by the Rapporteur on 26th January 2012 (some *ad hoc* papers were also included after that date). A draft of the evaluation was circulated to Industry for comment during summer 2012 and further information submitted in their response was incorporated into the final document. This evaluation is therefore an update of the 2010 report, summarising all the relevant new data available and considering their significance in relation to the PBT and vPvB criteria.

Based on the available information, D4 meets the Annex XIII criteria for both a ‘persistent, bioaccumulative and toxic’ (PBT) and a ‘very persistent and very bioaccumulative’ (vPvB) substance in the environment. This conclusion was endorsed by the ECHA PBT Expert Group in November 2012.

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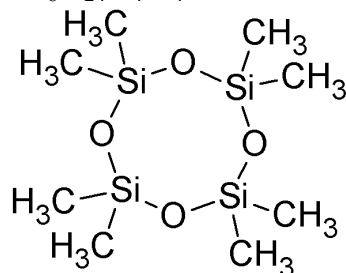
JUSTIFICATION

Note: A detailed review of existing information on the properties of D4 was published by EA (2009). In the following sections, the information from this previous review has been described only briefly under the heading *Summary of information from existing evaluation*. It is understood that these data have been included as robust study summaries in the Chemical Safety Reports submitted by the registrants under the REACH Regulation, although a comparison has not been done for the purposes of this report. New information that has become available since the EA (2009) report was completed is reported under the heading *New information*.

1 IDENTIFICATION OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifier of the substance

Name: Octamethylcyclotetrasiloxane
 EC Number: 209-136-7
 CAS Number: 556-67-2
 IUPAC Name: Octamethylcyclotetrasiloxane
 Molecular Formula: $C_8H_{24}O_4Si_4$
 Structural Formula:



Molecular Weight: 296.62 g/mole
 Synonyms (and registered trade names): Cyclic dimethylsiloxane tetramer, Cyclen D4/OMCTS, Cyclen D4/OMCTS WN, Cyclomethicone, Cyclotetrasiloxane, octamethyl-, Cyclotetrasiloxane, D4, Dow Corning 244, KF 994, DC 344, DC 244, Dow Corning 344, NUC silicone VS 7207, Oel Z020, OMCTS, SF 1173, Tetramere D4/OMCTS, Tetramere D4/OMCTS Silbione, TSF 404, Volasil 244 and VS 7207.

The abbreviation D4 will be used for the substance throughout this dossier.

1.2 Composition of the substance

The purity of D4 is between >96 per cent and >99 per cent. The major impurity is decamethylcyclopentasiloxane (D5; CAS no. 541-02-6). The actual amount of D5 present has not been reported, but based on the stated purity of D4 it is likely to range from 1 per cent or less to up to approximately 4 per cent. No additives are present in the commercial substance (EA, 2009).

1.3 Physico-chemical properties

The physico-chemical property data are summarised in Table 1.

Table 1 Summary of relevant physico-chemical properties

REACH ref Annex, §	Property	Value	Comments
V, 5.1	Physical state at 20°C and 101.3 kPa	Liquid	
V, 5.2	Melting / freezing point	17.7°C	Experimental value; EA, 2009
V, 5.3	Boiling point	175°C at 1,013 hPa	Experimental value; EA, 2009
V, 5.5	Vapour pressure at 25°C	132 Pa	Derived from a temperature-vapour pressure correlation using critically evaluated data; EA, 2009
V, 5.7	Water solubility at 20°C	0.056 mg/l (at 23°C)	Experimental value; EA, 2009
V, 5.8	Partition coefficient n-octanol/water (K_{ow} , log value) at 25°C	6.49	Experimental value (slow stirring method); EA, 2009
VII, 5.19	Dissociation constant (pKa)	Not relevant	EA, 2009

2 MANUFACTURE AND USES

Four companies produce or supply D4 in the EU (EA, 2009). The actual quantity produced or supplied by each company is confidential information. The main uses of D4 can be divided into four areas:

- Use as a site-limited chemical intermediate at the site of production.
- Use as an off-site chemical intermediate.
- Use in personal care products (e.g. cosmetic, skin- and hair-care products).
- Use in household products (e.g. cleaning products).

The total amount of D4 used in the EU is confidential. EA (2009) reports that in 2004, around 8,866 tonnes were used as an off-site intermediate for the production of silicone polymers and 579 tonnes were used in personal care products. The amounts used in the other applications are confidential.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Environment

R53: May cause long-term adverse effects in the aquatic environment.

Human health

Repro. Cat 3

R62: Possible risk of impaired fertility.

3.2 Classification in Annex VI of Regulation (EC) No. 1272/2008

Environment

Hazard class and category: Aquatic Chronic 4.

Hazard statement: H413: May cause long lasting harmful effects to aquatic.

Human health

Hazard class and category: Repr. 2.

Hazard statement: H361f: Suspected of damaging fertility.

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Abiotic degradation

4.1.1.1 Summary of information from existing evaluation

Atmospheric degradation

Degradation of D4 occurs in the atmosphere by reaction with atmospheric hydroxyl radicals. The half-life of this reaction is estimated to be 12.7 to 15.8 days (mean value ~14 days; EA, 2009) based on a hydroxyl radical reaction rate constant in the range 1.01×10^{-12} to 1.26×10^{-12} cm³/molecule/s (determined in studies by Atkinson (1991) and Sommerlade *et al.* (1993); both at 24°C) and an average atmospheric hydroxyl radical concentration of 5×10^5 molecule/cm³. The products of the reaction are expected to be silanols, which are

removed from the atmosphere by wet deposition (either adsorbed onto particulates or dissolved).

Hydrolysis

D4 undergoes hydrolysis. The rate of hydrolysis is dependent on the pH and temperature. The rate is slowest at near neutral pH (half-life around 69 to 144 hours at pH 7 and 25°C) but increases at higher and lower pHs (for example half-life ~0.9-1 hour at pH 9 and 25°C and half-life ~1.8 hours at pH 4 and 25°C). The rate of reaction also decreases with decreasing temperature and the following half-lives were recommended in the environmental evaluation by EA (2009).

- Hydrolysis half-life at pH 7 and 12°C (freshwater) = 16.7 days.
- Hydrolysis half-life at pH 8 and 9°C (marine water) = 2.9 days.

The main degradation product formed during the abiotic degradation of D4 is expected to be dimethylsilanediol and this is expected to undergo further degradation processes in the environment to ultimately form carbon dioxide and silicic acid and/or silica.

A preliminary study investigating the disappearance of ¹⁴C-D4 from a water-sediment system estimated the disappearance half-life to be around 131 days in a sandy sediment and 115 days in a sandy-silt sediment at a temperature between 20-25°C (EA, 2009). The disappearance resulted from a combination of volatilisation and hydrolysis.

4.1.1.2 New information

Atmospheric degradation

Xu and Kim (no year) estimated the atmospheric half-life of D4 in various locations taking into account the yearly average hydroxyl radical concentration measured in that location. The data are summarised in Table 2 (for comparison, the default hydroxyl radical concentration normally assumed in the EUSES model/REACH Guidance is lower, at 5×10^5 molecules/cm³). The atmospheric half-lives estimated (based on the reaction rate constant (k_{OH}) determined by Atkinson (1991)) ranged between 0.9 and 4.0 days for three urban areas, 8.0 days for a semi-rural area, 10 and 15 days for two rural areas and 10 days for a marine area. The authors pointed out that D4 is released mostly to urban and suburban atmospheres.

A series of studies by Navea *et al.* (2009a and b), Xu (no year), Kim *et al.* (2008) and Kim & Xu (2009a and 2009b) have investigated further the adsorption of D4 onto atmospheric aerosol components and the subsequent degradation of D4 on the aerosol. The results of these studies show that reaction of D4 with a number of mineral aerosols such as kaolinite, illite, mica and hematite can significantly contribute to the overall removal of D4 from the gas phase of the atmosphere, especially under dry conditions, and this removal can be promoted by ozone and sunlight.

Table 2 Locations and yearly hydroxyl radical concentrations used in the Xu and Kim (no year) study

Area	Location	Measured yearly average hydroxyl radical concentration (molecule/cm ³)	Reference used for hydroxyl radical concentration data	Estimated atmospheric half-life of D4 (days)
Marine	Finokalia, Greece	0.8×10^6	Mandalakis <i>et al.</i> (2003)	10
Rural	Kanto, Japan	0.53×10^6	Suzuki <i>et al.</i> (1984)	15
	Spring/Rock Spring, PA, USA	1.2×10^6	Ren <i>et al.</i> (2005)	10
Semi-rural	Italy	1×10^6	Hjorth <i>et al.</i> (1984)	8
Urban	Nashville, TN, USA ¹	9×10^6	Nunnermacker <i>et al.</i> (1998)	0.9
	Four Corners, USA ¹	7.1×10^6	Davis (1977)	1.1
	Schauinsland, Germany ¹	2×10^6	Kramp and Volz-Thomas (1997)	4.0

Note: 1) For these locations, measured data on the yearly average hydroxyl radical concentration were not available. The yearly average was estimated by Xu and Kim from the maximum concentration assuming the yearly average concentration = $0.75 \times$ the summer daily average concentration, and the summer daily average concentration = summer maximum concentration/4.

Overall the studies conclude that reaction of D4 with mineral aerosols is important to the atmospheric degradation of D4 and will contribute to its removal from the atmosphere. Navea *et al.* (2009a) estimated that the atmospheric lifetime² of D4, taking into account reaction with aerosols, could be around 6.6 days.

Hydrolysis

No new information is available.

² The atmospheric lifetime is the time for the concentration to fall to 1/e (around 1/2.7 or approximately 37 per cent) of its original value. The equivalent half-life would be approximately 4.6 days.

4.1.2 Biotic degradation

4.1.2.1 Summary of information from existing evaluation

The available standard biodegradation experiments show little evidence that D4 is readily biodegradable. However, D4 is highly volatile and will partition readily into the air from water, which makes it unavailable to the microorganisms in the test systems used. This makes it very difficult to test D4 for biodegradation (for example it is likely that in many of the tests carried out a major proportion of D4 was in the head space rather than the water phase). Thus, although the available data appear to indicate that D4 is not readily biodegradable, they do not provide absolute proof of this.

Degradation of D4 has been demonstrated in dry soils (e.g. Xu (1999) and Xu and Chandra (1999)), most probably by an abiotic process. Half-lives for the reaction were estimated in EA (2009) to be around 4.1 to 5.3 days for dry temperate soils in equilibrium with air of relative humidity of 50 to 90 per cent and 0.05 to 0.08 days for tropical soils in equilibrium with air of 50 to 90 per cent relative humidity. However, the presence of moisture significantly reduced the rate of degradation such that when the dried soil was equilibrated with a 100 per cent relative humidity atmosphere essentially no degradation was seen. EA (2009) concluded that although it is possible that such degradation in soils could occur in the environment (for example under low relative humidity or drought conditions) this was unlikely to be the typical case (particularly for agricultural soil where watering of crops during dry conditions may be expected)³.

4.1.2.2 New information

The degradation of D4 under anaerobic conditions has been studied using a modified version of OECD Test Guideline 308 (Xu, 2009a). The substance tested was ¹⁴C-labelled D4 with a radiochemical purity of 97.0 per cent.

The sediment used was collected from the top layer (to 15 cm) of a natural freshwater sediment in Lake Pepin, Minnesota, USA (this lake is known to receive inputs of D4 from urban sources upstream (for more details, see Section 4.3.3.2) and so the sediment was likely to have been pre-exposed to D4). The overlying water had a pH of 7.9 and an organic carbon content of 3.7 per cent. The test vessels used were designed to minimise the headspace and to minimise the volatile loss of D4 during the test. They consisted of 250 ml flasks containing 25 g of dry sediment (~2.5 cm layer). The flasks were completely filled with lake water and 40 ml of the water was removed giving a small headspace. The flasks were incubated in a nitrogen-filled glove box at 24°C for between one and four weeks prior to the addition of the test substance. Sterile controls were prepared by adding sodium azide to the flasks.

The tests were initiated by adding 40 to 50 µl of a solution of D4 in di(ethylene glycol) methyl ether) to multiple positions in the surface layer of the sediment. The initial D4 concentration was in the range 200 to 270 µg/kg dry sediment. The flasks were then sealed

³ A recent study by Sánchez-Brunete *et al.* (2010) found that D4 was detectable in only one out of 15 soil samples analysed. The soils sampled included agricultural soils, sludge-amended soils and industrial soils (D4 was detectable in one industrial soil). However, it is not possible to deduce a rate of degradation from these data.

and incubated at 24°C for up to 204 days. At various time points duplicate flasks were sacrificed for determination of the amount of ¹⁴C-D4 present in the samples (a total of nine sampling points for the active samples and eight sampling points for the sterile controls). During the incubation period, regular exchange of the headspace gases was carried out, whereby around 40 ml of the headspace was withdrawn (with nitrogen gas being drawn into the flask to replace the gas withdrawn). These samples were analysed for ¹⁴CO₂ and ¹⁴CH₄ and any ¹⁴C-containing volatile compounds 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.

The average (± standard deviation) recovery of ¹⁴C from all samples was 101.1±13.0 per cent. The majority (mean value of 97 per cent) of the D4 in the system was found to be adsorbed onto sediment. Degradation of D4 in the system was apparent; the disappearance of D4 was found to follow first order kinetics and the rate constant for the reaction was determined to be 0.0019 day⁻¹, giving a half-life of around 365 days. However a similar degradation was also evident in the sterile control (degradation rate constant 0.0018 day⁻¹, half-life around 385 days) indicating that the degradation was mainly abiotic in origin.

The degradation was thought to proceed by progressive hydrolysis of D4. The first step was thought to be ring-opening to form octamethyltetrasiloxane- α,ω -diol (tetramer diol) followed by hydrolytic depolymerisation to hexamethyltrisiloxane- α,ω -diol (trimer diol), tetramethyltrisiloxane- α,ω -diol (dimer diol) and finally dimethylsilanediol (monomer diol). Evidence for these intermediate products was obtained from HPLC analysis of the water samples.

Little evidence for mineralisation was evident in this study. The amount of ¹⁴CO₂ generated in the biotic samples amounted to <0.2 per cent of the total radiolabel present after a 6 month period. Similarly, the amount of ¹⁴CH₄ collected was minimal (<0.12 per cent of the total radiolabel).

Overall the degradation half-life obtained in this study was around 365 days at 24°C. The study is considered to be a good quality study as evidenced by the excellent mass balance obtained (although there may be some issues over the length of storage of the sediment (see below)). The half-life would be expected to be longer than 365 days at lower temperatures (for the PBT and vPvB assessment a temperature of around 12°C is normally considered).

Xu (2009b) used a similar test set-up to investigate the degradation of ¹⁴C-D4 under aerobic conditions, again using sediment from Lake Pepin. The main difference between this study and the anaerobic study was that the sediment was acclimated with aeration twice per day and during the test frequent exchange of the headspace air was made (with collection of volatile products (these were again reintroduced into the headspace) and ¹⁴CO₂) in order to maintain aerobic conditions. The initial D4 concentration was in the range 130-270 µg/kg dry weight. The spiked sediments were incubated at 24°C for up to 156 days.

The average recovery of ¹⁴C over the whole experiment was 90.3 per cent including the controls. For the D4 biotic samples the overall recovery of ¹⁴C averaged around 88.5 per cent (three pairs of samples with recovery rates <75 per cent were excluded from the data analysis).

The majority of the D4 (98 per cent or more) was found to be associated with the sediment fraction. The concentration of D4 present in the biotic samples was found to decrease with

increasing incubation time, with a concurrent increase in the amount of the major degradation product (dimethylsilanediol). The half-life for D4 degradation was estimated to be around 242 days at 24°C.

Degradation was also evident in the sterile controls. Two sets of sterile controls were used, one sterilised by autoclave and one sterilised by addition of sodium azide. The degradation in the autoclaved samples was found to be much higher than found in the biotic experiments and it was thought that this was an artefact resulting from changes to the sediments during the autoclave process (the fraction of D4 in the water phase was increased in these sediments over that found in the biotic experiments and the controls treated with sodium azide). Degradation of D4 was still apparent in the sodium azide-treated controls and the half-life was estimated to be around 425 days at 24°C in these samples. The slower rate found in these sterile controls than in the biotic experiments suggests that microbial activity may also play a role in the aerobic degradation of D4 along with hydrolysis.

Little or no $^{14}\text{CO}_2$ or $^{14}\text{CH}_4$ was found indicating that the complete mineralisation of D4 and its hydrolysis products was very slow. Analysis of the main degradation products formed suggested that degradation occurred via progressive hydrolysis of D4 leading ultimately to formation of dimethylsilanediol (as was also found under anaerobic conditions above).

Xu (2009b) indicates that the degradation rate for D4 seen in the Lake Pepin sediment was lower than that observed in a sediment from Michigan (Sanford Lake) tested using a similar test methodology (this refers to a study by Xu and Miller, 2008). The Sanford Lake sediment had a lower pH for the overlying water (pH 6.95 versus pH 7.9) and a lower organic carbon content (2.9 per cent versus 3.7 per cent) than for the Lake Pepin sediment experiments. The recovery of total ^{14}C in this system averaged 96.7 per cent and more than 95 per cent of the D4 was found to be associated with the sediment phase. The degradation half-life determined in the Sanford Lake sediment was 47 days at 24°C. Using the default temperature conversion for half-lives (as incorporated into EUSES 2.0.3), a half-life of 47 days at 24°C is equivalent to a half-life of around 123 days at 12°C.

It should be noted that the sediment used in the studies with Lake Pepin was collected on the 22nd May 2008 for both the Xu *et al.* (2009a) anaerobic study and Xu *et al.* (2009b) aerobic study but the degradation studies themselves were not initiated until 19th February 2009 (anaerobic study) or 6th August 2008 (aerobic study). Therefore the sediment was stored for around 9 months for the anaerobic study and just over 10 weeks for the aerobic study (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 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. The sediment used in the briefly reported study using sediment from Sanford Lake had a similar, but slightly lower organic carbon content of 2.9 per cent. The length of storage of the Sanford Lake sediment before the test was started is not currently known.

Although these deviations from the OECD Test Guideline are not ideal, the results of the study suggest strongly that degradation of D4 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 (for example similar results were obtained under aerobic conditions and anaerobic conditions despite the large differences in the storage time in the sediments used in the two tests). The effect of organic carbon content of the sediment on the degradation rate is currently unclear; the limited data available suggest that the rate of degradation may increase as the organic carbon content decreases.

4.1.3 Summary and discussion of persistence

The main degradation process for D4 in water is hydrolysis, with a half-life dependent on the pH and temperature of the water. The extrapolated hydrolysis half-lives are 16.7 days at pH 7 and 12°C, and 2.9 days at pH 8 and 9°C (as considered in the REACH TGD for freshwater and marine environments respectively).

The new data available on the degradation of D4 in sediment show that it has a relatively long half-life, of the order of 242 days at 24°C under aerobic conditions, and 365 days at 24°C under anaerobic conditions. The half-life at lower temperatures (e.g. 12°C) would be expected to be longer. The sediment half-life appears to depend on the sediment characteristics (e.g. pH and organic carbon content); for example, a half-life of 47 days at 24°C (equivalent to a half-life of 123 days at 12°C) was found in a second sediment.

The situation is less clear for soil. Although rapid degradation of D4 is evident in dry soils in equilibrium with air of relative humidity up to around 90 per cent, the rate of reaction reduces markedly with increasing moisture content. Therefore it is probable that under some situations rapid degradation of D4 may occur, but in other situations the degradation will be much slower.

When considering the persistence of D4 in the environment it is also important to note that D4 is volatile and will be lost from surface water and soil by volatilisation (see Section 4.2). The degradation half-life of D4 in the atmosphere is estimated to be around 14 days (although the half-life may be shorter in urban and suburban areas). Thus volatilisation followed by subsequent degradation in the atmosphere is an important process in the overall persistence of D4 in the environment.

4.2 Environmental distribution

4.2.1 Adsorption

4.2.1.1 Summary of information from existing evaluation

An organic carbon-water partition coefficient (K_{oc}) value of 1.7×10^4 l/kg ($\log K_{oc} = 4.22$) was recommended for D4 by EA (2009). This value was obtained from a high-quality experimental study using the OECD Test Guideline batch equilibrium method carried out by Miller (2007).

4.2.1.2 New information

The partitioning of D4 to activated sludge has been briefly reported in a poster presentation by van Egmond *et al.* (2010). The experiments were carried out by equilibrating the activated sludge with pure water for 24 hours and then determining the concentration of D4 in the water phase (via a headspace technique) and the total sediment phase. The samples used contained sufficient native D4 to carry out the investigation (i.e. no further D4 was added to the samples). The $\log K_{oc}$ value determined was 3.86 (mean of six determinations).

In addition to these data, further new information is available for the related substance D5 that could also be applicable to D4. The new studies for D5 are reviewed in the Evaluation Report for that substance and these found that it is strongly adsorbed to (or associated with) humic acids in water, which leads to a progressive increase in the predicted half-life in water (resulting from a combination of volatilisation and hydrolysis) with increasing humic acid/dissolved organic carbon content, with the half-life also depending on the depth of water. An association of D4 with dissolved organic carbon/humic acid could also be expected to lead to an increase in the overall half-life. However, it should be noted that the hydrolysis and volatilisation half-lives for D4 are markedly shorter than for D5 and so it is not possible to extrapolate the results for D5 quantitatively for D4.

4.2.2 Distribution modelling

4.2.2.1 Summary of information from existing evaluation

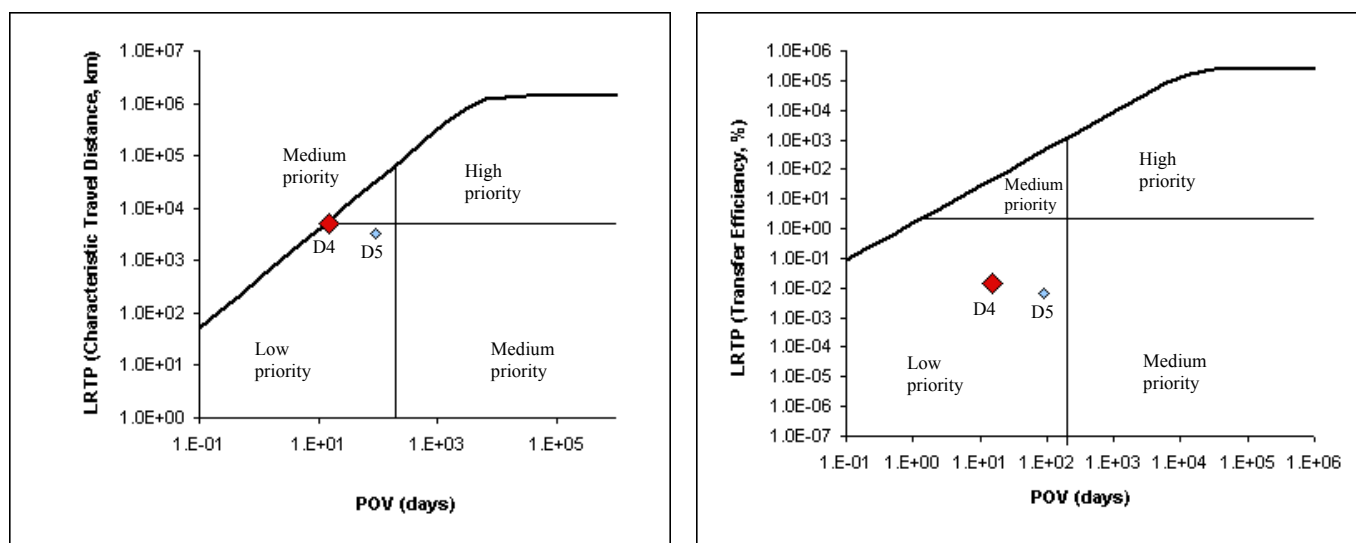
The high Henry's law constant for D4 (around 1.21×10^6 Pa m³/mol at 25°C (taken from EA, 2009)) means that it will volatilise rapidly from water and soil. EA (2009) estimated that the rate constant for volatilisation from soil would be around 2 day⁻¹ for agricultural soil and 4 day⁻¹ for grassland, corresponding to volatilisation half-lives of 0.35 and 0.17 days respectively.

EA (2009) estimated that the volatilisation half-life would be around 1.8 hours in a river (assumed to have a depth of 1 m, a current velocity of 1 m/s and a wind velocity of 5 m/s) and 164 hours in a shallow lake (assumed to have a depth of 1 m, a current velocity of

0.05 m/s and a wind velocity of 0.5 m/s). These estimates were carried out using the USEPA EPI estimation program.

A number of global modelling studies were also reported in EA (2009). In general, the studies suggest that although D4 has the potential to be transported long distances in the atmosphere, its properties mean that it has a low potential for redeposition in remote regions. The long-range transport potential using the OECD Screening Tool is summarised in Figure 1 (based on a study by Xu (2007b) reported in EA (2009)).

Figure 1 Summary of long-range transport potential using the OECD Screening Tool



4.2.2.2 New information

A series of modelling studies have been carried out looking at the behaviour of D4 in various aquatic systems using local and regional modelling approaches. The studies are summarised in Table 3. They were carried out using the best available measured data for the physico-chemical properties of D4 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 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 D4 into the water compartment of the model was generally based on a per capita release rate to waste water (taken from EA (2009); 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 exception⁴ no sensitivity analysis was carried out in the studies other than investigating the effect of temperature/season, and no predictions were made for known substances of concern. For the Whelan (2009d) study, a limited sensitivity analysis was

⁴ It is understood that further sensitivity analysis of the modelling studies is being carried out (CES, 2010b)

carried out in relation to the predictions for D5 only. 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.

The possibility of deposition of D4 from the atmosphere has been considered at an expert panel workshop held by the Global Silicones Counsel (Global Silicones Counsel, 2009). In general, it was thought that four main processes can contribute to atmospheric deposition:

- Vapour condensation.
- Gas absorption.
- Wet deposition.
- Dry particle deposition.

Vapour condensation was considered to be not relevant to D4 as this can occur only when the concentration in air exceeds the concentration corresponding to the saturated vapour pressure at any given temperature and the concentrations of D4 predicted in Arctic air are many orders of magnitude lower than the saturated vapour pressure.

Similarly, wet gaseous deposition at temperatures above freezing point was not considered to be a significant process for D4 owing to the high K_{AW} (air-water partition coefficient) for D4. Wet and dry deposition via organic and mineral aerosols was also not thought to be significant as, although D4 may be expected to partition to such aerosols, the aerosol/air partition coefficients for D4 are not sufficiently large to offset the low concentrations of such aerosols in the atmosphere (i.e. a significant flux of D4 to surface media would not be expected).

Global Silicones Counsel (2009) also considered the potential for deposition of D4 at or below freezing point adsorbed onto the surface of snow crystals. It was concluded that deposition of D4 is potentially possible if the snow-air partition coefficient is very high. However, the snow-air partition coefficient for D4 is relatively small (predicted to be around $0.01 \text{ m}^3/\text{m}^2$) and based on this value, and assuming an air concentration of $5 \text{ ng}/\text{m}^3$, the maximum concentration of D4 adsorbed by snow was estimated to be around $300 \text{ ng}/\text{m}^3$ or a maximum of about 1 per cent of the amount of D4 in the air compartment (assuming an atmosphere height of 6 km and a very high snow area index⁵ of $6,000 \text{ m}^2/\text{m}^2$; for more compacted snow (snow area index $1,000\text{-}3,000 \text{ m}^2/\text{m}^2$) the maximum concentration of D4 adsorbed was predicted to fall to $50\text{-}150 \text{ ng}/\text{m}^3$).

It is important to note that the D4 deposited in snow is only temporarily stored in the deposited snow. As the snow melts, the majority of D4 will volatilise from the water.

Overall, the expert panel workshop concluded that the ultimate deposition of D4 from the atmosphere to surface media is unlikely to be significant.

⁵ Snow area index is the vertically integrated surface area of snow crystals.

The results of a modelling assessment of the contribution from surface/air exchange to the deposition potential for D4 were presented and discussed at the EU Member States Siloxanes Workshop in June 2010 (Xu, 2010; Dow Corning, 2010). The study considered the partitioning of D4 from air to soil, plant biomass (rye grass and deciduous tree leaves) and aquatic suspended particulates using an equilibrium modelling approach. For the study, plant-air partition coefficients (K_{BA}) were estimated from the known octanol-air partition coefficient using the method developed by Kömp and McLachlan (1997) and the soil-air and suspended particulate-air partition coefficients (K_{SA} and K_{SPA} , respectively) were estimated from the known organic carbon-water partition coefficient (K_{OC}) and air-water partition coefficient (K_{AW}).

The log K_{BA} value for D4 was estimated to be 1.42 at 25°C (values ranged from 2.79 at -20°C to 1.30 at 30°C) which is around two log units or more lower than values estimated by Kömp and McLachlan (1997) for polychlorinated biphenyls. The log K_{SA} values estimated for D4 were between 0.14 and 0.45 (estimates for temperatures between 20 and 25°C and organic carbon contents of 1-2.6 per cent) and 1.70 and 0.26 (estimates for temperatures between -20°C and 30°C and an organic carbon content of 3 per cent) which are around 4.5 to 5.5 log units lower than estimated for more known persistent organic pollutants such as hexachlorobenzene and 2,4,4'-trichlorobiphenyl (PCB-28). The log K_{SPA} values estimated were between 2.26 (at -20°C) and 1.17 (at 30°C) assuming a 50 per cent organic carbon content. Based on these partition coefficients, Xu (2010) and Dow Corning (2010) estimated that surface/air exchange processes would make only a negligibly small contribution (<1 per cent of the total mass in air) to the deposition potential of D4 in remote regions even at low temperature (~0°C).

4.2.3 Other new information

A survey of the levels of D4 in eleven sediment samples from the Barents Sea (part of the Arctic Ocean located north of Norway and Russia) has been undertaken by Bakke *et al.* (2008). The samples were collected in 2006/2007 and included two samples from the Kola Transect (latitude 71,3683°N and 72,1833°N) one sample from the Shtokman structure (latitude 72,8667°N), three samples from the Pechora Sea (latitude 68,6633°N, 70,3817°N and 70,5983°N), three samples from Tromsøflaket (latitude 71,1580°N, 71,3138°N and 71,3193°N), one sample from Sternøysundet (latitude 70,2302) and one sample from Troms II (latitude 70,1357°N). D4 was detectable in one of the eleven samples (from the Kola Transect) at a concentration of 40 µg/kg dry weight. The source is unknown.

Table 3 Predicted persistence of D4 in water 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.	<p>Total D4 flux to lake 1.2-12 kg/year via waste water after waste water treatment (removal during waste water treatment assumed to be between 98 per cent and 99.8 per cent). The estimate was based on a population of 4,200,000 discharging into the river feeding the lake.</p> <p>Concentration of D4 in air was assumed to be constant at 10 ng/m³.</p> <p>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 1.6 days and a degradation half-life in sediment of 1.1 years (the sediment half-lives were estimated at a temperature of 8°C which was considered more appropriate for sediment than the mean annual water temperature).</p> <p>$\log K_{oc} = 4.22$ (at 25°C).</p> <p>$\log K_{ow} = 6.5$ (at 25°C) or 6.43 (at 14°C).</p> <p>$\log K_{aw} = 2.34$ (at 14°C).</p> <p>$\log K_{oa} = 4.09$ (at 14°C).</p>	<p>The predicted total concentration in water and sediment are 0.01-0.1 ng/l and 0.014-0.14 µg/kg dry weight respectively (for comparison the measured level of D4 in sediments from Lake Pepin is of the order of 0.4 µg/kg wet weight; see Powell <i>et al.</i> (2009a) in Section 4.3.3.2. Assuming the default water content of sediment from the REACH Guidance this concentration corresponds to around 1.8 µg/kg dry weight which is one to two orders of magnitude higher than the modelled data). The estimated fraction of the total steady state mass in the lake is estimated to be distributed 69 per cent in the water phase and 31 per cent in the sediment phase.</p> <p>The persistence² 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 104 days (approximate half-life 72 days). However it should be noted that recent sediment core data from Lake Pepin are suggestive of a longer half-life than this (up to around 2.5 years; see Section 4.2.3).</p> <p>The persistence in the water column was found to be 1.8 days (approximate half-life 1.2 days), and the overall persistence was estimated to be 2.51 days (approximate half-life 1.7 days). The main driving force for loss of D4 was estimated to be hydrolysis, presumably because of the short hydrolysis half-life expected at a pH of 8.</p> <p>As noted above, the predicted concentration in sediment appears to be much lower than actually measured in this lake. This suggests that the emission assumed in the model is too low and/or that the actual persistence of D4 is longer than estimated.</p>	Whelan (2009a)

System	Model used	Main assumptions ¹	Main findings	Reference
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.	<p>Total D4 flux via waste water 4.48 kg/year after waste water treatment (removal during waste water treatment was assumed to be 98 per cent for D4). This estimate was based on a population of 1,600,000 discharging into the catchment.</p> <p>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 3.9 days at pH 7 and 9.4 hours at pH 8. The equivalent values for sediment (at 25°C) were 3 years at pH 7 and 110 days at pH 8.</p> <p>$\log K_{oc} = 4.22$ (at 25°C). $\log K_{ow} = 6.5$ (at 25°C). Vapour pressure = 122 Pa at 25°C.</p> <p>Although the above properties refer to 25°C the actual modelling was carried out using the known seasonal temperature variation in 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).</p>	<p>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 concentrations in the water column were estimated to be below the levels that would be detectable analytically with current methods (<10 ng/l).</p> <p>The predicted concentrations of D4 in sediment were low, up to around 0.009 µg/kg dry weight with the Oslofjord POP model and a maximum of around 0.016 µg/kg dry weight with the CoZMo-POP model. These results are consistent with the monitoring study of Schlabach <i>et al.</i> (2007) (see EA, 2009) which found D4 was below the limit of detection (<4 to <38 µg/kg dry weight) in Inner Oslofjord but appear to be low compared with the recent study by Powell <i>et al.</i> (2009c and 2010b; reported in Section 4.3.3.2) which found mean levels of D4 of 0.8-0.9 µg/kg wet weight in Inner Oslofjord (these concentrations would increase to approximately 3.7-4.1 µg/kg when expressed on a dry weight basis using the default water content of sediment from the REACH Guidance).</p> <p>The persistence of D4 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 285 days, mainly as a result of sediment burial supplemented by loss via hydrolysis and volatilisation.</p> <p>Degradation (hydrolysis) was found to be the most important loss process from the water column, accounting for >60 per cent of the emissions, followed by volatilisation.</p>	Whelan (2009b)

System	Model used	Main assumptions ¹	Main findings	Reference
Lake Ontario	QWASI Model adapted to Lake Ontario	<p>Total D4 flux to lake 2 kg/year via waste water after waste water treatment (removal during waste water treatment assumed to be 99.8 per cent for D4). This estimate was based on a population of 7,135,800 discharging into the catchment.</p> <p>Concentration of D4 in air was assumed to be constant at 10 ng/m³.</p> <p>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 2.97 days and a degradation half-life in sediment of 1.13 years.</p> <p>$\log K_{oc} = 4.22$ (at 25°C).</p> <p>$\log K_{ow} = 6.5$ (at 25°C).</p> <p>Temperature correction was applied to partition coefficients assuming the following energies of phase transfer (ΔU) = 7.9 kJ/mole for octanol-water, -44 kJ/mole for octanol-air and 51.9 kJ/mole for air-water. These are the recommended values from the Whelan (2009d) study below³.</p>	<p>The predicted concentrations in water and sediment are 1.6×10^{-5} ng/l and 1.1×10^{-5} µg/kg dry weight respectively. The fraction of the total steady state mass in the lake is estimated to be distributed 98.6 per cent in the water phase and 1.4 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 4.6×10^{-5} ng/l and 2.7×10^{-5} µg/kg dry weight respectively, and the percentage steady state mass was distributed 98.8 per cent in the water phase and 1.2 per cent in the sediment phase. At 20°C the predicted concentrations were 4.1×10^{-6} ng/l in water and 3.1×10^{-6} µg/kg dry weight in the sediment, with 98.4 per cent of the steady state mass in the water phase and 1.6 per cent in the sediment.</p> <p>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 496 days (equivalent to a half-life of around 342 days) at all three temperatures.</p> <p>The persistence in the water column was found to range between 1 day at 20°C (summer) and 12 days at 2°C (winter) (the equivalent half-lives are approximately 0.7 days (summer) and 8.3 days (winter)). The overall persistence ranged between 1 days (summer) and 12 days (winter) (the equivalent half-lives are approximately 0.7 days (summer) and 8.3 days (winter)) reflecting the fact that most of the D4 in this system was predicted to be in the water column (dissolved).</p>	Whelan (2009c)

System	Model used	Main assumptions ¹	Main findings	Reference
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.	<p>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 D4 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 D4 to be compared directly with those for D5).</p> <p>Degradation in water takes place by hydrolysis in the dissolved phase only. This results in degradation half-lives in water (at 25°C) of 3.9 days for freshwater (at pH 7) and 9.4 hours 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 5.6 years for freshwater and 168 days for marine water.</p> <p>$\log K_{ow} = 6.5$ (at 25°C). $\log K_{aw} = 1.9$ (at 25°C). $\log K_{oa} = 4.6$ (at 25°C).</p> <p>Temperature correction was applied to partition coefficients assuming the following energies of phase transfer (ΔU) = 7.9 kJ/mole for octanol-water, -44 kJ/mole for octanol-air and 51.9 kJ/mole for air-water. These values were taken from a study by Xu (2007a)³ and are based on an estimate of the ΔU for octanol-air using a linear free energy relationship. The modelling was carried out using seasonal temperature profiles appropriate to the Baltic Proper.</p>	Only limited modelling was carried out for D4 (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 two years.	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 $\approx 0.69 \times$ persistence.

- 3) A more recent study by Xu (2009c) has determined the ΔU values for D4 to be -32.0 kJ/mole for octanol-water partition, 42.5 kJ/mole for octanol-air partition and -74.7 kJ/mole for air-water partition. These values were determined based on measurements of octanol/air/water three-phase equilibrium over the temperature range 6°C to 35°C. It should be noted that the values measured by Xu (2009c) are different from those used in the modelling. In particular, the sign (whether the energy change is positive or negative), as well as the actual values, are different in Xu (2009c) from those used in the modelling studies. CES (2010b) indicates that these differences in the sign result from different conventions for defining the terms in different studies and have no effect on the modelling results because these differences were taken into account in the model parameterisation. Further, both CES (2010b) and Xu (2009c) consider that the impact of the small differences in the actual values (ignoring the sign) on the predicted fate, transport and distribution should be small.

Another recent study has investigated the levels of D4 in sediments in remote regions (Campbell, 2010) (some of the results also appear in Warner *et al.* (2010)). The main focus of the study was on the levels of D4 in biota (these results are reported in Section 0) but a number of sediment samples were also collected. The samples were collected in 2009 from Adventfjorden (approximately 78°13'N 15°40'E) and Kongsfjorden (approximately 78°55'N 11°54'E) in Svalbard. Although these are considered to be remote regions it should be noted that there are potential local sources of emission of D4 in the area. 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 sampling sites as Longyearbyen (the capital of Svalbard with around 2,500 inhabitants) is located in the area.

The sediment samples were collected in a linear transect away from the waste water effluent pipe from the communities of Longyearbyen (surface sediment samples collected from Adventfjorden in front of the effluent pipe and 50, 100, 200 and 400 metres away from the pipe) and Ny Alesund (surface sediment samples collected from Kongsfjorden at distances of 90, 155, 220, 300 and 420 metres away from the pipe). The samples were subdivided into three subsamples and sent to three laboratories for analysis (giving a total number of 15 samples for each of Adventfjorden and Kongsfjorden). Precautions were taken during the sample collection, processing and analysis to avoid inadvertent contamination with D4.

The method detection limit was in the range 1.54 to 3.01 µg/kg dry weight for the samples from Adventfjorden and 1.54 to 6.27 µg/kg dry weight for the samples from Kongsfjorden. D4 was not detectable in any of the sediment samples analysed.

Powell (2009 and 2010a) reports the results from an evaluation of D4 in sediment cores from the depositional areas of Lake Pepin. The cores were taken from three locations (towards the upstream end, intermediate and towards the downstream end of the lake) in July 2006. The cores were dated based on correlation of the magnetic susceptibility of the core with that from reference cores that had previously been dated directly using ²¹⁰Pb measurements. The 80 cm-depth layer in the cores corresponded to deposition around 1972 in the upstream sample, 1975 in the intermediate sample and 1960 in the downstream sample. D4 was found to be detectable at all depths in the core down to 80 cm. The concentration of D4 was generally greater at a depth of around 30 cm, and the concentrations in the downstream core were generally lower than in the intermediate and upstream core (the concentrations in these two cores were generally similar). The peak concentrations of D4 corresponded to around 1997 and were in the range 2.4 to 3.5 µg/kg dry weight, which were only slightly above the limit of detection of the analytical method used. The rates of accumulation were found to be greater in the upstream and intermediate core samples than in the downstream core samples. The intermediate and downstream cores showed an increasing rate of accumulation of D4 from around 1985 to 1993 followed by a continually decreasing rate of accumulation. The rate of accumulation at the upstream site over time was more variable, showing two periods of increased accumulation (from around 1985 to 1991 and from 1997 to 2000). The pattern of accumulation appeared to track the known usage of D4, the known population growth of the Twin Cities metropolitan area, and the subsequent implementation of improved waste water treatment practices at the metropolitan waste water treatment plant in the area. The whole basin rate of accumulation of D4 between 2005 and 2006 was estimated to be around 1.09 kg/year for D4.

Powell (2010a) noted that part of the reason for declining concentrations of D4 in the more recent layers resulted from a replacement of D4 by D5 in personal care products. Furthermore, Powell (2010a) argued that as complete replacement of D4 by D5 had not occurred by the mid-1990s the rate of deposition of D4 to the sediment in 1994-1995 should have been comparable, if not greater than, the rate of deposition of D5 at the time. Based on a comparison of the rates of deposition of D4 and D5 estimated from the sediment core data, Powell (2010a) suggested that at least 95 per cent of the D4 originally deposited to the sediment had been removed/degraded by the time of collection of the sediment core (over an 11 year period), which is equivalent to a degradation half-life in the sediment column of up to 2.5 years. It should be noted, however, that this estimate does not appear to take into account the fact that D4 has a lower organic carbon-water partition coefficient than D5, and the effect that this may have on the expected deposition rate for D4 compared with D5 for a given unit emission to the water phase. Even so, the sediment core data do provide further evidence that D4 has a relatively long half-life in sediment, possibly longer than the 72 days estimated in the modelling exercise reported in Table 3.

A study by Genualdi *et al.* (2011) has investigated the global distribution of D4 in air samples collected at 20 sites worldwide, including five locations in the Arctic. The samples were collected between April and June 2009. Field blanks were also collected at each sampling location and on average the concentrations in the field blanks were around 4 per cent of those in the samples. All the D4 concentrations reported were individually blank corrected. At one location (Sable Island) the concentration of D4 in the blank was higher than the sample and so this point was excluded from the data set. D4 was detectable in the remaining nineteen samples at a concentration between 0.66 ng/m³ and 50 ng/m³. The highest levels were generally associated with source-dominated or urban areas (the highest concentration was measured in Paris, France). For the five more northerly (Arctic) locations, the D4 concentrations were 12 ng/m³ at Alert, Canada (82.45°N, 63.50°W), 16 ng/m³ at Ny Alesund, Norway (78.90°N, 11.89°W), 0.66 ng/m³ at Barrow, United States (71.32°N, 156.6°W), 0.94 ng/m³ at Storhofdi, Iceland (63.40°N, 20.28°W) and 18 ng/m³ at Little Fox Lake, Canada (61.35°N, 135.6°W). There was no significant difference between the concentrations of D4 found at urban and background sites but elevated concentrations were generally measured on sites on the west coast of North America and some high altitude sites. Genualdi *et al.* (2011) speculated that the D4 measured at these sites may have originated from sources in Asia.

Krogseth *et al.* (2012 & 2013) report measured atmospheric concentrations of D4 from samples collected at the Zeppelin observatory, Svalbard, Norway (79°N, 12°E) in late August through to early December 2011. A solid phase extraction active air sampling method was used, and concentrations were measured using GC/MS. The D4 concentrations ranged from not detected to 0.95 ng/m³ in summer, and not detected to 2.13 ng/m³ in winter. It was thought that these results were strongly influenced by *in situ* formation from captured D5 during storage.

4.2.4 Summary of environmental distribution

The properties of D4 mean that it is volatile and also adsorbs strongly onto soil and sediment. Therefore it is important that these properties are considered in relation to the environmental persistence of D4. A number of new modelling studies are available and the results of these studies are generally comparable. Although they generally predict a short persistence in water (owing to rapid hydrolysis and volatilisation), the models also predict that a significant fraction⁶ of D4 will distribute to the sediment phase and the persistence of D4 in the sediment may be much longer than found in the water column, depending on factors such as temperature, pH, sediment burial rate, etc. For the models recently investigated, the half-life of D4 in sediment was estimated to be around 72 days for Lake Pepin, 285 days for Inner Oslofjord and 342 days for Lake Ontario. In addition, sediment cores from Lake Pepin are suggestive of a half-life of D4 of up to 2.5 years, which is longer than predicted in the modelling exercise.

Transport to remote areas via air is likely to occur but the substance has a low potential for subsequent deposition to surface media in such regions.

4.3 Bioaccumulation

When considering the available information on bioaccumulation it is important to recognise that current bioaccumulation theories suggest that accumulation in an organism will depend on several factors, including the lipid content of the organism. Therefore in order to compare data from different studies it is usual to lipid normalise the data in order to try to factor out differences between studies resulting solely from differences in lipid contents between the species used⁷. Such normalisation is particularly important when considering field studies investigating biomagnification processes where comparisons are made between concentrations with species from different trophic levels. In the following sections lipid normalisation has been carried out where possible and appropriate. However it should be noted that such lipid normalisation assumes that D4 partitions primarily to the lipid compartment in an organism. Whilst it is thought that this is a good approximation for lipophilic chemicals in general, and so also highly likely to be the case for D4, this has not yet been unequivocally demonstrated for D4.

4.3.1 Screening data

D4 has a log K_{ow} of 6.49.

4.3.2 Measured bioaccumulation data

⁶ The actual fraction depends on a large number of assumptions, including the fraction released to water, sedimentation rate, etc.

⁷ Lipid normalization of accumulation factors such as biomagnification factors (BMFs) effectively results in the factor being expressed as a fugacity ratio (Woodburn, 2010).

4.3.2.1 Summary of information from existing evaluation

A number of bioaccumulation studies using D4 were reviewed in detail in EA (2009). A summary of the available studies is given in Table 4.

Table 4 Summary of available bioaccumulation data for D4 (taken from EA, 2009)

Species	Exposure concentration	Value	Value/comment	Reference
<i>Chironomus tentans</i> (midge)	2.6-54 mg/kg dry weight in sediment	Biota sediment accumulation factors (BSAF) 0.6-2.6	Use with care – no information is given as to whether steady state was reached – not clear if based on total ¹⁴ C or parent compound (most likely total ¹⁴ C)	Kent <i>et al.</i> (1994)
<i>Oncorhynchus mykiss</i> (rainbow trout)	457 mg/kg food	BMF = 0.18	Valid – steady state value on a wet weight fish/wet weight food basis – based on parent compound	Dow Corning (2007)
		BMF = 0.47	Valid – steady state value on a lipid normalised basis – based on parent compound	
		BMF = 1.8	Valid – kinetic, growth corrected value on a wet weight fish/wet weight food basis – based on parent compound	
		BMF = 4.6 ⁸	Valid – kinetic, growth corrected value on a lipid normalised basis – based on parent compound	
<i>Carassius auratus</i> (goldfish)	306-425 mg/kg food (mixture of oligomers)	Value not given but reported to be similar to that for <i>Poecilia reticulata</i>	Invalid – exposure concentration not well defined – based on parent compound	Opperhuizen <i>et al.</i> (1987)
	Saturated solution			
<i>Poecilia reticulata</i> (guppy)	1,008-1,044 mg/kg food (mixture of oligomers)	BMF = 0.06	Invalid – exposure concentration not well defined – based on parent compound	Opperhuizen <i>et al.</i> (1987)
	Saturated solution	BCF = 1,090 l/kg		
	Dietary study	No result obtained	Invalid – exposure concentration could not be maintained	Bruggeman <i>et al.</i> (1984)
<i>Pimephales promelas</i> (fathead minnow)	0.41-0.51 µg/l	BCF = 4,300 – 7,000 l/kg	Use with care – relatively short (6-day) exposure period; based on total ¹⁴ C	Fackler <i>et al.</i> (1995)
	0.23 µg/l	BCF = 12,400 l/kg	Valid – steady state value based on total ¹⁴ C – the estimated value based on parent compound is ≥11,495 l/kg	Fackler <i>et al.</i> (1995)
	20-80 µg/l	BCF = 2,500 – 10,000 l/kg	Use with care – exposure concentration varied during the test and was close to (and in some cases above) the water solubility of D4 – based on total ¹⁴ C	Annelin and Frye (1989)

⁸ RIVM (2012) presents further analysis of the kinetic data, and suggests that the lipid normalized BMF is 1.8.

Overall the available experimental data show that D4 bioconcentrates in fish and is taken up from food. The most reliable value for the steady state BCF is 12,400 l/kg in *P. promelas* based on total ¹⁴C measurements⁹. Although this value may contain a contribution from metabolites as well as parent D4, parent compound analysis indicated that a large proportion of the body burden (~93 per cent) was parent compound and so this value is considered to be appropriate for consideration in the PBT and vPvB assessment.

4.3.2.2 New information

Fish bioconcentration studies

Two new bioconcentration studies with common carp (*Cyprinus carpio*) (CERI, 2007 and 2010) have been reviewed for the purposes of this evaluation but have not been summarised because they are not yet publicly available. They appear to be well carried out, and show that the steady state BCF is in the range 3,000 – 4,000 l/kg (based on parent compound analysis). The kinetic BCF in one of the studies was in the range 4,100 - 5,500 l/kg (without growth correction; it is higher if growth is taken into account). It can therefore be concluded that the BCF in this species appears to be lower than fathead minnow (see Table 4), but is still well above 2,000 l/kg. It is understood that two further bioconcentration tests with D4 in carp have been performed in Japan (CES, personal communication), but they are not yet finalised.

Fish dietary studies

A GLP dietary accumulation test using D4 has been carried out in carp (*Cyprinus carpio*) using the draft version of the OECD TG 305 dietary exposure test (draft version 10 of August 31st 2010). The full study report (CERI, 2011) is currently available only in Japanese but the raw data allow for all of the reported bioaccumulation parameters to be verified. In the test carp were exposed to a diet containing¹⁰ D4 (mean concentration 219 µg/g), D5 (mean concentration 221 µg/g) and/or a reference substance (hexachlorobenzene at a mean concentration of 97.2 µg/g), for thirteen days (at a feeding rate of 3 per cent of body weight per day) followed by a 28-day depuration period. The food used had a lipid content of 16.1 per cent and the concentration of D4 in the food was found to be stable over the duration of the uptake phase. The fish were 6.6-7.2 cm in length at the start of the test. The test was carried out at a temperature of 24.6-25°C and a pH of 8.0-8.1. At various times during the uptake phase (day 4, 7 and 13) and depuration phase (days 1, 4, 7, 14 and 28) groups of four fish were sampled and individually analysed for the presence of D4 (the gut contents appear to have been removed prior to analysis). The weights of the fish were also determined at these timepoints to allow the growth rate constant to be determined. The mean lipid content of the test fish was found to be 5.77 per cent. The lipid contents were found to increase as the

⁹ RIVM (2012) noted that the concentration in fish was still increasing at the end of the uptake phase in this study, and was 31% higher at day 28 than at day 7. In addition, the average water concentration of 0.23 µg/l cited in the study report could not be reproduced from the available data. The data were therefore reanalysed with a kinetic model, using all data from the preliminary and definitive experiment and accounting for the variable water concentrations in the uptake phase. This results in an uptake rate constant of 1166 l/kg.d and a depuration rate constant of 0.0613/d, resulting in a BCF of 19,000 l/kg. Normalised to a fish containing 5% lipids, the best BCF that could be deduced by RIVM (2012) from this study was 14,900 l/kg (it is not indicated whether this value was corrected to take account of the contribution of metabolites).

¹⁰ It is not entirely clear from the Japanese report whether the exposure was to all three substances simultaneously or whether three separate experiments were carried out.

test progressed (from 4.16 per cent prior to the test to 7.98 per cent at the end of the depuration phase).

The mean concentration of D4 determined in the fish at the end of the uptake phase was 27.4 µg/g (standard deviation 8.4 µg/g). The key bioaccumulation parameters derived from the study are summarised in Table 5 (these parameters have all been verified from the raw data presented in CERI (2011)).

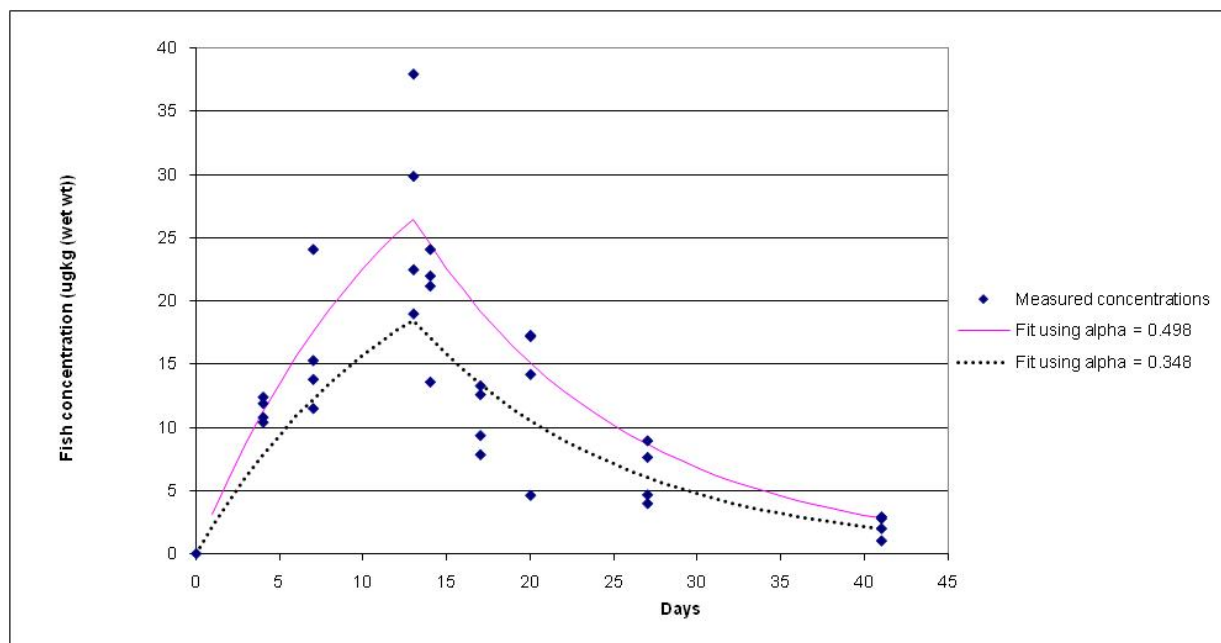
Table 5 Summary of bioaccumulation parameters from the CERI (2011) dietary accumulation test

Parameter	Value	Comment
Overall depuration rate constant (k_2)	0.0797 day ⁻¹ [depuration half-life 15.4 days]	Obtained from the slope of a plot of ln [Conc _{fish}] against time.
Growth rate constant (k_g)	0.0224 day ⁻¹	Obtained from the slope of a plot of ln [fish weight] against time for the test fish during the depuration phase [see note a].
Growth-corrected depuration rate constant ($k_{\text{growth-corrected}}$)	0.0573 day ⁻¹ [depuration half-life 30.8 days]	$k_{\text{growth-corrected}} = k_2 - k_g$ (rate constant subtraction method)
Growth-corrected depuration rate constant ($k_{\text{growth-corrected}}$), alternative method	0.0582 day ⁻¹ [depuration half-life 29.6 days]	Obtained from the slope of a plot of ln [amount fish] against time for the test fish during the depuration phase [see note b].
Assimilation efficiency	0.348 [0.498-0.515]	Determined from the intercept of the ln [Conc _{fish}] against time plot [see note c].
BMF	0.131	Estimated from the assimilation efficiency, feeding rate and overall depuration rate constant.
Growth-corrected BMF	0.182	Using the growth-corrected depuration rate constant of 0.0573 day ⁻¹ .
Growth-corrected and lipid-normalised BMF	0.509 [0.728-0.753]	Lipid normalised using the ratio of the lipid content in food and the mean lipid content in the test fish [see note c].

Note: a) The growth rate constant obtained during the depuration phase for the test population was used as there was a statistically significant difference between the growth rate constants during the uptake phase and depuration phase for both the control group and test group, and between the growth rate constant during the depuration phase for the test population and the control group (significance tested using the t-test with $\alpha=0.05$). This means that the control group should not be combined with the test group and the data for the uptake phase should not be combined with the data for the depuration phase. Thus the most appropriate growth rate constant is from the test population during the depuration phase. These differences, although statistically significant, were relatively small in magnitude, and did not necessarily indicate a toxic effect in the treatment group since although the growth rate constant for the treatment group during uptake phase was lower than for the control group (0.0272 day⁻¹ for the treatment group compared with 0.0338 day⁻¹ for the control group), the opposite was true for the depuration phase (0.0224 day⁻¹ for the treatment group compared with 0.0209 for the control group).

- b) As differences were evident in the growth rate constant obtained during the uptake and depuration phases of the experiment, an alternative method (based on the amount of substance present in fish during the depuration phase (Brooke and Crookes, 2012)) was used to estimate the growth-corrected depuration rate constant. This value is similar to that obtained using the rate constant subtraction method and provides further reassurance in the growth-corrected depuration rate constant.
- c) Using this method the concentration in fish estimated at the start of the uptake phase was 18.5 $\mu\text{g/g}$. This is markedly lower than the concentration in fish measured on day 13 of uptake (mean concentration 27.4 $\mu\text{g/g}$). If the mean measured concentration on day 13 is used, the assimilation efficiency can be estimated to be higher at 0.515. The equivalent lipid and growth corrected BMF is then 0.753 which is still well below 1. The assimilation efficiency can also be obtained by fitting (least squares) the relevant equation from the draft OECD 305 test guideline to the concentrations measured at each timepoint during the uptake phase. When this is done the assimilation efficiency is estimated to be 0.498 (similar to the value obtained from the day 13 concentration) and the equivalent lipid and growth corrected BMF is then 0.728. The fit to the measured data using this approach is shown in **Figure 2**.

Figure 2 Plot showing fit to the experimental data for the CERI (2011) dietary study



The growth-corrected and lipid-normalised BMF from this study is 0.509 [reported as 0.510 in CERI (2011); the small difference probably results from rounding] using the calculation method in the draft OECD 305 test guideline (a higher value of 0.728-0.753 is obtained if the measured concentrations in fish during the uptake phase are used). The equivalent lipid-normalised and growth-corrected BMF value for the hexachlorobenzene reference substance was 1.16. The CERI (2011) report also estimated the BMF by fitting the data directly using the Berkeley Madonna Software (version 8.3.18) and this gave a value of 0.530 for D4 and 1.24 for hexachlorobenzene [these values have not been re-verified for the purposes of this evaluation]. Overall the study appears to be well conducted and reliable, and demonstrates that the BMF value for D4 in carp is below 1.

The REACH Guidance (and also the revised OECD 305 test guideline) indicates that it is possible to estimate a BCF from the results of a feeding study if a rate constant for the uptake

from water (k_1) can be estimated. This k_1 value can be used in combination with the depuration rate constant measured in the feeding study to estimate a kinetic BCF. The method suggested in the REACH Guidance for estimating the k_1 value is based on Sijm *et al.* (1995) which estimates the k_1 value from the fish weight but other methods have also been evaluated (Environment Agency, 2011). The resulting BCFs estimated using these methods are shown below in Table 6. Estimates are based on the initial fish weight at the start of the test and the fish weight at the end of the uptake phase (day 13); a lipid content of 5.77 per cent was assumed where necessary. The method reference refers to the methods reviewed in Environment Agency (2011); only the recommended methods from that report have been used (see Environment Agency (2011) for further details).

The predicted growth-corrected BCF values using these methods are in the range 1,667 to 9,667 l/kg, which is in broad agreement with the experimental BCF values. However, it should be recognized that these are estimates and it is not known if the assumptions inherent in the calculations are appropriate for D4 (the calculations assume that the k_1 value can be reliably predicted for D4 and that the growth-corrected depuration rate constant obtained by dietary exposure is the same as the growth-corrected depuration rate constant following aqueous exposure).

Table 6 Estimates for BCF from the results of the CERl (2011) feeding study

Reference	Estimated non-growth corrected BCF (l/kg)		Estimated growth-corrected BCF (l/kg)	
	Day 0	Day 13	Day 0	Day 13
Sijm <i>et al.</i> (1995)	4,076	3,590	5,669	4,993
Hendriks <i>et al.</i> (2001)	3,512	3,181	4,885	4,424
Thomann (1989)	6,950	6,294	9,667	8,754
Barber (2001)	6,862	6,437	9,545	8,954
Barber <i>et al.</i> (1991)	6,535	6,079	9,090	8,456
Erickson and McKim (1990a)	6,699	6,294	9,317	8,754
Erickson and McKim (1990b)	5,112	4,669	7,110	6,495
Hayton and Barron (1990)	4,738	4,383	6,590	6,097
Streit and Sire (1993)	4,057	3,810	5,643	5,300
Barber (2003) (observed)	4,179	3,865	5,813	5,376
Barber (2003) (calibrated)	4,620	3,926	6,427	5,461
Spacie and Hamelink (1982)	1,198	1,198	1,667	1,667
Tolls and Sijm (1995)	1,952	1,952	2,715	2,715
Maximum	6,950	6,437	9,667	8,954
Minimum	1,198	1,198	1,667	1,667
Mean	4,653	4,283	6,472	5,957

Invertebrate studies

The uptake and accumulation of D4 from sediment by the oligochaete *Lumbriculus variegatus* have been determined (Krueger *et al.*, 2008). The test was carried out using a 28-day exposure period followed by a 23-day depuration period. The substance used in the test had a purity of 99.75 per cent.

The sediment used in the study was based on the recommendations of OECD Test Guideline 218 and was composed of 10 per cent peat moss, 70 per cent sand and 20 per cent silt and clay. The organic carbon content of the sediment was determined to be 3.2 per cent during the uptake phase and 3.4 per cent during the depuration phase. The dilution water used was well water (dissolved oxygen concentration ≥ 69 per cent saturation, pH 8.1 to 8.3 and temperature $23 \pm 1^\circ\text{C}$).

The test chambers consisted of 9 litre aquaria containing 1 litre of sediment and 5 litres of water. The system used was a flow-through system where the water flow-rate provided two volume additions per day. Two nominal test concentrations were used, 5.0 and 20 mg/kg dry weight, along with a control group. In order to spike the sediment, neat test substance was firstly mixed with the peat component of the sediment for around 18 hours. After this time, the sand and clay components of the sediment were added to the peat, and the sediment was mixed for a further 40 to 60 minutes before being added to the test chambers. Sufficient food for 28 days was also added to the sediment prior to the addition of the water. The sediment/water system was conditioned for 48 hours prior to introduction of the test organisms. Similar test chambers, but without the addition of D4, were prepared for the depuration phase.

The test was initiated by adding approximately one gram (wet weight) of oligochaetes to each test chamber. One replicate chamber (worms) or three replicates (sediment) in each treatment group and one replicate (worms) or two replicates (sediment) in the control were sacrificed for analysis on day 0, day 14 and day 28 of the uptake phase. At the end of the uptake phase, the organisms from three replicates of each treatment group and control group were sieved from the sediment, counted and transferred to three replicate chambers containing clean sediment and water for the depuration phase. One replicate (worms) of each treatment group and control was sacrificed for analysis on day 14 of the depuration phase, and a further replicate was sacrificed on day 23 of the depuration phase for determination of the lipid content of the organisms. The mean lipid content of the organisms was found to be 2.26 per cent.

The concentration of D4 in the sediment was found to decrease slightly during the course of the study. For the nominal 5 mg/kg dry weight treatment group the mean measured concentration was found to be 0.908 mg/kg dry weight on day 0 of the study, 0.818 mg/kg dry weight on day 14 of the study, and 0.845 mg/kg dry weight on day 28 of the study. The overall mean concentration over the entire 28-day period was 0.86 mg/kg dry weight, which corresponds to around 17 per cent of the nominal concentration. Similarly for the nominal 20 mg/kg dry weight treatment group, the measured concentration was 6.17 mg/kg dry weight at day 0, 3.97 mg/kg dry weight at day 14 and 2.04 mg/kg dry weight on day 28. The mean concentration over the 28-day period was 4.06 mg/kg, which corresponds to 20 per cent of the nominal. There are a number of factors that should be considered here.

- Only one replicate of worm samples was analysed at each time point and so the variability in the measurement of the concentration at each time point is unknown (qualitative read across from the equivalent D5 study has not been performed because the analytical variability will be substance specific).
- No special measures were taken to avoid loss from volatilisation during the spiking of the sediment. This explains why the measured concentrations are only 17-20 per cent of the nominal values.
- No specific measures were taken to avoid loss from volatilisation during the uptake phase. In addition, the test was carried out using a flow-through system. Under the conditions used, any D4 present in (i.e. partitioning to) the water phase would be continually lost from the system. This probably explains the apparent declining concentrations in the sediment during the study.

The concentrations found in the oligochaetes during the study are summarised in Table 7.

Table 7 Uptake and depuration of D4 by *Lumbriculus variegatus*

Time point (days)	Nominal sediment level (mg/kg dry weight)	Measured sediment level (mg/kg dry weight) ¹	Measured concentration in <i>Lumbriculus variegatus</i> (mg/kg) ¹	Bioaccumulation factor ²
0	5	0.908		
14	5	0.818	16.4	20.0
28	5	0.845	16.8	19.9
42 (depuration day 14)	0	0	0.959	
0	20	6.17		
14	20	3.97	66.1	16.6
28	20	2.04	27.2	13.3
42 (depuration day 14)	0	0	1.64	

Note: 1) Concentrations based on measurements in one replicate.

- 2) Bioaccumulation factor is estimated here as the ratio of the concentration in whole organisms (mg/kg) at the given time point divided by the concentration in sediment (mg/kg dry weight) measured at the same time point.

Based on these data, Krueger *et al.* (2008) estimated the BAF to be 19.6 for the 5 mg/kg dry weight (nominal) treatment group and 6.7 for the 20 mg/kg dry weight (nominal) treatment group. These values appear to be derived based on the mean measured exposure concentration over the 28 day uptake period and the measured concentration in the organisms measured on day 28. However, the validity of this approach, particularly at the higher concentration group can be questioned for the following reasons.

- The concentration of D4 in the sediment appeared to decrease during the test (particularly in the higher exposure group).

- The concentration of D4 in the organisms in the 20 mg/kg dry weight (nominal) treatment group was much higher on day 14 of the uptake than found on day 28 of the uptake. The reason for this is not clear (there is no discussion of this in the test report) and, as only one replicate was analysed at each time point, it is not clear whether the 14-day or 28-day value is an outlier.

To try to investigate these uncertainties further, the Environment Agency has performed a re-analysis using the data obtained at each time point separately. The results are summarised in Table 7. When this is done it can be seen that a) the bioaccumulation factors obtained at the two concentration levels are broadly similar, and b) the bioaccumulation factor obtained at the two time points in the 20 mg/kg dry weight (nominal) group are now reasonably consistent.

Krueger *et al.* (2008) also determined the kinetics of the uptake and depuration. The uptake (k_1) and depuration (k_2) rate constants were determined to be 4.02 day^{-1} and 0.21 day^{-1} for the 5 mg/kg dry weight (nominal) treatment group (giving a kinetic bioaccumulation factor of 19.7) and 1.35 day^{-1} and 0.20 day^{-1} for the 20 mg/kg dry weight (nominal) treatment group (giving a kinetic bioaccumulation factor of 6.7 mg/kg). Similar to the steady state bioaccumulation factors determined by Krueger *et al.* (2008) these values are determined assuming the mean measured concentration over the entire 28-day exposure period, and the uptake rate constant is determined from the measured concentration in the organisms on day 28 of the uptake phase (and so will be subject to the same uncertainties as outlined above). The depuration rate constants obtained correspond to depuration half-lives of 3.4 to 3.5 days.

CES (2010a) have recently re-analysed the kinetic data from this study. In this re-analysis the rate constants have been estimated from all of the available data for exposure days 14 and 28 along with clearance day 14. In this analysis the kinetic bioaccumulation factor was 19.7 for the low dose group and 11.5 for the high dose group.

A further possible source of uncertainty in this study is the fact that the organisms reproduced during the study. Therefore the offspring would have been exposed for a shorter period than the parents (and reproduction itself could provide an additional parental depuration mechanism). However, as it is not possible to analyse parent and offspring separately such complications are unavoidable in such a study. As steady state appears to have been reached quickly (within 14 days) and the study was carried out over 28 days, this uncertainty is probably of little overall consequence in interpreting the data.

Overall the study is considered to be a “use with care” study, owing to the limited amount of analysis that was carried out, and the apparent declining exposure concentrations. Nevertheless the results are considered relevant and usable for use in the PBT and vPvB assessment, as the substance has been shown to be persistent in sediment (see Section 4.1).

In order to consider these data in relation to the PBT and vPvB criteria it is necessary to consider how the bioaccumulation factors determined relate to the bioconcentration factors used in the criteria. One way to do this is to assume that the main route of exposure of the organisms during the test was via the sediment pore water. If this is the case then the concentration in the pore water can be related to the concentration in the sediment using the following equation.

$$\text{Conc}_{\text{water}} = \frac{\text{Conc}_{\text{sed,orgC}}}{K_{\text{OC}}}$$

Where $\text{Conc}_{\text{water}}$ = concentration in (pore) water (mg/l).

$\text{Conc}_{\text{sed, orgC}}$ = concentration in sediment on a mg/kg organic carbon basis.
The sediment organic carbon content was 3.2 per cent.

K_{oc} = organic carbon-water partition coefficient. This is 1.7×10^4 l/kg for D4 (EA, 2009).

This equation assumes that the pore water concentration is in equilibrium with the sediment and it is possible that this was not the case in the experiment (for example the D4 was initially added to the solid phase of the sediment and the time taken for the D4 to equilibrate with the water phase is not known). This therefore introduces some uncertainty in the derived pore water concentration.

Using this approach to obtain the concentration in pore water at each time point in Table 7, the equivalent BCF value can be estimated (the concentration in the organism at the time point (mg/kg) divided by the estimated concentration in pore water at the same time point (mg/l)) to be in the approximate range 7,000 to 11,000 l/kg. It is recognised that there are a number of assumptions, and hence uncertainties, inherent in these estimates. In particular, the estimated BCFs depend crucially on the assumption that exposure in these studies occurs mainly via pore water and the assumption that the pore water concentration is in equilibrium with the sediment concentration.

4.3.3 Other supporting information

4.3.3.1 Metabolism studies

Summary of information from existing evaluation

EA (2009) summarised the available toxicokinetic studies in mammals. These show that D4 is rapidly eliminated from mammalian systems (by exhalation and metabolism) and so it has a low potential for accumulation in mammals. However, it was noted that the pharmacokinetic behaviour after oral exposure is complex and does not appear to be as well understood as the inhalation and dermal routes of exposure (although rapid metabolism following oral exposure was thought to occur). There is no information on the behaviour in birds.

New information

The elimination and metabolism of D4 in rainbow trout (*O. mykiss*) following exposure via a single oral gavage dose of D4 has been investigated over a 96-hour period (Springer, 2008). The substance tested was ^{14}C -labelled D4 with a radiochemical purity of 99.95 per cent. In the study four mature male rainbow trout (weights in the range 0.967 to 1.377 kg) were administered a single oral dose of D4 dissolved in corn oil at a nominal concentration of 15 mg/kg body weight (the actual concentrations administered were in the range 9.5 to 14.2 mg/kg body weight). The fish were then maintained in well water (dissolved oxygen concentration >75 per cent saturation throughout) at a temperature of 13°C for 96 hours. During this time samples of blood and urine were periodically collected via an aortic canula

and urinary catheter. In addition, any faecal material excreted into the tanks was also collected. At the end of the 96-hour period the fish were sacrificed and various tissues (bile, digestive tract, milt, fat, liver and remaining carcass) collected for analysis.

The various samples were analysed for the presence of ^{14}C . In addition some of the samples were also analysed for the presence of parent D4 and metabolites. A further set of four fish were also dosed in a similar fashion and the urine collected to facilitate analysis of metabolites in the urine.

The average recovery of the administered dose in the study was around 79 per cent. The amount of the dose that was found to be absorbed by the fish was around 82 per cent of the recovered dose, with around 69 per cent of the recovered radioactivity present in the carcass, 12 per cent of the recovered radioactivity present in the excised tissues and bile and 1 per cent of the recovered dose present as metabolites in the urine. The remaining 18 per cent of the recovered dose was found to be eliminated via the faeces as parent D4.

For the various tissues analysed, metabolites of D4 were evident in the bile (94.5 per cent of the radioactivity present was metabolites), liver (40.1 per cent of the radioactivity was present as metabolites), milt (20.3 per cent of the radioactivity was present as metabolites) and digestive tract (1.9 per cent of the radioactivity was present as metabolites). However, in the fat samples the radioactivity present was solely parent compound (the highest concentrations of radiolabel were associated with the fat; lipid contents do not appear to be reported). The carcass samples were analysed only in terms of total radioactivity and so the percentage of metabolites in these samples is not known.

The blood samples were found to contain mainly parent D4. The calculated elimination half-life of the radiolabel in the blood was estimated to be around 39 hours based on the concentrations measured in blood at each time point.

Based on the known amount of metabolites found, it was estimated that approximately 2 per cent of the administered dose had been metabolised in the study. However, it should be noted that this figure does not take into account any possible metabolites that may have been present in the carcass.

A more detailed analysis of the blood samples from this study has been carried out by Domoradzki (2009). The approach used was to apply a first-order fish compartment model to determine the metabolism rate constant from the measured amounts of D4 and metabolites (i.e. the total radioactivity not identified as D4). Using this approach a metabolism rate constant of $0.00431 \text{ hour}^{-1}$ or 0.10 day^{-1} was calculated for D4, which is equivalent to a metabolic half-life of 6.7 days. The overall rate constant for elimination of D4 from the blood (taking into account elimination of D4 from the blood from processes such as gill ventilation, urine and faeces and transfer to fat storage) along with metabolism resulted in an estimated half-life in the blood of around 1.2 days.

Overall the study is of sufficient quality for use in the PBT and vPvB assessment (validity “use with care” owing to the overall mass balance of 79 per cent and the uncertainty over the presence of metabolites in the carcass). The results show that metabolism of D4 was limited in this test system, amounting to approximately 2 per cent of the administered dose over 96 hours. The elimination of D4 from the blood was found to occur with a half-life of around 1.2 days, however, as the time trends in the concentration in D4 in other tissues (for example fat) was not determined in this study, it is not clear how this half-life for blood relates to the whole body elimination half-life (for example the half-life would include processes such as

loss from blood due to fat storage). The metabolism half-life for D4 was estimated to be around 6.7 days based on the blood data.

4.3.3.2 Field bioaccumulation data

Summary of information from existing evaluation

No field bioaccumulation studies were reported in EA (2009).

New information

Field studies investigating the bioaccumulation of D4 have now been carried out. It should be noted that there is a lack of agreed guidelines¹¹ for carrying out and interpreting such studies, for example relating to the number of species and number of samples (of different life cycle stages) for each species that should be considered, how the feeding relationships and trophic levels within the food chain are best assigned, and how the statistical significance of the findings should be assessed. This therefore introduces some uncertainties when interpreting the results and assessing the significance of the findings in relation to the overall PBT or vPvB assessment. It should also be noted that although the REACH Guidance document indicates that the results from such field studies should be considered as part of the overall evaluation of the data, Chapter R.11.1.3.2 of the REACH Guidance¹² 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

Five food chains have been investigated in some detail.

1. The bioaccumulation of D4 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 km², 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/m²/year in the upstream end of the lake to 3-5 kg/m²/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

¹¹ In order to try to address some of these issues, an Expert Workshop on “Lab to Field Bioaccumulation” sponsored by the Health and Environmental Sciences Institute (HESI), the Society of Environmental Toxicology and Chemistry (SETAC) and the United States Environmental Protection Agency (USEPA) was held on 18-19 November 2009 to identify and discuss impacts of ecosystem and ecological variables on trophic magnification factors. The findings of this workshop have been recently published (e.g. Borgå *et al.* (2011) and Conder *et al.* (2011)).

¹² Page 25-26 of the Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT Assessment.

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 the 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 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 $\delta^{15}\text{N}$ measurements¹³ and ranged from TL ~2.0 (benthic detritivores 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 8. 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)¹⁴.

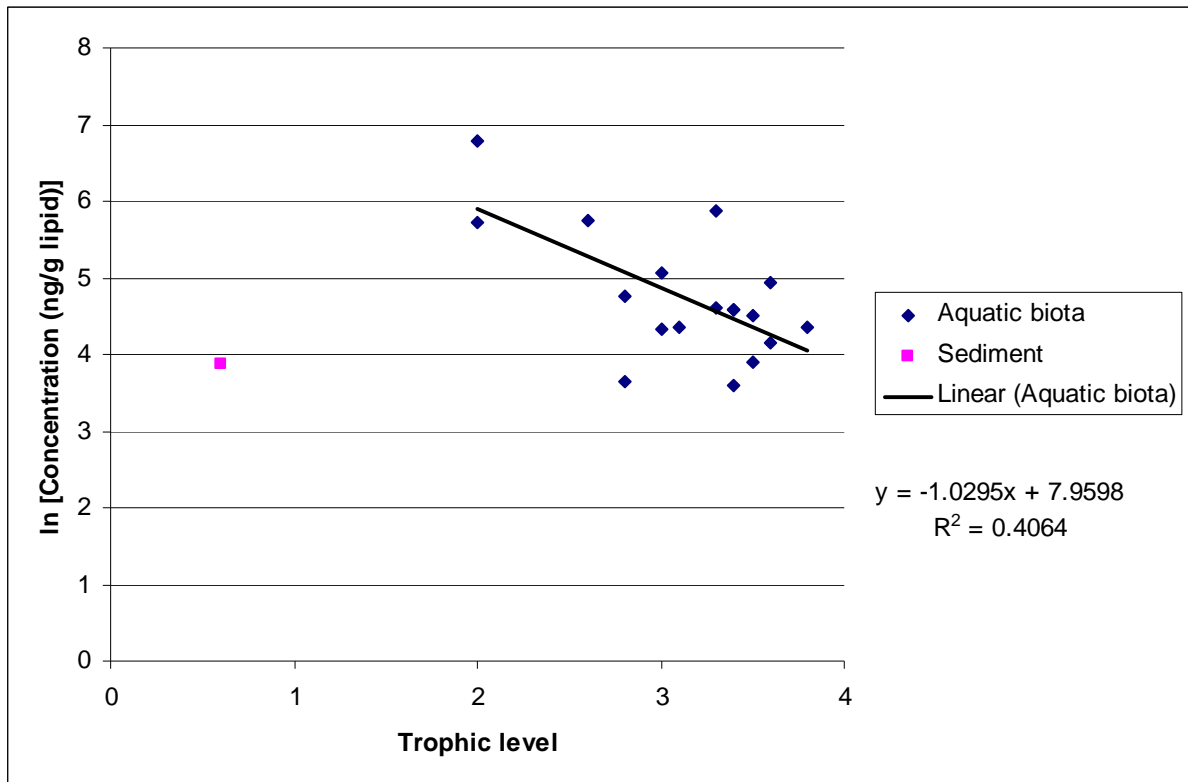
- The concentrations of D4 in the sediment were all less than the MDL.
- The concentrations of D4 in benthic macroinvertebrates were all above the MDL but were below the LOQ (midge and mayfly).
- The concentrations of D4 in fish were all above the MDL but below the LOQ in 8 out of 16 species.
- Concentrations that were less than the MDL but above the LOD were reported as the actual concentration measured. This however introduces some uncertainty over the actual concentration present, particularly for sediment.

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 3.

¹³ $\delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$ where R_{sample} is the $^{15}\text{N}/^{14}\text{N}$ abundance (in parts per thousand) in the sample and R_{standard} is the $^{15}\text{N}/^{14}\text{N}$ abundance of a standard (atmospheric nitrogen gas). The trophic level of a consumer is defined as follows, assuming the trophic level of midge larvae is 2: $\text{TL} = 2 + \frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{midge}})}{3.4}$.

¹⁴ 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).

Figure 3 Plot of \ln [mean concentration] (on a lipid weight basis¹⁵) against trophic level for the Lake Pepin food chain



Note: In the actual paper the plots are given with the error bars shown. For several of the species the error bars do not overlap with the regression line

¹⁵ The sediment concentration is on a ng/g organic carbon basis.

Table 8 Accumulation of D4 in the Lake Pepin food chain

Sample ³	Number of samples analysed	Trophic level	Mean measured D4 concentration (±standard deviation)	
			µg/kg wet weight	µg/kg lipid
Surface sediment - samples taken from whole lake	25	0.7	[0.4±0.0] ²	[48±5] ^{1,2}
Surface sediments - samples taken from where benthic macroinvertebrates were collected	5	0.6	[0.4±0.1] ²	[46±2] ^{1,2}
Midge (<i>Chironomus sp.</i>)	5 composites	2.0	7.3±2.6	888±304
Burrowing mayfly (<i>Hexagenia sp.</i>)	2 composites	2.0	7.8±0.2	309±36
White sucker (<i>Catostomus commersoni</i>)	1	2.6	6.4	314
Common carp (<i>Cyprinus carpio</i>)	3	2.8	4.9±0.6	38±3
Gizzard shad (<i>Dorosoma cepedianum</i>)	4	2.8	10.0±2.0	118±4
Gizzard shad (young of year) (<i>Dorosoma cepedianum</i>)	3 composites	3.0	2.8±1.1	76±31
Silver redhorse (<i>Moxostoma anisurum</i>)	3	3.0	10.9±1.7	160±56
Bluegill sunfish (<i>Lepomis macrochirus</i>)	3	3.1	3.6±0.6	78±18
River carpsucker (<i>Carpionodes carpio</i>)	1	3.3	59.1	351
Shorthead redhorse (<i>Moxostoma macrolepidotum</i>)	3	3.3	6.1±4.1	101±79
Freshwater drum (<i>Aplodinotus grunniens</i>)	3	3.4	1.7±1.1	36±24
Emerald shiner (<i>Nitropis atherinoides</i>)	4 composites	3.4	3.0±1.9	98±62
Black crappie (<i>Pomoxis nigromaculatus</i>)	3	3.4	6.7±1.5	97±24
White bass (<i>Morone chrysops</i>)	3	3.5	3.4±1.4	49±16
Smallmouth bass (<i>Micropterus dolomieu</i>)	3	3.5	5.3±3.5	91±57
Quillback carpsucker (<i>Carpionodes cyrinus</i>)	2	3.6	17.5±6.8	140±35
Walleye (<i>Stizostedion vitreum</i>)	3	3.6	4.4±0.6	64±14
Largemouth bass (<i>Micropterus salmoides</i>)	3	3.8	3.1±0.3	78±30

Note: 1) Sediment concentrations are expressed on a total organic carbon basis rather than a lipid basis.
2) Concentrations in square brackets are concentrations that were below the method detection limit but above the limit of detection, and are reported as the actual concentrations found.

The antilog of the slope¹⁶ of the regression line gives the Trophic Magnification Factor (TMF). The TMF for D4 in this food web can therefore be estimated as around 0.36 based on the mean measured lipid normalised concentrations. The TMF value quoted in Powell *et al.* (2009a) is slightly smaller than this value (TMF 0.31) and this value appears to have been 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 Table 8 as it minimises errors associated with unbalanced sampling (for example different numbers of organisms were collected for each species)¹⁷. Powell *et al.* (2009a) estimate a further TMF of 0.24 using trophic guilds (here the data were assigned to one of six trophic guilds¹⁸ and the mean value per trophic guild used in the regression). Based on these analyses, the TMF for D4 is less than 1 in this food web, and lies in the approximate range 0.24-0.36.

The paper also estimated the biomagnification factor (BMF) for various organisms, taking into account the composition of the diet of each organism¹⁹, 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, and an alternative method was used to correct for the trophic level (CES, 2010a). The equation used is shown below. This method effectively converts the BMF (that is defined for a specific predator-prey interaction) into a TMF (which is usually obtained from the antilog of the slope of a plot of ln [concentration] against trophic level).

$$\ln[\text{BMF}_{\text{TL}}] = \frac{\ln[\text{BMF}]}{\text{TL}_{\text{Pred}} - \text{TL}_{\text{Prey}}}$$

Where BMF_{TL} = corrected BMF. This is equivalent to the TMF.

BMF = the observed BMF for a given predator-prey interaction.

TL_{Pred} = the trophic level of the predator.

TL_{Prey} = the trophic level of the prey.

The resulting BMF, BMF_{TL} (using the method proposed in CES, 2010a) and BSAF values are summarised in Table 9.

¹⁶ The slope of the plot is statistically significant ($p < 0.05$) and the regression line had an R^2 of 0.4064. The slope of the plot was -1.0295 with a standard error of 0.3111. The lower and upper 95th percentile values of the slope were -1.689 and -0.370 respectively (equivalent to a TMF range of 0.18 to 0.69).

¹⁷ 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 3 in order to illustrate the findings. Given the different numbers of samples for each species it would have been preferable to reconstruct Figure 3 here using the individual data points for this evaluation report but this was not possible.

¹⁸ The six trophic guilds considered were detritivores, planktivores, omnivores, invertivores, carnivores and piscivores.

¹⁹ 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 predator. The concentrations in diet were calculated as 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.

As can be seen from Table 9, the BMF is one or above for the two benthic macroinvertebrates species at the bottom of the food chain and three fish species (gizzard shad (young of year and adults) and river carp sucker. However, for all other fish species the BMF is less than one and there is a general progressive reduction in the BMF with increasing trophic level. It should be noted that gizzard shad were considered to be pelagic planktivores, with adults occasionally ingesting some sand and associated detritivores (and hence are not particularly associated with the benthic food chain). Consequently the BMF for gizzard shad was estimated based on a diet consisting of 95 to 100 per cent plankton. As plankton was not sampled as part of this study the concentration in D4 that was used for the BMF calculation was estimated as the total organic carbon-normalised concentration in sediment, and hence there is a very high uncertainty associated with these BMF values. Similarly for the river carp sucker the diet was assumed to consist of 10 per cent plankton and 10 per cent sediment detritus along with invertebrates (70 per cent) and fish eggs (10 per cent); the concentration in sediment detritus was also estimated based on the total organic carbon-normalised concentration in sediment. The diet of the midge and burrowing mayfly was similarly assumed to consist of 75-80 per cent sediment detritus and 20-25 per cent plankton.

The BMF_{TL} follows the same general trend as the BMF (though some of the BMF_{TL} for the 'higher' trophic level species are greater than those for some of the 'lower' species, reflecting the variability in the data). These values are generally consistent with the TMF analysis that implies that trophic dilution of D4 appears to be occurring in this food chain.

A number of BSAFs obtained are above 1. The highest BSAF is obtained for the midge larvae although BSAFs are also above 1 for mayfly nymphs and fourteen out of the sixteen fish species. It should be noted that the BSAF values may be sensitive to the uncertainties over the actual concentration of D4 present in the sediment and this needs to be taken into account when considering the data (the sediment and invertebrates appear to have been collected together, unlike the fish).

Table 9 BMF, BMF_{TL} and BSAF values derived for D4 for the Lake Pepin food chain

Sample	Trophic level	BSAF	BMF ²	BMF _{TL}
Midge	2.0	19.2	19.2 ¹	9.2
Burrowing mayfly	2.0	6.7	6.7 ¹	4.1
White sucker	2.6	6.8	0.9	0.9
Common carp	2.8	0.8	0.1	0.1
Gizzard shad	2.8	2.6	2.0	1.5
Gizzard shad (young of year)	3.0	1.7	1.7	1.3
Silver redhorse	3.0	3.5	0.4	0.5
Bluegill sunfish	3.1	1.7	0.2	0.2
River carpsucker	3.3	7.6	1.0	1.0
Shorthead redhorse	3.3	2.2	0.3	0.4
Freshwater drum	3.4	0.8	0.1	0.1
Emerald shiner	3.4	2.1	0.9	0.9
Black crappie	3.4	2.1	0.3	0.3
White bass	3.5	1.1	0.2	0.2
Smallmouth bass	3.5	2.0	0.3	0.3
Quillback carpsucker	3.6	3.0	0.4	0.6
Walleye	3.6	1.4	0.2	0.2
Largemouth bass	3.8	1.7	0.3	0.4

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.

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 D4 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 D4 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.

Although the data show that D4 does not biomagnify 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 or not compared with dietary exposure. The reason for this is that the contribution from the water phase cannot be assessed due to the lack of data on the levels of D4 in water. In addition there is some uncertainty over the actual concentration of D4 present in the sediment phase. Although the concentrations appear to be higher in the organisms associated with the sediment, and so accumulation through sediment and diet appears to be a likely explanation, it cannot currently be ruled out that the concentration found in these organisms results from (or was contributed to by) exposure via sediment pore water or overlying water (i.e. bioconcentration processes). This is considered further in Section 4.3.3.3. It should 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 D4 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 would not affect the conclusions that can be drawn regarding the trends in concentration with trophic level in the fish samples. Indeed, when the TMF is calculated omitting the macroinvertebrates (plot not shown) the TMF is still below 1 (around 0.6 when estimated using the mean measured concentration for each species), but the correlation coefficient for the plot of \ln [concentration] against trophic level²⁰ is very low ($r^2 = 0.07$) and the slope of the plot is not statistically significant ($p > 0.05$). A 'leave one out' analysis was not performed, so the influence of any individual data point (i.e. individual species' trophic position or measured concentration) on the analysis is unknown. The placing of different species at particular trophic levels might not always reflect known ecological relationships, especially if diets differ slightly in different locations (e.g. there is some difference for the Oslofjord species depending whether they were sampled from the inner or outer estuary – see study 3 below).

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 D4 (Woodburn and Durham, 2009; Woodburn *et al.*, 2011). The samples (three males and one female) were collected from

²⁰ The slope of the plot was -0.503 with a standard error of 0.486. The lower and upper 95th percentile values of the slope were -1.545 and 0.539 respectively (equivalent to a TMF range of 0.21 to 1.7)

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 D4 found in the mink were all below the limit of detection (<1- <1.5 µg/kg lipid) in fat, liver and muscle. Comparing these concentrations with the concentrations measured in fish in Lake Pepin (Table 8) it can be seen that the lipid normalised concentrations in mink are much lower than found in the fish, providing further evidence that biomagnification is not 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 further follow-up to the Lake Pepin study has been carried out by Powell and Seston (2011). This investigated the bioaccumulation behaviour of polychlorinated biphenyls (PCBs) in the same food chain. These substances are known to biomagnify in the environment and so it was thought that the results for these reference chemicals could be used to benchmark the information available for D4 in the same food chain. Study samples of surface sediment, zooplankton, macroinvertebrates and fish (15 species) were collected and analysed for PCBs (the study included PCB-5+8, -18, -28, -44, -52, -66, -77, -101, -105, -118, -126, -128, -138, -153, -170, -180, -187, -195, -206 and -209). The sediment and benthic macroinvertebrates were collected from four locations along a shore-to-shore transect of the lake on the 20th May 2010. The zooplankton were collected on the 4th June 2010 by horizontal tow during an obvious *Daphnia* sp. bloom and the fish were collected on 19th July 2010 by electrofishing in near-shore areas on the Minnesota and Wisconsin borders of the lake. For most fish species, only one to three animals were collected (summarised in Table 10).

The trophic level of biota was estimated based on measurements of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$). In the previous study for Lake Pepin (described above) trophic levels were assigned using a trophic enrichment factor ($\Delta^{15}\text{N}$) of 3.4‰. However, when this value was used in the current study it resulted in walleye occupying a very high trophic level of 5.7, which was considered unlikely. Therefore, in addition to this value, trophic levels were also estimated using an enrichment factor of 4.642‰ (estimated assuming the trophic level separation between walleye and their diet was 1.0), 5.344‰ (estimated from the slope of a plot of $\delta^{15}\text{N}$ against relative trophic position assuming trophic levels of 2.0 for zooplankton, 3.0 for young-of-year gizzard shad, 4.1 for sauger and 4.3 for walleye) and 6.067‰ (estimated assuming the TMF for a reference material PCB was 4.65, as the mean value from the published literature).

Biota-sediment accumulation factors (BSAFs) for the PCB congeners were found to generally increase with increasing trophic level and were generally smallest in the benthic detritivores. The BSAF was also found to generally increase with the degree of chlorination in the PCB, being lowest for the least chlorinated PCBs (e.g. BSAFs were between around 0.7 to 1.1 for PCB-5+8 and PCB-18) and reaching around 11.3-15.8 for PCB-128, -138, -153, -180 and -187, before declining to around 1.1 to 3.8 for the most highly chlorinated congeners (e.g. PCB-195, -206 and -209).

The trophic level-corrected biomagnification factors were generally greatest in the species occupying the highest trophic level and followed a similar pattern to the BSAFs.

Table 10 Samples collected in the Lake Pepin PCB study

Sample	Number of samples	Trophic level			
		$\Delta^{15}\text{N}=3.4$	$\Delta^{15}\text{N}=4.642$	$\Delta^{15}\text{N}=5.344$	$\Delta^{15}\text{N}=6.067$
Surface sediment	4				
Zooplankton	4 composites	2.0	2.0	2.0	2.0
Burrowing mayfly (<i>Hexagenia sp.</i>)	4 composites	3.3	3.0	2.8	2.7
Midge (<i>Chironomous sp.</i>)	3 composites	3.4	3.0	2.9	2.8
Gizzard shad (young of year) (<i>Dorosoma cepedianum</i>)	1 composite	3.8	3.3	3.2	3.0
Bluegill sunfish (<i>Lepomis macrochirus</i>)	3	4.1	3.5	3.3	3.2
Emerald shiner (<i>Nitropis atherinoides</i>)	1	4.3	3.7	3.4	3.3
Common carp (<i>Cyprinus carpio</i>)	3	4.3	3.7	3.5	3.3
Gizzard shad (<i>Dorosoma cepedianum</i>)	1	4.6	3.9	3.6	3.5
Shorthead redhorse (<i>Moxostoma macrolepidotum</i>)	3	4.7	4.0	3.7	3.5
Quillback carpsucker (<i>Carpionodes cyrinus</i>)	2	4.7	4.1	3.8	3.6
Freshwater drum (<i>Aplodinotus grunniens</i>)	3	4.8	4.1	3.8	3.6
River carpsucker (<i>Carpionodes carpio</i>)	3	4.9	4.1	3.8	3.6
Black crappie (<i>Pomoxis nigromaculatus</i>)	4	5.0	4.2	3.9	3.7
White bass (<i>Morone chrysops</i>)	3	5.1	4.3	4.0	3.7
Silver redhorse (<i>Moxostoma anisurum</i>)	3	5.1	4.3	4.0	3.8
Smallmouth bass (<i>Micropterus dolomieu</i>)	3	5.2	4.3	4.0	3.8
Largemouth bass (<i>Micropterus salmoides</i>)	3	5.3	4.4	4.1	3.7
Sauger (<i>Sander Canadensis</i>)	3	5.4	4.5	4.2	3.9
Walleye (<i>Sander vitreus</i>)	2	5.7	4.7	4.4	4.1

TMF values were generally above one (range 1.5 to 5.1), but a few congeners did show TMF values below one including PCB-5+8, -18, -77, -126, -195 and -209. These were estimated using a $\Delta^{15}\text{N}$ of 6.067‰ as this was thought to be most appropriate to this food chain (it was estimated by calibrating the food chain to the known value for the reference chemical). However, it was noted that the value of $\Delta^{15}\text{N}$ chosen has a large impact on the estimated trophic level position and subsequent TMF calculation. Although this is the case, the $\Delta^{15}\text{N}$ effectively defines the “length” of the food chain in terms of the trophic

levels covered and it does not affect whether the TMF derived is above one (concentrations increasing with trophic level) or less than one (concentrations decreasing with trophic level) and so similar results were obtained when the other $\Delta^{15}\text{N}$ were considered. The study showed that, for the majority of PCBs considered, the TMF was greater than one in the Lake Pepin food chain, which contrasts with the situation for D4.

2. A second field study investigating the bioaccumulation of D4 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 km², a maximum depth of 49.4 m and a mean depth of 14.6 m. The lake is free from potential sources of D4 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 small yellow perch (*Perca flavescens*), small cisco (*Coregonus artedi*) and lake trout (*Salvelinus namaycush*) were collected on 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 thought 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.

The concentrations of D4 measured in the samples are summarised in Table 11. 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 0.47 to 0.90 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). 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.

For sediment and zooplankton the levels of D4 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.

The trophic level of each species was determined using $\delta^{15}\text{N}$ values. In this case the trophic level was determined relative to the $\delta^{15}\text{N}$ value for cisco, which was assumed to be in trophic level 3. The trophic level data are summarised in Table 11.

Table 11 Accumulation of D4 in the Lake Opeongo food chain

Sample	Number of samples analysed	Trophic level	Mean measured D4 concentration (\pm standard error)	
			$\mu\text{g}/\text{kg}$ wet weight	$\mu\text{g}/\text{kg}$ lipid
Surface sediment	9 (2 sediment cores and 7 surface sediments)		$[0.37\pm 0.05]^3$	$[34.4\pm 5.8]^{1,3}$
Zooplankton	3 pooled samples	2.0 ²	$[0.43\pm 0.04]^3$	$[10.9\pm 1.1]^3$
Cisco	7 composite samples and individuals	3.0	1.24 ± 0.07	25.6 ± 1.4
Yellow perch	7 composite samples and individuals	3.1	0.87 ± 0.06	21.0 ± 1.4
Lake trout	5 individuals	3.7	3.77 ± 0.57	48.7 ± 7.4

Note: 1) Sediment concentrations are expressed on a total organic carbon basis rather than a lipid basis.
 2) No $\delta^{15}\text{N}$ 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.

Based on the lipid normalised data, Powell *et al.* (2010a) estimated predator-prey BMF values²¹ for lake trout-perch and lake trout-cisco by bootstrap analysis using Monte-Carlo simulation. The mean BMFs estimated were 2.4 (95 per cent confidence interval 1.6 to 3.3) for the lake trout-perch relationship and 1.9 (95 per cent confidence interval 1.3 to 2.7) for the lake trout-cisco relationship. The bootstrap analysis indicated that there was a high probability that the BMF values were above 1.

The source of D4 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 D4 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 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.

²¹ 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.

- The levels found in the lowest 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) had indicated that further fish would be sampled (using an appropriate quality control programme) 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 no longer 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, CES (2010b) reported that other lakes were being evaluated as a substitute for Lake Opeongo. The criteria for selection of a suitable lake include that it must receive some waste water effluent and the food web must be comparable to that in Lake Opeongo (i.e. a pelagic food chain consisting of zooplankton, cisco and lake trout).

3. A further field study investigating the bioaccumulation potential of D4 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 trophic level of each species was determined based on $\delta^{15}\text{N}$ 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 D4 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 12.

Table 12 Concentrations of D4 measured in Oslofjord

Species	Inner Oslofjord				Outer Oslofjord			
	Number of samples	Trophic level	Concentration (\pm standard error)		Number of samples	Trophic level	Concentration (\pm standard error)	
			$\mu\text{g}/\text{kg}$ wet weight	$\mu\text{g}/\text{kg}$ lipid ¹			$\mu\text{g}/\text{kg}$ wet weight	$\mu\text{g}/\text{kg}$ lipid ¹
Sediment (0-1 cm depth)	7		0.8 \pm 0.2	86 \pm 20	5		0.3 \pm 0.2	45 \pm 27
Sediment (1-2 cm depth)	8		0.9 \pm 0.2	98 \pm 19	6		0.0 \pm 0.2 ²	1 \pm 25
Blue mussel (<i>Mytilus edulis</i>)	5	1.5	0.3 \pm 0.2	18 \pm 3				
Sea Urchin (<i>Brissopsis lyrifera</i>)					3	2.1	0.5 \pm 0.1	154 \pm 17
Worms	1	1.7	8.6	2,687	1	2.1	0.1	16
Jellyfish	1	2.0	0.1	2	1	2.2	0.0	2
Plankton	1	2.0	2.8	379	1	2.2	0.2	15
Mussels (species A)	2	2.6	0.2 \pm 0.0	21 \pm 4	3	3.1	0.2 \pm 0.0	14 \pm 2
Mussels (species B)	2	2.8	0.3 \pm 0.0	36 \pm 8	3	3.0	-0.3 \pm 0.0 ³	- ³
Atlantic herring (<i>Clupea harengus</i>)	6	3.0	10.2 \pm 2.2	115 \pm 22				
Northern shrimp (<i>Pandalus borealis</i>)	6	3.0	2.7 \pm 0.0	100 \pm 14	6	3.0	0.3 \pm 0.0	10 \pm 2
European plaice (<i>Pleuronectes platessa</i>)	6	3.1	22.1 \pm 5.8	414 \pm 77	5	3.4	3.2 \pm 0.6	141 \pm 3
Coalfish (<i>Pollachius virens</i>)	6	3.3	11.8 \pm 1.6	504 \pm 48	6	3.6	0.8 \pm 0.3	20 \pm 4
Common sole (<i>Solea vulgaris</i>)					3	3.4	2.9 \pm 0.3	61 \pm 18
Norway pout (<i>Trisopterus esmarkii</i>)	6	3.3	26.8 \pm 2.0	303 \pm 15	10	3.5	1.4 \pm 0.2	22 \pm 2

D4 PBT/vPvB EVALUATION

Species	Inner Oslofjord				Outer Oslofjord			
	Number of samples	Trophic level	Concentration (\pm standard error)		Number of samples	Trophic level	Concentration (\pm standard error)	
			$\mu\text{g}/\text{kg}$ wet weight	$\mu\text{g}/\text{kg}$ lipid ¹			$\mu\text{g}/\text{kg}$ wet weight	$\mu\text{g}/\text{kg}$ lipid ¹
European hake (<i>Merluccius merluccius</i>)	4	3.4	6.9 \pm 2.4	271 \pm 53				
Starry skate (<i>Amblyraja radiata</i>)					3	3.5	0.5 \pm 0.7	69 (standard error not given)
Haddock (<i>Melanogrammus aeglefinus</i>)	4	3.8	4.2 \pm 0.7	98 \pm 5	12	3.7	0.4 \pm 0.1	12 \pm 2
European whiting (<i>Merlangius merlangus</i>)	6	3.8	2.3 \pm 0.4	192 \pm 7				
Long rough dab (<i>Hippoglossoides platessoides</i>)	6	3.8	12.2 \pm 2.8	715 \pm 140	6	3.6	0.5 \pm 0.3	40 \pm 7
Vahl's eelpout (<i>Lycodes vahlii</i>)	6	3.8	1.7 \pm 0.2	174 \pm 22				
North Atlantic Pollock (<i>Pollachius pollachius</i>)	6	3.8	11.9 \pm 4.3	278 \pm 81				
Poor cod (<i>Trisopterus minutus</i>)	6	3.8	1.7 \pm 0.4	71 \pm 7				
Atlantic cod (<i>Gadus morhua</i>)	6	4.0	2.6 \pm 0.8	100 \pm 19	6	4.1	0.2 \pm 0.2	17 \pm 3

Note: 1) The concentrations in sediment are $\mu\text{g}/\text{kg}$ organic carbon.

2) This is how the datum was reported (i.e. the mean value is 0.0 to one decimal place).

3) Value is given as a negative number in the study report. No lipid normalised concentration is given.

It was found that the concentrations of total cyclic volatile methyl siloxanes (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).

¹³C-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 chains²². The dominant food chain (which included Atlantic cod²³) 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 D4 are summarised in Table 13 and were below 1 for both the inner and outer Oslofjord.

Table 13 Trophic magnification factors (TMF) and biomagnification factors (BMFs) for D4 in Oslofjord

Food web grouping	Location	Derived accumulation factor ³
Dominant food chain ² trophic magnification factor	Inner Oslofjord	Mean TMF = 0.6 ¹ (95% confidence interval 0.3 to 1.0; probability TMF >1 1.6%; mean fit of regression model (r ²) 9.2%)
	Outer Oslofjord	Mean TMF = 0.5 (95% confidence interval 0.2 to 1.1; probability TMF >1 3.5%; mean fit of regression model (r ²) 16%)
Atlantic cod-shrimp biomagnification factor	Inner Oslofjord	Mean BMF = 1.0 (95% confidence interval 0.4 to 2.1; probability BMF>1 37%)
	Outer Oslofjord	Mean BMF = 1.4 (95% confidence interval 0.4 to 4.2; probability BMF>1 55%)
Atlantic cod-herring biomagnification factor	Inner Oslofjord	Mean BMF = 1.0 (95% confidence interval 0.4 to 2.0; probability BMF>1 39%)
	Outer Oslofjord	No estimate possible

Note: 1) The TMF was 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 ¹³C flows.

3) Variability associated with the TMF and BMF was evaluated by bootstrap analysis using Monte Carlo simulation.

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. CES (2010b) reports some preliminary results from this further work. A pelagic-dominated food chain has been identified for the Inner Oslofjord based

²² Based on a significant difference in the signature compared with that for Atlantic cod, northern shrimp and Atlantic herring.

²³ 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.

on ^{13}C -signatures (a similar food chain could not be identified for the Outer Oslofjord owing to an insufficient number of species). Atlantic cod were again found to occupy the highest trophic level in this pelagic food chain. The mean TMF for D4 in this pelagic food chain was 0.7 with a probability of 21 per cent that the TMF was greater than 1 (estimated using Monte-Carlo simulation with bootstrap analysis). This is comparable with the TMF of 0.6 (with a probability of 7 per cent that the TMF was greater than one²⁴) estimated in Table 13 for the benthic dominated food chain. No further details of this analysis are currently available.

In addition to the TMFs, Powell *et al.* (2010b) also determined biomagnification factors (BMFs) for various predator-prey interactions. The BMF values determined for D4 were 1.0-1.4 (probability of a BMF >1 37-55 per cent) for Atlantic cod-shrimp and 1.0 (probability of a BMF >1 39 per cent) for Atlantic cod-herring. The data are also summarised in Table 13.

It should be noted that the BMFs were not corrected for differences in trophic level 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 D4 was not occurring in this food chain. However, the appropriateness of this conclusion is questionable as the BMF for the Atlantic cod-northern shrimp interaction was 1.0 for the inner Oslofjord and 1.4 for the outer Oslofjord. As noted above it was reported that at the time of sampling the Atlantic cod were feeding mainly on shrimp. The number of samples was also small, so the robustness of the conclusions is unclear.

In addition, RIVM (2012) noted that the choices made by the study authors with respect to the division of the species between pelagic and benthic food chains do not strictly follow the stable carbon isotope ratio ranking. The ratios were therefore re-analysed and species ranked on the basis of average stable carbon isotope ratios, species having a $\delta^{13}\text{C}$ lower than that of long rough dab (a flat fish associated with the benthic food web) being assigned to the pelagic food chain.

For the inner Oslofjord, this resulted in nine (groups of) species (worms, two mussel species other than blue mussels, Northern shrimp, European plaice, poor cod, Vahl's eelpout, long rough dab, and Arctic cod) in the benthic based food chain, and ten (groups of) species (blue mussel, zooplankton (netplankton and jellyfish), Atlantic herring, coalfish, Norway pout, European hake, North Atlantic pollock, European whiting and haddock) in the pelagic food chain. The TMFs that can be calculated for the benthic based food chain, the pelagic based food chain, and the whole ecosystem are 0.60, 3.27, and 1.66, respectively. None of the slopes is significantly different from zero, but the slope for the pelagic based food web is close to significant ($p=0.07$; 95% CI: -0.1371 to 2.504), leaving the 90% confidence interval of the TMF to vary from 0.87 to 12.2.

For the outer Oslofjord, the benthic food chain contains ten (groups of) species (sea urchin, worms, two mussel species other than blue mussels, Northern shrimp, European plaice, common sole, starry skate, long rough dab, and Arctic cod), while the pelagic food

²⁴ The original study report (Powell *et al.*, 2010b) indicated that the mean TMF was 0.6 with a 1.6 per cent probability that the TMF was greater than 1. CES (2010b) reports the same mean TMF but with a 7 per cent probability that the TMF was greater than 1.

web contains five (groups of) species: zooplankton (netplankton and jellyfish), coalfish, Norway pout, and haddock. The TMFs that can be calculated for the benthic based food chain, the pelagic based food chain, and the whole ecosystem are 0.83, 2.21, and 1.37, respectively, which are similar to those for the inner Oslofjord. However, none of the slopes is statistically significant.

4. Borgå (2012) reports the results of a further study investigating the TMF for D4. This study was carried out on a pelagic food chain in Lake Mjøsa in Norway (60°53'N, 10°41'E). 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) assumed that the influence of sampling location on contaminant exposure would be negligible.

Precautions were taken during the sampling and subsequent chemical analysis 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 $\delta^{15}\text{N}$ measurements and $\delta^{13}\text{C}$ measurements were used to identify whether the carbon source in the food web was predominantly from the same source for all organisms studied. A number of chlorinated and brominated compounds²⁵ were also analysed in the samples as benchmark substances.

The concentration of D4 was found to be above the limit of quantification in 22 per cent of the samples, but not in any of the invertebrates. The amount of D4 in field blanks was significant in some cases (the ratio of the sample to field blank was <5 in 22 out of 32 samples). The results are summarised in Table 14.

²⁵ 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 14 Accumulation of D4 in the Lake Mjøsa food chain

Sample	Number of samples analysed	Trophic level	Concentration range ($\mu\text{g}/\text{kg}$ wet wt.) ^a	Mean lipid normalised D4 concentration ($\mu\text{g}/\text{kg}$ lipid) (\pm standard deviation)
Zooplankton (predominantly <i>Daphnia galeata</i>) - epilimnion	4 pooled samples ^c	2.0	<1.4 to <3.7	<830
Zooplankton (predominantly <i>Limnocalanus macrurus</i>) - hypolimnion	4 pooled samples ^c	2.7	<1.6 to <2.5	<190
<i>Mysis relicta</i> - hypolimnion	4 pooled samples ^c	2.6	<0.59 to <1.0	<38
Vendace	5 muscle samples ^c	3.6	<1.6 to 4.0	<71 ^b
Smelt	5 muscle samples	4.1	<0.60 to 2.3	<150 ^b
Brown trout	5 muscle samples ^c	4.2	<0.55 to 4.5	190 (\pm 80)

Note: a) The detection limit was sample specific. Mean values are not given in the paper.

b) Although D4 was detected in at least one sample for each fish species, a “less than” value was reported in cases where more than half of the samples were below the limit of quantification. Effectively, the numerical value is a maximum possible concentration.

c) Two samples were analysed in duplicate for vendace and three samples were analysed in triplicate for brown trout. The zooplankton and *Mysis* samples were considered as pseudoreplicates.

The $\delta^{13}\text{C}$ measurements demonstrated that the organisms in the food web were predominantly feeding on a carbon source from a similar origin (the authors considered that they were indicative of a pelagic food chain) and the trophic level assignments were consistent with known feeding relationships in the food web. Borgå *et al.* (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. It is relevant to note that the smelt were collected from a different area of the lake than the other fish (four specimens from one location and a fifth from another) and so could potentially have been exposed to different concentrations of D4 than the other species. However, Borgå *et al.* (2012) assumed that as these fish species are pelagic and cover large areas in search of food, the influence of sampling location on contaminant exposure would be negligible.

As the concentration of D4 was below the limit of quantification in the majority of samples, it was not possible to estimate a TMF for D4 in this food chain. However, a possible positive trend in the D4 concentrations (on a lipid weight basis) with trophic level may be evident. The concentration in brown trout (occupying the highest trophic level; mean concentration 190 $\mu\text{g}/\text{kg}$ lipid) was higher than for mysids (mean concentration <38 $\mu\text{g}/\text{kg}$ lipid), vendace (mean concentration <71 $\mu\text{g}/\text{kg}$ lipid) and smelt (mean concentration <150 $\mu\text{g}/\text{kg}$ lipid). The low concentrations of D4 present in the other species (generally not detectable) mean that it is not possible to draw any definitive conclusions on any trend amongst these species. In addition, D4 was not detectable in zooplankton but the limit of quantification was higher than the other species (mean concentrations were reported as <830 $\mu\text{g}/\text{kg}$ lipid for zooplankton from the epilimnion

and <190 µg/kg lipid in samples from the hypolimnion) and so it is not possible to infer whether the concentrations in zooplankton were higher or lower than the other species. 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 also important to note that the number of samples analysed in this study was relatively small (four to five per species). Furthermore, the fish concentrations were determined from muscle samples rather than whole fish, and the relationship between the two is unknown. 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.

5. A preliminary study into the bioaccumulation of D4 in a pelagic marine food web in Tokyo Bay is reported by Powell (2012). The study incorporated two PCB congeners as a reference chemical (PCB-153) and a benchmark chemical (PCB-180). The samples used in the study were collected between October and November 2011. The aim was to generate information to guide the experimental design of a subsequent five-year monitoring program to be conducted in Tokyo Bay. The samples consisted of sediments and the following fish species: adult Japanese sea bass (*Lateolabrax japonicus*), adult red barracuda (*Sphyraena pinguis*), adult chub mackerel (*Scomber japonicus*), adult and juvenile dotted gizzard shad (*Konosirus punctatus*), juvenile Japanese anchovy (*Engraulis japonicus*), juvenile Japanese scaled sardine (*Sardinella zunasi*) and juvenile white croaker (*Pennahia argentata*).

Surface sediment samples (top 1 cm) were collected from 20 locations within the study area (approximately 500 km², sampled across the bay and about 30 km seaward) using a stratified random sampling design. Samples of fish were collected by commercial fishermen from the same study area. Powell (2012) indicates that a rigorous quality control program was followed which included reference samples, control samples and blank samples to verify that the samples were not contaminated by sample storage and processing procedures (although it is not entirely clear if this extended to the sampling by the commercial fishermen themselves).

The fish sampled were pelagic with the exception of white croaker (benthopelagic) and Japanese sea bass (demersal). The carbon isotopic signature ($\delta^{13}\text{C}$) indicated that all of the fish were feeding on the same or a similar carbon source but that this was different from that of the sediment. Therefore it was considered that the biota samples were representative of a pelagic food chain. Trophic levels for the biota were assigned based on $\delta^{15}\text{N}$ measurements assuming a trophic enrichment factor ($\Delta^{15}\text{N}$) of 3.2 (this value was estimated by defining the TMF of the benchmark chemical PCB-180 as 4.0 and using this to calibrate the food web, i.e. the $\Delta^{15}\text{N}$ value chosen is that which results in a TMF of 4 for PCB-180).

The sediment measurements showed a concentration gradient for D4 (no data were presented on the levels of PCB-153 and PCB-180 and so it is not clear whether the reference substance and benchmark substance were also subject to a concentration gradient in the study area), indicating that exposure of the organisms may vary within the study area. Powell (2012) considered that as most of the fish sampled were pelagic these

would actively move throughout the study area and so the impact of variable exposure would be minimal for these species. However, Powell (2012) noted that the Japanese sea bass was a demersal species that does not migrate as actively as other species and so it could be exposed to higher concentrations compared with other organisms in the sampled foodchain. To correct for this, the BSAF was used to correct the BMF and TMF for variable exposure based on the relationship that $TMF_{LIPID}=TMF_{BSAF}$ and $BMF_{LIPID}=BMF_{BSAF}$. The exact method used to carry out these corrections was not given in the paper. Furthermore, these corrections appear to have been applied only to the siloxane and not the reference and benchmark chemicals. (Powell (2012) noted that concentration gradients resulting from point source emissions are generally not a significant concern for chemicals with diffuse emissions such as PCBs; however, as noted above there were no data reported for these two substances for sediment to check that this was the case).

The sediment sampling design allowed mean concentrations (and hence BSAF values) to be calculated for each section of the study area (the study area was divided into four sections based on the gradient of D4 concentrations observed).

The concentrations reported in the sediment and biota samples are summarised in **Error! Reference source not found.** The sediment concentrations are reported as $\mu\text{g}/\text{kg}$ wet weight values but no units are given in the Powell (2012) paper for the biota samples. For **Error! Reference source not found.** it has been assumed that they are also $\mu\text{g}/\text{kg}$ wet weight values. The corresponding concentrations on a lipid weight or organic carbon weight basis have been estimated from the information on organic carbon and lipid contents given in the paper.

The BSAF, BMF and TMF values derived by Powell from the data are summarized in **Error! Reference source not found.** In all cases mean values, 95% confidence intervals and the probability that the value was greater than one were estimated by bootstrap analysis using Monte Carlo simulation.

For the BSAF, values above one were obtained for D4 for a number of species including Japanese sea bass (mean BSAF 1.4), juvenile Japanese anchovy (mean BSAF 1.2), juvenile Japanese scaled sardine (mean BSAF 2.6), juvenile white croaker (mean BSAF 1.1) and juvenile dotted gizzard shad (mean BSAF 1.0). The probability that the BSAF was greater than one for these species ranged between 41 and 95 per cent depending on the species. No BSAF values were calculated for the two PCB reference substances.

For the BMF, values above one were obtained for D4 for three out of the four predator – prey interactions involving Japanese sea bass (the probability that the value was greater than one was between 57 and 79 per cent for these three interactions). The remaining BMF values for the predator – prey interactions considered were all below one. For comparison, the BMF values obtained for PCB-153 and PCB-180 were in the range 3.5-8.9 and 3.9-10, respectively, for the four sea bass – prey interactions (BMFs were not calculated for other predator – prey interactions).

The TMF for D4 was calculated to be 0.6 when the Japanese sea bass data were included and 0.4 when they were excluded. The probability that the TMF was above one was low, at between 1.7 and 5.5 per cent. In contrast, the TMF for PCB-153 was 3.7 and the TMF for PCB-180 was 4.0 when the Japanese sea bass data were included (no analysis was done excluding the Japanese sea bass) and the probability of the TMF being above one was approaching 100 per cent in both cases.

On the face of it, these data suggest that the bioaccumulation potential of D4 is much lower than PCB-153 and -180 with a TMF in the range 0.4 – 0.6, although some individual BSAF and BMF values are above one. However, there are a number of potential uncertainties with the way the analysis of the data was carried out that warrant further consideration. These are outlined below.

- The trophic level assignments were based on the assumption that the TMF for PCB-180 was 4.0, so the system was effectively calibrated to the benchmark chemical. This affects the magnitude of the slope of the ln [concentration] versus trophic level plot, but not whether the gradient is positive or negative. If the trophic level assignments were different, a different TMF would have been derived, but it would still be below one.
- The calculation of the mean, 95% confidence intervals and probability of values being above one were all carried out by bootstrap analysis using Monte Carlo simulation. One of the inputs into such analysis is the standard deviation in the measured concentrations in the various species in the food web. For D4, these standard deviations were known for five of the eight species included in the food web. For the remaining three species, the standard deviations were estimated based on previous studies conducted on Lake Pepin. The standard deviations assigned are summarized in **Error! Reference source not found.** It is evident from these data that the standard deviations for the samples where they could be measured are much smaller (typically 7-42%) than in the samples where the standard deviations were estimated (typically 60-61%). Therefore, the calculations of the statistics in the study may have been influenced more by the samples for which the standard deviation was estimated than those for which the standard deviation was known.

Table 15 Concentrations of D4 in the Tokyo Bay food chain

Sample	Number of samples analysed	Lipid/organic carbon content (%)	Trophic level	Mean measured D4 concentration (\pm standard deviation)	
				$\mu\text{g}/\text{kg}$ wet weight	$\mu\text{g}/\text{kg}$ lipid or $\mu\text{g}/\text{kg}$ organic carbon
Surface sediment – Sector 1	2	0.86 \pm 0.021		2.3 \pm 0.64	267
Surface sediments – Sector 2	6	0.93 \pm 0.052		2.8 \pm 1.9	301
Surface sediments – Sector 3	6	0.78 \pm 0.36		1.3 \pm 0.74	167
Surface sediments – Sector 4	6	0.55 \pm 0.34		0.48 \pm 0.33	87
Dotted gizzard shad juvenile (<i>Konosirus punctatus</i>)	3 composites (each of 11 individuals)	7.9 \pm 0.76	3.0	14 \pm 1.6	177
White croaker juvenile (<i>Pennahia argentata</i>)	3 composites (each of 13 individuals)	5.9 \pm 1.0	3.1	11 \pm 0.81	186
Japanese scaled sardine juvenile (<i>Sardinella zunasi</i>)	3 composites (each of 48 individuals)	4.5 \pm 0.45	3.2	22 \pm 1.9	489
Japanese anchovy juvenile (<i>Engraulis japonicas</i>)	3 composites (each of 55 individuals)	3.9 \pm 0.42	3.5	8.9 \pm 0.62	228
Dotted gizzard shad adult (<i>Konosirus punctatus</i>)	1 composite (of 5 individuals)	17(\pm 6.8) ^a	3.9	9.4(\pm 5.7) ^a	55
Chub mackerel adult (<i>Scomber japonicas</i>)	1 composite (of 4 individuals)	20(\pm 8.0) ^a	4.2	8.4(\pm 5.0) ^a	42
Red barracuda adult (<i>Sphyraena pinguis</i>)	1 composite (of 5 individuals)	11(\pm 4.4) ^a	4.2	16(\pm 9.8) ^a	145
Japanese sea bass adult (<i>Lateolabrax japonicas</i>)	6 individuals	6.4 \pm 2.7	4.4	24 \pm 10	375

Note: a) The standard deviations for these samples were estimated from the 90th percentile coefficient of variation of replicate analyses of three or more samples from previous studies.

b) Estimated from the mean wet weight concentration and mean organic carbon contents given in Powell (2012).

Table 16 Bioaccumulation parameters derived for of D4 in the Tokyo Bay food chain by Powell (2012)

Parameter	Mean value ^a	95% confidence interval ^a	Probability the value is >1 ^a	Comment
BSAF for Japanese sea bass (adult)	1.4	0.7-2.5	78%	Units are g-total organic carbon/g-lipid
BSAF for red barracuda (adult)	0.8	0.2-2.1	25%	Units are g-total organic carbon/g-lipid
BSAF for chub mackerel	0.2	0.1-0.6	<1%	Units are g-total organic carbon/g-lipid
BSAF for dotted gizzard shad	0.3	0.1-0.8	<1%	Units are g-total organic carbon/g-lipid
BSAF for Japanese anchovy (juvenile)	1.2	0.8-1.9	68%	Units are g-total organic carbon/g-lipid
BSAF for Japanese scaled sardine	2.6	1.9-3.6	95%	Units are g-total organic carbon/g-lipid
BSAF for white croaker (juvenile)	1.1	0.6-1.7	56%	Units are g-total organic carbon/g-lipid
BSAF for dotted gizzard shad (juvenile)	1.0	0.7-1.3	41%	Units are g-total organic carbon/g-lipid
BMF for Japanese sea bass – Japanese anchovy	1.3	0.4-3.2	57%	Lipid normalised ^b .
BMF for Japanese sea bass – Japanese scaled sardine	0.6	0.2-1.0	3%	Lipid normalised ^b .
BMF for Japanese sea bass – white croaker	1.3	0.6-2.4	70%	Lipid normalised ^b .
BMF for Japanese sea bass – dotted gizzard shad (juvenile)	1.3	0.7-2.2	79%	Lipid normalised ^b .
BMF for red barracuda – Japanese anchovy	0.6	0.1-2.2	16%	Lipid normalised ^b .
BMF for red barracuda – Japanese scaled sardine	0.3	0.0-0.8	<1%	Lipid normalised ^b .
BMF for red barracuda – white croaker	0.8	0.1-2.1	23%	Lipid normalised ^b .

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Parameter	Mean value ^a	95% confidence interval ^a	Probability the value is >1 ^a	Comment
BMF for red barracuda – dotted gizzard shad (juvenile)	0.8	0.2-2.1	26%	Lipid normalised ^b .
BMF for chub mackerel – Japanese anchovy	0.1	0.0-0.5	<1%	Lipid normalised ^b .
BMF for chub mackerel – Japanese scaled sardine	0.1	0.0-0.4	<1%	Lipid normalised ^b .
BMF for chub mackerel – white croaker	0.3	0.0-0.7	<1%	Lipid normalised ^b .
BMF for chub mackerel – dotted gizzard shad (juvenile)	0.3	0.0-0.7	<1%	Lipid normalised ^b .
BMF for dotted gizzard shad (adult) – Japanese scaled sardine	0.1	0.0-0.3	<1%	Lipid normalised ^b .
BMF for dotted gizzard shad (adult) – white croaker	0.2	0.0-0.8	<1%	Lipid normalised ^b .
BMF for dotted gizzard shad (adult) – dotted gizzard shad (juvenile)	0.3	0.0-0.8	<1%	Lipid normalised ^b .
TMF – food web including Japanese sea bass	0.6	0.3-1.1	5.5%	Obtained from the slope of a plot of ln [Concentration in fish (lipid weight basis)] against trophic level.
TMF – food web without Japanese sea bass	0.4	0.1-0.9	1.7%	Obtained from the slope of a plot of ln [Concentration in fish (lipid weight basis)] against trophic level.

Note: a) Mean values, 95% confidence intervals and probabilities that the values were greater than one were estimated by bootstrap analysis using Monte Carlo simulation.

b) The BMF values were calculated for possible predator-prey relationships where the difference in trophic level between the two species was greater than 0.7. The values were then adjusted for this difference to effectively normalise the BMF to a trophic level difference of 1.

- The sediment levels show that there was a probable concentration gradient in the study area for D4. The Powell (2012) analysis corrects for this in the BMF and TMF calculations by using the information on the BSAF values. It is not clear from the test report how this correction was carried out. In addition, and more importantly, it is not clear whether such a correction is actually appropriate. Powell (2012) states in the report that “most of the sampled food web organisms were pelagic species that actively migrate throughout the study area feeding on nekton (free-swimming organisms), zooplankton, and phytoplankton” and it was assumed that the impact of variable exposure would be minimal for such species. The one species identified as potentially not migrating widely in the study area was Japanese sea bass. Correction for variable exposure was therefore probably not necessary for seven of the eight species in the study.
- The study assumes that there is no concentration gradient for the two PCB reference substances and so the TMF values were not corrected for this gradient in the same way as the TMF values for D4. There is no information provided to show whether or not this is appropriate.

To investigate the possible significance of some of the assumptions made by Powell (2012) in correcting the TMF for D4, the TMF has been recalculated for the purposes of this evaluation using the concentration and trophic level data as reported in the study but without correcting for the concentration gradient in sediment. The results of this analysis are shown in **Error! Reference source not found.** (including the data for Japanese sea bass) and **Error! Reference source not found.** (excluding the Japanese sea bass data). When the data are analysed in this way, a similar picture emerges in that the TMF value obtained from the slope of the regression is still below one, in both cases. In addition, several of the BMFs for individual predatory-prey interactions are also close to or above one. The relevant data are summarized in **Error! Reference source not found.** and **Error! Reference source not found.**. The significance of the BMFs above one for Japanese sea bass calculated using this method is unclear as this species is the one most likely to be influenced by concentration gradients within the sampled area and so the values presented in Powell (2012) would be preferred over these values (the Powell (2012) analysis also indicates BMFs above one for Japanese sea bass). Overall, the re-analysis carried out here generally confirms that the TMF for D4 in this food chain is below one.

Figure 4 Plot of ln (concentration in fish) against trophic level for the Tokyo Bay food chain including the data for Japanese sea bass (not corrected for concentration gradient in sediment)

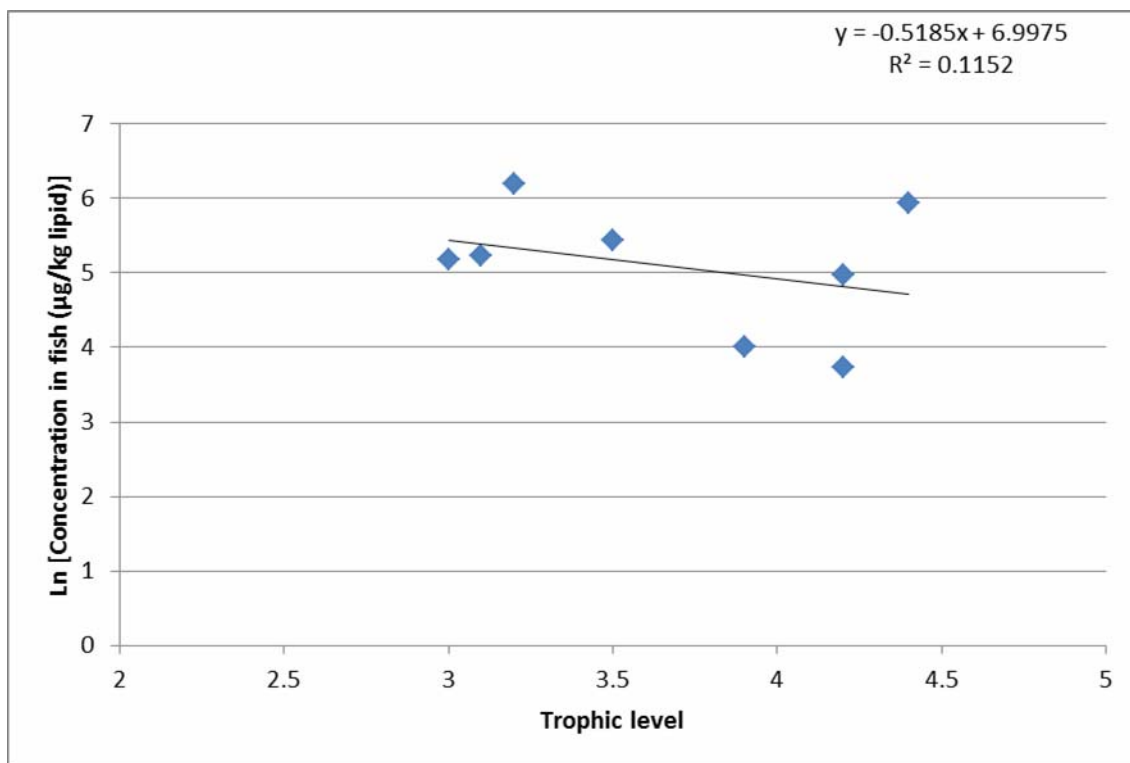


Figure 5 Plot of ln (concentration in fish) against trophic level for the Tokyo Bay food chain excluding the data for Japanese sea bass (not corrected for concentration gradient in sediment)

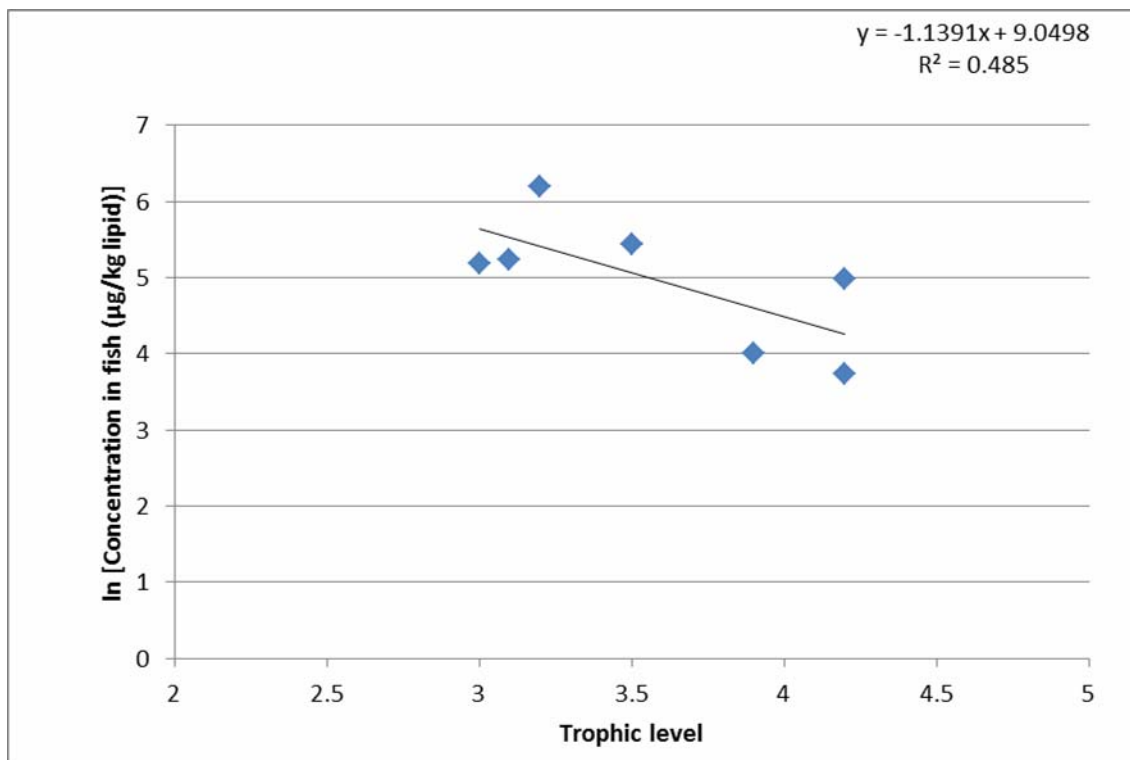


Table 17 Bioaccumulation parameters derived for of D4 in the Tokyo Bay food – BMF values reanalysed for this evaluation

Parameter	Value based on the ratio lipid normalised concentrations	Value corrected for differences in trophic level ^a
BMF for Japanese sea bass – Japanese anchovy	1.64	1.74
BMF for Japanese sea bass – Japanese scaled sardine	0.77	0.82
BMF for Japanese sea bass – white croaker	2.01	1.65
BMF for Japanese sea bass – dotted gizzard shad (juvenile)	2.12	1.71
BMF for red barracuda – Japanese anchovy	0.64	0.53
BMF for red barracuda – Japanese scaled sardine	0.30	0.33
BMF for red barracuda – white croaker	0.78	0.81
BMF for red barracuda – dotted gizzard shad (juvenile)	0.82	0.85
BMF for chub mackerel – Japanese anchovy	0.18	0.09
BMF for chub mackerel – Japanese scaled sardine	0.09	0.09
BMF for chub mackerel – white croaker	0.23	0.26
BMF for chub mackerel – dotted gizzard shad (juvenile)	0.24	0.30
BMF for dotted gizzard shad (adult) – Japanese scaled sardine	0.11	0.04
BMF for dotted gizzard shad (adult) – white croaker	0.30	0.22
BMF for dotted gizzard shad (adult) – dotted gizzard shad (juvenile)	0.31	0.27

Note: a) The values were corrected for the difference in trophic level using the equation outlined in CES, (2010a) and discussed earlier in this section. The actual method used by Powell (2012) was not given.

Table 18 Bioaccumulation parameters derived for of D5 in the Tokyo Bay food – BMF values reanalysed for this evaluation

Food web	Parameter ^a	Value
All fish species including Japanese sea bass	Slope of plot	-0.519
	TMF	0.59
	95% Confidence interval of the slope	-1.954 to 0.917
	95% Confidence interval of the TMF	0.14 to 2.50
	p-value of slope ^b	0.41
	R ² of regression	0.12
All fish species excluding Japanese sea bass	Slope of plot	-1.139
	TMF	0.32
	95% Confidence interval of the slope	-2.489 to 0.210
	95% Confidence interval of the TMF	0.08 to 1.23
	p-value of slope ^b	0.082
	R ² of regression	0.49

Note: a) The TMF value was estimated from the slope of a plot of ln [Concentration] against trophic level. The statistical values are derived by linear regression analysis.

b) The p-value indicates that the slope is not statistically different from zero.

In addition to the above five field studies summarised above, some preliminary results have been provided on the levels of D4 in pike (*Esox lucius*) and roach (*Rutilus rutilus*) obtained from the River Cam in the UK (van Egmond, 2012). 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 contents of the two pike were 0.44 per cent and 0.49 per cent and the lipid content of the roach sample was 0.62 per cent. The concentration of D4 in the roach sample was 2.5 mg/kg lipid (mean of duplicate analyses of the sample). The concentration of D4 in the pike was 2.8 mg/kg lipid in one sample (mean of duplicate analyses of the sample) and 3.4 mg/kg lipid (single analysis). Thus these results show that the levels in pike are similar to, but slightly higher than, those in roach. The significance of this finding is unclear given the very small sample size, and questionable lipid contents (they appear to be rather low). It is therefore not considered further in this report.

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 D4 (Lake Pepin, Oslofjord, Lake Opeongo, Lake Mjøsa and Tokyo Bay) it should be noted that there are limitations in terms of the sampling (in general only a small

number of samples were obtained for each species; in some cases just single samples) 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 from different areas within large water bodies.

CES (2010b) summarises 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 (and is 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 generated in the report were derived using the individual data points rather than the species means (for example in Lake Pepin).

CES (2010b) also suggests 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 (2010c) outlines 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 $\delta^{15}\text{N}$ and $\delta^{14}\text{C}$ 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), which may give rise to:
 - 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 D4 up to 2009 (i.e. minus the Lake Mjøsa and Tokyo bay data) were considered at an expert panel workshop organized by the Global Silicones Counsel (Global Silicones Counsel, 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 agreed that the TMF is the “gold standard” for evaluating bioaccumulation. However it was also noted that the available data for D4 do not allow a definitive assessment of the bioaccumulation potential to be made.

Other measures of accumulation

The accumulation of D4 in the Humber Estuary, UK, has been studied by Kierkegaard *et al.* (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 D4 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 D4 concentrations were less than 1-2 µg/kg dry weight in sediment, between not detectable and 20 µg/kg fresh weight in ragworm and between not detectable and 10 µg/kg fresh weight in flounder fillet. 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 D4 to that of PCB-180 was used to investigate the bioaccumulation potential of D4. 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 D4:PCB-180 approximates to the ratio of the sediment-biota bioaccumulation factors (BSAF) for D4 to that for PCB-180 in the same sample. Ratios for ragworms and flounder were calculated from the measured data in

cases where the D4 was detectable in the biota samples. For samples in which it could be calculated, the mean ratio was around 6 in ragworms and 14 in flounder, indicating that D4 was bioaccumulating to a greater extent than PCB-180 in these organisms. It should be noted that Kierkegaard *et al.* (2011) considered that these ratios were minimum values as the concentrations of D4 in the sediment were generally below the limit of quantification (the limit of quantification was used in the calculation of these ratios).

It should be noted that the concentration in flounder samples relates to fillet (i.e. muscle) rather than whole body, and the relationship between the two are unknown. In addition, it is not known if this relationship is the same for both D4 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. This may introduce some uncertainty into the flounder results from this study.

A field study from Japan has recently investigated the sediment-biota accumulation factor (BSAF) for D4 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 D4 (both whole fish and edible parts were analysed).

It should be noted that the method used for extraction of D4 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 D4 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 103 per cent with a standard deviation of 4.3 per cent (total of seven recovery samples) for D4 indicating that loss of D4 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 D4).

The results are summarised in Table 19.

Table 19 Summary of BSAFs derived from rivers in Japan

River	Location ¹	Sample ⁷	N ⁶	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	{0.56±0.16} ⁵	{370±110} ⁵	
		Pale chub	3	44±0.84	980±19	{2.6}
		Common carp	3	[23±0.35] ⁴	[590±8.8] ⁴	{1.6}
	Down-stream	Sediment	6	{2.9±2.3} ⁵	{770±230} ⁵	
		Yellowfin goby	3	28±1.9	1,000±67	{1.3}
		Flathead mullet	3	220±21	4,100±400	{5.3}
		Japanese seabass	3	28±0.98	1,200±43	{1.6}
Arakawa River	Mid-stream	Sediment	6	{2.4±1.9} ⁵	{970±880} ⁵	
		Pale chub	3	67±3.4	980±50	{1.0}
		Common carp	3	{7.7±0.48} ⁵	{310±19} ⁵	{0.3}
	Down-stream	Sediment	6	25±2.3	2,000±130	
		Yellowfin goby	3	62±5.5	2,900±260	1.5
		Flathead mullet	3	200±4.0	3,800±75	1.9
		Japanese seabass	3	120±10	4,200±340	2.1
Tone River	Mid-stream	Sediment	6	[3.0±0.7] ⁴	[260±37] ⁴	
		Pale chub	3	66±1.4	900±20	[3.5]
		Common carp	3	[13±0.51] ⁴	[520±21] ⁴	[2.0]
	Down-stream	Sediment	6	[2.0±0.5] ⁴	[240±32] ⁴	
		Yellowfin goby	3	[14±4.1] ⁴	[720±200] ⁴	[3.0]
		Flathead mullet	3	80±2.9	1,400±49	[5.8]
		Japanese seabass	3	35±3.5	400±41	[1.7]

Note: 1) These terms are used in the SIAJ (2011) 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) Number of samples: with the exception of the midstream sample from Tama River, three sediment samples were collected from each of two locations.
- 7) Latin names: Pale chub – *Zacco platypus*, common carp – *Cyprinus carpio*, yellowfin goby – *Acanthogobius flavimanus*, flathead mullet – *Mugil cephalus* and Japanese seabass – *Lateolabrax japonicus*.

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 biota/organic carbon-normalised concentration in sediment) were above 1 for fourteen of the fifteen biota samples. However, in many cases the concentrations of D4 in sediment were below the method detection limit (0.90 µg/kg wet weight) and the limit of quantification (2.7 µg/kg wet weight). For three samples, the concentrations in both fish and sediment were above the limit of quantification and the BSAFs estimated in these cases were 1.5 for yellowfin goby, 1.9 for flathead mullet and 2.1 for Japanese seabass (all samples from the Arakawa River). 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.

In addition to these data, samples of fish were also collected from Tokyo Bay. These showed generally lower concentrations of D4 (≤ 450 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.

It is relevant to note from this study that, although the concentrations in sediment were generally low (often close to or below the limit of quantification), D4 was still detectable in the biota samples from the area, particularly flathead mullet and Japanese seabass. $\delta^{13}\text{C}$ -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 foodchain (land origin or marine origin). The sediment from midstream and downstream locations generally showed the sediment to be of land origin (the midstream sample from the Arakawa River gave a $\delta^{13}\text{C}$ value midway between land and marine origin). The carp samples from midstream had $\delta^{13}\text{C}$ values typical of land origin but the pale chub from midstream showed a wider range of $\delta^{13}\text{C}$ values, with the pale chub from the Arakawa River having a value more consistent with marine origin than land origin (possibly reflecting the findings for sediment). The $\delta^{13}\text{C}$ values from the downstream biota samples reflected differences in habitat and food webs 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 the food of flathead mullet is likely to be highly influenced by the D4 in the sediment. Both the yellowfin goby and flathead mullet had $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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.

Kierkegaard *et al.* (2012) obtained samples of Grey Seal (*Halichoerus grypus*) blubber from three animals that had drowned in nets north of Västervik, Sweden in the autumn of 2008. The samples were taken from parts of the tissue that had not been exposed to air or packaging material. Three herring (*Clupea harengus*) from a nearby monitoring station that had been sampled in the same year (as part of the Swedish Marine Monitoring Program) were also analyzed. Although no special precautions had been followed during the collection and storage of the fish, dorsal muscle samples were excised without skin and measures were taken to reduce contamination during sample preparation and instrumental analysis. Extraction of the biological samples was performed with a purge and trap method, followed by immediate GC/MS analysis. A procedural blank and a control sample were analyzed with every extraction round of eight samples. D4 was detected in each of the three seal blubber samples, between the limits of quantitation and detection (reported range 2.3 – 3.0 ng/g ww). Although the lipid content of the blubber samples was not available, blubber is known to consist primarily of lipid, so the wet weight and lipid normalized concentrations were considered to be the same. The lipid normalized concentrations in the herring were also between the limits of quantitation and detection (reported range 171 to 1,010 ng/g lw). This may be due to the low lipid content of the herring (and thereby low lipid mass in the sample) (<0.1–0.43%), as well as the relatively high limit of quantitation (9.9 ng) as a result of the small amount of fish extracted. For comparison, the median concentration of D4 in herring collected from the same site collected in 2007 (~11 ng/g lw) was four times higher than the median concentration in the seal blubber. Despite the lack of blank correction, the small sample size and the fact that the concentrations are not based on whole body homogenates, these results suggest that D4 is a contaminant throughout the food chain, but does not biomagnify in Grey Seals (herring accounts for ~80% of the diet of Grey Seals in the Baltic food web).

4.3.3.3 Modelling studies

Summary of information from existing evaluation

EA (2009) reports the results of physiologically-based pharmacokinetic modelling studies considering both inhalation and dermal exposure of D4 in mammalian systems (but not oral exposure). The models were developed by Anderson (2005) and Reddy *et al.* (2004 and 2005) and were based on a comprehensive data set developed using both single and repeated inhalation studies in rats, a single inhalation exposure study in humans and both *in vitro* and *in vivo* percutaneous absorption studies. The model included a sequestered pool of D4 (presumed to be in lipoproteins) released from the liver, distributed by the blood, and cleared from the blood into fat. The inhalation model showed that metabolism and exhalation are important mechanisms for elimination of D4 and that the rapid clearance by these two routes means that D4 does not accumulate, despite a high predicted blood-to-fat partitioning behaviour.

Using the dermal absorption model, absorption of D4 was thought to be very limited with only around 0.3 per cent of the dose predicted to be systemically adsorbed. Furthermore, the dermally absorbed dose is predicted to enter the venous circulation and move directly to the lungs, from which ~80 per cent is eliminated via exhalation prior to it being available systemically.

New information

A modelling study for D4 has been carried out to compare the predicted bioaccumulation with the bioaccumulation observed in both laboratory experiments and in the field situation (HydroQual Inc., 2009). The bioaccumulation model used was the Thomann-Farley food chain model (Thomann *et al.*, 1992) and takes into account accumulation from both dietary and aqueous exposure. The aim of the study was to try to reconcile the aqueous and dietary accumulation measured for D4 in the laboratory (Domoradzki *et al.*, 2006) (see EA (2009) for a summary of the laboratory studies) with the field measurements found in the Lake Pepin study summarised in Section 4.3.3.2 (Powell *et al.*, 2009a).

The model was firstly applied to the laboratory data. The laboratory data were used to calibrate the key parameters in the model (such as gill and dietary chemical assimilation efficiencies). The model was found to describe the observed laboratory data reasonably well. The laboratory-calibrated model was then used to predict the field data generated in the Lake Pepin study (most of the results are only presented graphically in the report). In order to simplify the modelling the fish species were grouped into two general feeding classes: forage fish (which were assumed to consume a diet consisting 100 per cent of benthic invertebrates) and piscivorous fish (which were assumed to consume a diet consisting of 25 per cent small fish and 75 per cent benthic invertebrates). The model was run by specifying the concentrations in the diet species (benthic invertebrates and young-of-year fish) to be the mean concentration in these species from the field data.

Under these conditions the model was found to predict the general trends of the D4 concentrations in fish reasonably well, with the forage fish generally showing higher concentrations than piscivorous fish, consistent with trophic dilution. In addition the model predicted that the concentrations within fish would decrease with the size of the fish as a result of growth and elimination rates that are faster than the rates of accumulation from diet and water exposures. The model calculations also suggested that the primary route of exposure was through the diet (>50 per cent for D4). A key uncertainty in the modelling data is the assumption of a single elimination rate to take account of metabolism and the various excretion mechanisms within the fish. As noted by HydroQual Inc. (2009) such elimination rates can vary substantially between different fish species.

It is possible that the finding over the percentage contribution from diet may be influenced by some of the initial parameters assumed in the model, but no sensitivity analysis was performed. In particular the concentration in benthic macroinvertebrate (~7.8 µg/kg wet weight) and young-of-year fish (2.6 µg/kg wet weight) were based on the Lake Pepin field data (and not predicted within the model) whereas the freely dissolved concentration for each cyclic siloxane was set at 0.1 ng/l. As the actual concentration of freely dissolved D4 was not known in the Lake Pepin study this may have biased the predictions towards accumulation from diet over accumulation from water.

This issue has been considered further in CES (2010a). The modelling carried out by Whelan (2009a) (reported in Table 3) estimated that the total concentration of D4 in Lake Pepin would be of the order of 0.01-0.1 ng/l and the dissolved concentration would be expected to be lower than this owing to adsorption onto suspended matter and association with dissolved organic carbon. CES (2010a) estimates that around 43 per cent of the D4 in water will be in the dissolved phase. Therefore the assumption used in the HydroQual Inc. (2009) modelling may have overestimated slightly the actual concentration of D4 in water, and hence the contribution from water uptake.

Overall the modelling carried out on the Lake Pepin data set provides strong evidence that uptake in this food chain was primarily by dietary exposure with bioconcentration processes making a smaller contribution to the uptake seen. It should however be noted that sediment concentrations measured in the lake (around 48 µg/kg organic carbon (standard deviation ±5 µg/kg organic carbon)) are higher than would be expected from a freely dissolved concentration of 0.1 ng/l (e.g. assuming the K_{oc} of 1.7×10^4 l/kg reflects the partitioning between the dissolved water phase and the sediment phase, a sediment concentration of around 1.7 µg/kg organic carbon would be expected; the concentration would be proportionately lower if lower freely dissolved water concentrations were assumed). Therefore the sediments in Lake Pepin appear to be more highly contaminated with D4 than might be expected from the predicted concentration in the water phase and this may partly explain the pattern of uptake seen in this food chain.

Whelan and Breivik (2013) also investigated pelagic food chain transfer of D4 in the Inner Oslofjord using two dynamic models (the Oslofjord POP Model and the aquatic component of ACC-HUMAN). Initial predicted concentrations in zooplankton, herring (*Culpea harengus*) and cod (*Gadus morhua*) were significantly lower (379, 115 ± 22 and 100 ± 19 ng/g lipid, respectively) than measured concentrations. When measured zooplankton concentrations were used to estimate the dissolved aqueous concentration, the model overestimated the fish concentrations. This was thought to be due to the use of a metabolism rate constant (for D5) that was too low for D4. Trophic dilution was predicted, principally due to a combination of *in vivo* metabolism and reduced gut absorption efficiency (as a consequence of the high K_{ow}).

4.3.3.4 Measured concentrations in biota

Summary of information from existing evaluation

The available monitoring data for D4 in general are summarised in EA (2009). Of most relevance to the PBT and vPvB assessment are data on the occurrence of D4 in biota from marine areas and from remote regions. The available relevant data are briefly summarised below.

- D4 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).
- TemaNord (2005) reports levels of D4 of <5 to 70 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 of 70 µg/kg fresh weight was for cod liver from the Inner Oslofjord in Norway.
- Schlabach *et al.* (2007) investigated the levels of D4 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). D4 was detectable in all

samples. The highest concentrations were found in cod liver (81-134 µg/kg wet weight).

- EVONIK Industries (2007) carried out a survey of the levels of D4 in freshwater and marine fish from Europe. The analytical detection limit was 20 µg/kg wet weight. For the marine fish D4 was not detectable 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 close to Estonia. For the freshwater fish, D4 was not detectable in three species from Lake Nipgård, Denmark and in three species from Lake Constance, Germany. In contrast to these data, D4 was found at much higher concentrations (between 100 and 900 µg/kg wet weight) in samples of roach, ide and eel from the River Rhine, Germany (close to the Dutch border) showing that relatively high concentrations of D4 can occur in biota in some environments, presumably close to sources of release.
- A preliminary screening study of the levels of D4 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 D4 found were below the method detection limit (<6 µg/kg) in all of the samples analysed.

New information

The available new information on the levels of D4 in biota, including biota samples from remote regions is summarised in Table 20. 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. In addition to the data in Table 20, other monitoring data for biota have been generated in investigations of food chain accumulation (see Section 4.3.3.2).

Of most relevance to the PBT and vPvB assessment are the studies by Campbell (2010; very brief details of this study are also given in an interim report by Campbell (2009)) and Evenset *et al.* (2009) of the levels of D4 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. Some of the data are also reported in Warner *et al.* (2010).

- 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 and Liefdefjorden within the Svalbard archipelago, Bjørnøya (Svalbard) and off the west coast of Sweden. The 2009 samples were collected mainly from Adventfjorden, Kongsfjorden and Liefdefjorden within the Svalbard archipelago, with some additional zooplankton samples collected from Nordkappsundet. 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 Campbell (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 Table 20. In addition to biota, as indicated above, sediment samples were also collected from some locations. These results are reported in Section 4.2.3 (and show that D4 was not detectable in the sediment). For the data in Table 20, where D4 was not detected in one or more of the samples the method detection limit was given. The limit of quantification was generally set as three times the method detection limit²⁶. D4 was detectable in some samples of Atlantic cod (*Gadus morhua*) liver, sculpin²⁷ liver and whole body minus liver, zooplankton and glaucous gull (*Larus hyperboreus*) liver (the Warner *et al.* (2010) paper shows that D4 was not detectable in any of the ten samples of Atlantic cod liver, ten samples of sculpin liver or zooplankton samples collected in 2009 and analysed by that particularly laboratory). D4 was not detectable in the other species sampled. Where detectable, the concentration of D4 was generally low. The highest concentrations found were in Atlantic cod liver from Adventfjorden (up to 9.2 µg/kg wet weight, with D4 being detectable in 10 out of 11 samples analysed), sculpin liver from Adventfjorden (up to 3.38 µg/kg wet weight, with D4 being detectable in 6 out of 16 samples) and glaucous gull liver from Bjørnøya (up to 6.5 µg/kg wet weight with D4 being detectable in 2 out of 8 samples). The levels found in samples from Adventfjorden may reflect a local source of emission.

It is interesting to note that in this study some of the higher concentrations are found in fish such as Atlantic cod and sculpin rather than invertebrates (in contrast with some of the field bioaccumulation studies reported in Section 4.3.3.2). However the lack of information on predatory-prey relationships and lipid contents, and limited numbers of samples etc. precludes a detailed evaluation of the bioaccumulation potential for D4 in this food chain.

²⁶In many of the samples, although D4 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.

²⁷ Species name not given.

The Evenset *et al.* (2009) study showed that D4 was detected frequently in samples of Atlantic cod (*Gadus morhua*) and polar cod (*Boreogadus saida*). D4 was also detectable in four out of five samples of seabird liver (kittiwake (*Rissa tridactyla*)) from Kongsfjorden but was not detectable in samples of kittiwake liver from Liefdefjorden or eider liver from Kongsfjorden. D4 was not detectable in sediment samples collected on the west coast of Spitsbergen. The source of exposure is not known.

Overall the Campbell (2010) and Evenset *et al.* (2009) studies confirm that D4 is present in some biota samples from remote regions, generally at very low concentrations (close to the limit of detection). When considering these data, it is important to note that local sources of D4 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 of D4 even in remote locations means that the interpretation of these data in terms of long-range transport potential for D4 is difficult.

An interlaboratory comparison of the levels of D4 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 D4 was found in all samples at concentrations between around 5 and 280 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)).

Table 20 Measured concentrations of D4 in biota

Species	Location	Measured concentration	Comment	Reference
Arctic char (<i>Salvelinus alpinus</i>)	Samples from urban lakes in Sweden	<1.2-1.6 µg/kg wet weight (detected in 6 out of seven samples from 2007/2008 from Lake Vättern)	Samples analysed were skin-free dorsal muscle samples.	Kierkegaard <i>et al.</i> (2010b)
Atlantic cod (<i>Gadus morhua</i>) – liver	Samples from remote region around Svalbard (Kongsfjorden) ¹	2.9-3.9 µg/kg wet weight or 6.9-13 µg/kg lipid (detected in 5 out of 5 samples)	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Evenset <i>et al.</i> (2009)
	Samples from remote region around Svalbard (Kongsfjorden) ⁴	0.51-1.38 µg/kg wet weight (detectable in 7 out of 19 samples ² in 2009; method detection limit was 0.19 to 2.60 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Adventfjorden) ⁴	1.84-9.2 µg/kg wet weight (detectable in 10 out of 11 samples ³ in 2009; method detection limit 0.19 to 2.60 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region (the exact location is unclear but was probably either Kongsfjorden or Liefdefjorden)	0.88-1.13 µg/kg wet weight (detected in 2 out of 3 samples from 2008; method detection limit 0.75 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Seventeen samples from inner Oslofjord.	5-280 µg/kg wet weight (detected in all seventeen samples)	Part of an interlaboratory comparison study (see text)	Durham <i>et al.</i> (2009)

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Species	Location	Measured concentration	Comment	Reference
Bivalve (<i>Astarte borealis</i>)	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Not detectable (single sample from 2008; method detection limit 0.93 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Bivalve (<i>Chlamys islandies</i>)	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Not detectable in 3 samples from 2008 (method detection limit 0.56-0.63 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 3 samples in 2008 (method detection limit 0.67-0.87 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 4 samples in 2008 (method detection limit 0.67-0.84 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Bivalve (<i>Mya truncate</i>)	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 4 samples in 2008 (method detection limit 0.67-0.96 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 2 samples in 2008 (method detection limit 0.80-0.91 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)

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Species	Location	Measured concentration	Comment	Reference
Bivalve (<i>Serripes groenlandica</i>)	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 2 samples in 2008 (method detection limit 0.68-1.01 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 2 samples in 2008 (method detection limit 0.73-0.78 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Black guillemot (<i>Cephus grille</i>) – liver	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 2 samples in 2008 (method detection limit 2.6 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Black guillemot (<i>Cephus grille</i>) – muscle	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 2 samples in 2008 (method detection limit 2.6 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Black guillemot (<i>Cephus grille</i>) – plasma	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 10 samples in 2008 (method detection limit 7.93-8.16 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Black guillemot (<i>Cephus grille</i>) – blood cells	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 10 samples in 2008 (method detection limit 6.02-17.0 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)

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Species	Location	Measured concentration	Comment	Reference
Common eider (<i>Somateria mollissima</i>) – liver	Samples from remote region around Svalbard (Kongsfjorden) ¹	Not detectable (<3.8 µg/kg wet weight or <228 µg/kg lipid) (5 samples)	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Evenset <i>et al.</i> (2009)
Glaucous gull (<i>Larus hyperboreus</i>) – liver	Samples from remote region - Bjørnøya	3.0-6.5 µg/kg wet weight (detectable in 2 out of 8 samples ² in 2008; method detection limit was between 0.51 and 2.6 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Glaucous gull (<i>Larus hyperboreus</i>) – muscle	Samples from remote region - Bjørnøya	Not detectable in 5 samples in 2008 (method detection limit 2.6 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Herring ³	Samples from west coast of Sweden (Skagerrak)	Not detectable in 6 samples from 2008 (method detection limit 1.08-1.74 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Herring ³	Samples from 10 locations, Baltic Sea.	Up to around 30 µg/kg lipid in archived specimen from 2007. The highest levels were found in samples from the Swedish west coast.	Poster presentation. Few other details available.	Kierkegaard <i>et al.</i> (2010a).
Herring (<i>Clupea harengus</i>) – dorsal muscle	Samples from ten sites along the Swedish coast from the Baltic to the North Sea (three individuals per site)	Detected in all samples from archived specimens collected in 2007, at a mean concentration of 12 ng/g lw (reported range 0.6 – 30 ng/g lw).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Kierkegaard <i>et al.</i> (2010a & 2012)
Grey seal (<i>Halichoerus grypus</i>) – blubber	Three individuals that drowned in fishing nets north of Västervik, Sweden in the autumn of 2008	Detected in all samples of blubber, at concentrations between the limit of detection and quantification (reported range 2.3 – 3.0 ng/g ww).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Kierkegaard <i>et al.</i> (2012)

D4 PBT/vPvB EVALUATION

Species	Location	Measured concentration	Comment	Reference
Herring gull (<i>Larus argentatus</i>) – liver	Samples from remote region around the west coast of Sweden	Not detectable in 9 samples ² in 2008 (method detection limit was between 1.41 and 2.6 µg/kg wet weight where reported).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Herring gull (<i>Larus argentatus</i>) – muscle	Samples from remote region around the west coast of Sweden	Not detectable in 9 samples ² in 2008 (method detection limit was between 1.39 and 2.6 µg/kg wet weight where reported).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Kittiwake (<i>Rissa tridactyla</i>) – liver	Samples from remote regions around Svalbard (Kongsfjorden and Liefdefjorden) ¹	Not detectable – 3.5 µg/kg wet weight or not detectable to 125 µg/kg lipid (detected in 4 out of 9 samples; the detection limit for the non-detectable samples range between <1.1 and <3.6 µg/kg wet weight or <50 to <139 µg/kg lipid).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Evenset <i>et al.</i> (2009)
Kittiwake (<i>Rissa tridactyla</i>) – blood	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 13 samples in 2008 (method detection limit in the range 3.07-9.43 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Polar cod (<i>Boreogadus saida</i>) – liver and whole fish	Samples from remote regions around Svalbard (Liefdefjorden, Billefjorden and close to Moffen) ¹	<3.4-9.2 µg/kg wet weight or <9.2-26 µg/kg lipid (detected in 5 out of 6 liver samples) 3.6-7.8 µg/kg wet weight or 129-231 µg/kg lipid (detected in 5 out of 5 whole fish samples)	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Evenset <i>et al.</i> (2009)
Sculpin ³ – liver	Samples from remote region around Svalbard (Liefdefjorden)	0.35 µg/kg wet weight (detectable in 1 out 18 samples ² in 2009; method detection limit was 0.19 to 2.60 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Adventfjorden) ⁴	0.45-3.38 µg/kg wet weight (detectable in 6 out of 16 samples ² in 2009; method detection limit was 0.19 to 2.60 µg/kg wet weight).		

D4 PBT/vPvB EVALUATION

Species	Location	Measured concentration	Comment	Reference
	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	2.6 µg/kg wet weight (detected in 1 out of 5 samples in 2008; method detection limit 1.32-2.21 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	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 2.6 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Sculpin ³ – whole body minus liver	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	0.55 µg/kg wet weight (detected in 1 out of 5 samples in 2008; method detection limit 0.35-0.39 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Sea urchin ³	Samples from remote region (the exact location is unclear but was either Kongsfjorden or Liefdefjorden)	Not detectable in 3 samples in 2008 (method detection limit 0.35-0.48 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Seal ³ blubber	Samples from remote region around Svalbard (Kongsfjorden) ⁴	Not detected in 10 samples ² in 2009 (method detection limit 1.36 to 2.60 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Shrimp (<i>Pandulus borealis</i>)	Samples from remote region around Svalbard (Kongsfjorden)	Not detectable in 3 samples (method detection limit 0.65-0.93 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)

D4 PBT/vPvB EVALUATION

Species	Location	Measured concentration	Comment	Reference
Shrimp ³	Samples from remote region around Svalbard (Liefdefjorden)	Not detected in 2 samples ² from 2008 (method detection limit 0.92-2.6 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	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 0.99-1.28 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Sprat ³	Samples from west coast of Sweden (Skagerrak)	Not detectable in 4 samples from 2008 (method detection limit 1.43-1.85 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
Zooplankton	Samples from remote region around Svalbard (Liefdefjorden)	Not detectable in 9 samples ² in 2009 (method detection limit was in the range 0.19 to 2.60 µg/kg wet weight).	Precautions were taken during sampling and analysis to avoid contamination with cyclic siloxanes.	Campbell (2010)
	Samples from remote region around Svalbard (Kongsfjorden) ⁴	Not detectable in 9 samples ² in 2009 (method detection limit was in the range 0.19 to 2.60 µg/kg wet weight).		
	Samples from remote region around Svalbard (Kongsfjorden)	0.98-1.5 µg/kg wet weight (detected in 3 out of 3 samples in 2008).		
	Samples from remote region (Nordkappsundet)	Not detected in 4 samples in 2009 (method detection limit was in the range 0.19 to 1.36 µg/kg wet weight).		

Note: 1) Marine sediment samples were also collected in Kongsfjorden and Liefdefjorden, Smeerenburgfjorden. D4 was not detected in any of the sediment samples (concentration typically <4 µg/kg dry weight).

- 2) The total number of samples here refers to the total number of sample 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.
- 3) The species name was not given.
- 4) Marine sediment samples were also collected in Kongsfjorden and Adventfjorden in 2009. D4 was not detected in 15 sediment samples from Kongsfjorden or 15 sediment samples from Adventfjorden (method detection limit 0.62 to 2.52 µg/kg wet weight).

4.3.4 Summary and discussion of bioaccumulation

A large amount of data is available on the bioaccumulation potential for D4. These are summarised below.

- A fish BCF of 12,400 l/kg was measured for fathead minnow. The BCF also appears to be above 2,000 l/kg for common carp, with a reported steady state BCF in the range 3,000 – 4,000 l/kg in two studies (and a kinetic BCF in the range 4,100 – 5,500 l/kg (without growth correction; higher if growth is taken into account)).
- The measured dietary BMF is 0.47 (steady state lipid normalised value) or 1.8 (growth corrected kinetic value; not lipid normalised) in rainbow trout. A growth corrected and lipid normalised BMF of between 0.509 and 0.753 has been measured in carp.
- Laboratory accumulation studies with invertebrates (*Lumbriculus variegatus*) imply bioaccumulation factors of the order of 6.7 to 20 (based on the concentration in whole organisms (mg/kg) divided by the concentration in sediment (mg/kg dry weight)). If it is assumed that exposure is mainly via pore water, the equivalent BCF for D4 is in the range 7,000-11,000 l/kg; however there is considerable uncertainty in these estimates.
- BSAF values (based on the lipid normalised concentration in biota/organic carbon normalised concentration in sediment) above one have been determined in several fish samples from rivers in Japan. In addition, a benchmarking study suggests that the BSAF for D4 is higher than that for PCB-180 in ragworm and flounder in a UK estuary.
- A mixed picture is presented by field monitoring studies:
 - The Lake Pepin field study implies that the trophic magnification factor (TMF) of D4 is less than one in this food web. The levels of D4 are highest in benthic invertebrates (BSAFs >1 are derived for the benthic invertebrates and also 14 out of 16 fish species) and the results suggest that uptake from food rather than bioconcentration is the dominant uptake route in this food chain. However, specific BMFs for three fish species were one or above. In addition, when invertebrates are removed from the data set, the TMF findings for fish are less conclusive (the TMF is in the range of 0.21 to 1.7; the slope of the plot of ln [concentration] against trophic level is not statistically significant ($p > 0.05$) and the correlation coefficient is very low (it should be noted however that by necessity this analysis was carried out on the mean concentrations per species and it would have been better to carry out the analysis on the individual data points).
 - The Lake Opeongo field study suggests that biomagnification may be occurring in a pelagic food web, although the analytical background concentrations were relatively high and variable, and it is also possible that contamination might have occurred during sampling. Further work is needed to clarify this issue. Powell *et al.* (2010a) indicated that it was originally intended that further fish from Lake Opeongo 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 for logistical reasons. A repeat study has not been performed.

- The Oslofjord field study shows that the overall TMF for D4 is below one in this food web. There is only a small probability that the TMF could be one or above for the Inner Oslofjord food chain (around a 7 per cent probability for a benthic food chain and around a 21 per cent probability for a pelagic food chain). However, there is some uncertainty associated with this conclusion. For example, RIVM (2012) re-analysed the data and estimated TMFs of 0.60, 3.27, and 1.66 for the benthic based food chain, the pelagic based food chain, and the whole ecosystem, respectively. Although none of the slopes is significantly different from zero, the slope for the pelagic based food web is close to significant ($p=0.07$; 95% CI: -0.1371 to 2.504), leaving the 90% confidence interval of the TMF to vary from 0.87 to 12.2. In addition, the BMF for Atlantic cod-shrimp was found to be 1.0 for Inner Oslofjord and 1.4 for Outer Oslofjord. This is a significant finding as it was known that at the time of sampling the Atlantic cod were feeding mainly on shrimp.
- Although it was not possible to determine a TMF for D4 in the Lake Mjøsa field study, it is relevant to note that there was a possible positive trend in D4 concentrations with the concentrations found in brown trout (highest trophic level) being higher than for mysids, vendace and smelt. The situation with zooplankton, and the relative concentrations within mysids, vendace and smelt is not clear owing to the large number of samples for which D4 was not detectable. This was a pelagic food chain.
- The Tokyo Bay field study shows that the overall TMF for D4 is below one in this pelagic food web. However, some individual BSAF and BMF values were above one in this study.

RIVM (2012) suggested that the apparent differences between studies could possibly be the result of a deviation from thermodynamic equilibrium between sediment and water for those systems that receive the substance adhered to suspended particles from a sewage treatment plant (rather than from atmospheric deposition or direct emission). In food chains that originate from the pelagic environment, a different picture is obtained, as suggested for the pelagic part of the food chain in the Oslofjord and Lake Mjøsa (and to some extent Lake Opeongo), but not Tokyo Bay.

- Two new studies are available on the levels of D4 present in biota in remote regions. These two studies are important because specific precautions were taken to avoid possible contamination of the samples with D4 during sampling, processing and analysis (such inadvertent contamination could have adversely affected the findings from earlier studies).

In one of the studies (Evenset *et al.*, 2009), D4 was detected in samples of fish (Atlantic cod and polar cod) and birds (Kittiwake liver). Although the levels are generally low (often close to the limit of detection), some samples had higher levels (up to 125 µg/kg lipid in Kittiwake liver and 231 µg/kg lipid in samples of polar cod). It should be noted that the overall sample numbers were also small.

The second study (Campbell, 2010) found that D4 was detectable in some samples of Atlantic cod liver, sculpin liver and whole body minus liver, zooplankton and glaucous gull liver. D4 was not detectable in the other species sampled. Where detectable, the concentration of D4 was generally low. The highest concentrations found were in Atlantic cod liver from Adventfjorden (up to 9.2 µg/kg wet weight, with D4 being detectable in 10 out of 11 samples analysed), sculpin liver from Adventfjorden (up to 3.38 µg/kg wet weight, with D4 being detectable in 6 out of 16 samples) and glaucous gull liver from Bjørnøya (up to 6.5 µg/kg wet weight with D4 being detectable in 2 out of 8 samples). The levels found in samples from Adventfjorden may reflect a local source of emission.

- The levels of D4 in biota are generally highest in samples collected from close to sources of emission at levels up to 900 µg/kg wet weight in fish (i.e. close to 1 mg/kg or 1 ppm). Although the data generally show that overall trophic dilution is occurring in the food chains studied it is important to note that D4 is detectable in a wide range of species and trophic levels in the food chains that have been sampled (for example Lake Pepin and Oslofjord; see Section 4.3.3.2), where sources of D4 exist.
- Accumulation in mammals appears to be lower than in other aquatic organisms, based on limited field data. For example D4 was not detectable in three mink in the Lake Pepin study. No information is available for birds from similar food chains (though as noted above, D4 has been detected in bird livers in the Arctic).

Overall the available field data show that D4 is detectable in biota in the environment, particularly in areas close to sources of release, but in some cases in samples from more remote regions. Although overall TMFs below one are obtained in three of the four food chains studied where a TMF could be derived (the third study needs to be repeated because the samples might have been contaminated during collection; it was not possible to derive a TMF for D4 in a fifth study although it was detectable in all fish samples from the highest trophic position), some BMFs are above one for these food chains and the TMF could be greater than one for the fish studied in Lake Pepin and also Oslofjord, depending on how the data are interpreted. In particular, the BMF for Atlantic cod-shrimp was found to be 1.0 for Inner Oslofjord and 1.4 for Outer Oslofjord. This is a significant finding as it was known that at the time of sampling the Atlantic cod were feeding mainly on shrimp.

4.4 Secondary poisoning

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

A review of information related to human health is included in EA (2009) and a more recent evaluation by the Scientific Committee on Consumer Safety is also available (SCCS, 2010). The currently agreed classification under Directive 67/548/EEC of D4 for health hazard is as follows.

- Repro. Cat 3.
- R62: Possible risk of impaired fertility.

The equivalent classification under Regulation (EC) No. 1272/2008 is as follows.

- Hazard class and category: Repr. 2.
- Hazard statement: H361f: Suspected of damaging fertility.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES

Not relevant for this dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Summary of information from existing evaluation

D4 is not toxic to fish when they are exposed for short durations (e.g. up to 96 hours) at concentrations up to the water solubility limit. Following longer exposure toxicity to fish is apparent and the NOEC for *Oncorhynchus mykiss* was determined to be 4.4 µg/l in a 14-day prolonged acute study and ≥4.4 µg/l (the highest concentration tested; no adverse effects were seen at this concentration) in a 93-day fish early life stage study (EA, 2009).

New information

As part of a GLP bioconcentration study, a limit test was carried out to investigate the toxicity of D4 to Japanese medaka (*Oryzias latipes*) over 96 hours (CERI, 2007; details of the bioconcentration study are reported in Section 4.3.2.2). Very few details of this test were provided. The test substance had a reported purity of 100.0 per cent. The nominal

concentration was 5.6 mg/l; the solution was prepared with a dispersant (hydrogenated castor oil) and a solvent (N,N-dimethylformamide; present at around 1,120 mg/l in the test solution which was around 1/10th of its 96-h LC₅₀ value) with renewal of test water every 8 to 16 hours. Under these conditions, the 96-h LC₅₀ for D4 was >5.6 mg/l at 24°C. This result is consistent with the previous data on the short-term toxicity of D4 to fish, where no adverse effects have been seen at concentrations up to the water solubility limit.

7.1.1.2 Aquatic invertebrates

Summary of information from existing evaluation

D4 is not toxic to aquatic invertebrates when they are exposed for short durations (e.g. up to 96 hours) at concentrations up to the water solubility limit. Following longer exposure toxicity is apparent and the long-term (21-day) NOEC for *Daphnia magna* is 7.9 µg/l (EA, 2009).

New information

No new information is available.

7.1.1.3 Algae and aquatic plants

Summary of information from existing evaluation

The available results from toxicity tests with algae were not considered valid for use in risk assessment (EA, 2009). Although there is some uncertainty over the actual algal NOEC EA (2009) considered that the available QSAR estimates for algae suggest that they should not be significantly more sensitive to D4 than fish and invertebrates.

New information

No new information is available.

7.1.1.4 Quantitative structure-activity relationships (QSARs)

Summary of information from existing evaluation

EA (2009) carried out some QSAR estimates for the algal toxicity using both the methods given in the REACH Guidance Document and also the USEPA EPI (v3.12) program. The

estimates for the 72- and 96-hour EC₅₀ were in the range 5.7×10^{-3} mg/l to 0.27 mg/l and the algal NOEC (96-h Chv²⁸) was 0.16 mg/l.

New information

No new information is available.

7.1.1.5 Sediment organisms

Summary of information from existing evaluation

EA (2009) summarises the available sediment toxicity data. Long-term (28-day) sediment toxicity studies were available for *Chironomus riparius*. The NOEC determined for this species was 44 mg/kg dry weight. The sediment used in this study had an organic carbon content of 4.1 per cent and normalising the NOEC to a standard organic carbon content of 5 per cent gives a NOEC_{standard} of 54 mg/kg dry weight.

Sediment toxicity studies have also been carried out with *Chironomus tentans* over a shorter period of 14 days. The lowest NOEC from these studies was 54 mg/kg dry weight for a sediment with 4.1 per cent organic carbon content.

New information

The results of further recent toxicity tests with sediment organisms are reported in Environment Canada (2011). The test reports for these studies have not been provided to the rapporteur and so a brief summary of the results as reported in Environment Canada (2011) is provided below.

- A 28-day toxicity test with *Lumbriculus variegatus* was carried out using the OECD 225 Test Guideline (Picard, 2009). The sediment used was a natural sediment with an organic carbon content of 2.2 per cent and a pH of 6.5. The test was carried out under static conditions and the concentrations measured in the sediment were found to decline slightly over the course of the experiment (the mean concentration measured was used for reporting of the results). A statistically significant reduction in the mean number of surviving organisms was seen in the two highest exposure groups (19 and 32 mg/kg dry weight) when compared with the control groups but no treatment related effects were seen on mean biomass. The 28-d EC₅₀ for survival was determined to be >32 mg/kg dry weight. No NOEC was reported but, taking 19 mg/kg dry weight as the LOEC, the NOEC would be the next lowest concentration of 13 mg/kg dry weight. Based on this value, the 28-d NOEC_{standard} would be 30 mg/kg dry weight.
- A second 28-day toxicity test with *Lumbriculus variegatus* was carried out by Krueger *et al.* (2009). The method used again followed the OECD 225 Test Guideline but in this case a flow-through test system was used for the overlying water. The

²⁸ This is actually estimated as a chronic value (Chv) which most probably represents the geometric mean of the lowest observed effect concentration (LOEC) and the NOEC.

sediment used was artificial sediment with an organic carbon content of 2.4 per cent and a pH of 7.3 and the mean measured sediment concentrations used in the study were 0.73, 1.5, 3.1, 5.8, 11 and 38 mg/kg dry weight. The endpoints considered were survival/reproduction (based on the total number of organisms present at the end of the test) and growth (dry weight). No treatment-related effects on growth were seen. Significant differences were found in all treatment groups compared with the control group based on the mean number of worms per replicate (survival/reproduction) and the 28-d EC₅₀ for this effect was 9.32 mg/kg dry weight. The NOEC was therefore <0.73 mg/kg dry weight (the 28-d NOEC_{standard} would be <1.5 mg/kg dry weight).

7.1.1.6 Other aquatic organisms

No data.

7.1.1.7 Summary of aquatic toxicity data

The long-term NOEC for water exposure of fish is ≥ 4.4 $\mu\text{g/l}$ from a long-term (93-day) toxicity study with *Oncorhynchus mykiss* (this was the highest concentration tested in the study and no adverse effects were observed). However, a 14-day NOEC with the same species also gave a NOEC of 4.4 $\mu\text{g/l}$: although normally considered to be a prolonged acute toxicity test, this is consistent with the limit NOEC obtained in the 93-day fish early life stage study and so the overall NOEC for fish is assumed to be around 4.4 $\mu\text{g/l}$. It is noted that this substance has effects on mammalian reproduction (see below), and no data are available to determine whether it affects fish reproduction.

For invertebrates the 21-day NOEC with *Daphnia magna* is 7.9 $\mu\text{g/l}$.

No reliable data are available on the toxicity of D4 to algae but consideration of QSAR data suggests that algae should not be more sensitive to D4 than fish or invertebrates.

Long-term sediment toxicity data are also available. The lowest NOEC is <0.73 mg/kg dry weight, obtained in a 28-day study with *Lumbriculus variegatus* (although it should be noted that a higher NOEC of 13 mg/kg dry weight was found for this species in a second study). Normalising this value to a standard organic carbon content gives a NOEC_{standard} of <1.5 mg/kg dry weight (for comparison with the pelagic organisms, the equivalent pore water concentration, assuming that the effects seen occur via exposure via pore water, is estimated to be around <2 $\mu\text{g/l}$ using the methods outlined in the REACH Guidance). This value is well below the solubility limit of the substance in pure water.

8 PBT AND vPvB

8.1 Comparison with criteria from Annex XIII

Persistence

A substance is considered to be persistent (P) if it has a half-life >60 days in marine water or >40 days in fresh or estuarine water, or >180 days in marine sediment or >120 days in freshwater or estuarine sediment or soil. A substance is considered to be very persistent (vP) if it has a half-life >60 days in marine, fresh or estuarine water, or >180 days in marine, freshwater or estuarine sediment, or soil.

D4 is not readily biodegradable but it does degrade in water by hydrolysis. The half-life for hydrolysis is dependent on the pH and temperature. EA (2009) reviewed the available data and recommended the following half-life values.

- Hydrolysis half-life at pH 7 and 12°C (freshwater) = 16.7 days.
- Hydrolysis half-life at pH 8 and 9°C (marine) = 2.9 days.

The main product from the hydrolysis reaction is known to be dimethylsilanediol, which itself is unlikely to possess PBT or vPvB properties. Therefore, based on its hydrolysis half-life in water, D4 would not meet the P or vP criteria.

However, D4 is highly adsorptive to organic matter in suspended solids, sediment and soils, so the relevance of hydrolysis for such a hydrophobic substance is low. D4 has a very long degradation half-life in sediment, of the order of 242 days at 24°C under aerobic conditions and 365 days at 24°C under anaerobic conditions. The half-life at lower temperatures (e.g. 12°C) would be expected to be longer than these values. D4 therefore meets the Annex XIII criteria for a persistent (P) and very persistent (vP) substance. Persistence in sediment is also supported by the sediment core data from Lake Pepin.

When considering the persistence of D4 in any one medium it is important to recognise that it is highly volatile and so can be lost from water bodies (and soil) by this mechanism (and this is likely to be the major removal mechanism in some water bodies and soil). Therefore to take account of these factors it is necessary to consider the persistence of the substance in the whole environment rather than just the water, sediment or soil compartment alone.

Various modelling approaches have been used to estimate the expected environmental distribution and overall persistence of D4. Although these generally predict a relatively short persistence in the water column (owing to loss from volatilisation and hydrolysis) the models also predict that a significant proportion of D4 will distribute to the sediment phase and that the persistence of D4 in sediment may be much longer than found in the water column. The actual fraction of D4 distributed to sediment and its persistence in sediment in any one system will depend on a number of site-specific factors including the pH, the water depth, the temperature, the sediment deposition rate, the concentration of particulate and dissolved organic carbon, etc. For the systems recently investigated the effective half-life of D4 in sediment was estimated to be around 72 days (Lake Pepin), 285 days (half-life in Inner Oslofjord) and 342 days (Lake Ontario). However, sediment cores from Lake Pepin suggest a half-life of D4 of up to 2.5 years in sediment, which is longer than predicted in the modelling exercise.

The available modelling studies on long-range transport potential of D4 (reported in both this evaluation and the EA (2009) report) suggest that although D4 can be transported to remote regions to some extent via the atmosphere, significant deposition in remote regions is unlikely.

It should also be noted that although the models used generally predict an overall short persistence in water and air (and the environment as a whole²⁹), D4 has been found in samples from remote regions (for example sediment from the Barents Sea and biota from Svalbard). The interpretation of the monitoring data in remote regions is complicated by two main issues: firstly, the possibility of inadvertent contamination of the samples with D4 during collection and analysis unless adequate controls are taken to limit this and secondly, the likelihood of local sources of emission in some remote areas. Thus, although the actual transport process is not clear (local sources, sediment transport, food chain transfer and/or aerial deposition) these data do suggest that D4 is sufficiently persistent to allow occurrence in biota in remote regions.

An expert panel workshop hosted by the Global Silicones Counsel has considered the relative importance of overall persistence compared with compartment-specific persistence for D4 (Global Silicones Counsel, 2009). The workshop participants agreed that “it is not appropriate to imply that overall persistence is more important than compartment-specific persistence because the overall persistence is derived by adding the persistence from each of the relevant environmental compartments” and that “persistence should be based on a compartment of concern, not persistence in each compartment”.

Overall the available data suggest that D4 can be considered to meet the Annex XIII criteria for a persistent (P) and very persistent (vP) substance based on the measured and predicted half-lives in sediment.

Bioaccumulation

According to Annex XIII of REACH, a substance is considered to be bioaccumulative (B) if it has a bioconcentration factor (BCF) >2,000 l/kg or very bioaccumulative (vB) if it has a BCF >5,000 l/kg. However, the REACH Annex XIII criteria are currently being discussed in terms of using a weight of evidence approach in the assessment of B and vB. Given the large amount of data available for D4, a weight of evidence approach is considered appropriate in this case. In order to facilitate this, the available evidence has been categorised in terms of providing unequivocal/strong support, equivocal support or not support for the substance being considered to be B or vB.

- i) Information providing **unequivocal** support for B or vB under the current Annex XIII criteria:
 - The steady state BCF for D4 has been determined as 12,400 l/kg in fathead minnows. Therefore D4 clearly meets the Annex XIII criteria for B and vB. This is supported by additional BCF data for common carp, which significantly exceed the B criterion.

²⁹ The overall persistence estimated in global-type models can be considered as effectively the weighted average of the persistence in the individual environmental compartments. Since, at steady-state, a high proportion of D4 in the model is expected to be in the atmosphere, the overall persistence is governed mainly by the persistence in air, and to a lesser extent by the persistence in water. Thus an overall relatively short environmental persistence does not preclude a high persistence of D4 in sediment.

- ii) Information providing **unequivocal** support that D4 is bioaccumulative or very bioaccumulative in the broader sense:
- No unequivocal evidence to support this.
- iii) Information providing **equivocal** support that D4 is bioaccumulative or very bioaccumulative in the broader sense:
- Laboratory accumulation studies with invertebrates (*Lumbriculus variegatus*) imply bioaccumulation factors of the order of 6.7 to 20 (based on the concentration in whole organisms (mg/kg) divided by the concentration in sediment (mg/kg dry weight)). If it is assumed that exposure is mainly via pore water, the equivalent BCF for D4 is in the range 7,000-11,000 l/kg, however there is considerable uncertainty in these estimates. In addition, BSAF values of one or above are derived for benthic invertebrates and also 14 out of 16 fish species in the Lake Pepin field study.
 - BSAF values (based on the lipid-normalised concentration in biota/organic carbon-normalised concentration in sediment) above 1 have been determined in several fish samples from rivers in Japan. In addition, a benchmarking study suggests that the BSAF for D4 is higher than that for PCB-180 in ragworm and flounder in a UK estuary.
 - A fish feeding accumulation study is available for D4 (reviewed in detail in EA (2009) and summarised in Section 4.3.2.1). Although the steady-state BMF is below one, a significant proportion of the depuration seen in this study appears to result from growth dilution. Therefore the BMF for D4 could be above one in fish that are not growing rapidly, as shown by the growth-corrected values in Section 4.3.2.1.
 - Field studies provide mixed information on the bioaccumulation behaviour of D4, which could be linked to different sources of the substance that in turn might lead in some cases to deviation from thermodynamic equilibria. For example, there is some evidence from pelagic based food chains (Oslofjord, and to some extent Lakes Mjøsa and Opeongo, but not Tokyo Bay) that the TMF may exceed one, depending how the data are interpreted. In contrast, food chains dominated by benthic exposure (related to the introduction of the substance adsorbed to suspended matter from sewage treatment works) show TMFs below one. Individual feeding relationships should not be overlooked. For example, the BMF for Atlantic cod-shrimp was found to be 1.0 for Inner Oslofjord and 1.4 for Outer Oslofjord. This is a significant finding as it was known that at the time of sampling the Atlantic cod were feeding mainly on shrimp. In Lake Pepin, the levels of D4 are highest in benthic invertebrates and the results suggest that uptake from food rather than bioconcentration is the dominant uptake route in this food chain. However, specific BMFs for three fish species were also one or above (there is some uncertainty in these values). Similarly, although the TMF is below one in the Tokyo Bay field study, BMFs above one were obtained for a number of predator-prey interactions when Japanese sea bass are considered as the predator.
 - D4 has been found to be present in a wide range of organisms, particularly fish and aquatic invertebrates, but also birds. Levels are generally highest in

samples collected from close to sources of emission (levels of up to 900 µg/kg wet fish have been determined in such locations). D4 is also found in biota in regions with low background levels in abiotic media (e.g. Svalbard). The concentrations found in biota from remote regions are generally very low (close to the analytical detection limit or not detectable) but higher levels have also be noted (up to 125 µg/kg lipid in Kittiwake liver and 231 µg/kg lipid in samples of polar cod in one study). It should be noted that in these studies in remote regions a significant number of the samples had not detectable levels of D4 and it is possible that these elevated concentrations reflect local sources in remote regions rather than long-range transport of D4 to remote regions (although it is not clear if such local sources can explain all of the findings).

iv) Studies providing contrary/non-supporting information that D4 is bioaccumulative or very bioaccumulative in the broader sense:

- A growth-corrected and lipid-normalised dietary BMF of between 0.509 and 0.753 has been measured in carp.
- The bioaccumulation potential for D4 in mammals appears to be much lower than may be expected based on the fish BCF or log K_{ow} alone, particularly in relation to inhalation exposure. The available data (reviewed in detail in EA (2009)) show that D4 is rapidly eliminated from mammalian systems (by exhalation and metabolism) and so it has a low potential for accumulation in mammals. However, it was noted that the pharmacokinetic behaviour after oral exposure is complex and does not appear to be as well understood as the inhalation and dermal routes of exposure (although rapid metabolism following oral exposure was thought to occur). Although the accumulation in mammals appears to be lower than in other aquatic organisms, the top predator in some food chains may not be air breathing, and no information is available for birds.

One of the principal concerns around bioaccumulative substances is the likelihood that they will increase in concentration up the food chain. In this case, there is no *unequivocal* evidence that D4 is biomagnifying in the environment. At the same time, whilst trophic magnification factors above one might be considered to be the ultimate proof of a substance's ability to bioaccumulate significantly (e.g. Weisbrod *et al.* (2009) and Gobas *et al.* (2009))³⁰, field studies must be treated with caution due to the limitations of sampling, uncertainties in food chain relationships and analytical variation. Five food webs have been studied, and all provide some indication that the BMF may be greater than one for some predator-prey relationships (e.g. the BMF for Atlantic cod-shrimp was found to be 1.0 for Inner Oslofjord and 1.4 for Outer Oslofjord - at the time of sampling the Atlantic cod were feeding mainly on shrimp; the BMFs for Japanese sea bass as predator were above one for three of the four predator-prey relationships considered for Tokyo Bay). D4 is clearly present in a variety of species even in regions with low background levels in abiotic media (e.g. in Svalbard, up to

³⁰ It should be noted that in relation to biomagnification potential the current REACH Guidance states that "However, because food chain transfer and secondary poisoning are basic concerns in relation to PBT and vPvB substances, an indication of a biomagnification potential can on its own right be considered to conclude that a substance meets the B or vB criteria but absence of such a biomagnification potential cannot be used to conclude that these criteria are not fulfilled". Taken from Section R.11.1.3.2 of the Guidance on information requirements and chemical safety assessment, Chapter R.11: PBT Assessment.

125 µg/kg lipid in Kittiwake liver and 231 µg/kg lipid in samples of polar cod), although local sources of emission in such areas are possible. Accumulation in sediment invertebrates has been demonstrated. Finally, whilst accumulation in mammals appears to be lower than in other aquatic organisms, the top predator in some food chains may not be air breathing, and no information is available for birds.

In conclusion, D4 meets the Annex XIII criteria for B and vB based on the fish BCF and overall weight of evidence.

Toxicity

A substance fulfils the toxicity criterion (T) when:

- the long term no observed effect concentration (NOEC) for marine or freshwater organisms is less than 0.01 mg/l (10 µg/l); or
- the substance is classified as carcinogenic (category 1 or 2), mutagenic (category 1 or 2) or toxic for reproduction (category 1, 2 or 3)³¹; or
- there is other evidence of chronic toxicity, as identified by the classifications T, R48, or Xn, R48, according to Directive 67/548/EEC³².

D4 has a long-term fish NOEC of around 4.4 µg/l and a long-term NOEC of 7.9 µg/l with *Daphnia magna*. In addition, it is classified as toxic to reproduction category 3. Therefore it can be concluded that D4 meets the Annex XIII criteria for toxicity (T) based on both aquatic and mammalian end points.

8.2 Assessment of substances of an equivalent level of concern

Not relevant for this dossier.

³¹ The CLP Regulation (EC) No 1272/2008 amends this to be substances classified as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2).

³² The CLP Regulation (EC) No 1272/2008 amends this to be “there is evidence of chronic toxicity, as defined by the classifications STOT (repeated exposure), category 1 (oral, dermal, inhalation of gases/vapours, inhalation of dust/mist/fume) or category 2 (oral, dermal, inhalation of gases/vapours, inhalation of dust/mist/fume, according to Regulation (EC) No 1272/2008”.

8.3 Emission characterisation

Since this dossier relates to evaluation of the data in the context of whether the PBT criteria are met, emission characterisation is not relevant. A detailed assessment of the emissions of D4 throughout the lifecycle is included in EA (2009).

8.4 Conclusion of PBT and vPvB or equivalent level of concern assessment

Based on the available data, D4 meets the REACH Annex XIII criteria for a PBT substance, and also a vPvB substance.

INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS

Information on the uses, exposure and environmental risks of D4 throughout its lifecycle are included in EA (2009). No information has been sought on alternatives.

OTHER INFORMATION

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APPENDIX 1 – OTHER NEW DATA AND ON-GOING/PLANNED STUDIES

This appendix outlines other data that have become available since EA (2009) was published. These studies are considered to be more relevant to the quantitative risk assessment aspects (i.e. PEC/PNEC-type assessment) than the PBT and vPvB evaluation and so they have not been reviewed in detail. In some cases, they provide similar information to that already considered in EA (2009) (e.g. journal publications of industry test reports). In addition, information has been received from Industry on a number of on-going or planned research initiatives. Brief details of these studies are also given. Other relevant papers may be found in the summary of additional studies for D5.

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