Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Appendix 2 ADDENDUM to D5 PBT Evaluation Fact Sheet of February 2013 (EA, 2013)

Environmental fate and behaviour studies are still being conducted by the producers of D5, academic research groups and other regulators. This addendum summarises relevant studies that have been produced or published since 2011, which was the cut-off date for the previous version of the PBT fact sheet. Most of these have been brought to the attention of the dossier submitter by the D5 producers, but a targeted literature search was also carried out using PUBMED covering the years 2012 and 2013. The focus of the search was on papers relevant to the PBT assessment (particularly bioaccumulation). The references summarised below are included in the main reference list. This appendix also briefly considers additional papers highlighted during the public consultation (PC) by the Member State Committee.

Biodegradation

As part of a study into the fate and behavior of D5 in a municipal waste water treatment plant in Beijing City, China, Xu *et al.* (2013) carried out an *in vitro* study on the anaerobic degradation of D5. The test used a batch system consisting of sealed glass vials containing 40 mL of an activated sludge-liquid mixture obtained from the anaerobic tank of the waste water treatment plant. The sludge mixture had a dry solids content of 10 g/L and a pH of 6.5-6.8. D5 was added to the vial at either 2, 5 or 10 µg/L and then incubated at 30°C with shaking for up to 60 hours under a nitrogen-carbon dioxide headspace (approximately 20 mL). The amount of D5 present in the liquid phase and the headspace was determined at intervals (0, 10, 20, 40 and 60 hours). Sterile sludge was used as a control.

Degradation of D5 in this test system was around 9.1-32.7% after 10 hours and 44.4-62.8% after 60 hours (the figures refer to both D4 and D5 combined). D5 was found to be relatively stable in the sterile control. Xu *et al.* (2013) concluded that degradation of D5 during anaerobic waste water treatment would contribute to its removal. (This study is not mentioned in the October 2014 update of the CSRs.)

Bioaccumulation

Studies performed by the Japanese regulatory authorities

A GLP bioconcentration study with Common Carp (*Cyprinus carpio*) has been carried out using D5 (purity 97.3 per cent) according to the OECD TG 305 method (CERI, 2010). It is currently available only in Japanese but the raw data presented allow the reported bioconcentration parameters to be verified. A pre-test with Japanese Medaka (*Oryzias latipes*) gave a 96-h LC_{50} for D5 of >45 mg/L. The bioconcentration test was carried out using two nominal ^{14}C-D5 exposure concentrations (1 µg/L and 0.1 µg/L) in a continuous-flow system. A dispersant (hydrogenated castor oil) and possibly a solvent were used to prepare the test solutions. A control containing the dispersant/solvent was also prepared. The total duration of the test was 101 days, consisting of a 60-day uptake phase followed by a 41-day depuration phase.

The fish had a length of between 6.2 and 12.0 cm at the start of the test and were fed at a rate around 2 per cent of body weight per day over the duration of the study. The test was carried out at a temperature of 24-25 °C and test water had a pH between 7.7 and 7.9 and a dissolved oxygen concentration of between 6.4 and 7.7 mg/L throughout the test.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

The concentrations in water were analysed on day 4, 7, 21, 35, 49 and 60 of the uptake phase. The concentrations were found to be stable, with the mean concentrations (±standard deviation) at the two exposure levels being 1.03 (±0.047) µg/L and 0.0981 (±0.013) µg/L. The concentration in fish was determined on the same days as above, and steady state was found to be reached by day 35. The analytical method used was GC-MS and so presumably determined the concentration of parent compound. Mean measured steady-state concentrations in whole fish were 13,070 µg/kg for the 1.03 µg/L treatment group and 1,247 µg/kg for the 0.0981 µg/L treatment group. The mean measured concentration at day 41 of depuration ranged from 3860-4800 µg/kg for the 1.03 µg/L treatment group, and 298-470 µg/kg for the 0.0981 µg/L treatment group.

The steady state BCFs determined for the last three sampling times were 11,932 L/kg (day 35), 12,336 L/kg (day 49) and 13,584 (day 60) at the 1.03 µg/L treatment level and 11,278 L/kg (day 35), 11,769 L/kg (day 49) and 13,100 (day 60) at the 0.0981 µg/L treatment level. The mean BCF at steady state was 12,617 L/kg at the 1.03 µg/L treatment level and 12,049 L/kg at the 0.0981 µg/L treatment level. The lipid content of the fish was 5.96 per cent at the start of the test and 5.45 per cent at the end of the test (mean over the test is therefore 5.71 per cent). Normalising the steady state values to a "standard" lipid content of 5 per cent would reduce the BCFs to 11,048 L/kg for the 1.03 µg/L treatment and 10,550 L/kg for the 0.0981 µg/L treatment.

The test substance was found to depurate only relatively slowly, with the concentration declining to around 24-38 per cent of the steady state concentration by day 41 of depuration. The depuration half-life was estimated to be between 19 and 22 days. No other kinetic parameters were determined in the CERI (2010) report but the raw data given in the report allow a more detailed kinetic analysis to be undertaken. When this is done for the 1.03 µg/L exposure level (see Figure A2.1), the uptake rate constant (k_1) can be estimated as 503.6 L/kg/day and the overall depuration rate constant (k_2) can be estimated as 0.0315 day^{-1}, giving a kinetic BCF of 15,998 L/kg. Similarly, for the 0.0981 µg/L group, the k_1 value determined is 519.5 L/kg/day, and the k_2 value is 0.0362 day^{-1} giving a kinetic BCF of 14,350 L/kg[1].

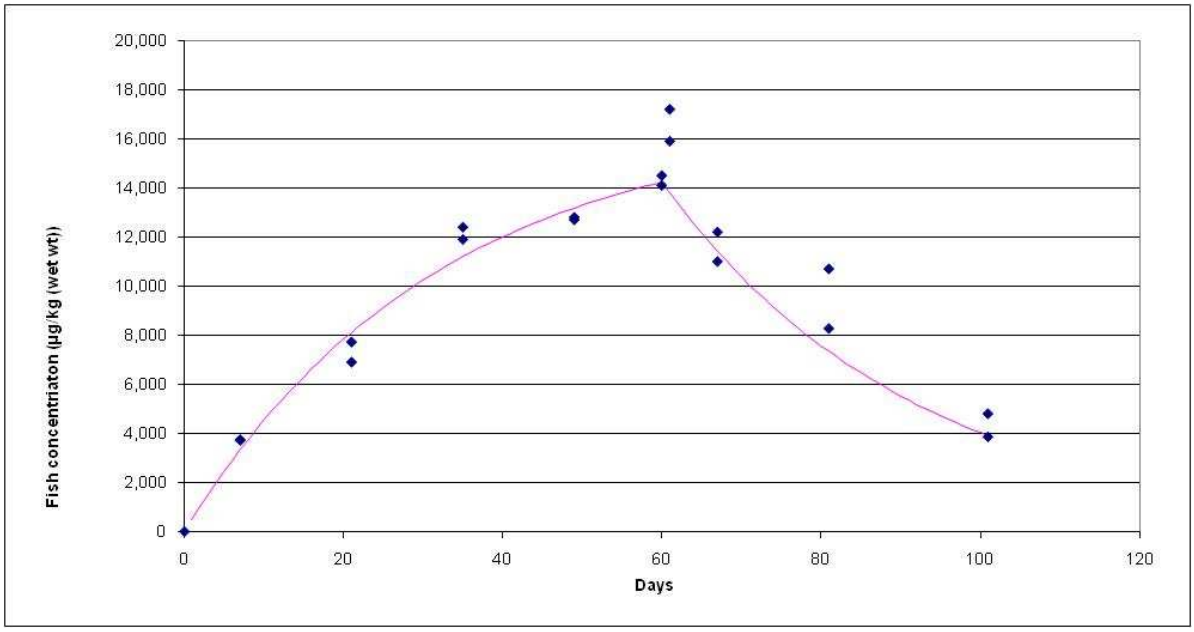
Normalising these kinetic BCFs to a 5 per cent lipid content gives kinetic BCFs of 14,009 L/kg and 12,566 L/kg respectively. In both cases the concentration in fish measured on day 1 of depuration was slightly higher than that measured on day 60 of uptake.

No data on the growth of fish were reported in the CERI (2010) study but, by comparison with a similar study with D4 (CERI, 2007), it would be expected that significant fish growth would have occurred. The rate constant for growth dilution estimated from the CERI (2007) was around 0.016-0.017 d^{-1}, which is around half of the overall depuration rate constant measured in the study with D5. Thus, if the fish in the CERI (2010) D5 study were growing at a similar rate to those in the D4 study, the growth corrected kinetic BCFs would be around a factor of two higher than those

[1] One of the papers submitted during PC ["Does D5 meet PBT or vPvB Criteria? *Regulation in the context of developments in science.* A review by CES 25 November 2014"] includes a brief summary of this study. This gives the measured concentration of the nominal 1 µg/L treatment group as 0.103 µg/L. The authors of this summary estimate the depuration rate constants (obtained from the slope of a plot ln [Cfish] against time) as 0.031 d^{-1} for the higher treatment group and 0.0355 d^{-1} for the lower treatment group. As the authors state that no data appears to be reported on fish weight or length, these depuration rates were not growth corrected. A crude estimate for the uptake rate was made from a visual fit of a one compartment, first order kinetic model to the uptake phase Cfish data. The uptake rate constant was thought to be in the region of 500 d^{-1} for both treatment groups. On this basis, the kinetic BCF would be approximately 14,000-16,000 L/kg.

given above. In addition, the reported "steady state" BCF may be misleading.

Figure A2.1 Plot showing fit to the experimental data for the CERI (2010) bioconcentration study for the 1.03 µg/L exposure level



Overall the CERI (2010) study appears to be well carried out. The results show that the BCF for D5 in carp is above 10,000 L/kg. (This study is not mentioned in the October 2014 update of the CSRs.)

Dietary bioaccumulation in Rainbow Trout

Documents submitted during PC cite a study in Rainbow Trout *Oncorhynchus mykiss* by Woodburn *et al*. (2013). This is a formal publication of a study report already evaluated by the DS and summarised in the main report (Dow Corning, 2006b; full details of this study are provided in EA, 2009). The DS has not evaluated the published article, but notes that although some of the derived BMF values are different to those quoted in this report, the overall conclusion is the same (i.e. the lipid-normalised steady state BMF is below 1, but the kinetic BMF is above 1 when growth is taken into account). The values cited in this report are consistent with those in the CSRs (October 2014 update).

BSAF in marine fish

Industry documents submitted during PC cite a study by Hong *et al*. (2014), which estimated a BSAF value for D5 of 0.103 ± 0.077 in a marine fish (*Hexagrammos otakii*) sampled from a site northeast of China. This has not been evaluated by the DS. However, the registrants suggest that whilst this species may feed on benthic organisms, it does not appear to live within the sediment. (This study is not mentioned in the October 2014 update of the CSRs.)

Study of allometric relationships for Atlantic Cod liver concentrations
A further study briefly summarised in industry documents submitted during PC is that of

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Warner *et al*. (2014). This study has not been evaluated by the DS. However, it indicates that D5 concentrations in Atlantic Cod (*Gadus morhua*) livers (n=20) collected at two locations near Tromsø, Norway in November 2010 and April 2011 were negatively correlated with fish length and weight, indicating a greater elimination capacity compared to uptake processes with increasing fish size. D5 was detected in all livers (including from Nipøya, considered a remote location in this study). The arithmetic mean liver concentration was 582 (range 200 – 1,110) µg/kg ww at the first location and 164 (range 13.8 – 831) µg/kg ww at the second. Lipid normalized concentrations for D5 were around one order of magnitude greater than those observed for PCB-153 and -180 (generally considered to be bioaccumulative substances), suggesting efficient uptake of D5 from the surrounding environment. Stomach contents of fish collected at the two sites were similar, so the difference in concentration was not linked to dietary feeding (more likely it was linked to distance from the pollution source). This study suggests that relationships between allometric measurements and D5 concentrations should be taken into account in future field studies of bioaccumulation potential. (This study is not mentioned in the October 2014 update of the CSRs.)

Norwegian lake food chain accumulation study

Börga *et al.* (2013a and 2013b) carried out a study of the pelagic food web in Lake Mjøsa, Norway (to replicate the study reported by Borgå *et al*. (2012), which was summarised in EA, 2013) and extended it to include a similar lake in the same area (Lake Randsfjorden) and a lake thought to be remote from any known sources of emission (Lake Femunden)[2]. All three lakes are deep and contain well-defined pelagic food webs including zooplankton, planktivorous fish and Brown Trout (*Salmo trutta*) as a top predator. (This study is included in the October 2014 update of the CSRs, and is indicated as 'reliable with restrictions'.)

Lake Mjøsa has a pelagic food web with Brown Trout (*Salmo trutta*) as the top predator, Smelt (*Osmerus eperlanus*) and Vendace (*Coregonus albula*) as primary planktivorous prey, and an invertebrate community consisting of cladocerans, copepods and *Mysis relicta*. Lake Randsfjorden has some similarities to Lake Mjøsa and has a well-defined pelagic food web with Brown Trout and Arctic Char (*Salvelinus alpinus*) as top predators, and Whitefish (*Coregonus lavaretus*) and Smelt as planktivorous prey. Lake Femunden has a pelagic fish community of Brown Trout, Arctic Char and Whitefish. The main food web difference between the lakes is that Lake Mjøsa includes *Mysis relicta* in the invertebrate community, Vendace among the planktivorous fish, and excludes Arctic Char as top predator. Whitefish is assumed to be a benthic feeding species in Lake Mjøsa but assumed to replace Vendace in the pelagic food web of Randsfjorden and Femunden.

The samples were collected between July and September 2012. Fish and invertebrates were sampled from the pelagic zone in all three lakes. In addition benthic fish (Whitefish, Perch *Perca fluviatilis* and Burbot *Lota lota*) were sampled from Lake Mjøsa. As well as biota samples, samples of surface sediments were also collected from all three lakes along with surface water and effluent samples from Lake Mjøsa and Lake Randsfjorden. The majority of biota samples in Lake Mjøsa (zooplankton, *Mysis reticta*, Vendace and Smelt) were collected mid-lake in an area south of the town of Helgøya. Brown Trout were collected from close to the town of Gjøvik but as this species uses the entire lake in search of food it was thought that these samples were representative of a larger geographical area. In Lake Randsfjorden the biota samples were all collected mid-lake from an area south of Brandu and in Lake Femunden the biota samples were collected

[2]Although Lake Femunden was considered to be a remote lake with low human impact, the map given in the paper shows a small village close by and so point sources of emission cannot be totally ruled out.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

from the southern basin.

The fish samples consisted of skinless fillets from one individual except for small Smelt where five or six skinless fillets were pooled for each sample. For Burbot, both fillets and liver were sampled. Pre-cleaned field blanks were handled in the same way as the biota samples. Sediment samples were taken from the surface layer (0-1 cm depth) in areas close to the discharge from waste water treatment plants where this was possible. Each sample consisted of a pool of three cores from each sampling area. Deeper sediments (typically from 30 cm or deeper) were also collected to act as reference samples. Water samples from Lake Mjøsa were collected from a depth of 15 m[3]. Grab samples of effluent were collected from the outlets of three waste water treatment plants in each of Lake Mjøsa and Lake Randsfjorden. Precautions were taken during sampling to avoid inadvertent contamination of the samples (for example all personnel avoided the use of personal care products).

The trophic level of each species was assigned based on $\delta^{15}N$ measurements and the carbon source for the organism was determined based on $\delta^{13}C$ measurements. The zooplankton from the epilimnion was defined as the baseline consumer and assigned a trophic level of 2. The other trophic levels were assigned relative to this using an enrichment factor (ΔN) of 3.4‰ TL^{-1}.The number of samples collected and trophic level assigned are summarised in Table A2.1.

The samples were analysed for the presence of D4, D5 and D6 (cyclic volatile methylsiloxanes, cVMS). In addition known bioaccumulative substances (polychlorinated biphenyls (PCB-153 and PCB-180) and dichlorodiphenyldichloroethylene (p,p'-DDE) in Lake Mjøsa and Lake Randsfjorden, and polybrominated diphenyl ethers (PBDE-47 and PBDE-99) in Lake Mjøsa) were analysed in the sample to act as reference substances. Procedural blanks, field blanks and an internal matrix control (homogenate of herring from the Baltic Sea for biota samples and a sediment sample from Lake Mjøsa for abiotic samples) were also analysed at intervals along with the samples. The limit of quantification (LOQ) for biota was set to the mean plus 10×standard deviation of the procedural blanks and the LOQ for sediment was set at 3×maximum quantity measured in the reference sediments. The levels found are summarised in Table A2.1. The levels were not blank-corrected[4].

The levels of cVMS found in Lakes Mjøsa and Randsfjorden were generally higher than found in Lake Femunden, reflecting the local sources of release into the lakes. The concentration of D5 was above the LOQ in 98% of the biota samples (a total of 91 samples were analysed) and 80% of the sediment samples (a total of 18 samples were analysed). In Lake Femunden, all cVMS were below LOQ in all samples analysed[5] except for a few trout in which D5 was above the LOQ.

All of the effluent water samples contained all cVMS above the LOQ, with the exception

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

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

[5] As low levels in this lake were foreseen, sediments and samples of the top predators Brown Trout and Arctic Char were analysed first. As only low levels were found, the remaining samples collected in Lake Femunden (zooplankton, Whitefish, Arctic Char) were not analysed.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

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

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

Table A2.1 Summary of levels of D5 in samples collected from Lakes Mjøsa, Randsfjorden and Femunden

Lake	Sample	Food web	No. of samples analysed	Mean trophic level (±standard error)	Mean D5 concentration (ng/g lipid) (±standard error)
Lake Mjøsa	Zooplankton (epilimnion)	Pelagic	3	2.0±0.0	342±33
	Zooplankton (hypolimnion)	Pelagic	4	2.6±0.2	1,664±296
	Mysis relicta	Pelagic	4	2.8±0.1	927±116
	Vendace (*Coregonus albula*)	Pelagic	7	3.9±0.0	14,160±2,446
	Smelt, small (*Osmerus eperlanus*)	Pelagic	5	3.8±0.1	3,533±224
	Smelt, large (*Osmerus eperlanus*)	Pelagic	5	4.4±0.0	5,256±737
	Brown Trout (*Salmo trutta*)	Pelagic	5	4.4±0.0	5,629±1,041
	Whitefish (*Coregonus lavaretus*)	Benthic	5	3.6±0.1	1,027±325
	Perch (*Perca fluviatilis*)	Benthic	6	4.0±0.1	403±47
	Burbot, liver (*Lota lota*)	Benthic	6		5,296±1,019
	Burbot, muscle (*Lota lota*)	Benthic	6	4.4±0.1	1,507±244
Lake Rands-fjorden	Zooplankton (epilimnion)	Pelagic	4	2.0±0.0	251±5
	Zooplankton (hypolimnion)	Pelagic	3	3.0±0.3	2,251±39
	Whitefish (*Coregonus lavaretus*)	Bentho-pelagic	9	3.2±0.1	112±39
	Smelt (*Osmerus eperlanus*)	Pelagic	5	3.5±0.1	969±71
	Brown Trout (*Salmo trutta*)	Pelagic	5	3.8±0.1	2,579±806
Lake Femunden	Arctic char (*Salvelinus alpinus*)	Pelagic	1	-[a]	<20
	Brown Trout (*Salmo trutta*)	Pelagic	6	-[a]	39±14

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Note: The trophic level of the fish from Lake Femunden was not reported.

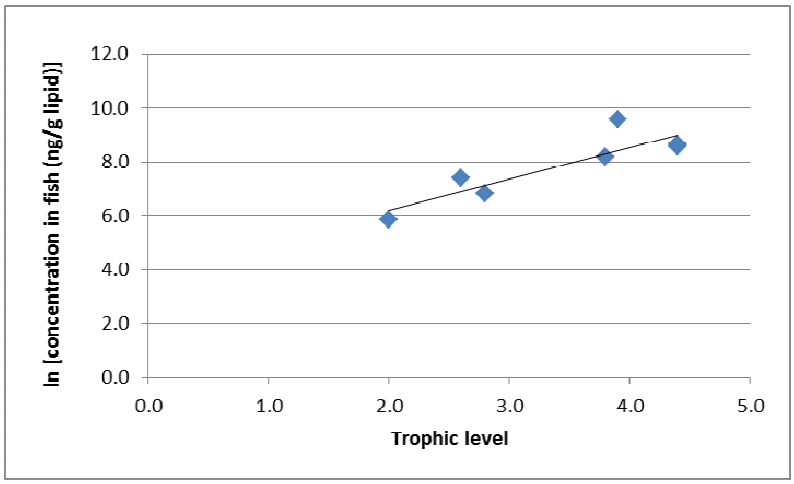
The $\delta^{13}C$ measurements showed a clear separation of the pelagic feeding fish from the benthic feeding fish in Lake Mjøsa. In Lake Ransfjorden, a relatively high variation in the $\delta^{13}C$ value was found in Whitefish, suggesting that there was some variation in the diet of this species.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

The TMF was estimated from the slope of a plot of the natural logarithm of lipid normalised concentration in biota versus trophic level. The benthic fish (from Lake Mjøsa) and sediment samples were not included in the analysis. For Lake Randsfjorden, one hypolimnion zooplankton sample was identified as a multivariate outlier and so was excluded from the analysis. A plot showing the mean concentrations against the trophic level for Lake Mjøsa is shown in Figure A2.2. The TMFs derived from the data are summarised in Table A2.2 (these values were derived in the actual publications from plots of the individual data points rather than the mean data points).

The TMF for D5 was found to be similar between Lakes Mjøsa and Randsfjorden regardless of whether whitefish were included or excluded. The TMF for D5 was in the range 2.13-3.12 indicating that trophic magnification was occurring. However, the statistical significance of the TMF being above 1 was reduced for Lake Randsfjorden when Whitefish were included compared with the situation when they were omitted (for example see the 95% confidence intervals and p-values in Table A2.2), although the actual magnitude of the TMF was similar in both cases. The lower significance of the TMF in Randsfjorden when Whitefish were included resulted from the fact that the D5 concentrations in this species in this lake were lower compared with other species at the same trophic level, suggesting that the source of D5 in Whitefish may have been different from the other, purely pelagic species considered. For example, this could have been as a result of feeding in the littoral zone on terrestrial and benthic prey.

In Lake Mjøsa, benthic feeding fish (Perch, Whitefish and Burbot) generally had lower levels of D5 than pelagic fish of a similar trophic level (these species were not included in the TMF derivation for the pelagic food web).

Figure A2.2 Plot of ln [mean concentration in biota (ng/g lipid)] versus trophic level for Lake Mjøsa



Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Table A2.2 Summary of TMFs derived by Börga *et al*. (2013)

Lake	Number of data points	TMF	95% confidence interval	p-value[a]	R^2 of regression	Comment
Lake Mjøsa	33	3.12	2.28-4.29	<0.0001	0.64	Not including whitefish
Lake Randsfjorden	17	2.74	1.70-4.41	0.0004	0.58	Not including whitefish
	26	2.13	0.76-5.98	0.144	0.09	Including whitefish
Combined Lake Mjøsa and Lake Randsfjorden	51	2.91	2.11-4.02	<0.0001	0.60	Not including whitefish
	59	2.79	1.86-4.20	<0.0001	0.57	Including whitefish for Lake Randsfjorden

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

The levels of D5 in the pelagic food webs were also found to correlate with the levels of known biomagnifying substances, for example PCB-153 and p,p'-DDE. The TMFs for these reference substances were higher in Lake Mjøsa than Lake Randsfjorden but were above 1 in both lakes.

As is the case with the previous study by this research group, there are a number of uncertainties associated with these results, including the following:

- The Brown Trout in Lake Mjøsa were sampled from a different area of the lake than the other biota samples. The trout were sampled near to Grjøvik and the sediment samples suggested that this area may have been more heavily contaminated than other parts of the lake. However, it was noted that this species use the entire lake for feeding and so the levels found are probably more reflective of the levels in the whole lake rather than the specific area sampled. In addition, a similar level of trophic magnification was evident in the food webs of both Lake Mjøsa and Lake Randsfjorden.
- The fish samples analysed were skinless fillets (with the exception of Burbot livers), so the reported concentrations do not necessarily reflect the levels present in whole fish. The Burbot data show that the levels of D5 (and the halogenated reference substances) were generally higher in liver than in fillets, although the liver will contribute only a relatively small fraction of the total weight of the fish (this presumably varies between individual fish). The concentration (or amount) of D5 present in other, non-fillet, portions of the fish is unknown.
- The total number of samples for each species is low (3 – 9), so the representivity and variation of the concentrations is unclear.

Despite these limitations, this study provides evidence that D5 biomagnifies in pelagic food webs of both Lake Mjøsa and Lake Randsfjorden. The TMF determined in both lakes was similar and the overall combined TMF was 2.91 with a 95% confidence interval of 2.11-4.02. In addition, the levels of D5 in the pelagic food chain correlated with the reference substances that are known to biomagnify. This study confirms the findings of the previous study at Lake Mjøsa (Borgå *et al*., 2012), which derived a TMF of 2.28 for the whole food chain, 1.62 when Smelt were omitted and 3.58 when Brown Trout were omitted.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Tokyo Bay food chain accumulation study

A further study of the bioaccumulation of D5 is currently in the process of being published (Powell *et al.*, 2014)[6]. (This study is partially reported in the October 2014 update of the CSRs, and is indicated as 'reliable without restriction'.) A pre-publication draft of the study has been made available to the dossier submitter. The study was of a pelagic marine food web in Tokyo Bay. The samples for the study included sediment and fish collected between 4th and 15th November 2011 from a defined 500 km^2 area covering approximately 55% of inner Tokyo Bay. The area was defined using a two-dimensional probability design based on 25 km^2 square grids extending seaward from the head of the bay to the narrows between Cape Kannon and Cape Futtsu. Sediments were collected from 20 locations by systematically sampling each 25 km^2 grid and fish were collected within the northern part of the study area. Precautions were taken during sampling, storage and analysis to avoid unintentional contamination of the samples and loss from evaporation and degradation. As well as D5, the study included PCB-180 as a benchmark chemical and PCB-153 as a reference chemical.

The trophic positions of the organisms were determined based on $\delta^{15}N$ measurements and $\delta^{13}C$ measurements were used to assess the sources and flow of dietary carbon in the food web. The trophic levels assigned to the organisms (using a $\Delta^{15}N$ of 3.4‰ TL^{-1}) are shown in Table A2.3 along with the measured concentrations of D5. In all cases the concentration of D5 was above the method detection limit[7].

Table A2.3 Summary of levels of D5 in samples collected from Tokyo Bay

Sample	Number of samples analysed	Trophic level (based on a $\Delta^{15}N$ value of 3.4‰ TL^{-1})	Mean lipid content (%)	Mean D5 concentration (ng/g lipid) (±standard deviation)[a]
Dotted Gizzard Shad (juvenile) (*Konosirus punctatus*)	3 composites (each of 11 individuals)	3.0	8.0	3,140±194
Silver Croaker (*Pennahia argentata*)	3 composites (each of 13 individuals)	3.1	5.9	3,290±720
Japanese Sardinella (*Sardinella zunasi*)	3 composites (each of 48 individuals)	3.1	4.5	6,300±563
Japanese Anchovy (*Engraulis japonicas*)	3 composites (each of 55 individuals)	3.5	3.9	3,640±540
Dotted Gizzard Shad (adult) (*Konosirus punctatus*)	1 composite (of 5 individuals)	3.8	17.0	840±(168)
Chub Mackerel (*Scomber japonicas*)	1 composite (of 4 individuals)	4.1	20.0	1,030±(207)
Red Barracuda (*Sphyraena pinguis*)	1 composite (of 5 individuals)	4.1	11.0	3,040±(607)
Japanese Sea Bass	6 individuals	4.4	6.3	3,780±1,200

[6] A further related report was highlighted during PC (ECC, 2013), but this has not been reviewed by the DS.

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

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Sample	Number of samples analysed	Trophic level (based on a $\Delta^{15}N$ value of 3.4‰ TL^{-1})	Mean lipid content (%)	Mean D5 concentration (ng/g lipid) (±standard deviation)[a]
(*Lateolabrax japonicas*)				

Notes: a) For the species where only one sample was analysed the standard deviation (given in brackets) was estimated using sampling variances from other studies conducted on cVMS.

The concentration of D5 (and also PCB-153 and PCB-180) in sediment varied spatially across the area, generally decreasing with distance from the inner part of the estuary (close to the mouths of the Arakawa River and the Edogawa River). The $\delta^{15}N$ and $\delta^{13}C$ measurements in sediment also appeared to be related to the proximity of the rivers entering the bay but no significant trends were apparent. As a result of the existence of this concentration gradient in the sediment, the study area was stratified and mean concentrations in sediments were calculated using appropriate methods for a stratified experimental design.

The $\delta^{13}C$ measurements indicated that all fish species were feeding on a similar carbon source, and that this carbon source was different to that in the sediment. The $\delta^{15}N$ measurements suggested that the food web covered around 1.4 trophic steps with planktivorous forage species at the base of the food web (e.g. juvenile Dotted Gizzard Shad (*Konosirus punctatus*), Silver Croaker (*Pennahia argentata*) and Japanese Sardinella (*Sardinella zunasi*)) and piscivorous predatory species at the top of the food web (e.g. Red Barracuda (*Sphyraena pinguis*), Chub Mackerel (*Scomber japonicus*) and Japanese Sea Bass (*Lateolabrax japonicas*)). Examination of the gut contents indicated that the Japanese Sea Bass were feeding exclusively on Japanese Anchovy (*Engraulis japonicas*) and Japanese Sardinella at the time of sampling. With the exception of Japanese Sea Bass the species sampled were thought to actively migrate throughout the estuary (Japanese sea bass were not thought to migrate as actively as other species).

Several approaches were used to estimate the TMF, and the results are summarised in Table A2.4.

Table A2.4 Summary of bioaccumulation parameters derived for Tokyo Bay

Parameter		D5	PCB-153	PCB-180
Biota-sediment accumulation factor (BSAF)	Dotted Gizzard Shad (juvenile)	1.1	1.0	0.44
	Silver Croaker	0.91	0.87	0.57
	Japanese Sardinella	1.4	1.3	0.65
	Japanese Anchovy	0.83	1.4	0.94
	Dotted Gizzard Shad (adult)	0.31	2.6	1.5
	Chub Mackerel	0.36	3.3	1.8
	Red Barracuda	0.50	2.5	1.6
	Japanese Sea Bass	0.49	5.4	3.3
TMF using the standard method; $\Delta^{15}N$ = 3.4‰ TL^{-1}	TMF	1.0	2.7	2.8
	95% Confidence Interval	0.5-1.9	1.4-5.3	1.4-5.6
	TMF statistically significantly different from 1	No (p=0.90)[a]	Yes (p=0.01)[a]	Yes (p=0.01)[a]
Probabilistic TMF;	Median TMF	0.6	2.2	2.2

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Parameter		D5	PCB-153	PCB-180
$\Delta^{15}N$ = 3.4‰ TL^{-1}	95% Confidence Interval	0.4-0.8	1.7-2.9	1.7-3.0
	Probability TMF >1	0.1%	>99.9%	>99.9%
Benchmark TMF[b]; $\Delta^{15}N$ = 5.9‰ TL^{-1}	Median TMF	0.4	3.9	4.0
	95% Confidence Interval	0.2-0.7	2.4-6.3	2.4-6.9
	Probability TMF >1	0.1%	>99.9%	>99.9%
Corrected benchmark TMF; $\Delta^{15}N$ = 3.9‰ TL^{-1}	Median TMF	0.4	3.6	4.0
	95% Confidence Interval	0.3-0.5	2.6-4.9	2.9-5.7
	Probability TMF >1	<0.1%	>99.9%	>99.9%

Note: a) The p-value indicates the statistical significance of the regression. Statistically significant difference is usually taken as a value of p≤0.05.
b) PCB TMFs were the median values from log normal distributions of TMF values reported in the literature for PCB-180 (n=22) and PCB-153 (n=26).

i) As the sediment data also indicated the existence of concentration gradients within the sampled area, and hence the possibility of variable exposure of the fish sampled, an analysis was undertaken to correct for this based on estimated migration patterns for each species (based on their known ecology) and the concentrations in sediment (used as an indicator of exposure based on the assumption that the concentrations in water and sediment were in equilibrium over the long-term). This was carried out by estimating BSAF values for each species based on the mean concentration in each species (ng/g lipid) by the relative exposure concentration in sediment (ng/g total organic carbon) for that species. The BSAFs derived are summarised in

ii) Table A2.4. The BSAF for D5 was >1 in some cases but it was found to generally decrease with increasing trophic level, which was in contrast to the BSAFs calculated for PCB-153 and PCB-180[8].

The BSAFs for PCB-180 were then used to apply an exposure correction to the food web. Using this approach an exposure-corrected $\Delta^{15}N$ value of 3.9‰ TL^{-1} was calculated using the benchmarking approach outlined above. This was then used to estimate the TMF for D5 and PCB-153 using the probabilistic approach. The exposure-corrected median TMF for D5 was 0.4 (95% confidence interval 0.3-0.5, probability of TMF >1 <0.1%). The median TMF for PCB-153 was estimated to be 3.6. This method was considered by Powell *et al*. (2014) to provide the best estimates of the TMFs for this food chain.

iii) Trophic magnification factors were firstly estimated from the fish data from the slope of a plot of ln [concentration in fish (ng/g lipid)] versus trophic level. The TMF for D5 was 1.0 with a 95% confidence interval of 0.5 to 1.9 and was not statistically different from 1 (p=0.90). The TMFs derived for PCB-153 and PCB-180 were 2.7 and 2.8 respectively.

iv) The TMFs were also estimated from the same data using a multivariate probabilistic method (to take account of bias resulting from experimental design). This resulted in a median TMF for D5 of 0.6 (95% confidence interval 0.4-0.8, probability of TMF >1 0.1%). The median TMFs derived for PCB-153 and PCB-180 were both 2.2 using this method.

[8] Similar observations were made by Kierkegaard *et al*. (2011), who found that D5 was bioaccumulating to a greater extent than PCB-180 in ragworm and Flounder in a UK estuary. Powell (2014) (and subsequent correspondence with the DS) provides arguments based on lipid solubility to explain why D5 should have a higher bioaccumulation potential relative to PCB-180 at lower trophic levels.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

v) Next the data were analysed using a benchmarking approach combined with the probabilistic method, using PCB-180 as the benchmarking chemical. For this approach the TMF for PCB-180 was assumed to be 4.0 and this was used to calibrate the food web, resulting in a benchmarked $\Delta^{15}N$ value of 5.9‰ TL^{-1}. This value was then used to derive the TMF for D5 and PCB-153. Using this approach the median TMF for D5 was 0.4 (95% confidence interval 0.2-0.7, probability of TMF >1 0.1%). The median TMF for PCB-153 was 3.9. Although this approach resulted in a TMF value for PCB-153 that was in line with the expected value for this substance the $\Delta^{15}N$ value derived was outside the accepted range for aquatic food webs (generally taken to be between 3.0‰ TL^{-1} and 5.0‰ TL^{-1}). Powell *et al.* (2014) suggested that this was indicative of variable exposure in the current food web.

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

- The species sampled covered 1.4 trophic levels, which is smaller than in some of the other studies available, although similar when only fish are considered (for example the Lake Erie study (see below) sampled fish between trophic level 3.1 and 4.2, compared with fish samples between trophic level 3.0 and 4.4 in the Tokyo Bay study).
- The exposure correction was based on data for PCB-180. It is possible that the distribution of D5 throughout the estuary may have been different to that for PCB-180. No detailed analysis of this was given in the paper but, from visual inspection of the sediment data, it would appear that the concentrations of D5 followed a similar pattern to that of PCB-180.
- In principle, "correction" to take account of concentration gradients is a more reasonable approach than assuming homogeneous exposure in such a large water body. However, by necessity this involves data manipulation and further assumptions. For example, fish home range may not be simply related to body size (as was assumed in the study). It is possible that other factors could also have influenced exposure (D5 concentrations in the water column were not measured).
- The choice of a single TMF for the PCB benchmarks directly affects the magnitude of the TMF derived for the substance of interest when the correction is applied. Borgå & Starrfelt (2014) point out that adjusting the enrichment factor only scales the extent to which the estimated TMF deviates from 1; the larger the enrichment factor, the further the TMF will be 'pushed' away from 1[9]. The reliability of the selected benchmark TMFs has not been assessed, and the variability in the underlying datasets might be important (e.g. it is possible that other values would be derived if they were corrected for exposure). The apparent differences in bioaccumulation behaviour between D5 and PCB-180 at lower trophic levels cast some doubt as to whether it is an appropriate benchmark. It is not known whether other benchmarks would give different values.

[9] The choice of $\delta^{15}N$ value does not affect whether or not the TMF is above or below 1, because it only affects the size of the slope of the ln [concentration] versus trophic level plot, not whether the slope is positive (TMF >1) or negative (TMF <1).

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

- Borgå & Starrfelt (2014) also observed that the probabilistic method used in the report uses probability distributions for contaminant levels in the different species and uses samples drawn from these distributions to estimate TMFs (instead of using the actual observed data). This approach has the merit of correcting for sampling design (as the approach weights each species equally, which is not usually the case for studies with different number of samples from different species), but also introduces some complicating aspects. In particular, the choice of distribution ignores variability and underestimates uncertainty. Caution is therefore needed when interpreting the reported "confidence bounds", as they may give a false impression of the precision of the TMF estimates.

Overall, the results of this study suggest that the TMF for D5 in this marine pelagic food web was ≤1.

Lake Erie food chain accumulation study

McGoldrick *et al.* (2014) investigated the biomagnification of D5 in the western basin of Lake Erie, Canada. (This study is included in the October 2014 update of the CSRs, and is indicated as 'reliable with restrictions'.) The biota used in the study were collected in the summer/autumn of 2009[10] in the vicinity of Middle Sister Island and included zooplankton, mayflies (*Hexagenia* sp.), Common Shiner (*Luxilus cornutus*), Yellow Perch (*Perca flavescens*), Emerald Shiner (*Notropis atherinoides*), Trout Perch (*Percopsis omiscomaycus*), White Perch (*Morone americana*), Freshwater Drum (*Aplodinotus grunniens*) and Walleye (*Sander vitreus*). The fish were analysed as whole fish samples (Walleye and Freshwater Drum were analysed as individual fish, the other species were analysed as composite samples of between 2 and 60 individuals with each composite being divided into 5 subsamples). Precautions were taken during sampling and analysis to avoid inadvertent contamination of the samples.

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

The concentration of D5 measured in each species, along with the assigned trophic levels and lipid contents are summarised in
The study also included analysis of PCB-180 as a reference substance that is known to bioaccumulate. The TMF derived for this substance was 1.2 when all species were included, 1.7 when mayfly were excluded, 0.55 when zooplankton were excluded, 2.1 when both mayfly and Walleye were excluded and 0.58 when both zooplankton and Walleye were excluded. This suggests that the TMF is dependent on the food web structure.

There are some uncertainties with this study resulting, for example, from the relatively small sample sizes and the inclusion of species with a relatively high contribution from pelagic carbon sources in what was essentially a benthic food web. It is also relevant to note that the recoveries of the $^{13C\text{-}D5}$ used as analytical standard range from 25% to

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

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

115%, were highest for the zooplankton samples and generally decreased as the lipid content of the fish increased. This may have introduced some bias into the results as the fish at the higher trophic levels generally had higher lipid contents than the fish at lower trophic levels, e.g. the lipid contents for the fish in trophic levels between 3.7 and 4.2 were in the range 3.4 to 13% compared to lipid contents between 0.7% of 3.5% for fish at lower trophic levels. This could potentially lead to an underestimation of the concentrations in fish at the higher trophic levels compared with lower trophic levels.

Table A2.5. The TMFs were estimated from the data using the lipid equivalent concentrations and various assumptions over the food web composition. The TMF for D5 was determined to be 0.75 (95% confidence interval 0.50-1.1; probability of TMF >1 7.9%) when all species were included, 0.68 (95% confidence interval 0.41-1.0; probability of TMF >1 7.0%) when mayfly were excluded, 0.91 (95% confidence interval 0.52-1.4; probability of TMF >1 35%) when the zooplankton were excluded, 0.80 (95% confidence interval 0.45-1.3; probability of TMF >1 19%) when both mayfly and Walleye were excluded and 1.2 (95% confidence interval 0.64-1.9; probability of TMF >1 65%) when both zooplankton and Walleye were excluded.

The study also included analysis of PCB-180 as a reference substance that is known to bioaccumulate. The TMF derived for this substance was 1.2 when all species were included, 1.7 when mayfly were excluded, 0.55 when zooplankton were excluded, 2.1 when both mayfly and Walleye were excluded and 0.58 when both zooplankton and Walleye were excluded. This suggests that the TMF is dependent on the food web structure.

There are some uncertainties with this study resulting, for example, from the relatively small sample sizes and the inclusion of species with a relatively high contribution from pelagic carbon sources in what was essentially a benthic food web. It is also relevant to note that the recoveries of the ^{13}C-D5 used as analytical standard range from 25% to 115%, were highest for the zooplankton samples and generally decreased as the lipid content of the fish increased. This may have introduced some bias into the results as the fish at the higher trophic levels generally had higher lipid contents than the fish at lower trophic levels, e.g. the lipid contents for the fish in trophic levels between 3.7 and 4.2 were in the range 3.4 to 13% compared to lipid contents between 0.7% of 3.5% for fish at lower trophic levels. This could potentially lead to an underestimation of the concentrations in fish at the higher trophic levels compared with lower trophic levels.

Table A2.5 Summary of levels of D5 in samples collected from Lake Erie

Sample	Estimated diet composition	Number of samples analysed	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D5 (ng/g wet weight) (±standard deviation)
Zooplankton		1	2.0±0.32	0.3	5.2
Mayfly (*Hexagenia* sp.)		1	2.2±0.08	1.3	11
Common Shiner (*Luxilus cornutus*)	13% pelagic – 87% benthic	2	3.1±0.08	3.5	15±5.6
Yellow Perch (*Perca flavescens*)	15% pelagic – 85% benthic	5	3.4±0.1	1.6	14±3.6
Emerald Shiner (*Notropis atherinoides*)	40% pelagic – 60% benthic	5	3.6±0.07	2.1	17±5.7

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Sample	Estimated diet composition	Number of samples analysed	Mean trophic level (±standard deviation)	Mean lipid content (%)	Mean concentration of D5 (ng/g wet weight) (±standard deviation)
Trout Perch (*Percopsis omiscomaycus*)	49% pelagic – 51% benthic	5	3.6±0.08	0.7	23±5.3
White Perch (*Morone americana*)	3% pelagic – 97% benthic	4	3.7±0.05	5.3	26±5.4
Freshwater Drum (*Aplodinotus grunniens*)	28% pelagic – 72% benthic	5	4.0±0.12	3.4	23±12
Walleye (*Sander vitreus*)	20% pelagic – 80% benthic	15	4.2±0.12	13	36±15

Borgå & Starrfelt (2014) noted similar caveats about the use of the probabilistic method and estimating fish home range as for the Tokyo Bay study (see above). In addition, they noted that the study suffers from low sample size at the base of the food web, with unknown variance. The TMF (both range and mean) is sensitive to the species included in the regression, and the study did not consider the impact of including/excluding species that have a benthipelagic feeding regime (rather than benthic only). Lack of information on lipid normalisation and associated statistics makes it impossible to evaluate the significance of the approach used to take lipid into account.

Overall the results of this study suggest that trophic magnification of D5 was not occurring in this predominantly benthic food chain, although a TMF above 1 was suggested from one of the food web configurations (with a 65% probability that the TMF is above 1 when both zooplankton and a top predator (Walleye) were excluded). PCB-180 (a known bioaccumulative substance) was also found to have a TMF below 1 for some food web configurations.

Lake Champlain food chain accumulation study

Powell (2014a) (supplemented by personal communication with the DS) reported the interim results of an investigation of a pelagic food web in Lake Champlain, USA (a final report is expected before the end of 2014). The lake is long (200 km), narrow (19 km at widest point) and deep (maximum depth 122 m; average depth 19.5 m), with a surface area of 1,130 km^2. Surface sediments (top 1 cm) were collected at 59 locations across the 800 km^2 study area from water depths of 6.4 to 114 m (one sample per site). Biota samples (ten species of fish, plus zooplankton and mysid shrimp *Mysis relicta*) were collected during October 2012 from thirteen locations across six sites. Five to eleven samples (pooled or individual) were collected for each species, although not all species were collected at each site.

The interim report does not provide concentration data. Some samples were excluded from the TMF calculations due to either lack of analysis (White Perch *Morone americana*), inclusion in a separate study (Brown Trout *Salmo trutta;* large Rainbow Smelt *Osmerus mordax*; large Alewife *Alosa pseudoharengus*), or stable isotope results indicating that samples were not part of the same food web (zooplankton) (see further discussion below). Biota concentrations were highly variable, with around 65% of the variability appearing to be due to fish lipid content and around 30% to trophic position. In addition,

variation appeared to be related to sample collection location for some though not all species.

Stable isotope analysis indicated trophic level positions that were subject to a consistently high bias compared to expectations from FishBase®, suggesting that the recommended $\Delta^{15}N$ value of 3.4 ‰ TL^{-1} that was used to calculate trophic level may not have been a good estimate for the sampled food web. In addition, Yellow Perch occupied a lower trophic level position than expected, presumably due to dietary preferences and availability rather than sample collection bias. The length of the sampled food web was estimated to be about 1.3 to 1.8 trophic steps, depending upon the $\delta^{15}N$ value used for the calculation.

PCB-180 was used as a benchmark chemical to "calibrate" the food web as done for the Tokyo Bay study (see above). Concentration gradients also existed across the study area (the pattern of sediment contamination was different between D5 and PCB-180), so it was assumed that relative exposure concentrations were proportional to the organic carbon-normalised sediment concentrations within the home ranges of the species sampled at each site. Exposure "correction" was therefore performed using a number of models and estimates (e.g. of home range). The results of the various approaches to estimate the TMF are summarised in Table A2..

Table A2.6 Summary of TMFs derived for Lake Champlain

Chemical	End-point	Standard ($\delta^{15}N$=3.4)	Benchmark ($\delta^{15}N$=4.0)	Corrected Standard ($\delta^{15}N$=3.4)	Corrected Benchmark ($\delta^{15}N$=3.0)
PCB-180	TMF	3.2	4.0	4.5	4.0
	95% CI	2.5-4.1	3.0-5.3	2.5-9.0	3.1-5.1
	R-square	62.9%	62.9%	70.3%	70.3%
	P value	<0.001	<0.001	<0.001	<0.001
D5	TMF	2.0	2.3	0.5	0.5
	95% CI	1.5-2.7	1.6-3.3	0.3-0.7	0.4-0.6
	R-square	29.3%	29.3%	56.4%	56.4%
	P value	<0.001	<0.001	<0.001	<0.001

The TMF for D5 was above 1 (2.0 or 2.3) using the standard method with and without benchmark-correction, but below 1 (0.5) when exposure correction is applied.

Stable isotope analysis suggested that zooplankton occupied the highest trophic level (presumably due to the presence of detritus), so the data for this trophic group were excluded from the TMF calculations summarised in Table A2.. Further analysis showed that their inclusion would increase residual bias but improve the fit of the TMF regression model, giving a TMF of 1.6 (standard approach) or 0.6 ("corrected" approach).

The results of multivariate probabilistic analysis were also presented in follow-up correspondence. The TMF was calculated by bootstrap regression analysis using Monte-Carlo sampling of probability density functions for measured concentrations (ng/g lipid) or exposure corrected concentrations (i.e. BSAF; g-TOC/g-lipid), which were used to correct for variable exposure across concentration gradients. This gave median TMFs for D5 of 1.8 (95% CI 1.2-2.8) (standard method), 2.1 (95% CI 1.2-3.6) (benchmark correction) and 0.5 (95% CI 0.4-0.8) (exposure-corrected benchmark method).

As a final report is not yet available, the results of this study should be treated with

caution. Concerns about the data interpretation are similar to those of the Tokyo Bay study summarised above.

A further report was highlighted during PC (Powell, 2014b), but this has not been reviewed by the DS. The median TMF was calculated as 1.6 – 2.1, and the probability that the TMF exceeded 1.0 was in the region of 99%. However, the variability was high, and the median r^2 value was in the range 16-19%. Attempts were made to adjust the fish concentrations for likely exposure, and this gave a median TMF of 0.2 – 10. The report's conclusion was that a reliable TMF could not be obtained for D5. (This study is not mentioned in the October 2014 update of the CSRs.)

Lake Ontario food chain accumulation study

A study using samples collected from Lake Ontario, Canada/USA since 2011 is underway but full results have not been reported yet[11]. (This study is not mentioned in the October 2014 update of the CSRs.) CES (2014) indicated that concentrations in fish towards the top of the food chain are around one order of magnitude higher than invertebrates on a wet weight basis: mean D5 concentrations were ~180 to 220 ng/g ww in Lake Trout and 20-45 ng/g ww in mysids. No information was given on sample number or locations and the relevance of this information in unknown.

CES (personal communication, 25 April 2014) stated that the results of this study are confounded by variable exposure, concentration gradients and "dietary switches resulting from invasive species", which casts some doubt on the usefulness of the data. Nevertheless, the same source provides an assessment of TMF in the Lake Ontario food web based on samples collected in November 2011, although the original data are not provided. Trophic level (TL) was calculated using an assumed $\Delta^{15}N$ enrichment factor of 3.40‰ TL^{-1}. The evaluated food web consisted of mysid shrimp (TL=3.0), Alewife (TL=3.1), small 'goby' (TL=3.5), large 'goby'[12] (TL=3.7), Rainbow Smelt (TL=3.9), and Lake Trout (TL=4.6). The resulting TMF based on log (mean lipid weight concentrations) was stated to be 1.3 (r^2=9.2%; standard error=0.052; p=0.03). The positioning of Alewife below Round Goby is somewhat surprising given its expected diet of mysids and small fish (whereas Round Goby eats aquatic insects and molluscs). In addition, the influence of any overlap in concentration amongst the species is not discussed.

However, CES (personal communication, 25 April 2014) goes on to state that the TMF is "anomalous" if not corrected for exposure, presumably on the basis of lower than expected TMFs derived for PCB-180 and -153 in the same food chain (1.8 and 1.5, respectively). As for the Tokyo Bay study reported above, additional calculations were therefore performed, to both 'benchmark' against PCB-180 (median benchmark TMF=4.0, relative trophic levels based on a ^{15}N enrichment factor of 3.31) and "correct" for exposure across concentration gradients (by estimating BSAFs; the data are not presented, but it is stated that BSAFs for Lake Trout and 'goby' were based on measured concentrations in sediment collected from the Niagara Delta (mid-water, near shore location; TOC=0.95% ww), and BSAFs for the other species were based on measured concentrations in sediment collected from the Niagara Basin (deep-water, offshore location; TOC=0.86% ww)). Using the 'concentration gradient-correction' approach, the TMF is 1.7 (with a 95% confidence interval of 1.0-2.8, and a 98% probability that the TMF exceeds 1). However, benchmarking against PCB-180 gave a TMF of 0.5 (r^2=49%; standard error=0.042; p<0.01), or 0.4 (95% confidence interval of 0.3-0.7, with

[11] A relevant report was highlighted during PC (Seston *et al*., 2014), but this has not been reviewed by the DS.

[12] This species is understood to be Round Goby, based on a more detailed report for D4.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

<0.01% probability that the TMF exceeds 1) when 'concentration gradient-correction' was applied.

As a full study report is not currently available, the results must be treated with caution. Concerns about data interpretation are similar to those of the Tokyo Bay study summarised above. The BSAFs may or may not be appropriate, depending on the sampling locations, variation in sediment concentrations, and whether the biota concentrations are indeed linked to sediment sampled at the selected sites, especially for widely foraging species.

Comparison of field studies

Powell *et al.* (2014) carried out a comparison of the TMFs derived for cVMS from the various studies. (This study is not mentioned in the October 2014 update of the CSRs, although the broad principles are included.) This included recalculation of the TMF for the food chain using the probabilistic approach with a $\Delta^{15}N$ of 3.4‰ TL^{-1} and species-specific probability density functions for $\delta^{15}N$ and the lipid-normalised concentrations defined by the means and standard deviations reported in each study. The probabilistic approach was considered by Powell *et al*. (2014) to be the most appropriate method of analysing the data to minimise bias resulting from experimental design. The results of this analysis for D5 are summarised in
Table A2.77. The analysis did not consider the data from Lake Opeongo, Lake Champlain or Lake Ontario.

Table A2.7 Summary of TMFs derived for D5 in field studies (based on Powell *et al*., 2014)

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

Note: a) An earlier unpublished preliminary study of Tokyo Bay suggested a TMF of 0.5 for D5.

Based on this analysis, median TMFs for D5 are in the range 0.2-3.1, and TMFs >1 are derived for the two studies in Lake Mjøsa (the TMF was similar in both cases) and the study in Lake Randsfjorden.

These findings were considered further by Powell *et al*. (2014). The probabilistic TMFs determined for the two Norwegian lakes were statistically significantly higher than for the other study areas. For the other study areas no significant difference was evident between the values obtained in benthic food webs compared with pelagic food webs for D5. Powell *et al*. (2014) considered that the findings in the Norwegian lakes may be related to variable exposure resulting from non-uniform migration patterns of some species and food web dynamics. Powell *et al*. (2014) noted that the range of $\delta^{13}C$ across the food web was larger in both Lake Mjøsa and Lake Randsfjorden than in other study areas suggesting that omnivorous feeding by consumers may have occurred or that samples were inadvertently collected from trophically distinct food webs. In addition Powell *et al.* (2014) considered that variable exposure resulting from concentration gradients may be a confounding factor in these studies (as is potentially a case with most studies).

It is relevant to note that this paper was attempting to find scientific explanations for the difference between the TMF found in Lakes Mjøsa and Randsfjorden and the other studies, and so concentrated on the potential uncertainties in the Norwegian study. However, there are potential uncertainties with all of the other field studies and these were not discussed in the same level of detail. Overall, although the concerns raised by Powell *et al.* (2014) are reasonable, it is not currently possible to assess the significance of the various uncertainties on the TMFs derived in Lake Mjøsa and Lake Randsfjorden.

Comparison of laboratory bioconcentration data between substances

Table A2.8 compares the available fish laboratory bioconcentration data for D4 and D5 with substances that are agreed to meet the vB criterion following submission of Annex XV dossiers to the Member State Committee[13]. Wet weight whole fish concentrations have been estimated from the cited BCF and aqueous exposure concentrations (unless otherwise stated), and do not take account of lipid content. Polyaromatic hydrocarbons other than anthracene have not been considered for the purpose of this exercise.

Table A2.8 Summary of BCF data for vB substances

Substance	CAS No.	BCF, L/kg	Maximum fish conc., mg/kg ww	Comment	Reference
Anthracene	120-12-7	>6,000	-	Exposure concentrations are not stated so whole fish concentrations cannot be derived.	EC (2008b)
Alkanes, C_{10-13}, chloro (short chain chlorinated paraffins)	85535-84-8	ca. 7,273	ca. 240	Data are for a C_{10-12} 58% wt Cl substance based on parent compound analysis. Fish lipid content not stated.	ECHA (2008b)
2-(2H-Benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol (UV-328)	25973-55-1	4,590	0.4	Based on average BCF at study end. Fish lipid content 4.2%.	UBA (2014a)

[13] Comparisons of concentrations actually measured in wildlife have not been included because of the size of the task and variability of use patterns and quantities leading to very different exposures.

Appendix to the UK-CA's report on the identification of PBT and vPvB substance results of evaluation of PBT/vPvB properties of D5

Substance	CAS No.	BCF, L/kg	Maximum fish conc., mg/kg ww	Comment	Reference
2-Benzotriazol-2-yl-4,6-di-*tert*-butylphenol (UV-320)	3846-71-7	9,265	0.9	Fish lipid content 3.6%.	UBA (2014b)
5-*tert*-Butyl-2,4,6-trinitro-m-xylene (musk xylene)	81-15-2	3,730 and 10,500	9.9 and 33 (estimated)	Steady state not reached – plateau fish concentrations were estimated using a one-compartment model. Fish lipid content 3.4%. Another study resulted in slightly lower fish concentrations (but still >1 mg/kg).	ECHA (2008c)
Hexabromocyclo-dodecane (HBCDD)	25637-99-4	18,100 and 13,085	110 and 4.4	Fish lipid content not specified.	ECHA (2008a)
Henicosafluoro-undecanoic acid	2058-94-8	ca. 2,700 and 3,700	ca. 1.3 and 0.4	BCF in first study based on carcass only. Lipid normalisation not appropriate.	ECHA (2012b)
Pentacosafluoro-tridecanoic acid	72629-94-8	ca. 18,000 and ca. 13,000	ca. 3.6 and ca. 1.3	BCF in first study based on carcass only; second study cited as a BCF range so estimated fish concentration is based on the average. Lipid normalisation not appropriate.	ECHA (2012c)
Heptacosafluoro-tetradecanoic acid	376-06-7	ca. 23,000 and ca. 16,500	ca. 0.3 and ca. 1.6	BCF in first study based on carcass only; second study cited as a BCF range so estimated fish concentration is based on the average. Lipid normalisation not appropriate.	ECHA (2012d)
Octamethylcyclo-tetrasiloxane (D4)	556-67-2	≥11,495	≥2.6	Fish lipid content 6.4%.	EA (2009a)
Decamethylcyclo-pentasiloxane (D5)	541-02-6	≥5,860 and ca. 12,600	≥24 and ≥13	In the first study, fish lipid content varied from 2.9 to 4.1% during the uptake phase. In the second study, the variation was less and the mean lipid content was 5.71%.	EA (2009b) and EA (2014)
Pentabromo-diphenyl ether	32534-81-9	PentaBDE ca. 17,700 HexaBDE ca. 5,640	PentaBDE ca. 42 HexaBDE ca. 1.4	The analysis is complicated because several congeners were tested at the same time, and some corrections have to be made to the data. The cited data are for one pentaBDE and one hexaBDE constituent, respectively. Fish lipid content was 4.8%.	EC (2001)

Whole fish concentrations associated with a high BCF depend on the dissolved concentration achieved in the experiment as well as (usually) the size and lipid content of the test organisms, species-specific factors (such as metabolism, which may change with life stage), and growth dilution, etc. Comparisons between studies using the same substance can therefore be complicated, and comparisons between substances should be treated with caution. Nevertheless, it can be seen that substances with vB properties can generally achieve whole fish concentrations in the laboratory in the range of 0.9 –

ca. 50 mg/kg ww, with only one substance below this range[14]. A benchmark of 1 mg/kg ww might therefore be suitable as an indicator of high bioaccumulation potential.

The maximum whole fish concentrations for both D4 and D5 exceed 1 mg/kg ww, and so are comparable to substances such as UV-328 and UV-320, long chain perfluorocarboxylic acids, musk xylene, hexaBDE and HBCDD. Molar concentration is inversely proportional to the molecular weight (MW). The MW of D4 (297 g/mole) and D5 (371 g/mole) are lower than some of these substances (e.g. henicosafluoroundecanoic acid, 564 g/mole; HBCDD, 642 g/mole), so there will be more D4/D5 molecules present in the fish compared to these substances when concentrations are the same.

A similar comparative exercise could be performed for dietary bioaccumulation tests, but this has not been done for the purposes of this evaluation.

Ecotoxicity

The October 2014 update of the REACH registrations includes the results of a 22-week OECD TG 206 reproduction test using Japanese Quail (*Coturnix coturnix japonica*) (Smithers Viscient, 2013). EA (2009) contains a summary of the preliminary range-finding test. The DS has not evaluated the original test report of the main study, but the registrants' summary is replicated below.

Exposure to D5 began at 24 days of age with doses of 0, 250, 500, and 1000 mg/kg (ppm) in the diet. The parental birds were put in the same cage for two weeks and then separated into adjoining cages for the remainder of the study with one visit together allowed per day. The NOEC for adult and hatchling body weight, adult feed consumption and all reproduction endpoints[15] was determined to be ≥1,000 mg/kg feed (ppm) (143.5 mg/kg body weight/day). Seven adult birds died during the study: one control female, three females at the 250 ppm level, one male and one female at the 500 ppm level, and one female at the 1,000 ppm level (out of 18 animals per sex, per dose). Post-mortem was conducted on all fatalities and the study authors concluded that because the findings associated with the mortalities were not consistent across all of the fatalities, not observed in other birds and there was no dose-response pattern associated with these findings, they cannot be attributed to exposure to the test substance.

In conclusion, D5 did not cause treatment-related effects at concentrations up to 1,000 mg/kg feed (143.5 mg/kg bw/day). The registrants consider the study to be reliable without restriction.

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

[15] The following reproductive parameters were determined: number of laying pairs at the start of egg collection, eggs laid, eggs cracked, eggs set, viable embryo, live embryos, eggs hatched, hatchling survivors (14 days old), mean hatchling weight, mean hatchling survivor weight (14 days old), mean eggshell thickness.