IDENTIFICATION OF PBT AND VPVB SUBSTANCES

RESULTS OF EVALUATION OF THE PBT/vPvB PROPERTIES

Substance name: 2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol

EC number: 204-279-1

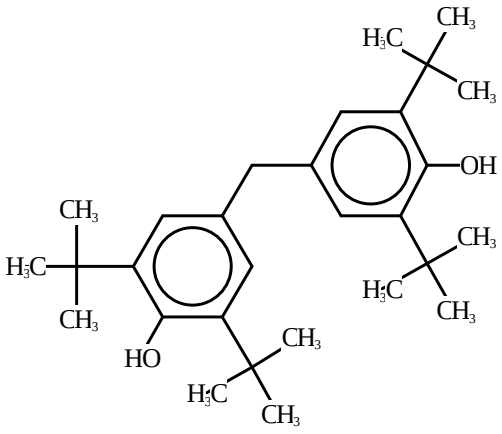
CAS number: 118-82-1

EINECS name: 2,2’,6,6’-tetra-tert-butyl-4,4’-methylenediphenol

IUPAC Name: 4-[(4-hydroxy-3,5-di-tert-butylphenyl)methyl]-2,6-di-tert-butyl-phenol

Molecular formula: $C_{29}H_{44}O_2$

Structural formula:



Summary of the evaluation:

2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol (TBMD) was discussed by the EU PBT Working Group. As a result of these discussions industry carried out a fish bioaccumulation study (OECD TG 305), which was requested for TBMD under Commission Regulation (EC) No. 465/2008 (Bioconcentration study on fish (OECD 305 or dietary study) within 18 months). This study has now been completed (Blankinship et al. 2009 and 2010[1]) and is summarised in the following factsheet.

In the bioconcentration study (Blankinship et al. 2009, 2010), the growth rate constant (0.017 day^{-1}, Environmental Risk Evaluation Report: TBMD) is close to the overall depuration rate constant (0.020 day^{-1}) indicating that growth dilution is the main "depuration" process. Due to the significant fish growth (from 5.88 g to around 16 g), the kinetic BCF corrected for growth is preferred over the steady-state BCF. The growth-corrected and lipid-standardized BCF value is around 14,100 l/kg (EA, 2011 in preparation) based on C^{14}-analysis. Approximately 60% of the ^{14}C in fish at steady-state is parent compound; this would imply a revised BCF for the parent substance of TBMD of 8,640 L/kg (lipid- and growth-corrected) (EA, 2011 in preparation). In another study, the measured steady state BCF values from the NITE website (4,600 – 9,200 l/kg) indicate a high bioaccumulation potential. A dietary study yielded a BMF value of 0.95, which is higher than the BMF for hexachlorbenzol used within the same study. The BCF may be derived from the dietary data resulting in BCF values > than 5,000.

At the current state, it is concluded, that the BCF value of **TBMD is ≥ 5,000 L/kg**, so the substance **meets** the **bioaccumulative (B)** and the **very bioaccumulative (vB) criterion** according to Annex XIII of REACH (see chapter 4.3).

As described in chapter 4.1 the substance undergoes rapid primary degradation; however several major potential persistent metabolites arise.

Only limited data on toxicity is available and the available data mostly refers to acute toxicity is of limited reliability. Based on the available data and considering the missing information on long term toxicity no assessment of the T criteria is possible (chapter 5 and 6). Currently a reproduction test using *Daphnia* has been commissioned by industry. Results to assess the T criterion are expected soon.

Currently, no final conclusion regarding the P and T-criterion can be drawn. Further investigations are needed to conclude on the P and T properties of the substance.

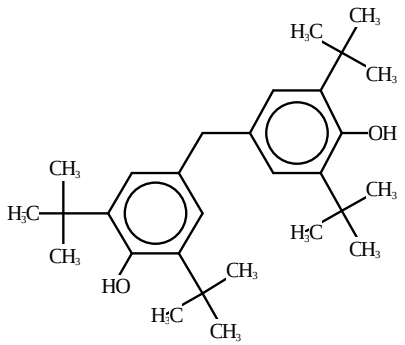
[1] The study report Blankinship et al. 2009 was amended 2010 with modified calculations for lipid content, estimation of t_{90}, $t_{1/2}$, k_1 and k_2.

Contents

JUSTIFICATION

Note: A detailed review of existing information on the properties of TBMD was compiled by the Environment Agency in the UK (EA, 2011 – in preparation). In the following sections, the relevant information from this report was considered.

1 IDENTIFICATION OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

Substance name:	2,2',6,6'-tetra-tert-butyl-4,4'-methylenediphenol
EC number:	204-279-1
CAS number:	118-82-1
EINECS name:	2,2’,6,6’-tetra-tert-butyl-4,4’-methylenediphenol
IUPAC Name:	4-[(4-hydroxy-3,5-di-tert-butylphenyl)methyl]-2,6-di-tert-butyl-phenol
Molecular formula:	$C_{29}H_{44}O_2$
Structural Formula:	
Molecular Weight:	424.67
Synonyms:	4,4’-Methylenebis[2,6-bis(1,1-dimethylethyl)phenol] 4,4’-Methylenebis(2,6-di-tert.-butyl-phenol) 4,4’-Dihydroxy-3,3’,5,5’-tetra-tert.-butyl-diphenylmethan 4,4’-Methylenebis(2,6-di-tert.butylphenol) TBMD Ethanox 702 and Ionox WTE

In the following the substance 2,2’,6,6’-Tetra-*tert*-butyl-4,4’-methylenediphenol is abbreviated with TBMD.

1.1 Purity/Impurities/Additives

No data available.

1.2 Physico-Chemical properties

Table 1: Summary of physico-chemical properties

REACH ref Annex, §	Property	Value	Comments
V, 5.1	Physical state at 20°C and 101.3 Kpa	solid	IUCLID, 2000
V, 5.2	Melting / freezing point	156.4 °C Decomposition occurs >150°C ≈125°C (estimated)	MSDS Albemarle[1] IUCLID, 2000 EA, 2011, in preparation
V, 5.3	Boiling point	Decomposition occurs >150°C ≈491°C estimated at atmospheric pressure	IUCLID, 2000 EA, 2011, in preparation
V, 5.5	Vapour pressure	< 10 hPa (< 1,000 Pa) at 15°C $2x10^{-7}$ hPa at 50°C $2.3x10^{-8}$ Pa at 20°C and $5.5x10^{-8}$ Pa at 25°C (estimated)	IUCLID, 2000 BG CHEMIE, 1990 EA, 2011, in preparation
V, 5.7	Water solubility	< 10 mg/L at 20 °C 0.032 µg/L at 20°C 0.109[2] - 0.02[3] µ/L (estimated)	IUCLID, 2000 Lezotte and Nixon, 2007 EA, 2011, in preparation
V, 5.8	Partition coefficient n-octanol/water (log K_{ow})	6.24 at 20 °C 7.4 8.99	IUCLID, 2000 Measured (cited in USEPA, 2007) Estimate (KOWWIN v1.67)[4]
VII, 5.19	Dissociation constant	Not relevant	

[1] Available via: http://www.albemarle.com/TDS/Antioxidants/ETHANOX_4702_4710.pdf [Dec. 30, 10]

[2] Estimated with WSKOWv1.14 (cited in EA, 2011, in preparation)

[3] Estimated with OECD (Q)SAR toolbox (cited in EA, 2011, in preparation)

[4] KOWWIN v1.67 based on a fragment method (USEPA 2004), (cited in EA, 2011, in preparation)

2 MANUFACTURE AND USES

According to the draft Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1) by the Environment Agency TBMD is used as an antioxitant in lubricants in Europe. The substance is used at less than 1,000 tonnes per year and not yet registered under REACH[5].

3 CLASSIFICATION AND LABELLING

The substance is not included in Annex VI of the Regulation (EC) No 1272/2008.

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Abiotic degradation

No experimental data regarding atmospheric degradation have been measured. According to the draft Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1) by the UK-Environment Agency the rate constant for the reaction (k_{OH}) has been estimated for the substance with AOPWIN (v1.91) (USEPA 2004) according to the TGD for estimating the value of k_{OH} from the chemical structure. The calculated rate constant is 3.6×10^{-11} cm^3/molecule/s and the estimated atmospheric half-life for TBMD (using an average atmospheric hydroxyl radical concentration of 5×10^5 molecules/ cm^3) is 10.7 hours.

Regarding aquatic degradation, information on hydrolysis, oxidation and photolysis should be taken into account. As the substance does not have any functional groups susceptible to hydrolysis in the environment, hydrolysis is therefore not likely to be a significant degradation process. Regarding oxidation, no data have been located on the reaction of the substance itself with oxidants present in water. Based on possible reactions with phenols and atmospheric hydroxyl radicals, TBMD presumably reacts with hydroxyl radicals in freshwater corresponding to primary removal only (half-lives for biodegradation concern ultimate mineralisation to carbon dioxide and water). There is no information available on potential reaction products. No information with respect to photolysis has been found.

4.1.2 Biotic degradation

QSAR output with BIOWIN v4.02 provides the following predictions for degradability: BIOWIN2 (non-linear model prediction) = 0.0005; BIOWIN3 (ultimate biodegradation (time)) = 1.45; BIOWIN6 (MITI Non-linear model prediction) = 0.0014. TBMD is predicted to be not readily biodegradable, as degradation takes weeks to months, using the BIOWIN v4.02 computer program (USEPA 2004) (see draft Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1) by the UK-Environment Agency).

[5] Request via: http://apps.echa.europa.eu/registered/registered-sub.aspx [16.02.2011]

A study performed according to the modified Sturm method (equalling OECD 301B) resulted in 0% degradation in 28 days. It is noted, that the test concentration of 20 mg/L was far above the expected water solubility. At 148 µg/l (the highest concentration tested), no inhibitory effects were observed (Degussa AG 1993, unpublished report).

An activated sludge die-away biodegradation test using both adapted and non-adapted sludge was carried out with radiolabelled test substance (Hamwijk 2007). The test design was based on the draft OECD guideline for simulation tests to assess the primary and ultimate biodegradability of chemicals discharged to wastewater (OECD 2006).

The substance used in the test was ^{14}C-labelled TBMD with the radiolabel uniformly distributed in the aromatic rings. The substance had a radiochemical purity of 99.4%. The substance was mixed with non-radiolabelled test substance (purity 99.54%) for use in the test.

Two samples of activated sludge and raw sewage were collected from an oxidation ditch used to treat domestic waste water. One sample of the sludge was used for the non-adapted treatment and the abiotic control (by sterilisation using heat and mercury chloride). The other sample was used for the pre-adapted treatment by using a Husmann unit. This was carried out over a three-week period during which increasing concentrations of unlabelled test substance were gradually introduced to the unit. The starting concentration was 0.0001 mg/L and the final concentration was 0.01 mg/L.

The tests were carried out by incubating the test substance (concentration around 0.01 mg/L) in the dark with samples of the activated sludge in a closed system at 20°C for 28 days. Each test vessel had a total volume of around two litres and the volume of sludge used was 1 litre per vessel (the dry weight concentration in the sludge was 2.5-3.0 g/L). A total of three test vessels were used: one containing the non-adapted activated sludge, one containing the adapted activated sludge and one containing the sterile activated sludge. The test substance was administered as a solution in ethanol (around 0.1 g of ethanol was added to the test vessels). The biotic vessels were connected to gas trapping systems. The air passing through the system was led through a soda lime column to remove CO_2. The gas flow was connected before dosing and was temporarily disconnected when samples were taken.

During the test the biotic systems were fed continuously with CO_2-free moisturised air and any $^{14}CO_2$ or volatile metabolites formed were collected and analysed. In addition, the primary degradation products in the sludge/solid phase were monitored by HPLC analysis with radiochemical detection.

Preliminary Test

A preliminary test was carried out before the definitive test. Samples were taken from the biotic activated sludge system after 0.5 hours, 1 and 7 days. These three sampling points were selected to (i) determine if recovery of the radioactivity was within 85 and 110% TAR, (ii) verify if the sampling schedule was appropriate, (iii) develop a suitable separation technique for the sludge and aqueous phase and for the extraction of the sludge solids (different solvents were tested: methanol, dichloromethane, hexane and acetonitrile), (iv) develop a concentration method for the extracts and (v) test a simple HPLC method using an Inertsil ODS column and a gradient with (mixtures of) water and acetonitrile as eluent. The preliminary test found that little or no $^{14}CO_2$ or volatile metabolites were formed after seven days incubation. However, primary degradation had occurred (around 80% of the TBMD had degraded after seven days) with two main metabolites being evident. The concentration of these metabolites was increasing at the end of the test, which indicates that they are rather

persistent. The half-life of TBMD was estimated to be approximately three days in this system.

Definitive Test

In the definitive test, samples were taken from the biotic activated sludge systems after 1 and 4 hours and after 1, 2, 3, 7, 14, 21 and 28 days and form the abiotic sludge after 1 and 4 hours and after 1, 7 and 28 days. A similar rapid primary degradation of the substance was also evident in the definitive tests including the abiotic control. The main findings for each test system are summarised below.

For the **non-adapted biotic system** the total recovery of radioactivity was around 149% at the first sampling point (after one hour) and between 92.7 and 109.8% at the subsequent sampling point[6]. The high recovery at the first sampling point may reflect the fact that the substance may not have been fully equilibrated within the test system at this time point. The majority of the radiolabel was associated with the solids present in the test system but the amounts of radiolabel in the aqueous phase and present as bound (non-extractable) residues both increased during the test (for the aqueous phase the amount of radiolabel present increased from around 4% at the start of the test to around 15% at day 28 and similarly for the bound residues the amount of radiolabel present increased from around 4% to around 16%). With a maximal amount of $^{14}CO_2$ of 2.8% TAR (after 28 days), it can be concluded that mineralisation was negligible.

The HPLC analysis of the solid extracts indicated that a total of four metabolites were present, with no TBMD left at any sampling point (it had degraded completely by one hour). One of these metabolites (M1) was present only at the first sampling point (after incubation for one hour; where it accounted for around 17% of the total radiolabel present) and was not detectable after this time. The second metabolite (M2) was present from one hour to day seven (accounting for around 61-81% of the total radiolabel present) but was not detectable from day fourteen onwards. The third metabolite (M3) was present at all sampling points (accounting for around 28-78% of the total radiolabel present). The final metabolite (M4) was not present until day fourteen, after which the amount present increased from around 21% of the total radiolabel (day fourteen) to around 36% of the total radiolabel present (day 28). The results are presented in Table 2. It was considered that M1, M2 and M3 represented primary degradation products (with M1 and M2 being transient and M3 being more persistent) and that M4 represented a more persistent secondary degradation product.

[6] These figures are based on the total amount of radiolabel present in the mixed liquor and the volatile traps. Slightly higher total recovery figures (~124-152 and ~121-173% for the biotic test with non-adapted and adapted sludge, respectively) were given when based on the radioactivity present in the different phases (water, extractable solids and non-extractable solids). This may reflect difficulties in separation/analysis of the various phases or differences in efficiency of the liquid scintillating counting between the fractionated phases and unfractionated mixed liquor.

Table 2: Results of HPLC analysis of organic extracts of the sludge solids in the biotic test system with non-adapted sludge, expressed as % TAR. n.d. = not detected (Source: Hamwijk 2007)

Time (days)	Parent Rt ~ 28.5 min	M1 Rt ~ 20.9 min	M2 Rt ~ 24.6 min	M3 Rt ~ 26.4 min	M4 Rt ~ 25.4 min
0.04	n.d.	17.1	68.0	58.0	n.d.
0.17	n.d.	n.d.	74.9	44.4	n.d.
1	n.d.	n.d.	67.4	48.2	n.d.
2	n.d.	n.d.	61.0	47.5	n.d.
3	n.d.	n.d.	81.1	27.8	n.d.
7	n.d.	n.d.	73.5	31.6	n.d.
14	n.d.	n.d.	n.d.	77.7	20.9
21	n.d.	n.d.	n.d.	58.9	33.8
28	n.d.	n.d.	n.d.	52.2	36.1

Similar rapid degradation was also evident in the **biotic test system with pre-adapted sludge**. In this experiment the total recovery of ^{14}C-label was around 142% at the first sampling point (after one hour) and 101-148% at the remaining time periods[4]. Again the majority of the radiolabel was associated with the solids present in the test system, with the amounts associated with the aqueous phase and present as bound residues accounting for 6-20% and 2-22% respectively. The maximum amount of CO_2 collected amounted to around 4% of the radiolabel administered, indicating that mineralisation was negligible.

The HPLC analysis of the solid extracts showed that TBMD was degraded over the first seven days of the experiment, with no TBMD being detectable from day fourteen onwards. The half-life for primary degradation of TBMD was estimated to be less than four days. Two major transient primary degradation products were evident (M2 and M7) along with three minor transient degradation products (M4, M5 and M6). In addition, two more persistent degradation products (M3 and M8) were found to be formed. The degradation products M2, M3, M5, M6 and M7 appeared from one hour onwards (with no M2 detectable after fourteen days, M3 being detectable at all time points, no M5 detectable after two days, no M6 detectable after four hours and no M7 detectable after fourteen days). The metabolite M4 was only detectable in the sample collected after one day incubation and the metabolite M8 was detectable in the day-one sample and in the samples from day seven onwards. Both M4 and M8 were considered to be secondary metabolites. It was noted that M2 and M4 appeared to be degraded more rapidly in this test system using adapted sludge compared with the test system using non-adapted sludge. The exact test results are presented in Table 3.

Table 3: Results of HPLC analysis of organic extracts of the sludge solids in the biotic test system with pre-adapted sludge, expressed as % TAR. n.d. = not detected (Source: Hamwijk 2007)

Time (days)	Parent Rt ~ 28.4 min	M2 Rt ~ 24.6 min	M3 Rt ~ 26.4 min	M4 Rt ~ 25.2 min	M5 Rt ~ 23.8 min	M6 Rt ~ 29.3 min	M7 Rt ~ 30.6 min	M8 Rt ~ 25.7 min
0.04	92.6	6.5	19.6	n.d.	4.2	6.2	6.4	n.d.
0.17	65.5	13.9	29.8	n.d.	7.8	n.d.	7.2	n.d.
1	80.7	16.7	25.8	5.1	7.2	n.d.	10.9	12.3
2	89.1	n.d.	15.6	n.d.	n.d.	n.d.	11.4	n.d.
3	59.4	10.2	25.5	n.d.	n.d.	n.d.	14.8	n.d.

Time (days)	Parent Rt ~ 28.4 min	M2 Rt ~ 24.6 min	M3 Rt ~ 26.4 min	M4 Rt ~ 25.2 min	M5 Rt ~ 23.8 min	M6 Rt ~ 29.3 min	M7 Rt ~ 30.6 min	M8 Rt ~ 25.7 min
7	12.5	15.2	36.9	n.d.	n.d.	n.d.	12.3	18.6
14	n.d.	n.d.	39.2	n.d.	n.d.	n.d.	n.d.	36.9
21	n.d.	n.d.	41.5	n.d.	n.d.	n.d.	n.d.	31.7
28	n.d.	n.d.	45.5	n.d.	n.d.	n.d.	n.d.	24.1

Similar to the two biotic test systems, TBMD was found to be rapidly degraded in the **abiotic control system**, indicating that the primary degradation of TBMD was essentially an abiotic process. The overall recovery of radiolabel in this system was between 99% and 114% at all time points[7]. The maximum amount of $^{14}CO_2$ evolved during the study amounted to 0.3% of the total radiolabel, indicating that mineralisation was minimal. The majority of the radiolabel was again associated with the solids, with between 2-9% of the radiolabel found in the aqueous phase and 2-23% of the radiolabel being found as bound residues.

The HPLC analysis of the extracts from the sludge solids found that TBMD was not detectable at any time point, indicating that primary degradation was rapid (half-life < 1 hour). A total of five metabolites were found. M2 and M3 were found to be formed within one hour and were still detectable after 28 days (the amount present after 28 days was less than at day 7). M4 was detectable only at the samples collected after one hour and 28 days. M7 and M9 were detectable only in the sample collected after 28 days. M2, M3 and M4 can be regarded as primary degradation products, whereas M7 and M9 can be regarded as secondary degradation products. No samples were collected after 14 days in this particular experiment. The exact test results are presented in Table 4.

Table 4: Results of HPLC analysis of organic extracts of the sludge solids in the abiotic test system, expressed as % TAR. n.d. = not detected (Source: Hamwijk 2007)

Time (days)	Parent Rt ~ 28.4 min	M2 Rt ~ 24.6 min	M3 Rt ~ 26.4 min	M4 Rt ~ 25.2 min	M7 Rt ~ 30.6 min	M9 Rt ~ 25.7 min
0.04	n.d.	66.8	27.5	23.6	n.d.	n.d.
0.17	n.d.	78.4	35.9	n.d.	n.d.	n.d.
1	n.d.	86.3	37.9	n.d.	n.d.	n.d.
7	n.d.	76.6	34.8	n.d.	n.d.	n.d.
28	n.d.	22.5	14.3	11.9	31.4	13.7

Overview of the test results

Overall, the study found that TBMD undergoes rapid primary degradation, probably by an abiotic mechanism. Several metabolites were identified, depending on the conditions used.

7 These figures are based on the total amount of radiolabel present in the mixed liquor and the volatile traps. Slightly higher total recovery figures (~123-132%) were given when based on the radioactivity present in the different phases (water, extractable solids and non-extractable solids). This may reflect difficulties in separation/analysis of the various phases or differences in efficiency of the liquid scintillating counting between the fractionated phases and unfractionated mixed liquor.

Some of these metabolites were transient intermediates, whereas others appeared to be more persistent. It was noted in the study that the HPLC retention times of the metabolites were, in many cases, close to that of the parent compound (metabolites M1 to M5, M8 and M9 had shorter retention times than TBMD and metabolites M6 and M7 had longer retention times than TBMD). This indicates that the degradation steps involved may have introduced only relatively small changes to the molecule (for example de-methylation). Unfortunately, no further analysis of the degradation products was undertaken. The results of the study are summarised in Table 5.

Table 5: Overview of degradation products in various test systems. n.d. = not detected. * = indicative, the concentrations at the end of the test were above 10%, but the peaks in the chromatograms had areas close to the background (Source: Hamwijk 2007)

Test system	Approximate retention time (min.)	Biotic system, with non-adapted sludge	Biotic system, with pre-adapted sludge	Abiotic sludge
M1	20.9	Major, transient	n.d.	n.d.
M2	24.6	Major, transient	Major, transient	Major, transient
M3	26.4	**Major, potential persistent**	**Major, potential persistent**	Major, but seems to degrade slowly
M4	25.4	**Major, potential persistent**	Minor, transient	**Major, potential persistent***
M5	23.8	n.d.	Minor, transient	n.d.
M6	29.3	n.d.	Minor, transient	n.d.
M7	30.6	n.d.	Major, transient	**Major, potential persistent**
M8	25.7	n.d.	**Major, potential persistent**	n.d.
M9	27.2	n.d.	n.d.	**Major, potential persistent***

It should be noted that the test concentration (0.01 mg/L) was around three orders of magnitude higher than the measured water solubility of TBMD (0.032 μg/L) which may have reduced its availability for degradation. Nevertheless, rapid primary degradation of TBMD was apparent in all test systems. In addition, a large proportion of the radiolabel present in the system was adsorbed onto the sludge solids which indicates that the primary degradation process may not depend on TBMD being in the dissolved phase.

Due to the fact that in the biotic system with non-adapted sludge and in the abiotic system no test substance was detected at the first sampling point, an empirical DT_{50} of < 1 hour is estimated. In the biotic system with pre-adapted sludge, the concentration of the test substance during the first three days was too variable to carry out reliable kinetic calculations. Based on the concentrations from day 0-3 (59.4-92.6% TAR) and the concentration at day 7 (12.5% TAR), it is estimated that the DT_{50} in this system was < 4 days. An overview of estimated DT_{50} and DT_{90} values is given in the Table 6.

Table 6: Overview of estimated degradation rates of the test substance in various test systems. n.d. = not determined. (Source: Hamwijk 2007)

Test system	Preliminary test	Biotic system, with non-adapted sludge	Biotic system, with pre-adapted sludge	Abiotic sludge
DT_{50}	Appr. 3 days	< 1 hour	< 4 days	< 1 hour
DT_{90}	n.d.	< 1 hour	Appr. 7 days	< 1 hour
k	0.2 $days^{-1}$	< 0.7 $hour^{-1}$	< 0.2 $days^{-1}$	< 0.7 $hour^{-1}$

The study by Hamwijk (2007) showed that TBMD undergoes rapid primary degradation in water. However, the products tend to be more persistent and in some cases have chromatographic properties not very different from the parent compound (implying similarities in chemical structure). The substance is considered to be not readily degradable in waste water treatments plants but that there is enough evidence of degradation in the environment to consider the substance inherently biodegradable in surface water. The rate constants used in the assessment on this basis are zero for the waste water treatment plant, 4.7×10^{-3}/d for surface water (corresponding to a half-life of 150 days) and 2.3×10^{-5}/d for sediment (corresponding to a half-life of 30,000 days).

4.1.3 Other information

No data regarding degradation in soil have been located. However, the draft Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1) by the UK-Environment Agency indicates that degradation in the environment might occur, and so assumes for the purposes of risk assessment that the substance is inherently biodegradable in soil. The rate constant used is 2.3×10^{-4}/d (corresponding to a half-life of 3,000 days).

4.1.4 Summary and discussion of persistence

Based on the BIOWIN-predictions, the substance is expected to be persistent in the environment. A former study on ready biodegradability (OECD 301) (Degussa AG, 1993, unpublished report) showed no degradation of the substance within 28 days as evidenced by CO_2 formation. However, it was noted that the concentration used was far above water solubility. The study by Hamwijk (2007) showed that the substance undergoes rapid primary degradation in the environment, however, some of the degradation products tend to be more persistent under the same conditions, and their identities are unknown. These degradation products have similarities in chemical structure with the parent compound. Therefore more information regarding the identity and the degradation pathway of the degradation products is necessary. This is also outlined in the REACH Guidance on information requirements and chemical safety assessment Chapter R.7c: Endpoint specific guidance (ECHA 2008b): When a substance is not fully mineralised, but degraded to more persistent degradation products, the PBT/vPvB properties of these should be evaluated before a final judgment of whether a substance fulfils the persistence criteria can be drawn.

4.2 Environmental distribution

4.2.1 Adsorption

No experimental data are available. However in the draft Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1) by the UK-Environment Agency the partition coefficients were estimated according to the TGD II (EC 2003). Although some uncertainties remain (equation only valid for phenolic substances with log K_{ow} between 1 and 5) these values were used in the UK-Risk Assessment (Table 7).

Table 7: Estimated partition coefficients (Source: Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1))

PCKOCWIN v1.66 computer program (USEPA 2004)	Koc: $8.34x10^{-7}$
TGD II (EC 2003): Equation: log K_{oc} = 0.63 × log K_{ow} + 0.90	

Partition coefficient	Symbol	Value
Organic carbon–water partition coefficient	K_{oc}	68,000 L/kg
Solids–water partition coefficient for soil	Kp_{soil}	1,400 L/kg
Solids–water partition coefficient for sediment	Kp_{sed}	3,400 L/kg
Solid–water partition coefficient for suspended matter	Kp_{susp}	6,800 L/kg
Soil–water partition coefficient	$K_{soil\text{-}water}$	2,000 m^3/m^3
Sediment–water partition coefficient	$K_{sed\text{-}water}$	1,700 m^3/m^3
Suspended matter–water partition coefficient	$K_{susp\text{-}water}$	1,700 m^3/m^3

Currently industry is carrying out an adsorption/desorption test.

4.2.2 Volatilisation

According to the draft Environmental Risk Evaluation Report: 2,2',6,6'-Tetra-tert-butyl-4,4'-methylene-diphenol (TBMD) (CAS No. 118-82-1) by the UK-Environment Agency TBMD has a low Henry's law constant (0.31 Pa m^3/mole at 20°C calculated using the bond method in USEPA HENRY v3.10). The substance is therefore expected to remain mainly in the water phase rather than volatilise to air.

4.2.3 Long-range environmental transport

No data available.

4.3 Bioaccumulation

4.3.1 Screening data

- **Fish:**

As outlined in the UK-Environment Agency draft Report the Technical Guidance Document (TGD II, EC 2003) provides equations for the prediction of a fish BCF from the log K_{ow} value: the result for TBMD is 38,900 L/kg based on a log K_{ow} of 6.24.

The estimated BCF is 4,516 L/kg using the BCFWIN v2.15 computer program (also based on log K_{ow}: 6.24) (EA, 2011 in preparation).

- **Earthworm:**

A value for the earthworm BCF has also been calculated using the TGD method (from the log K_{ow} value 6.24) giving a value of 2.09×10^4 L/kg.

4.3.2 Measured bioaccumulation data

4.3.2.1 Bioaccumulation in fish: Aqueous Exposure

A bioconcentration study on fish (OECD 305 or dietary study) was requested by the EU PBT Working Group, and this requirement was included in Regulation 465/2008. The study was carried out with rainbow trout (*Oncorhynchus mykiss*) according to the OECD test guideline 305 (OECD 2006) with ^{14}C-labelled substance (radiochemical purity 99.4%).

Study summary

The bioconcentration test (Blankinship et al. 2009 and 2010[8]) consisted of a 35-day uptake phase followed by a 60-day depuration phase.

During the uptake phase, two groups of the test organisms were exposed to:

1) a solvent (0.1 mL dimethylformamide (DMF)/L) control;

2) a nominal concentration of 0.025 µg/L of 2,2',6,6'-Tetra-tert-butyl-4,4-methylenediphenol (TBMD).

Due to analytical limitations two test concentrations could not be achieved. Prior to the initiation of the test, a preliminary trial was performed to determine the functional solubility of 2,2',6,6'-Tetra-tert-butyl-4,4-methylenediphenol (TBMD) in water in a diluter system. Based on this trial it was determined that the test substance could not effectively be delivered at a concentration higher than 0.025 µg/L. Therefore, the concentration of 0.025 µg/L was selected for the test.

8 The study report Blankinship et al. 2009 was amended 2010 with modified calculations for lipid content, estimation of t_{90}, $t_{1/2}$, k1 and k2.

The water used in the test had a hardness of 132-140 mg/L as $CaCO_3$, a pH of 8.0-8.3 and the dissolved oxygen concentrations remained at or above 6.4 mg/L throughout the test. The test was carried out at 15°C.

Each test chamber contained 85 rainbow trout at test initiation. At the start of the depuration phase, stock flow to the treated group and solvent control was stopped and the rainbow trout were exposed to dilution water without TBMD or DMF for the remainder of the test.

Test organism

All fish used in the test were from the same source and year class, and the total length of the longest fish was not more than twice the length of the shortest. Measurements of the 5 fish were obtained from fish collected for lipid analysis prior to test initiation. The mean total length of the 5 fish was 65.6 mm with a range of 64 to 67 mm and the average wet weight (blotted dry) was 3.02 g with a range of 2.79 to 3.30 g. Loading was defined as the total wet weight of fish per liter of test water that passed through the test chamber in 24 hours, and was determined to be 0.51 g fish/L/day. Instantaneous loading was 3.21 g/L.

All fish were observed daily to evaluate the number of mortalities and the number of individuals exhibiting signs of abnormal behaviour. There were no mortalities in the solvent control and the 0.025 µg/L treatment group during the uptake phase and all fish in these groups appeared normal with no treatment-related signs of toxicity.

On the 35th day of depuration 13 fish were missing in the solvent control group and 2 fish in the treatment group. Due to the lengths of the study which resulted in the fish growing to a size of approximately 10 g by day 31 depuration, the missing fish may have been lost during handling or due to aggression from other fish in the tank. The overall mortality in the solvent control (including the missing fish) during the study was 15%. The mortality in the treatment group was 2.4%.

Fish were collected on day 0, day 35 of uptake and day 60 of depuration for lipid analysis. Each fish was then dissected into non-edible (head, fins, viscera) and the remaining edible tissue fractions.

Analytical method and sampling

The concentrations of the ^{14}C-labelled substance (radiochemical purity 99.4%) in freshwater and fish tissue were verified by liquid scintillation counting (LSC):

Water samples

Water samples were collected on

- day -1 (pretest), on uptake days 0, 3, 7, 13, 21, 28 and 33 and on depuration days 3, 7 and 10 of the test and analysed for *TBMD based on total radioactivity*.
- day 33 of uptake and analysed for parent *TBMD.*

Tissue samples

Tissue samples were collected and analysed

- for *TBMD based on total radioactivity* at the same water sample collection periods during uptake and also during depuration days 17, 31, 45 and 60.
- for *parent TBMD* on days 21, 28, 33 and depuration day 60.

The limit of quantitation (LOQ)

LOQ for each sample set was calculated from the following equation:

- For freshwater, LOQ = (1.5 x mean of all background samples (dpm)) / sample volume (L) / specific activity x 1000.

  The mean of the daily calculated limits of quantitation (LOQ) for freshwater based on total radioactivity was 0.000969 µg/L.

- For tissue, LOQ = (1.5 x mean of all background samples (dpm)) / sample mass (g) / specific activity x 1000.

  The mean of the daily calculated limits of quantitation (LOQ) for tissue based on total radioactivity was 0.234 µg/kg.

Calculation of steady-state BCF values

Whole fish concentrations were calculated based on the sum of edible and non-edible tissue concentrations for each fish. The steady-state bioconcentration factor (BCF) values were determined from the mean tissue concentrations at apparent steady-state divided by the average water concentration. Tissue concentrations were considered to be at apparent steady-state if three or more consecutive sets of tissue concentrations were not significantly different ($p > 0.05$). Tissue concentrations were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively.

Since the data passed the assumptions of normality and homogeneity, analysis of variance (ANOVA) was used to determine whether or not statistically significant differences existed between days at the end of the uptake phase of the test ($p = 0.05$). Treatment means that were significantly different between days were identified using Tukey's test ($p \leq 0.05$).

Calculation of kinetic BCF values and equations for reaching 90% steady-state and 50% clearance

Test guidelines OECD 305 (OECD 1996) and OPPTS 850.1730 (USEPA 1996) give two methods for calculating rate constants (uptake rate (k_1) and depuration rate (k_2)) to determine kinetic BCF (BCFK) values. One method is the graphical method; the other method is non-linear regression. Both guidelines also provide equations for calculating the half-life for clearance in tissue (t½), time to reach 90% of steady-state (t90) and BCFK values using the equations and graphical methods.

Kinetic BCF values for edible, non-edible and whole fish tissue based on total radioactivity were calculated using the equations outlined in the OPPTS 850.1730 (USEPA 1996) using day 33 of uptake and day 60 of depuration.

The non-linear regression method uses the entire uptake and depuration data in a computer program to build a regression equation to fit the data. This method was used for total radioactivity data since this was the method used to analyse samples throughout the study.

The kinetic uptake rate (k_1) and depuration rate (k_2) were calculated for edible, non-edible and whole fish using SAS computer code described by Newman (cited in Blankinship et al. 2010). These rate constants were used to calculate a kinetic bioconcentration factor (BCFK = k_1/k_2) and also to calculate half-life for clearance in tissue (t½), time to reach 90% of steady-state (t90) and BCFK values using the equations outlined in test guideline OECD 305 (OECD 1996) and OPPTS 850.1730 (USEPA 1996).

Two non-linear regression procedures were applied:

- In the simultaneous procedure, nonlinear regression was used to simultaneously solve for k_1 and k_2 using fish tissue data from the uptake phase.
- In the sequential method, data from the depuration (elimination) phase were used to first estimate k_2, and then using both the k_2 estimate and fish tissue from the uptake phase to estimate k_1. Both methods generally give similar results.

However, in this study the results were different, and the simultaneous method was used because the residual error from regression was lower than in the sequential method.

Additionally, the simultaneous method appeared to give results more consistent with both the graphical method and steady-state BCF values. Therefore kinetic parameters estimated by the simultaneous method based on non-linear regression were assumed to be better estimates of rate constants than the sequential method and were used to calculate half-lives and time to 90% of steady-state for total radioactivity data.

CONCLUSIONS reported by Blankinship et al. (2010)

Because declining concentrations of the test material in tissues is not consistent with the assumption of first order kinetics (required for valid kinetic estimates of BCF), and it is clear that a steady-state had been achieved in this study, the steady-state estimates of the BCF were considered to be the most accurate of the available BCF estimates from this study.

Steady-state concentrations of 2,2',6,6'-Tetra-tert-butyl-4,4-methylenediphenol (TBMD) were achieved in the tissues of rainbow trout (*Oncorhynchus mykiss*) after 21 days. The mean measured water concentration based on total radioactivity was 0.016 µg/L, and on parent TBMD was 0.018 µg/L.

Steady-state BCF values based on total radioactivity TBMD concentrations were 815, 1644 and 1146 in edible, non-edible and whole fish tissue, respectively. TBMD depurated slowly in fish tissue and was 25-38% of steady-state values by depuration day 60. Steady-state BCF values based on parent TBMD concentrations were 552, 666 and 600 in edible, non-edible and whole fish tissue, respectively.

Estimates derived with the simultaneous method based on total radioactivity were as follows: Time to reach 90% steady-state was 30, 28 and 28 days and time to reach 50% clearance was 9.1, 8.4 and 8.4 days for edible, non-edible and whole fish tissue, respectively. Kinetic BCFK factors derived by this method were 920, 1807 and 1265 for edible, non-edible and whole fish tissue, respectively.