Substance Name: Terphenyl, hydrogenated

EC Number: 262-967-7

CAS Number: 61788-32-7

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT FOR IDENTIFICATION OF TERPHENYL, HYDROGENATED AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS vPvB[1] PROPERTIES (ARTICLE 57E)

Adopted on 1 June 2018

[1] vPvB means very persistent and very bioaccumulative

CONTENTS

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

Substance Name: Terphenyl, hydrogenated

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- The substance is identified as very persistent and very bioaccumulative (vPvB) according to Article 57 (e) of Regulation (EC) No 1907/2006 (REACH).

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

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to assess the PBT/vPvB properties of the substance. All available information (such as the results of standard tests, modelling and (Q)SAR results) was considered together in a weight-of-evidence approach.

According to the ECHA guidance (ECHA 2017a, R.11), the *Weight-of-Evidence* determination by expert judgement enables the use of all (screening and assessment) information types listed in Section 3 of Annex XIII to the REACH Regulation in the PBT/vPvB assessment for comparing with the criteria, although not all of these information types can be directly (numerically) compared with the criteria.

Persistence

A substance fulfils the persistence criterion (P) in any of the following situations:
(a) the degradation half-life in marine water is higher than 60 days;
(b) the degradation half-life in fresh or estuarine water is higher than 40 days;
(c) the degradation half-life in marine sediment is higher than 180 days;
(d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days;
(e) the degradation half-life in soil is higher than 120 days.

A substance fulfils the “very persistent” criterion (vP) in any of the following situations:
(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days;
(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days;
(c) the degradation half-life in soil is higher than 180 days.

For the persistence assessment of terphenyl, hydrogenated, most weight is given to half-lives measured in standard simulation tests or simulation tests which are considered comparable to standard tests in terms of reliability and test conditions. Half-lives from such tests can be directly compared with the P/vP criteria. Results from simulation tests with conditions differing from standard tests (or with insufficient documentation), screening tests, QSAR predictions, and microbial culture studies, are used as supporting information.

Based on the weight-of-evidence assessment of available relevant information, terphenyl, hydrogenated fulfils the P and vP criteria. The relevant findings are summarised below:

- Based on available information, abiotic degradation is expected to occur at such a low rate that it is not considered a relevant route of degradation for P/vP assessment

- In a soil simulation test, dissipation half-lives in soil of ≥218 days (temperature-corrected to 12°C) were determined for terphenyl and >224 days quaterphenyl (Monsanto Company 1989) thus fulfilling the P and vP criteria. These half-lives were determined for a mixture of terphenyls, quaterphenyls, and polyphenyls (the proportions of the different isomers are not known). Quaterphenyls and terphenyls are relevant constituents of the UVCB substance.
- In a seawater simulation test with hydrocarbon mixtures (ExxonMobil Biomedical Science, Inc., 2009) primary degradation half-live (temperature-corrected to 12°C) of >182 days was reported for o-terphenyl and half-lives of 32 d and 108 d for m-terphenyl, suggesting that o-terphenyl and m-terphenyl fulfil the P/vP criterion in marine water.
- In an OECD 307 soil simulation test a dissipation half-life of 2-10 days (NOTOX 2009a) for p-dicyclohexylbenzene (HT2) was detected during the test when the half-lifes are calculated for the whole test duration using bi-phasic models. Assuming that all non-extractable residues (NER) are parent substance, the half-life is 6-18 days in two soils whereas for one soil no exact half-life can be determined and it is estimated that the half-life for this soil is above test duration, i.e., >120 days. When the second phase ('slow phase') from bi-phasic models is used the half-lives were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k_2 parameter was not statistically significant and as the half-life obtained from temperature conversion is longer than the experimental period) whereas for one of the soils, no reliable second-phase half-lives could be determined. In this study a significant part of applied radioactivity partitioned to soil and was quantified as NER, which has a strong influence on the shape of the dissipation curve, which causes uncertainty for the determination of the degradation half-life. The results indicate that p-dicyclohexylbenzene (HT2) is potentially P or vP. Definitive P/vP conclusion has not been drawn in this assessment due to limited data on NER.
- In non-standard biodegradation ultimate biodegradation tests (Monsanto report ES-80-SS34, Monsanto 1977a), degradation of UVCB substances (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) based on CO_2 evolution was at the most 14 % within 35 days, suggesting that the tested substances are not readily biodegradable and therefore potentially P or vP.
- In a river die-away test, when tested separately, o- and p-terphenyl showed no or neglible degradation during 28 days whereas m-terphenyl started to degrade after 16 days. When tested in a mixture of m-, o-, and p-terphenyls, o- terphenyl and m-terphenyl started to biodegrade after 30 days. A HT3 constituent showed no degradation in 30 days whereas HT1 and HT2 constituents were more degradable (Mic 1983a). The results suggest that the tested o-T, p-T, and HT3 constituents are potentially P or vP whereas for the constituents with higher degradation, m-T, HT1, and HT2, no conclusion can be drawn as only primary degradation was measured and, in the case of m-T, as the results were different when tested in mixture or as individual compound.
- A shake-flask carbon dioxide evolution test with a hydrocarbon-adapted inoculum (Mic 1983b) showed relatively low (9-38%) mineralization for o-T, m-T, p-T, p-HT2, p-HT3, and p-Q) in 55 days, suggesting that o-T, m-T, p-T, p-HT2, p-HT3, and p-Q are potentially P or vP. No conclusion can be drawn from this study for p-HT1 as its higher degradation (63%) may be explained by the adapted inoculum.
- In a semi-continuous activated sludge (SCAS) study (Monsanto 1973) the mean disappearance of hydrogenated quaterphenyls (HQ) was 16% at the end of the SCAS study (with negligible volatilization), in a test system considered to be favourable for microbial adaptation. The presence of a detectable amoung of HQ at the end of the following die-away procedure is in line with the results of the SCAS study. The test substance (HQ40) was a mixture of approximately 80 % quaterphenyls with a degree of 40 % hydrogenation (the residual 20 % consists of terphenyl and higher (> 5-ring) phenyl structures). The results suggest that HQ is potentially P or vP.
- P-terphenyl persisted in an SCAS test system (Monsanto 1974) despite the possible

adaptation during the test and in a die-away procedure conducted with an inoculum from the SCAS system. Test substance was a mixture containing mainly o-, m-, and p-terphenyls. The results suggest that p-terphenyl is potentially P or vP whereas for m- and o-terphenyl no conclusions can be done due to different concentrations of the isomers in the test substance and possible abiotic losses.

- In a shake-flask carbon dioxide evolution test (Monsanto 1991) with an inoculum pre-exposed to p-terphenyl, p-terphenyl showed no significant mineralisation or primary degradation in 42 days. The CO_2 production after 42 days was 8-9% in the active test and 7% in sterile control. The mean residue recovery after 42 days was 78.0-81.1% of initial level in the active test and 82.1 in sterile control. The results suggest that p-terphenyl is potentially P or vP.

- In a microbial culture study (Ohmori et al 1973) the amounts and properties of microbial strains isolated from environmental samples using terphenyl or other hydrocarbons as a sole carbon source suggest that terphenyl is a less favourable growth substrate compared to other hydrocarbons tested (*n*-paraffin, biphenyl, diphenylmethane, diphenylethane, *trans*-stilbene) and therefore the ultimate degradability of terphenyl in the environment may be limited. The results indicate presence of terphenyl utilizing microorganisms but also suggest that microorganisms able to utilise other hydrocarbons are not necessarily able to utilise terphenyl. The results suggest that o-, m-, and p-terphenyl are potentially P or vP.

- BIOWIN models 3 and 6 in combination indicate that o-T, m-T, p-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4, are potentially P or vP, as the P/vP screening criteria for this model combination are fulfilled. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BIOWIN models 2 and 3 in combination indicate that o-T, m-T, P-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4 do not screen as P or vP. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BioHCwin model predicts primary degradation half-lives of 315 days for HT1, 470 days for HT2, 69 days for HT3, 68 days for HQ1, 809 days for HQ2, and 305 days for HQ3, exceeding the P and vP criteria in water (HT1, HT2, HT3, HQ1, HQ2, and HQ3) and in soil and sediment (HT1, HT2, HQ2, HQ3). No conclusion could be done for o-T, m-T, p-T, and Q (for which half-lives were 7-8 days and thus below the P and vP criteria) because BioHCwin model gives a primary biodegradation half-life estimate and because data obtained with mixtures has been used in its training set. Half-lives used to derive the BioHCwin model include results obtained from water, soil, and sediment studies.

Table below summarises the conclusions on P/vP for the selected constituents of terphenyl, hydrogenated.

P/vP conclusion of selected constituents of terphenyl, hydrogenated

	Persistence
o-T	P and vP
m-T	potentially P or vP
p-T	P and vP
p-HT1	potentially P or vP
p-HT2	potentially P or vP

p-HT3	potentially P or vP
p-Q	P and vP
p-HQ1	potentially P or vP
p-HQ2	potentially P or vP
p-HQ3	potentially P or vP

Bioaccumulation

A substance fulfils the B criterion when the bioconcentration factor in aquatic species is higher than 2000, and the vB criterion when the bioconcentration factor in aquatic species is higher than 5000. A weight-of-evidence determination using expert judgement is applied by comparing all relevant and available information. For the bioaccumulation assessment of terphenyl, hydrogenated most weight is given to valid measured BCF-values, because these are directly comparable with the criteria. Measured BMF-values and BCF-values derived from these are used as supporting information as well as QSAR predictions.

Based on the weight-of-evidence assessment of available relevant information, terphenyl, hydrogenated fulfils the B and vB criteria because:

- A measured BCF value in Rainbow trout above the vB criterion, 12 993, is determined for o-terphenyl (o-T), a relevant constituent of the UVCB substance (Schlechtriem 2016). This study result is supported by measured BMF values in Rainbow trout, 0.59 (OECD 2012) and 0.2 (ExxonMobil 2010a), which predict BCF-values of 6219 ±1647 and4887 ± 1611, respectively. Based on these data, it is concluded that this constituent is B and vB.
- Measured BCF values for o-terphenyl (o-T) in Carp, 1900 ± 300 and 1100 ± 200, (NITE 2012) are close to the B criterion. (It is noted that these BCF values might be underestimations due to growth dilution.) They are supported by measured BMF values of 0.09 – 0.25, (OECD 2012, Inoue et al. 2012) leading to estimated BCF values of 1575 ± 420 and 1482 ± 549. Based on these data, it is concluded that this constituent is B.
- Partially hydrogenated terphenyls (HT1, HT2) show high measured BCF-values (1551 – 12 436) in Carp and Bluegill (NITE 2004, Monsanto 1983) exceeding the vB criterion. Based on these data, it is concluded that these constituents are B and vB.
- Based on the BCF values measured for m,m-quaterphenyl (Q), 2273 – 3259, (NOTOX 2009b), it can be concluded that this constituent fulfils the B criterion but not the vB criterion. QSAR predictions are 9646 (regression model) and 1499 (Arnot-Gobas), thus supporting this conclusion. Based on these data, it is concluded that this constituent is B.

For some constituents (m-T, p-T, HT3, HQ1, HQ2, HQ3 a definitive conclusion is not possible due to lacking or contradictory data,

- For p-T, HT3, HQ1, HQ2, HQ3 no experimental data on bioaccumulation is available.
- Based on log Kow values (> 4.5), it is concluded that p-T, HT3, HQ1, HQ2 are potentially B and vB.
- For HQ3 the predicted logKow exceeds 10 and the predicted BCF values drop below 500. According to ECHA guidance (ECHA 2014), the aquatic BCF of a substance is probably lower than 2000 if the calculated Log K_{ow} is higher than 10. Therefore, it is concluded that the constituent in probably not B or vB.
- For m-terphenyl QSAR predictions and logKow value indicate that the substance is

potentially B. A dietary biomagnification study, on the other hand, shows rapid depuration in rainbow trout ($T_{1/2}$ = 0.52), which corresponds to estimated BCF-values of 636 ± 199. As the information is scarce and contradictory, it is not possible to conclude.

B/vB conclusion of selected constituents of terphenyl, hydrogenated

	Bioaccumulation
o-T	B and vB
m-T	not possible to conclude
p-T	potentially B and vB
p-HT1	B and vB
p-HT2	B and vB
p-HT3	potentially B
p-Q	B
p-HQ1	potentially B and vB
p-HQ2	potentially B and vB
p-HQ3	probably not B or vB

Conclusion: It can be definitively concluded that at least o-terphenyl fulfils both vP and vB criteria. As o-terphenyl occurs in significant concentrations in the UVCB substance (> 0.1 % w/w), terphenyl, hydrogenated is considered to fulfil the vPvB criteria.

In conclusion, terphenyl, hydrogenated meets the criteria for a vPvB substance according to Article 57 (e) of REACH.

Overall conclusion:
In conclusion, terphenyl, hydrogenated meets the criteria for a vPvB substance according to Article 57 (e) of REACH by comparing all relevant and available information according to Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

Registration dossiers submitted for the substance: YES

Justification

1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1. Substance identity

EC number:	262-967-7
EC name:	Terphenyl, hydrogenated
CAS number (in the EC inventory):	61788-32-7
CAS number:	61788-32-7
CAS name:	Terphenyl, hydrogenated
IUPAC name:	-
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	not applicable (n.a.) (UVCB)
Molecular weight range:	230 - 306 g/mol
Synonyms:	HB40/00 Partially hydrogenated terphenyls *Therminol 66*

Structural formula: n.a. (UVCB)

1.2 Composition of the substance

Name: Terphenyl, hydrogenated

Description: Substances obtained from the hydrogenation of terphenyl, containing thus unhydrogenated, partially hydrogenated and totally hydrogenated constituents of terphenyl.

Substance type: UVCB[2]

[2] Substances of Unknown or Variable composition, Complex reaction products or Biological materials

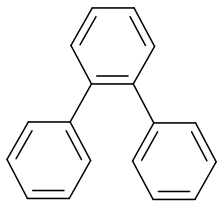
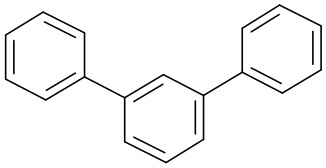
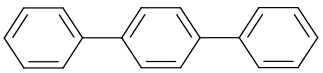
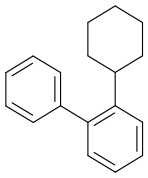
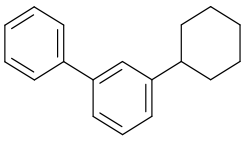
Table 2: Constituents

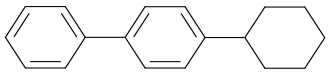
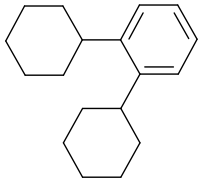
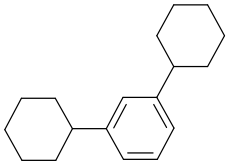
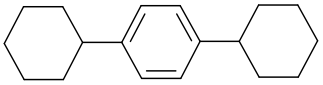
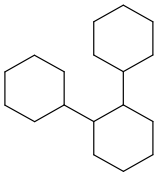
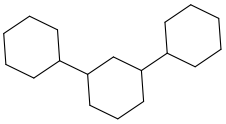
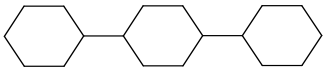
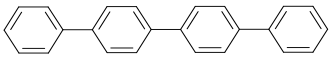
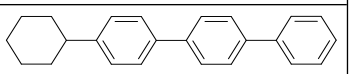
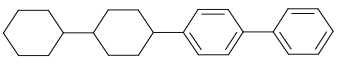
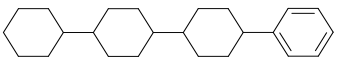
Constituents	Typical concentration	Concentration range
Terphenyl, hydrogenated	-	70 – 85 %*
Terphenyl	-	< 5 %*
Quaterphenyls and higher polyphenols, partially hydrogenated	-	-

* Exemplifying information from Safety Data Sheets at:
https://www.therminol.com/resources/therminol-heat-transfer-fluid-information-library#SDS

For the PBT/vPvB assessment representative structures from identified groups of constituents were identified in order to create QSAR predictions for these structures (Table 3). The structures were selected to cover constituents of terphenyl, hydrogenated. The structures include unhydrogenated structures as well as partially and completely hydrogenated structures of ortho, meta- and para-forms of terphenyls and quaterphenyls.

Table 3. Representative structures selected for the PBT/vPvB assessment

Group	Contains	Representative structure		
		name (CAS)	smiles	structure
o-T	ortho-terphenyl (1,1:2,1-Terphenyl)	1,2-terphenyl (1,1:2,1 -Terphenyl) (CAS 84-15-1)	c(c(c(cccc1)c1)ccc2)(c(ccccc3)c3)c2	
m-T	meta-terphenyl (1,1:3,1-Terphenyl)	1,3-terphenyl (1,1:3,1 -Terphenyl)	c(c(cccc1)c1)(cccc2c(cccc3)c3)c2	
p-T	para-terphenyl (1,1:4,1-Terphenyl)	1,4-terphenyl (1,1:4,1 -Terphenyl) (CAS 92-94-4)	c(c(cccc1)c1)(ccc(c(cccc2)c2)c3)c3	
o-HT1	1-ring hydrogenated terphenyls	2-cyclohexylbiphenyl	C1CCC(CC1)c2ccccc2c3ccccc3	
m-HT1	1-ring hydrogenated terphenyls	3-cyclohexylbiphenyl	C1CCC(CC1)c2cccc(c2)c3ccccc3	

p-HT1	1-ring hydrogenated terphenyls	4-cyclohexylbiphenyl	C1CCC(CC1)c2ccc(cc2)c3ccccc3	
o-HT2	2-ring hydrogenated terphenyls	1,2-dicyclohexylbenzene	C1CCC(CC1)c2ccccc2C3CCCCC3	
m-HT2	2-ring hydrogenated terphenyls	1,3-dicyclohexylbenzene	C1CCC(CC1)c2cccc(c2)C3CCCCC3	
p-HT2	2-ring hydrogenated terphenyls	1,4-dicyclohexylbenzene (CAS 1087-02-1)	C1(c2ccc(C3CCCCC3)cc2)CCCCC1	
o-HT3	3-ring hydrogenated terphenyls	o-tercyclohexyl	C1CCCCC1C1CCCCC1C1CCCCC1	
m-HT3	3-ring hydrogenated terphenyls	m-tercyclohexyl	C1CCCCC1C1CCCC(C2CCCCC2)C1	
p-HT3	3-ring hydrogenated terphenyls	p-tercyclohexyl	C1(C2CCC(C3CCCCC3)CC2)CCCCC1	
p-Q	Quaterphenyls	para-quaterphenyl	c4ccccc4c1ccc(c2ccc(c3ccccc3)cc2)cc1	
p-HQ1	1-ring hydrogenated quaterphenyls	4-cyclohexylterphenyl	C4CCCCC4c1ccc(c2ccc(c3ccccc3)cc2)cc1	
p-HQ2	2-ring hydrogenated quaterphenyls	dicyclohexylbiphenyl	C4CCCCC4C1CCC(c2ccc(c3ccccc3)cc2)CC1	
p-HQ3	3-ring hydrogenated quaterphenyls	tercyclohexylbenzene	C4CCCCC4C1CCC(C2CCC(c3ccccc3)CC2)CC1	

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

No relevant degradation products identified. The PBT/vPvB assessment is on the parent substance.

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

Table 4: Structurally related substance identity

EC number:	247-477-3
EC name:	Terphenyl
SMILES:	n.a.
CAS number (in the EC inventory):	26140-60-3
CAS number:	26140-60-3
CAS name:	Terphenyl
IUPAC name:	-
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	n.a. (UVCB)
Molecular weight range:	-
Synonyms:	Santowax R Therminol 75

Substance type: UVCB

Structurally related substance(s) formula: -

1.5 Physicochemical properties

Physico-chemical properties of the selected representative constituents, mainly predicted by Episuite QSARs, are presented in Table 5. The differences between predictions from different QSAR models (and measured values) for the same constituent and property are significant. Nevertheless, it can be stated that all constituents are scarcely water soluble. Many of the constituents can volatilise from water solutions (Henry's law constant > 1 Pa m^3 / mol). Therefore, losses due to volatilisation can be significant in test systems and need to be taken into account.

Table 5. Physico-chemical properties of selected constituents (Episuite QSAR-prediction unless otherwise stated)

Constituent (see Table 3)	MW	Water solubility (mg/l) (Episuite WatSol and WSKOW v.1.41)	logKow (KOWWIN)	Henry's law constant Pa –m3/mol (Episuite[1])	Boiling point °C (Episuite, Adapted Stein and Brown method)

o-T	230	0.06; 0.58 (1.24[2])	5.52	3.22 – 9.5 (6.19[2])	382.97
m-T	230	0.06; 0.58 (1.51[2])	5.52	0.86 – 3.42 (6.19[2])	382.97
p-T	230	0.05; 0.21 (0.018[2])	6.03	0.05 – 3.42 (6.19[2])	382.97
p-HT1	236.4	0.063; 0.068	6.57	7.75 – 84.1	359.52
p-HT2	242.4	0.008; 0.07	7.63	298 - 2200	335.74
P-HT3	247.4	0.00035; 0.0025	8.55	34 200 – 151 000	321.72
p-Q	306	0.00028; 0.0068	7.28	0.00023 – 0.27	481.20
p-HQ1	301 - 302	0.00031; 0.00079	8.34	0.51 – 6.46	457.75
p-HQ2	296 - 298	0.00011; 0.00035	9.26	27.7 - 203	430.94
p-HQ3	292 - 293	0.000018; 0.00040	10.18	699 - 6380	404.13

[1]Range of three QSAR values (bond method, group method, via vapour pressure/water solubility)
[2]Experimental values (EpiSuite)

Table 6: Overview of physicochemical properties of the UVCB substance[3]

Property	Description of key information	Value
Physical state	Clear pale yellow liquid (Newport plant specifications for Therminol 66, HB-40)	liquid at 20°C and 101.3 kPa
Melting / freezing point	EU method A1 ISO 3016 The reported value is the pour point.	ca. -24 °C at 101.3 kPa
Boiling point	EPA OPPTS 830.7220 Boiling range from 10% to 90 % volume distilled at atmospheric pressure.	342 – 400 °C at 101.3 kPa
Relative density	The average relative density at 20°C over several measurements was 1.013.	1.013 at 20°C
Vapour pressure	Value calculated from experimental data at higher temperature using the derived Antoine equation.	0.002 hPa at 20 °C
Partition coefficient n-octanol/water (log value)	The Log Pow of Therminol66 was determined with a HPLC method (OECD 117). The Pow and log Pow values of the main peak of the test substance were	5.3 – 6.5

[2]Experimental values (EpiSuite)

Property	Description of key information	Value
	above 3.2 x 10e+06 and >6.5. Additionally 19 other test substance peaks were detected with Log Pow values ranging from 5.3 to > 6.5.	
Water solubility	The water solubility of hydrogenated terphenyls (Santotherm 66) was determined to be 0.061 mg/L as a maximum value. Santotherm 66 is a complex mixture and the minor components constituted the bulk of the water soluble components.	0.061 mg/L at 20 °C
Flash point	The flashpoint was determined using a Pensky-Martens closed cup test apparatus. The flashpoint averaged over five samples was 171°C. Another flashpoint of 170°C was obtained using the DIN EN 22719 method.	170 °C at 1013 hPa

2. Harmonised classification and labelling

No harmonised classification.

3. Environmental fate properties

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

Not considered relevant based on the lack of hydrolysable functional groups.

3.1.1.2 Oxidation

There is no information available regarding abiotic oxidation in environmentally relevant conditions (with the exception of atmospheric reactions described in 3.1.1.3).

3.1.1.3 Phototransformation/photolysis

Phototransformation in air

The overall atmospheric gas phase reaction constants (and corresponding half-lives) are presented in Table 7. AOP v1.92 does not predict the reaction rate with ozone for these constituents (only olefins and acetylenes are estimated). The QSAR predicted atmospheric half-lives with hydroxyl radicals are between 3 and 14 hours for the compounds assessed.

Table 7. Overall atmospheric gas phase reaction constants (and corresponding half-lives) between photochemically produced hydroxyl radicals and selected constituents of terphenyl, hydrogenated. OH rate constants (25 deg C) are predicted by AOP v1.92 (EPI Suite v.4.11).

Group	Compound	Overall OH rate constant (cm^3/molecule-sec)	Half-life (hours)
o-T	ortho-terphenyl	9.1946 E-12	13.959
m-T	meta-terphenyl	12.6593 E-12	10.139

p-T	para-terphenyl	9.1946 E-12	13.959
p-HT1	4-cyclohexylbiphenyl	17.4639 E-12	7.350
p-HT2	1,4-dicyclohexylbenzene (CAS 1087-02-1)	25.5673 E-12	5.020
p-HT3	p-tercyclohexyl	34.2230 E-12	3.750
p-Q	para-quaterphenyl	11.6145 E-12	11.051
p-HQ1	4-cyclohexylterphenyl	19.8838 E-12	6.455
p-HQ2	dicyclohexylbiphenyl	30.3362 E-12	4.231
p-HQ3	tercyclohexylbenzene	40.3709 E-12	3.179

Phototransformation in water

A photolysis study in water on o-, m-, and p-terphenyl is available. The method information is: similar to the method described by Saeger V.W. and Adams W.J. “Method for conducting Sunlight photolysis screening of organic chemicals in aqueous solution”, Monsanto Report ES-81-M-23

In the dissemination site the study is described as follows:

“Three terphenyl isomers were tested for direct photolysis with sunlight during a 29 day period. The o- and p-isomer showed no significant decrease in concentration after 29 days of sunlight exposure, indicating that they are not susceptible to direct aqueous photolysis by sunlight. For m-terphenyl the half-life was calculated to be 140 days. For none of the three terphenyl isomers photolysis is expected to be a significant pathway for transformation in aqueous solution.

Polyphenyls are strong absorbers of UV light. It is well established that many compounds that absorb visible and UV light undergo rapid photodegradation in the environment. The purpose of this study was to examine the photolytic behavior of some compounds in the polyphenyl family to determine if

1) they are likely to photodegrade in the environment and

2) a relationship exists between compound structure and activity.

For the six compounds tested, biphenyl, cyclohexylbenzene, bicyclohexyl, o-, m- and p-terphenyl photolysis is not expected to be a significant pathway for transformation in aqueous solution. “

The full study report has not been available for the present assessment. Therefore, the dossier submitter currently considers the reliability of this study ‘not assignable’ (reliability score = 4).

Based on the available study, phototransformation in water is not expected to be significant for the purpose of PBT assessment.

Phototransformation in soil

This information is not available. Based on the available information and phototransformation in water, phototransformation in soil is not expected to be significant for the purpose of PBT assessment.

3.1.1.4 Summary on abiotic degradation

There is no information available regarding abiotic oxidation in environmentally relevant conditions, with the exception of reaction with hydroxyl radicals in air.

Hydrolysis is not considered a relevant route of degradation for terphenyl, hydrogenated.

The QSAR predicted atmospheric half-lives with hydroxyl radicals are between 3 and 14 hours for the compounds assessed. Based on the photolysis study on o-, m- and p-terphenyl,

photolysis is not expected to be a significant pathway for transformation in aqueous solution. No phototransformation studies in soil are available. However, based on the available information on phototransformation in water, phototransformation in soil is not expected to be significant for the purpose of PBT assessment.

In summary, based on available information, abiotic degradation in compartments relevant for determining a degradaiont half-life for P/vP assessment is expected to occur at such a low rate that it is not considered a relevant route of degradation for P/vP assessment. Reaction with hydroxyl radicals in the atmosphere is relatively fast based on QSAR predictions.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

3.1.2.1.1 Estimated data

Estimation using Biowin models 1-6 Epi Suite (Version 4.11)

Biowin modelling using Biowin models 1-6 was performed for the selected constituent groups (see Table 3). The results and conclusions are presented in Table 8. The reliability of the models was evaluated by considering the ability of the models to recognise the molecular fragments of the structures, and comparing structurally similar chemicals among the data used for deriving the models. The evaluation on model applicability is presented in Table 9.

Biowin reliability assessment

For most of the constituents, the Biowin models 1-6 recognise a part of the molecular fragments included in the representative structures while a part of the fragments is not recognised (Table 9). Therefore, the structure is only partially considered in the prediction. For Biowin 5 and 6 for three of representative structures (HT2, HT3, HQ3) all fragments are recognised. For the representative structure of HT3, Biowin 1-4 do not recognise any fragments at all and therefore the predictions are solely based on molecular weight and considered not reliable. For Biowin 1-4 for HT1 and HT2, the structure is covered by the recognised fragments, with the exception that the Biowin 1-4 models are not able to differentiate between different “alkyl substituents on aromatic ring”, e.g., non-cyclic or cyclic, or the number of rings, although these factors can have substantial effect on biodegradability. In addition, Biowin models 1-4 do not recognise any difference in structure between HT1, HQ1, HQ2, and HQ3; only the differences in molecular weight are considered.

It can be expected that the reliability of the prediction is higher when all fragments are recognised by the model. Alicyclic hydrocarbons (i.e. hydrocarbons with non-aromatic ring(s)) have been considered to be less suspectible to biodegradation compared to paraffins, isoparaffins, and the aromatics (Perry et al. 1984). Alicyclic hydrocarbons are also frequently unable to serve as the sole carbon source for microbial growth unless they have a sufficiently long aliphatic side chain (Atlas and Bartha 1998). Therefore Biowin models 5 and 6, which recognise both aromatic and non-aromatic ring, can be expected to be more reliable for the representative structures with non-aromatic rings (HT1, HT2, HT3, HQ1, HQ2, and HQ3) than Biowin 1-4.

The reliability of the predictions can be less accurate for compounds outside the molecular weight range of the training set compounds, and/or that have more instances of a given fragment than the maximum for all training set compounds (Biowin Help, Chapters 7.1.3, 7.2.3., and 7.3.2). For the selected constituents, the number of fragments does not exceed the maximum for all model training set compounds with the exception of (HT3 and HQ3 for Biowin 5 and 6) (Table 9). In those exceptions, the number of fragments is 13-14, and the maximum in training set is 12. This if considered a relatively small difference and it is considered that “reliable of restrictions” is an appropriate reliability score. The molecular weights of the selected constituents

are within the range of the training set compounds used for the Biowin models 1-6.

In general, the chemicals used for the derivation of the Biowin models 1-6 include relatively few compounds with high similarity to the selected constituents. Structural features not frequently found in the model derivation sets include non-aromatic rings, the C-C bond between the aromatic rings, compounds with both aromatic and non-aromatic rings, and three-ring aromatic compounds (Annex III).

Biowin models 1-6 are considered “reliable with restrictions” for the selected representative structures (with the exception of Biowin 1-4 for HT3) (Table 8, Table 9). For structures with non-aromatic rings, Biowin 5-6 are considered more reliable than Biowin 1-4.

Comparison to PBT screening criteria based on Biowin

The results of Biowin 2, 3, and 6 are discussed below in more detail as these models are used for the PBT screening criteria (ECHA guidance R.11, Version 2.0, 2014) (For HT3 the Biowin 2 and 3 predictions are not used as explained above).

For Biowin 2 the prediction ranges from 0.67 to 0.97, indicating “biodegrades fast” and therefore does not fulfill the cut-off value of <0.5 used in PBT screening. For Biowin 3 the prediction ranges from 2.42 to 2.73 (“weeks-months)” and therefore does not fulfill the cut-off value of <2.25 (“months”) used in PBT screening; however it is mentioned that when the value is 2.25-2.75 more degradation relevant information is generally warranted (ECHA guidance R.11, Version 2.0, 2014). According to the Biowin 6 model predictions none of the representative constituents is readily biodegradable and the model results (0.02-0.25) are below the cut-off value of <0.5 used in PBT screening.

It is noted that Biowin 2 and 3 give contradictory results compared to Biowin 6. As mentioned above, based on the fragments recognised, for structures with non-aromatic rings (HT1, HT2, HT3, HQ1, HQ2, and HQ3) Biowin 6 can be expected to be more reliable than Biowin 2 and 3 as Biowin 6 recognises both aromatic and non-aromatic ring fragments. The Biowin 5 results are in line with Biowin 6.

Therefore it is concluded that according to the individual Biowin models, HT1, HT2, HT3, HQ1, HQ2, and HQ3 are not readily biodegradable in the OECD 301 C test, suggesting that these constituents are potentially P. For o-T, m-T, p-T, and Q, no conclusion could be based on individual models done as the models give contradictory results, have uncertainties but no apparent differences in reliability could be identified.

According to the ECHA guidance R.11 combination of Biowin 2 and Biowin 3 and a combination of Biowin 3 and Biowin 6 can be used to screen substances with potential P/vP properties. As mentioned above, the Biowin 2 cut-off value is not fulfilled for any of the studied constituents and therefore the result for the combination of Biowin 2 and Biowin 3 does not indicate persistence (for HT3 the comparison is not relevant as the predictions are not reliable). Regarding the combination of Biowin 3 and Biowin 6, the results indicate “Potentially P or vP” (with the exception of HT2); however, the predictions are between 2.25 and 2.75, indicating according to the guidance that more degradation relevant information is warranted.

However, also the model derivation affects the reliability of the prediction and uncertainties in the prediction of similar structures were recognised for all models including Biowin 5 and 6 (Annex III). Therefore, the predictions must be interpreted with caution and together with other available data. It should also be noted that Biowin 5 and 6 are based on MITI test and the treatment of the inoculum according to the MITI test seriously impacts the diversity of the microbes (ECHA guidance R.7b, Version 2.0, 2014, p. 193) which may affect the result.

Table 8. Biowin (Epi Suite (Version 4.11) estimations for the selected constituents

Group	Compounds assessed[a]	Results of Biowin models 1-6							
		1	2	3	4	5	6	Overall conclusion based on Biowin models	Overall conclusion based on Biowin combinations (Screening information)
T	ortho-terphenyl (1,1 :2 ,1 -Terphenyl) ; meta-terphenyl (1,1 :3 ,1 -Terphenyl) ; para-terphenyl (1,1 :4 ,1 -Terphenyl)	0.8941 (Biodegrades fast)[b]	0.9657 (Biodegrades fast)[b]	2.7342 (Weeks-Months)[b]	3.5252 (Days-Weeks)[b]	0.1420 (Not Readily Degradable)[b]	0.0801 (Not Readily Degradable)[b]	no conclusion (conflicting results between models; significant uncertainties in all models but no apparent differences in reliability)	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and Biowin 6: Potentially P or vP[d]
HT1	1-ring hydrogenated terphenyls (o-, m-, and p-isomers with a terminal cyclohexyl group)	0.8178 (Biodegrades Fast)[b]	0.8838 (Biodegrades fast)[b]	2.6240 (Weeks-Months)[b]	3.4431 (Days-Weeks)[b]	0.1718 (Not Readily Degradable)[b]	0.1454 (Not Readily Degradable)[b]	not readily biodegradable in OECD 301 C test (Biowin 5 and 6), thus potentially P	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and Biowin 6: Potentially P or vP[d]
HT2	2-ring hydrogenated terphenyls (o-, m-, and p-isomers with a phenyl group in the center	0.7415 (Biodegrades Fast)[b]	0.6728 (Biodegrades Fast)[b]	2.5138 (Weeks-Months)[b]	3.3609 (Days-Weeks)[b]	0.2016 (Not Readily Degradable)[a]	0.2494 (Not Readily Degradable)[a]	not readily biodegradable in OECD 301 C test (Biowin 5 and 6), thus potentially P	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and

	position)								Biowin 6: Potentially P or vP[d]
HT3	3-ring hydrogenated terphenyls (o-, m-, and p-isomers)	0.6293 (Biodegrades Fast)[c]	0.3730 (Does Not Biodegrade Fast)[c]	2.6501 (Weeks - Months); however, the prediction is not reliable[c];	3.4893 (Days-Weeks)[c]	0.2989 (Not Readily Degradable)[a]	0.1355 (Not Readily Degradable)[a]	not readily biodegradable in OECD 301 C test (Biowin 5 and 6), thus potentially P	not applicable (Biowin 2 and Biowin 3 are not reliable)
Q	Quaterphenyls (all positional isomers)	0.8579 (Biodegrades fast)[b]	0.9052 (Biodegrades fast)[b]	2.5661 (Weeks - Months)[b]	3.4154 (Days-Weeks)[b]	0.0515 (Not readily biodegradable)[b]	0.0154 (Not readily biodegradable)[b]	no conclusion (conflicting results between models; significant uncertainties in all models but no apparent differences in reliability)	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and Biowin 6: Potentially P or vP[d]
HQ1	1-ring hydrogenated quaterphenyls (positional isomers with a terminal cyclohexyl group)	0.7816 (Biodegrades fast)[b]	0.7207 (Biodegrades fast)[b]	2.4558 (Weeks - Months)[b]	3.3333 (Days-Weeks)[b]	-0.0217 (Not Readily Degradable)[b]	0.0297 (Not Readily Degradable)[b]	not readily biodegradable in OECD 301 C test (Biowin 5 and 6), thus potentially P	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and Biowin 6: Potentially P or vP[d]
HQ2	2-ring hydrogenated quaterphenyls (positional isomers with two adjacent cyclohexyl groups located at an end of the four-ring chain)	0.7787 (Biodegrades Fast)[b]	0.7031 ((Biodegrades Fast)[b]	2.4425 (Weeks - Months)[b]	3.3246 (Days-Weeks)[b]	0.0312 (Not Readily Degradable)[b]	0.0306 (Not Readily Degradable)[b]	not readily biodegradable in OECD 301 C test (Biowin 5 and 6), thus potentially P	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and Biowin 6: Potentially P or

									vP[d]
HQ3	3-ring hydrogenated quaterphenyls (positional isomers with a terminal phenyl group)	0.7758 (Biodegrades Fast)[b]	0.6849 (Biodegrades Fast)[b]	2.4291 (Weeks - Months)[b]	3.3158 (Days-Weeks)[b]	0.0842 (Not Readily Degradable)[a]	0.0316 (Not Readily Degradable)[a]	not readily biodegradable in OECD 301 C test (Biowin 5 and 6), thus potentially P	Biowin 2 and Biowin 3: screening criteria not fulfilled Biowin 3 and Biowin 6: Potentially P or vP[d]

[a]It is noted that the Biowin models are not able to differentiate between isomers of terphenyls or quaterphenyls within a same level of hydrogenation in these selected cases, when these isomers differ only between the binding position(s) of hydrocarbon ring(s). For such isomers, exactly the same results are obtained as the number of fragments and molecular weight are the same. In Biowin Help (Chapter 9.0 'Known problems') it is stated that "Group contribution models like BIOWIN generally lack the sophistication required to consider the effects of neighboring substituents and substituent position."

Table 9: Molecular fragments recognised by Biowin models and conclusions on model reliability

		Biowin models 1,2,3, and 4			Biowin models 5 and 6		
Group	Compounds assessed[a]	Fragments recognised by the models	Remarks on fragments recognised by the model	Conclusion on model reliability	Fragments recognised by the models	Remarks on fragments recognised by the model	Conclusion on model applicability
o-T	ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	2x [Unsubstituted phenyl group (C6H5-)]	The models do not recognise the middle ring.[b] The models are not able to differentiate between the o-T, m-T and p-T. The structural differences between o-T, m-T, p-T, and Q are not recognised and for these compounds the predictions differ only on the basis of molecular weight.	Reliable with restrictions	14 x [Aromatic-H]	The models do not recognise the four carbon atoms forming the C-C bonds between the aromatic rings. The models are not able to differentiate between the o, m, and p-	Reliable with restrictions
m-T	meta-terphenyl (1,1 :3 ,1 - Terphenyl)	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Reliable with restrictions	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Reliable with restrictions
p-T	para-terphenyl (1,1 :4 ,1 - Terphenyl)	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Reliable with restrictions	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Like ortho-terphenyl (1,1 :2 ,1 - Terphenyl)	Reliable with restrictions
HT1	1-ring hydrogenated terphenyls (o-, m-, and p-isomers with a terminal cyclohexyl group)	1x [Alkyl substituent on aromatic ring], 1x [Unsubstituted phenyl group (C6H5-)]	The fragments recognised by the models cover the whole structure. However, the models recognise the alkyl part as [Alkyl substituent on aromatic ring] but do not identify the substituent (e.g., non-cyclic or cyclic, or the number of rings). The structural differences in HT1, HQ1, HQ2, and HQ3 are not recognised and for these compounds the prediction differs only on the basis of molecular weight.	Reliable with restrictions	1x [Aromatic-CH]; 9x [Aromatic-H], 5x [-CH2-[cyclic]]	The models do not recognise the two carbon atoms forming the C-C-bonds between the aromatic rings.	Reliable with restrictions

HT2	2-ring hydrogenated terphenyls (o-, m-, and p-isomers with a phenyl group in the center position)	2x [Alkyl substituent on aromatic ring]	The fragments recognised by the models cover the whole structure. However, the models recognise the alkyl part as [Alkyl substituent on aromatic ring] but do not identify the substituent (e.g., non-cyclic or cyclic, or the number of rings).	Reliable with restrictions	2 x [Aromatic-CH]; 4x [Aromatic-H], 10x [-CH2-[cyclic]]	The models recognise all fragments in the structure.	Reliable with restrictions
HT3	3-ring hydrogenated terphenyls (o-, m-, and p-isomers)	No fragments recognised by the models.	No fragments recognised by the models. Predictions are based solely on molecular weight.	Not reliable	14x [-CH2-[cyclic]], 4x[-CH- [cyclic]]	The models recognise all fragments in the structure. The models recognise 14 [-CH2- [cyclic]] fragments, which exceeds the maximum for all training set compounds (12).	Reliable with restrictions
Q	Quaterphenyls (all positional isomers)	2x [Unsubstituted phenyl group (C6H5-]	The models do not recognise the two rings in the middle of the structure.[b] The structural differences between o-T, m-T, p-T, and Q are not recognised and for these compounds the predictions differ only on the basis of molecular weight	Reliable with restrictions	18 x [Aromatic-H]	The models do not recognise the six carbon atoms forming the C-C-bonds between the aromatic rings.	Reliable with restrictions
HQ1	1-ring hydrogenated quaterphenyls (positional isomers with a terminal cyclohexyl group)	Like the assessed s 1-ring hydrogenated terphenyl compounds	The models do not recognise one of the aromatic rings (the middle one).[b] The models recognise the alkyl part as [Alkyl substituent on aromatic ring] but do not identify the substituent (e.g., non-cyclic or cyclic, or the number of rings). The structural differences in HT1, HQ1, HQ2, and HQ3 are not recognised and for these compounds the prediction differs only on the basis of molecular weight.	Reliable with restrictions	1x [Aromatic-CH], 13x [Aromatic-H], 5x [-CH2-[cyclic]]	The models do not recognise the four carbon atoms forming the C-C-bonds between the aromatic rings.	Reliable with restrictions
HQ2	2-ring hydrogenated quaterphenyls (positional isomers with two	Like the assessed 1-ring hydrogenated terphenyl compounds	The same remarks apply as for the assessed 1-ring hydrogenated terphenyl compounds (HT1).	Reliable with restrictions	1x [Aromatic-CH], 9x [Aromatic-H], 9x [-CH2-[cyclic]], 2x [-CH - [cyclic]]	The models do not recognise the two carbon atoms forming the C-C-bond between the aromatic rings.	Reliable with restrictions

	adjacent cyclohexyl groups located at an end of the four-ring chain)						
HQ3	3-ring hydrogenated quaterphenyls (positional isomers with a terminal phenyl group)	Like the assessed 1-ring hydrogenated terphenyl compounds	The same remarks apply as for the assessed 1-ring hydrogenated terphenyl compounds (HT1).	Reliable with restrictions	1x [Aromatic-CH], 5x [Aromatic-H], 13x [-CH2-cyclic]], 4x [-CH - [cyclic]]	The models recognise all fragments in the structure. The models recognise 13 [-CH2- [cyclic]] fragments, which exceeds the maximum for all training set compounds (12).	Reliable with restrictions

[a*] It is noted that the Biowin models are not able to differentiate between isomers of terphenyls or quaterphenyls within a same level of hydrogenation in these selected cases, when these isomers differ only between the binding position(s) of hydrocarbon ring(s). For such isomers, exactly the same results are obtained as the number of fragments and molecular weight are the same. In Biowin Help (Chapter 9.0 'Known problems') it is stated that "Group contribution models like BIOWIN generally lack the sophistication required to consider the effects of neighboring substituents and substituent position."

[b]In Biowin 1 and 2, counting the middle aromatic ring(s) as either an [Unsubstituted phenyl group (C6H5-)] or [Unsubsituted aromatic (3 or less rings)] would increase the predicted probability of biodegradation (the coefficients for these fragments are positive). In Biowin 3 and 4, counting the middle aromatic ring(s) as an [Unsubstituted phenyl group (C6H5-)] would cause a small increase in the predicted biodegradability whereas counting as [Unsubsituted aromatic (3 or less rings)] would decrease it. However, it should be noted that according to the Biowin guidance, the model "assumes additivity of fragments no matter what their type and number" and that "wrong predictions become more likely even for positive fragments if their frequency is high" (Biowin User Guide(v4.10) Chapter 9.0 Known problems with Biowin models (Biowin 1-7)).

Estimation using BioHCwin model

Epi Suite (Version 4.11) BioHCwin modelling was performed for the selected constituent groups (Table 10). BioHCwin model has been developed for determining quantitative primary biodegradation half-lives for individual petroleum hydrocarbons. This model uses a fragment-based approach that is similar to several other biodegradation models, such as those within the Biowin estimation program. It is concluded that the use of BioHCwin model for the selected constituents of terphenyl, hydrogenated, is generally applicable, due to the following reasons:

-the model includes in its training set structural fragments relevant to the selected constituents
-the fragments recognised by the model cover a substantial amount or all of the fragments included in the constituents
-the molecular weights of the selected constituents are within the range of the training set compounds used for the model (70.14 - 478.94)

However, in some cases, the number of instances of a fragment exceeds the maximum for all training set compounds (Table 10) and it is possible that in these cases the prediction is less accurate.

The primary biodegradation half-lives of 6.7-809 d were obtained by BioHCwin (Table 10). The shortest half-lives (6.7-8.1 d) were obtained for the constituents with aromatic rings only (o-T,m-T, p-T, and Q) whereas longer half-lives (68-808 d) were obtained for the constituents including non-aromatic ring(s).

It is noted that the model recognised all fragments in the structures of HT1, HT2, HT3, HQ2, and HQ3, which can improve the accuracy of the prediction compared to the other constituents. In addition, for o-T, m-T, and p-T, HT3, Q, HQ1, and HQ3, the amount of certain fragment(s) exceed the maximum amount for all training set compounds, which can decrease the accuracy.

In summary, the BioHCwin predictions suggest that the structures with non-aromatic rings (HT1, HT2, HT3, HQ1, HQ2, HQ3) are “potentially P/vP” whereas for the structures with only aromatic rings (o-T, m-T, p-T, and Q) the half-lives are below the P criterion. Although the BioHCwin model is generally suitable for the selected constituents, its relevance to the present assessment is limited because the BioHCwin model gives a primary biodegradation half-life estimate and because data obtained with mixtures has been used in its training set (Howard et al. 2005). Since it is known that cometabolism affects hydrocarbon biodegradation (See3.1.2.1.3, ExxonMobil Biomedical Science Inc., 2009. Primary bioegradation in seawater study), BioHCwin half-lives below the P or vP criterion should not be used as indicator of “not P” or “not vP”.

Table 10: BioHCwin estimations for the selected ten constituents

Group	Compounds assessed*	BIOHCWIN primary degradation half-life (d)	Remarks	Conclusion on model applicability
o-T	ortho-terphenyl (1,1 :2 ,1 - Terphenyl); ; meta-terphenyl (1,1 :3 ,1 -	6.694	The model does not recognise the two bonds between the aromatic rings. The model is not able to differentiate between the o-T, m-T, and p-T. The model recognises 3	Reliable with restrictions

	Terphenyl); para-terphenyl (1,1 :4 ,1 - Terphenyl)		[Benzene] fragments, which exceeds the maximum for all training set compounds (1).	
HT1	1-ring hydrogenated terphenyls (o-, m-, and p- isomers with a terminal cyclohexyl group)	315	The model recognises all fragments in the structure.	Reliable with restrictions
HT2	2-ring hydrogenated terphenyls (o-, m-, and p- isomers with a phenyl group in the center position)	469.5	The model recognises all fragments in the structure.	Reliable with restrictions
HT3	3-ring hydrogenated terphenyls (o-, m-, and p-isomers)	69.41	The model recognises all fragments in the structure. The model recognises 14 [-CH2-[cyclic]] fragments, which exceeds the maximum for all training set compounds (12).	Reliable with restrictions
Q	Quaterphenyls (all positional isomers)	8.123	The model does not recognise the three bonds between the aromatic rings. The model recognises 18 [Aromatic-H] fragments and 4 [Benzene] fragments, exceeding the maximum amounts for all training set compounds (14 and 1, respectively).	Reliable with restrictions
HQ1	1-ring hydrogenated quaterphenyls (positional isomers with a terminal cyclohexyl group)	68.03	The model does not recognise the two bonds between the aromatic rings. The model recognises 3 [Benzene] fragments, exceeding the maximum amounts for all training set compounds (1).	Reliable with restrictions

HQ2	2-ring hydrogenated quaterphenyls (positional isomers with two adjacent cyclohexyl groups located at an end of the four-ring chain)	808.8	The model recognises all fragments in the structure.	Reliable with restrictions
HQ3	3-ring hydrogenated quaterphenyls (positional isomers with a terminal phenyl group)	304.6	The model recognises all fragments in the structure. The model recognises 13 [-CH2-[cyclic]] fragments, which exceeds the maximum for all training set compounds (12).	Reliable with restrictions

* It is noted that the BioHCwin model is not able to differentiate between isomers of terphenyls or quaterphenyls within a same level of hydrogenation in these selected cases, when these isomers differ only between the binding position(s) of hydrocarbon ring(s). For such isomers, exactly the same results are obtained as the number of fragments and molecular weight are the same. In Biowin Help (Chapter 9.0 'Known problems') it is stated that "Group contribution models like BIOWIN generally lack the sophistication required to consider the effects of neighboring substituents and substituent position." The BioHCwin model uses a fragment-based approach that is similar to the BIOWIN models and therefore has the same limitation regarding the substituent position.

3.1.2.1.2 Screening tests

In the registration dossier (ECHA 2017c), there are several biodegradation screening tests available (Table 11). None of these tests has been done according to current international standards. In the two tests which appear to resemble OECD ready biodegradation tests (similar test substance concentration, inoculum not adapted, CO_2 production measured) (Monsanto report ES-80-SS34 and MONSANTO 1977a) different commercial products of terphenyls, hydrogenated have been studied. No specific information on the composition of the products is explicitly available. It is noted that test substance concentrations used are well above the estimated water solubility of the main constituents in the tested UVCB products. In the tests, degradation based on CO_2 evolution was at the most 14 % within 35 days, suggesting that the tested substances are not readily biodegradable. However, it is not possible to draw any information on individual constituents from these tests on the UVCB substance.

Table 11. Screening level biodegradation tests on the UVCB-substance.[4]

Method	Results	Reliability	Remarks	Reference
Inoculum: activated sludge (adaptation not specified) Method: ASTM Draft No.3 for the "Proposed Standard Practice for the Determination of Ultimate Biodegradability of Organic Chemicals". ASTM E35.24 subcommittee, Aquatic Biotransformation Task Group, 2/80; test substance conc. 20.9 mg/l	CO_2 evolution 14% of theory in 35 days % Degradation of test substance: 14 after 35 d (CO2 evolution) (Range of quadruplicate flasks = 1-41%; std.dev. 19%)	2	Test material (Common name): XA-2020	Monsanto report ES-80-SS34.
Inoculum in SCAS study: activated sludge (adaptation/ origin not specified) (According to the standard method (Snow et al. (1965) activated sludge obtained from a sewage treatment plant is used and if sludge is not acclimated to the test substance then an incremental surfactant feed schedule is used. The results are calculated starting with the 4th day on which the test susbstance feed is 20 mg/liter). Inoculum in RDA study: sludge from SCAS study after 31 weeks Method: Semi-continuous activated sludge (SCAS) combined with River die away (RDA), according to the standard method for measuring surfactant biodegradability as described in JAOCS 1965 vol. 42 p. 986 (Snow et al. (1965) and JAOCS 1969 vol. 46 p. 432 (Mausner et al. 1969).	SCAS study: The mean disappearance rate within a 24-hour cycle and 95 % confidence limits obtained during the 8th through 31st week of testing were 16 +/- 9 %. RDA study: At the conclusion of the 5 mg test period (after 31 weeks), a die-away procedure with the inoculum from the SCAS procedure was carried out for approximately 3 months. At the end of this period, a detectable amount of HQ-40 (approx. 1 mg) was still present in the unit.	2	Study on constituent of UVCB substance. Test material (Common name): HQ-40	Monsanto (1973)
Inoculum: activated sludge (adaptation/origin not specified) Method: Semi-Continuous	% Degradation of test substance: 49 Citation from	4	Test material (Common name):	Monsanto Report (1970) (secondary source; reference originates from

Method	Results	Reliability	Remarks	Reference
activated sludge test; no reference to specific guidelines. 7 ppm (10 mg) feed level over a 36-week period using a 24-hour cycle	dissemination site "The primary biodegradation rate of 49 +/- 7 was obtained only during the latter stage of the test, significantly lower rates were obtained during the first 12 weeks of the test indicating that the acclimation period was an important factor. Examination of the chromatograms from this test showed differences in degradation rate of the various components, but gave no evidence of highly resistant components."		Santosol 340 or HB-40	Monsanto (1977))
Inoculum: origin/adaptation not specified; however, according to method description in Monsanto (1977), river water is obtained either from the Meramec or Mississippi river and no adaptation prior to testing is included Method: RDA (River Die Away); No reference to specific guidelines. Initial conc. 1 ppm	% Degradation of test substance: 80 Citation from dissemination site "80% decrease in the Santosol 340 level at the conclusion of the 50-day test was observed. Furthermore, examination of the chromatograms from this test showed differences in degradation rate of the various components, but gave no evidence of highly resistant components." (It is noted that in the dissemination site there is a degradation rate of 49% reported for the RDA study; however, according to Monsanto (1977) degradation was 49% in the SCAS study and 80% in the RDA study in Monsanto Report (1970),	4	Test material (Common name): Santosol 340 or HB-40	Monsanto Report (1970) (secondary source; reference originates from Monsanto (1977))

Method	Results	Reliability	Remarks	Reference
Test type: screening test Inoculum: Specific bacterial culture for Hydrocarbon Degrading in Fresh Water Method: MIC Environmental Sciences Method for conducting shake flask CO_2 evolution ultimate biodegradation screening of organic chemicals (1)	CO_2 evolution at day 55: 20±34 (range 0-60)	2	Study on constituent of UVCB substance. Test material (EC name): o-terphenyl	MIC (1983b)
Inoculum: Specific bacterial culture for Hydrocarbon Degrading in Fresh Water MIC Environmental Sciences Method for conducting shake flask CO_2 evolution ultimate biodegradation screening of organic chemicals (1)	CO_2 evolution at day 55: 63±18 (range 19-53)	2	Study on constituent of UVCB substance. Test material (EC name): p-cyclohexylbiphenyl	MIC (1983b)
Inoculum: Specific bacterial culture for Hydrocarbon Degrading in Fresh Water MIC Environmental Sciences Method for conducting shake flask CO_2 evolution ultimate biodegradation screening of organic chemicals (1)	CO_2 evolution at day 55: 16±22 (range 3-41)	2	Study on constituent of UVCB substance. Test material (EC name): p-dicyclohexyl benzene	MIC (1983b)
Inoculum: Specific bacterial culture for Hydrocarbon Degrading in Fresh Water MIC Environmental Sciences Method for conducting shake flask CO_2 evolution ultimate biodegradation screening of organic chemicals (1)	CO_2 evolution at day 55: 9±9 (range 0-18)	2	Study on constituent of UVCB substance. Test material (EC name): trans-p-tercyclohexyl	MIC (1983b)
Inoculum: Specific bacterial culture for Hydrocarbon Degrading in Fresh Water MIC Environmental Sciences Method for conducting shake flask CO_2 evolution ultimate biodegradation screening	CO_2 evolution at day 55: 7±9 (range 0-16)	2	Study on constituent of UVCB substance. Test material (EC name): p-quaterphenyl	MIC (1983b)

Method	Results	Reliability	Remarks	Reference
of organic chemicals (1)				
inoculum: natural water Method: River die away, no reference to specific guidelines.	o- and m-isomer degrade after acclimatisation, p- does not significantly degrade % Degradation of test substance: ca. 100 after 42 d (Test mat. analysis) (for o-terphenyl, based on graphic data in test report) ca. 100 after 42 d (Test mat. analysis) (for m-terphenyl, based on graphic data in test report) ca. 20 after 42 d (Test mat. analysis) (for p-terphenyl, based on graphic data in test report)	2	Study on constituent of UVCB substance. Test material (EC name): o-terphenyl	MIC (1983a)
inoculum: natural water Method: River die away, no reference to specific guidelines.	% Degradation of test substance: ca. 100 after 14 d (Test mat. analysis) (for p-cyclohexylbiphenyl, based on graphic data in test report)	2	Study on constituent of UVCB substance. Test material (EC name): p-cyclohexylbiphenyl	MIC (1983a)
inoculum: natural water Method: River die away, no reference to specific guidelines.	% Degradation of test substance: ca. 100 after 28 d (Test mat. analysis) (p-dicyclohexylbenzene, based on graphic data in test report)	2	Study on constituent of UVCB substance. Test material (EC name): p-dicyclohexyl benzene	MIC (1983a)
inoculum: natural water Method: River die away, no reference to specific guidelines.	% Degradation of test substance: ca. 80 after 14 d (Test mat. analysis) (for the substance as tested, based on graphic data in test report)	2	Study on constituent of UVCB substance. Test material (EC name): p-tercyclohexyl	MIC (1983a)
Inoculum: obtained from	% CO_2 production in 43 days;	2	Study on	MONSANTO

Method	Results	Reliability	Remarks	Reference
an SCAS unit fed for 8 weeks with p-terphenyl Method: Method for Conducting Shake Flask Ultimate Biodegradation Testing of Organic Chemicals. MCC Environmental Sciences Method Report Number ES-90-M-12 (MSL-10910). Saeger V.W.	11.3 mg/l: 9 ± 3% 20.73 mg/l: 8 ± 5% sterile control: 7 % Mean residue recovery, % of initial dose at day 42: 78.0-81.1% (sterile control: 82.1%)		constituent of UVCB substance. Test material (EC name): p-terphenyl	(1991)
Inoculum; activated sludge; origin/adaptation not specified. According to the standard method (Snow et al. (1965) activated sludge obtained from a sewage treatment plant is used and if sludge is not acclimated to the test substance then an incremental surfactant feed schedule is used. The results are calculated starting with the 4th day on which the test susbstance feed is 20 mg/liter). Method: patterned after the standard semi-continuous activated sludge (SCAS) method for surfactants (JAOCS 42, (1965), JAOCS 46, 432 (1969))	% Degradation of test substance: ca. 11.5 after 1 wk (Test mat. analysis)	2	Study on constituent of UVCB substance. Test material (EC name): terphenyl (Therminol 88)	MONSANTO (1974)
Inoculum: activated sludge (adaptation not specified) Method: Although no specific guideline was mentioned, a description to the test method followed is given in 'Analytical Chemistry Method 71-32' (document not available for the present assessment).	% Disappearance of test substance: ca. 68 after 21 d (Test mat. analysis) (RDA test) (disappearance in distilled water control was 13% during the same period)	2	Test material (Common name): HB-40	MONSANTO (1970a)
Inoculum: activated sludge (According to the standard method (Snow et al. (1965) activated sludge obtained from a sewage treatment plant is used and if sludge is not acclimated to the test substance then an incremental surfactant	% Degradation of test substance: ca. 19.1 (Test mat. analysis) (95% CI: +- 20.8; sampling period 1; 10 mg feeding experiment) ca. 55 (Test mat. analysis) (95% CI:	2	Test material (Common name): HB-40	MONSANTO (1970b)

Method	Results	Reliability	Remarks	Reference
feed schedule is used. The results are calculated starting with the 4th day on which the test susbstance feed is 20 mg/liter). Method: The SCAS test method used is patterned after the standard test method for surfactants (Snow et al. (1965). A description of the test method followed is given in 'Analytical Chemistry Method 71-32' (document not available for the present assessment).	+- 12.9; sampling period 2; 10 mg feeding experiment) ca. 25 (Test mat. analysis) (95% CI: +- 81.2; sampling period 3; 10 mg feeding experiment) ca. 48.6 (Test mat. analysis) (95% CI: +- 6.9; sampling period 4; 10 mg feeding experiment)			
Inoculum: SCAS supernatant (non-acclimated and Santosol 340-acclimated (16th week of SCAS test period)) Method: Thompson Duthie Sturm method. See Sturm (1973); Thompson and Duthie (1968)	% Degradation of test substance: 1 after 35 d (CO2 evolution) (15.1 mg/L (non-acclimated supernatant)) 3 after 35 d (CO2 evolution) (10.3 mg/L (non-acclimated supernatant)) 50 after 46 d (CO2 evolution) (16.7 mg/L (acclimated supernatant))	2	Test material (Common name): Santosol 340; Contains 40% hydrogenated terphenyls	MONSANTO (1977a)
Inoculum: The bacterial seed was prepared using the 'standard 2-week acclimation period' (information from study report). It is noted that on dissemination site there is conflicting information as it is also mentioned that a 'non-acclimated SCAS supernatant was used') Method: Monsanto Shake Flask procedure. Ultimate biodegradability (conversion to carbon dioxide, water and inorganic salts). Test report mentions that the shake flask system is similar to that described by Gledhill (1975).	% Degradation of test substance: 3 after 35 d (CO2 evolution) (45.8 mg/L (non-acclimated supernatant))	2	Test material (Common name): Santosol 340; Contains 40% hydrogenated terphenyls	MONSANTO (1977a)
Inoculum: activated sludge, domestic	Primary degradation rate, 95% CL	2	Test material	MONSANTO (1977a)

Method	Results	Reliability	Remarks	Reference
(adaptation not specified; however, the general method description in Appendix of the report does not include any adaptation prior to test) Method: SCAS test method; patterned after the standard test method for surfactants (JAOCS 42, 986, 1965) (Snow et al. (1965)). A description of the test method followed is given in 'Analytical Chemistry Method 71-32'	Week 1 to 15: Santosol 340: 39±6 Week 16 to 31: Santosol 340: 64±5 Component 1 (3-ring hydrogenated): - Component 2 (2-ring hydrogenated (63%), terphenyl (37%): 54±5 Component 3 (2-ring hydrogenated): 58±7 Component 4 (1-ring hydrogenated): 78±3 Component 5 (1-ring hydrogenated): - Component 6 (1-ring hydrogenated): 79±7 Component 7 (1-ring hydrogenated): 77±7		(Common name): Santosol 340; Contains 40% hydrogenated terphenyls	
Inoculum: Sludge from SCAS study (Monsanto 1972b) after 31 weeks feeding with Santosol 300 Method: A die-away procedure with unit from the SCAS study (Monsanto 1972b). Feeding of the Santosol 300 was stopped; synthetic sewage was fed to the unit daily, but draining of the supernatant liquid was carried out on a weekly basis to minimise solubility and mechanical losses. Analysis of the drained supernatant was also carried out to check on such losses.	% Degradation of test substance: 95 after 14 d (Test mat. analysis) 100 after 28 d (Test mat. analysis)	2	Test material (Common name): Santosol 300	MONSANTO (1972a)
Inoculum: origin/adaptation not specified. (According to the standard method (Snow et al. (1965) activated sludge obtained from a sewage treatment plant is used and if sludge is not acclimated to the test substance then an incremental test substance feed schedule is used. The results are calculated starting with the 4th day on which the test substance feed is 20 mg).	% Degradation of test substance: ca. 68.1 after 19 wk (% Disappearance rate) (5 mg dose) ca. 65.6 after 31 wk (% disappearance rate) (20 mg dose)	2	Test material (Common name): Santosol 300	MONSANTO (1972b)

Method	Results	Reliability	Remarks	Reference
Method: The SCAS test method used is patterned after the standard test method for surfactants (JAOCS 42, 986, 1965) (Snow et al. (1965)). A description of the test method followed is given in 'Analytical Chemistry Method 71-32'				

Some information on individual constituents of the UVCB substance can be drawn from five screening level biodegradation tests (MIC 1983a, MONSANTO 1973, MONSANTO 1974, MIC 1983b, and MONSANTO 1991), which are thus described in more detail below. Also MONSANTO (1977a) includes information on degradation of different constituent groups (Table above).

MIC 1983a

The registrant considers the study reliable with restrictions and adequate for use as a supporting study.

The degradation of the two-ring compounds biphenyl, dicyclohexyl and cyclohexylbenzene, and terphenyls and hydrogenated terphenyls was studied in river water collected from the Mississippi river. The aim of the study was to 1) determine the relative biodegradation rates of similar compounds in natural waters and 2) to determine if any relationship exists between the molecular structure and ease of biodegradability. Two different River Die Away experiments were conducted. The first experiment was run using only one compound per bottle, each at 50 ppb. In the second experiment, ortho-, meta- and para-terphenyls (o-T, m-T and p-T) were run as a mixture, each at 20 ppb. The tests were conducted in ambient temperature.

By GC analysis it was verified that all tested compounds were > 95 % pure except for trans-para-tercyclohexyl which consisted of approximately 25 % of tercyclohexyl (HT3) and 75 % of a two ring saturated terphenyl (HT2) that had the same retention time as p-dicyclohexylbenzene. Analysis of the test substances was performed by GC-FID.

The accuracy and precision of the technique were evaluated for each compound at 5 and 50 ppb. Accuracy (%recovery) varied 86-144% (at 50 ppb) and 89-164% (at 5 ppb) between the compounds. Precision (% rel. std. dev.) varied 2.4-10 (at 50 ppb) and 3.1-12 (at 5 ppb) (at 5 ppb precision studied only for 3 compounds as only one extraction was done at this level for the other compounds). On each sampling day a freshly spiked sample of river water was analysed to monitor the accuracy of the analysis. Recoveries of these quality assurance samples varied between 92 - 130 % (%rel std. dev. 6-18).

In the first die-away experiment (the experiment with river water A), cyclohexylbenzene and m-terphenyl were run in duplicate. Difference between duplicate solutions was 0-23 % for m-terphenyl active tests and 6-25 % for m-terphenyl sterile tests (measurements on five days). Difference between duplicate solutions was 0.1-19% for cyclohexylbenzene active tests and 5.4-30% for cyclohexylbenzene sterile tests (measurements on four days except the active sample which had three measurement days as one sample was lost).

Losses were observed in the control solutions. At the end of the experiments the controls averaged 76% and 59% of the day 0 concentration for the two ring and saturated three ring compounds, respectively. After 28 days the terphenyl controls averaged 96% and 86% of day 0 in the separate and mixed solutions, respectively. According to the test

report these losses could have been caused by nonbiological processes or by incomplete sterilization by the mercuric chloride.

Biodegradation was determined as the ratio of the concentration of the test substance in the river water and the concentration of the substance in sterile control.

Two different sets of river water were used (Table 12), which might explain part of the variation in the results.

When tested separately, Half-lives were roughly estimated from the graphic data. It is noted, that these half-lives are based only on a few data points (4 -6) without kinetic analyses and related with considerable uncertainty and should not be directly compared with the P criteria. They have been derived in order to be able to compare the relative degradation of the constituents.

Table 12 Characteristics of Mississippi river water used in the tests (MIC 1983a)

	A (collected 7/22/83)	B (collected 9/5/83)
Suspended solids (mg/l)	51	72
pH	8.25	8.24
Microbial population (Colony forming units/ml)	5.5×10^4	6×10^4

Table 13 Half-lives (T50) of primary biodegradation (determined as concentration in river water / concentration in sterile control) estimated from graphic data by the dossier submitter (MIC 1983a)

River water	Test substance	T50 (days) roughly estimated from graphic data Test substance concentration 50 µg/l	T50 (days) roughly estimated from graphic data Test substance concentration 20 µg/l (mixture)
A	biphenyl	8	-
A	dicyclohexyl	6 - 7	-
A	cyclohexylbenzene	8	-
A	o-terphenyl (o-T)	>30	35
A	m-terphenyl (m-T)	22	33
A	p-terphenyl (p-T)	> 30	> 40
B	p-cyclohexylbiphenyl (HT1)	10	-
	p-dicyclohexylbenzene (HT2)	14	-

B	"trans-p-tercyclohexyl" containing of - 25 % HT3 (tercyclohexyl) - 75 % HT2	No degradation indicated for tercyclohexyl (HT3) within 30 days. < 10 (HT2)	-

Reliability: This study is considered reliable with restrictions (reliability score = 2) due to following reasons:

The method is referred to as “Standard method for measuring surfactant biodegradability as described in JAOCS 1965 vol. 42 p. 986 and JAOCS 1969 vol. 46 p. 432”. These are publically available documents (Snow et al. 1965, Mausner et al. 1965). The study is generally well documented and scientifically acceptable with some reservations due to the uncertainty of the test results due to the relatively large variation in recovery rates and lack of replicate test vessels for most of the compounds. In addition, the test report presents the results only as the percent remaining on a given day obtained by dividingthe concentration in the active river water by the concentration in the sterilised control. The test report does not include raw data and therefore it is not possible to reproduce the calculated biodegradation results. Regarding possible volatilisation, it is noted that the biodegradation results were calculated by taking into account sterile controls (separately for each constituent) and hence a possible loss of test substance due to volatilisation (if occurring at all) is not expected to affect the biodegradation results. In addition, considering that p-HT3 has a higher (predicted) Henry’s law constant compared to the p-isomers of the other tested constituents (Table 5) and no decrease in concentration was detected for HT3, it is unlikely that volatilisation caused any significant error on the observed biodegradation results.

Relevance: The study is considered relevant to be used as a supporting study for the purpose of PBT assessment. The results can be used to indicate differences between the biodegradability of the studied constituents by natural freshwater microorganisms. However, the results are not applicable for direct comparison to P/vP criteria.

In conclusion, it can be stated that the 2-ring compounds seem to be more readily biodegradable as compared to the three ring compounds. Based on the results from river water B, the fully hydrogenated compounds seem to degrade slower than partially hydrogenated compounds. The results indicate that o- and p-terphenyl have significant persistence, whereas m-terphenyl seems to be more susceptible to biodegradation. When tested separately, o- and p-terphenyl showed no or neglible degradation during 28 days whereas m-terphenyl was started to degrade after 16 days. When tested in a mixture with m-, o-, and p- terphenyls, also o- terphenyl started to biodegrade after 30 days (data not shown). It is noted that there is considerable uncertainty related to the test results due to large variation in recovery rates, and possible losses due to volatilisation.

MONSANTO 1973

Biodegradability of HQ40 was studied according to a standard method for measuring surfactant biodegradability. The test substance (HQ40) was a mixture of approximately 80 % quaterphenyls with a degree of 40 % hydrogenation (the residual 20 % consists of terphenyl and higher (> 5-ring) phenyl structures). The study comprised of two parts: a 31 week semi-continuous activated sludge test and a "river die-away test".

Semi-continuous activated sludge (SCAS) test (31 weeks)

Feeding of the HQ-40 test material was initiated at an addition rate of 1 mg per 24-hour cycle. The rate was increased to 3 mg the second week and to 5 mg the third week. The rate was maintained at 5 mg during the remainder of the test. 50 ml samples of the mixed

liquor were withdrawn approximately 30 minutes after feeding and at the end of the aeration cycle. The mixed liquor samples were extracted with nanograde hexane. The level of test material in the concentrated extracts was determined using FID-GC. The recovery of HQ-40 from the mixed liquor samples was demonstrated by spiking blank samples at three different levels in duplicate (100, 200 and 300 µg). The mean per cent recovery was 106.0 +/- 6.2. Scrubbing of the off-gases from the unit showed that no detectable amount of HQ-40 was volatilised during the cycles.

Comparison of the chromatograms taken at the beginning and at the end of an aeration cycle shows that there were only minor changes in the distribution of HQ-40 components.

Weekly monitoring of the suspended solids concentration during the testing demonstrated that there were no apparent inhibition of the sludge growth rate.

The mean disappearance rate within a 24-hour cycle and 95 % confidence limits obtained during the 8th through 31st week of testing were 16 +/- 9 %.

"River die-away test" (3 months)

At the conclusion of the 5 mg test period (after 31 weeks), a die-away procedure was carried out in which feeding of the HQ-40 was stopped but sampling and analysis continued on a periodic basis. Synthetic sewage was fed to the unit daily, but draining of the supernatant liquid was carried out only on a weekly basis to minimise solubility and mechanical losses. Analysis of the drained supernatant was also carried out as a check on such losses. The die-away procedure was carried out for approximately 3 months. The test report includes a semi-logarithmic plot of the HQ-40 concentration during the first 14 days of the die-away period. From this plot, a die-away half-life is calculated. The initial level of 26.1 mg/l HQ-40 in the die-away procedure decreased by 50% in 8.6 days. The test report emphasises that such a half-life calculation has limited validity and should server only as a relative basis of comparison. It is noted by the dossier submitter that the linear fit in the semi-logarithmic plot may be questionable as the concentration at the three last points (particularly the last one) in the plot are underestimated. Therefore, the half-life may be underestimated. At the end of the die-away period, a detectable amount of HQ-40 (approx. 1 mg) was still present in the unit.

Reliability: The study is considered reliable with restrictions (reliability score = 2) due to following reasons:

-The method is referred to as “standard method for measuring surfactant biodegradability as described in JAOCS 1965 vol. 42 p. 986 and JAOCS 1969 vol. 46 p. 432.” These are publically available documents (Snow et al. 1965, Mausner et al. 1965). The study is generally well documented and scientifically acceptable with some reservations due lack of information reqarding the origin/adaptation of inoculum in the SCAS test and apparent lack of replicate vessels (replication is not mentioned in test report). In addition, the test report does not include raw data and therefore it is not possible to reproduce the calculated biodegradation results. It appears that test substance loss due to volatilization was not an issue as it is reported for the SCAS test that the amount of HQ-40 volatilised was not detectable.

Relevance: The study is considered relevant for the purpose of PBT assessment as supporting information. In guidance R.7b (ECHA 2017b) it is stated that the value of an SCAS test for assessment purposes is low because of the strong potential for adaptation of micro-organisms to the substance in this kind of test. Therefore, due to adaptation, it is considered that any biodegradation in this type of study (both the SCAS part and the die-away part) should not be used to support that the test substance is not P/vP. However, the limited biodegradation can be used as supporting information for P/vP.

The mean disappearance of 16% at the end of the SCAS study (with negligible volatilisation), in a test system considered to be favourable for microbial adaptation suggests limited biodegradability of hydrogenated quaterphenyls. The presence of a detectable amoung of HQ at the end of the “die-away” procedure is in line with the results of the SCAS study, taking into account the adaptation during SCAS study. Regarding the estimated half-life, it is noted, in addition to the reservations described above, that the die-away study is not comparable to a standard simulation test e.g. due to its high test substance concentration and use of adapted inoculum. Therefore, the reported half-life is not directly comparable to P/vP criteria.

MIC 1983b

Ultimate biodegradations of ortho-, meta and para-terphenyl (T), para-quaterphenyls (Q) and hydrogenated para-terphenyls (HT1 - HT3) were measured with a 55-day shake flask carbon dioxide evolution procedure using a commercial, specific bacterial culture adapted to hydrocarbons (plate count of 1.3×10^8 colony forming units per ml). The concentration of each test substance was 20 mg/l and three replicates were used. Each flask in the test series contained an open reservoir containing 10 ml of 0.15 N barium hydroxide suspended via a glass tube inserted in a neoprene stopper. Periodic removal and titration of the barium hydroxide solution were used to determine the CO_2 evolved. Barium hydroxide solutions removed from the shake flask reservoir were analysed by titration with standard 0.1 N HCl to a pH 8.5 endpoint using a Fisher Automatic Titrimeter II Titration System.

The medium was a modification of Standard BOD medium containing the standard levels of magnesium sulfate and calcium chloride, twice the standard level of phosphate buffer, four times the standard level of ferric chloride and 40 mg/l ammonium sulfate per liter of purified water. One liter of media was charged to the requisite number of two-liter Erlenmeyer flasks and sparged with 70% oxygen in nitrogen.

The reported test substance concentrations are apparently nominal concentrations, and therefore losses due to volatilisation cannot be overruled. Glucose was used as a positive control. The results show that although inoculum adapted to hydrocarbons and a high plate count was used, mean degradation within 55 days was less than 40 % for all substances except para-HT1 (Table 14). It is noted that deviations between the replicate measurements was considerable.

Reliability: The study is considered reliable with restrictions (reliability score = 2) due to following reasons:

The study was conducted according to a specific method of the test laboratory. The guideline and its validation information were not available for this SVHC proposal report. Therefore, the study cannot be evaluated in relation to the testing guideline. The study is nevertheless generally well documented and scientifically acceptable, with restrictions regarding the lack of measured concentrations of test substance, possible losses due to volatilization, and lack of sterile controls. In addition, the test report does not include raw data and therefore it is not possible to reproduce the calculated biodegradation results. Regarding the possible volatilization it is noted that a high CO_2 production was recorded for para-HT1. Therefore, at least for o-T, m-T, p-T, and Q it seems unlikely that a loss of test substance due to volatilization (if occurring at all) would have affected the biodegradation results, as these constituents have a lower HLC than para-HT1 (Table 5).

Relevance: The study is considered relevant for the purpose of PBT assessment as supporting information. However, due to the hydrocarbon-adapted inoculum the results cannot be used to support “not P/vP”. Neither should differences between the degradability of the constituents be concluded based on this study alone as the pre-exposure history of the inoculum could possibly explain some of the differences. It is not reported to which hydrocarbons the inoculum was pre-exposed to. In addition, if test substances have

volatilised from test vessels, the reported degradation (based on CO_2 production) could be underestimated.

Table 14 Results from a 55 day shake flask carbon dioxide evolution test (MIC 1983b)

	Concentration mg/l	Carbon dioxide evolved (% of theoretical at day 55 calculated from 3 replicates))		
	mean	Mean	Std. Dev.	Range
ortho-T	20.0	20	34	0 - 60
meta-T	20.0	38	18	19 - 53
para-T	20.0	10	14	0 - 26
para-HT1	20.0	63	18	51 - 84
para-HT2	20.0	16	22	3 - 41
para-HT3	20.0	9	9	0 - 18
para-Q	20	7	9	0 - 16
Glucose positive control	20.1	69	9	62 - 79

MONSANTO 1991

Biodegradation screening of p-terphenyl (para-T) was carried out using a 42-day shake flask carbon dioxide evolution test with inoculums obtained from a semi-continuous activated sludge (SCAS) unit (plate count 6.1 x 10^5 colony-forming units per ml.) fed for 8 weeks with p-terphenyl. The test material contained 97.9 % p-terphenyl, 1.7 % m-terphenyl and 0.3 % o-terphenyl. Analytical measurements were conducted using iso-octane as extracting solvent and reverse phase HPLC with UV detection.

The medium was a modification of Standard BOD medium containing the standard levels of magnesium sulfate and calcium chloride, twice the standard level of phosphate buffer, four times the standard level of ferric chloride and ammonium sulfate at 40 mg/l per liter of medium. One litre of medium was charged to the requisite number of uniquely identified 2-liter flasks and sparged with 70% oxygen in nitrogen.

Mean CO_2 evolution expresses as a percent of theoretical indicate no significant mineralization (Table 15). Sodium benzoate positive controls yielded 104 % evolution with a 3-day half-life and no significant lag time indicating active inoculums.

The high recoveries and absence of any difference between test substance concentrations in active and sterile flasks indicate that primary biodegradation did not occur to a significant degree. The relatively high recoveries suggest that losses due to volatilisation (if occurring at all) did not significantly affect the biodegradation results.

Reliability: The study is considered reliable with restrictions (reliability score = 2) due to following reasons:

The method is referred to as MCC Environmental Sciences Method Report Number ES-90-M-12 (MSL-10910). The guideline and its validation information have not been available

for the present report. Therefore, the study cannot be subsumed under a testing guideline. However, the study is nevertheless well documented and scientifically acceptable.

Relevance: The study is considered relevant for the purpose of PBT assessment as supporting information. However, due to the use of an inoculum pre-exposed to p-T (during an SCAS study), any biodegradation in the shake flask carbon dioxide evolution test would not be appropriate to support “not P/vP”.

Table 15 Results from a 42-day shake flask carbon dioxide evolution test (MONSANTO 1991)

	Concentration mg/l	Carbon dioxide evolved (% of theoretical)			
	Mean	mean	Std. Dev.	Range	Mean Residue Recovery, % of initial dose at day 42
p-T	11.3	9	3	5-12	81.1
p-T	20.73	8	5	4-15	78.0
p-T (sterile, single flask)	11.77	7	-	-	82.1
Sodium benzoate	20.01	104	6	999-113	-

Monsanto 1974

Biodegradation testing using the semi-continuous activated sludge (SCAS) procedure was carried out on Therminol 88 (Santowax terphenyl mixture). This test was conducted according to the standard semi-continuous activated sludge (SCAS) method for surfactants (JAOCS 42, (1965), JAOCS 46, 432 (1969)). A description of the test method as applied for this study is given in Analytical Chemistry Method 71-32 of Monsanto.

Duration of test (contact time) was 27 weeks.

The dominant components in the test material were o-terphenyl (7-9%), m-terphenyl (45-50%) and p-terphenyl (30-33%). Minor components included biphenyl, 3- and 4-fused ring compounds, quaterphenyls and quinquiphenyls. SCAS testing was initiated at an addition level of 1 mg per 24-hour cycle. The feed level was increased to 3 mg the second week and to 5 mg the third week. Thereafter, the addition level was maintained at 5 mg until the end of the study. Therminol 88 levels were determined using flame ionization gas chromatography.

Recovery experiments were performed at three levels. At the 100, 200 and 300 µg/l levels the percent recovery was 103.8 ± 12.1.

At the 5mg per 24-hour cycle feed level, the disappearance rate and 95% confidence limit obtained with Therminol 88 was 11.5% ±7.2. Although the disappearance rate was relatively low, significant changes in the distribution of the components in this mixture occurred during the test period. It is mentioned in the test report that during the test cycle the o-terphenyl degraded almost completely while the m-terphenyl showed a much greater decrease than the p-terphenyl. The data for the constituents is not presented in numeric form but chromatograms for standard, initial, and final samples are shown are shown (with no peak areas). In the initial sample, p-terphenyl has the largest peak, m-terphenyl approximately 60% of the height of p-terphenyl, while o-terphenyl peak is very small compared to the other p- and m-terphenyls.

It is mentioned that because of the build-up of the more slowly degradable p-terphenyl isomer, the disappearance rate of Therminol 88 measured under the SCAS steady-state conditions is lower than it would be for the original or starting composition. These changes

indicate according to the Registrant(s) that the o- and m-terphenyl components are much more degradable than p-terphenyl. They mention that the material at the end of the biodegradation test has a higher proportion of the more slowly degradable p-isomer.

At the end of the SCAS testing, a die-away procedure was carried out in which feeding of the test material was stopped but sampling and analysis of the mixed liquor was continued on a periodic basis. Synthetic sewage was fed to the units daily, but draining of the supernatant liquid was only carried out on a weekly basis to minimise solubility and mechanical losses. For Therminol 88, the o-terphenyl an m-terphenyl disappeared more rapidly than p-terphenyl. The die-away was carried out for approximately three months, but considerable difficulty was encountered differentiating between the Therminol 88 and background interferences. As a result the data are not highly significant.

The test report mentions that a slight degree of inhibition on the normal sludge growth was noted for Therminol 88. However, data regarding sludge growth is not presented.

The relatively low mean disappearance of the test substance suggest persistence of the test substance. In addition, the observation that during the test cycle the o-terphenyl degraded almost completely while the m-terphenyl showed a much greater decrease than the p-terphenyl, suggests differences in degradabilities of these isomers. However, it should be noted that the test substance was a mixture including o-, m-, and p-terphenyl, and the concentrations of these constituents differed. In addition, possible volatilization is not discussed in test report and although recovery experiments showed high percent recovery, the duration of these experiments is not mentioned. It is noted that estimated Henry's law constant is higher for o-terphenyl than for m- and p-terphenyls (Table 5). Sterile controls were not mentioned in test report and thus it is assumed that there were no sterile controls in this study. Thus it is unclear whether abiotic factors such as volatilization could contribute to the dissipation of the terphenyl constituents. It should also be noted that the test system favours adaptation. Considering the above, it is considered that the study is not suitable for comparison of degradabilities of o-, m-, and p-terphenyl in context of PBT assessment. However, the fact that p-terphenyl persisted in the test system (despite the possible adaptation during the test) is relevant for P/vP assessment.

Reliability: The study is considered reliable with restrictions (reliability score = 2). The method is referred to as the standard semi-continuous activated sludge (SCAS) method for surfactants (JAOCS 42, (1965), JAOCS 46, 432 (1969)). These are publically available documents (Snow et al. 1965, Mausner et al. 1965). The study is generally well documented and scientifically acceptable, however with reservations due lack of information reqarding the origin/adaptation of inoculum in the SCAS test, apparent lack of replicate vessels (replication is not mentioned in test report) and lack of sterile controls. In addition, the test report does not include raw data (with the exception of chromatograms) and therefore it is not possible to reproduce the calculated biodegradation results. It is also noted that a description of the test method as applied for this study is given in Analytical Chemistry Method 71-32 of Monsanto and this document has not been available for the present assessment.

Relevance: The study is considered relevant for the purpose of PBT assessment as supporting information. In guidance R.7b (ECHA 2017b) it is stated that the value of an SCAS test for assessment purposes is low because of the strong potential for adaptation of micro-organisms to the substance in this kind of test. Therefore, due to adaptation, any biodegradation in this type of a study (both the SCAS part and the die-away part) should not be used to support that the test substance is not P/vP. However, the limited biodegradation of p-terphenyl (both in the SCAS and in the die-away parts) can be used as supporting information for P/vP.

3.1.2.1.3 Simulation tests (water and sediments)

One seawater simulation test is available (ExxonMobil Biomedical Science Inc., 2009). There are no sediment simulation tests available.

ExxonMobil Biomedical Science Inc., 2009. Primary bioegradation in seawater study.

This study was conducted to determine the primary biodegradation half-life in seawater of a series of hydrocarbons. The compounds tested in this study represent the principal aliphatic and aromatic hydrocarbon classes comprising petroleum products and refinery streams. There are no references to any biodegradation test guidelines in the test report. It is mentioned in the test report that there are currently no applicable test guidelines for measuring the primary biodegradation rates of multiple compounds with very low aqueous solubilities in water, in a single study.

Primary biodegradation half-lives were determined in natural seawater in two separate studies with two different hydrocarbon mixtures (also third study was included but that is not relevant for the present assessment). One of the studies consisted of a mixture of 35 "mostly liquid" hydrocarbons (later “liquid test”) and the other one consisted of a similar number of mostly solid hydrocarbons (later “solid test”). Different types of hydrocarbon were included in the studies, among these were compounds belonging to groups T, HT1, HT2, and HT3. The results for these compounds are reported here (Table 16), as well as the result for bicyclohexyl, due to its similarity to the constituents of terphenyl, hydrogenated.

Natural seawater was obtained from the Atlantic Ocean at Sandy Hook, NJ (Gateway National Recreation Area), USA. Seawater was collected within one hour after mean high tide. The seawater was not expected to contain any contaminants at levels which would interfere with the studies. The seawater was permitted to settle and then coarse filtered through #4 Whatman filter paper under slight vacuum and aerated prior to use. The DOC concentration was 2.5 mg/l in unfiltered water, 2.8 mg/l in filtered water and 2.5 mg/l in nutrient amended water which was used for the experiments. Seawater blank samples, filtered and amended with nutrient solution, were analyzed at each interval to measure the extent of potential background relative to the concentrations of the test substances. The microbial population present in the seawater served as the bacterial inoculum. The inoculum was not acclimated or amended with any additional microbial population. It is mentioned that the test substances represent hydrocarbons typically found in petroleum products that may be present in seawater if released into the environment. The temperature was 20(±1)ºC. The primary degradation of hydrocarbons was monitored by GC/MS. The quantification of absolute concentrations of the individual hydrocarbons was not performed. Hexachlorobenzene was used as internal standard.
The primary biodegradation half-life (t1/2) and rate constant (k) were determined for each test compound using the calculated percentages relative to the internal standard normalised responses for each test compound in the corresponding poisoned controls at each interval. These amounts were then normalised to the amount of the internal standard measured in each sample. In general, data were included through time intervals in which at least 10% of the initial concentration remained. Results were plotted using Microsoft Office Excel 2003 from which first order decay curves were fitted. Degradation rate constants for each compound were determined using the following equation:
$y = y_0 e$
where:
- y = concentration at time
- y_0 = initial (day 0) concentration
- k = first order primary biodegradation rate ($days^{-1}$)
- t = time (in days)
- Half-lives were calculated using k determined above and the following equation:

$t_{(1/2)}$ = 0.693 / k

In the test report there is no further information on the kinetic analysis, how the use of first order fit is justified, or whether other kinetic models were considered. The raw data is not presented in the report and therefore it was not possible to perform a kinetic analysis of the data.

For comparing with the P/vP criteria the results were converted to 12°C according to EFSA (2007) (page 7-32 (Eqn 3)):

$$DT50_{(12oC)} = DT50_{(20oC)} \exp(((65.4/0.008314*((1/285,15)-(1/293,15))))$$

The values used are:

activation energy, E_a: 65.4 kJ/mol
gas constant, R,: 0.008314 (kJ K^{-1} mol^{-1})
temperature 1: 285.15 K
temperature 2; 293.15 K

Test results for the selected compounds relevant to the present assessment are presented in Table 16. (60 days). It is noted that comparing the half-lives temperature-corrected to 12°C indicates that for m-terphenyl, the half-lives differ between the “liquid test” and “solid tests”.

For alicyclic hydrocarbons co-metabolism with *n*-alkanes has been reported (Kirkwood et al. 2008, Ko and Lebeault 1999, Koma et al. 2005.). For polyaromatic hydrocarbons co-metabolism with other polyaromatic hydrocarbons has been reported (Dean-Ross et al. 2002). The presence of PAHs in a mixture produces interactive effects which can either increase or decrease the rate of utilization of utilization of individual PAHs (Dean-Ross 2002). Enhancement in the rate of utilization of one polyaromatic hydrocarbon in the presence of a growth substrate has been frequently observed and attributed to cometabolism (Dean-Ross 2002 and references therein)

Considering the composition of the study mixtures, cometabolic degradation of both alicyclic and aromatic structures may have occurred in the test. *n*-Alkanes (dodecane, hexadecane, eicosane) and hydrocarbons with *n*-alkane side chain (e.g., *n-heptyl*) were present in the studied liquid hydrocarbon mixture. In the solid hydrocarbon mixture hydrocarbons with *n*-alkane side chain (e.g., decylbenzene; 1,1’, biphenyl, 4-pentyl) were present. Several PAHs were present in both the liquid (e.g., alkyl substituted naphthalenes) and solid (e.g., pyrene, phenanthrene) study mixture.

Reliability: the reliability of this study is currently not assignable (reliability score = 4) due to following reasons:

There are no references to any biodegradation test guidelines in the test report. It is mentioned in the test report that there are currently no applicable test guidelines for measuring the primary biodegradation rates of multiple compounds with very low aqueous solubilities in water, in a single study. The study appears to be well documented and scientifically acceptable. However, raw data is not included in the test report. Therefore, it has not been possible to verify whether the half-lives are reproducible from the raw data. In the test report there is no further information on the kinetic analysis, how the use of first order fit is justified, or whether other kinetic models were considered.

Relevance: The study is considered relevant for the purpose of PBT assessment; however, it should be used only as supporting information. The relevance of the observed biodegradabilities and half-livees from this study for the purpose of persistence

assessment of terphenyl, hydrogenated is compromised by the facts that there were several hydrocarbons present and that degradation rates of individual hydrocarbons may be influenced by "mixture effects" such as co-metabolism and bioavailability. Because cometabolism can increase degradation rates the half-lives are not used to support the conclusion 'not P/vP' but only to support 'P/vP'.

It is also noted that no information of transformation products is available in this study.

Table 16: Results from primary biodegradation study in sea water with hydrocarbon mixtures (ExxonMobil Biomedical Science Inc., 2009)

Compound	CAS	Group	CAS	Study	time (day)	primary degradation rate constant (%/day)	half-life (days) at 20°C	half-life (days) at 12°C**
o-terphenyl	84-15-1	T	84-15-1	solid	182	0.00008	>182	>182
m-terphenyl	92-06-8	T	92-06-8	liquid	191	0.0136	51	108
m-terphenyl	92-06-8	T	92-06-8	solid	89	0.0471	15	32
hexahydro-terphenyl (cyclohexylbiphenyl)	n/a	HT1	n/a	liquid	191	0.0103	67	142
3-phenylbicyclohexyl	33460-02-5	HT2	33460-02-5	liquid	62	0.0303	23	49
dodecahydro-terphenyl (dicyclohexylbenzene)	1087021	HT2	1087021	liquid	90	0.03	23	49
perhydroterphenyl (tricyclohexyl, m-tercyclohexane)	1706-50-9	HT3	1706-50-9	liquid	90	0.0291	24	51
perhydro-terphenyl (tricyclohexyl), MRD-08-158*	1706-50-9	HT3	1706-50-9	solid	28	0.113	6,1	13
bicyclohexyl	92-51-3	-	92-51-3	liquid	35	0.0458	15	32

*A number of the compounds tested were synthesised by ExxonMobil Research and Engineering (EMRE) Corporate Strategic Research (CSR) laboratory. The synthesised compounds were primarily fully or partially saturated aromatics (naphthenes) produced by catalytic hydrogenation of the corresponding diaromatic or polyaromatic hydrocarbon.
The synthesised compounds are those listed that include an MRD number identifier in addition to the chemical name. The products of these syntheses typically yielded varying degrees of saturation and isomeric positions. The identity of specific naphthenic compounds in these products was determined by GC-MS and the characterisation data is maintained in the testing facilitiy's compound preparation records. Test characterisation data is not reported.
**Temperature conversion

Birch et al. 2018. Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of Kow and Kaw

This study employed a partitioning-based experimental platform to determine biodegradation kinetics of 53 hydrocarobons at ng/L to mg/l concentrations covering C8-C20, 11 structural classes, and several orders of magnitude in hydrophobicity and

volatility. P-terphenyl was among the studied compounds. Biodegradation kinetics were determined in activated sludge filtrate, seawater, and lake water at 20°C. The DT50 values (20°C) for p-terphenyl in this study were 7.6 days in activated sludge filtrate, 20 days in seawater, and 20 days in lake water (Birch et al. 2018, supporting information available at journal website).

This study has been published in January 2018, i.e., just before the submission of the Annex XV report for terphenyl, hydrogenated. However, the dossier submitter considers that the results for p-terphenyl in this study are not relevant for a direct comparison with P/vP criteria in the assessment of terphenyl, hydrogenated. The main reason is that the half-lives for p-terphenyl were determined in a test system in which other hydrocarbons were present and, as explained above (3.1.2.1.3., Chapter concerning ExxonMobil Biomedical Science Inc., 2009.), results from such studies are not used to support “not P or vP” in this case.

In addition, regarding the results for activated sludge filtrate, these results are of limited relevance for PBT/vPvB assessment due to the fact that wastewater treatment plant is not a relevant compartment for PBT/vPvB assessment. There are no P/vP criteria for degradation in a wastewater treatment plant. Regarding the results for seawater, it is noted that degradation in the seawater samples in this study may be of limited relevance for PBT/vPvB assessment due the possible preadaptation of the seawater microorganisms at sampling site. The article mentions that “seawater samples were taken in the vicinity of a trafficked shipping port, which likely implied pre-exposure of the natural bacterial consortia to petroleum hydrocarbons”.

3.1.2.1.4 Other data on biodegradation in water

Prosser et al. 2016. Evaluating persistence of petroleum hydrocarbons in aerobic aqueous media

Prosser et al. (2016) reports a half-life for ortho-terphenyl of 364 days referring to CONCAWE (2012). However, the half-life of 364 days is not found in CONCAWE (2012) and there is no further information on that half-life in Prosser et al. (2016) (including supplementary data available at journal website) or in CONCAWE (2012). Therefore, the reliability score for this half-life is currently 4 (not assignable).

3.1.2.2 Biodegradation in soil

Monsanto Company 1989 Soil dissipation study

A soil dissipation study "Fate testing for Therminol Biodegradation" was conducted according to an internal method “Fate Screening Test in Soil” Ecova Protocol P87/803206.D28:1t/5 Dated and accepted February 8. 1989, and under GLP conditions. The aim of the test was to measure the change in concentration of terphenyls, quaterphenyls and polyphenyls relative to initial concentration and/or sterile controls. The used test material was a mixture of “Quatraphenyls - Santowax”, "terphenyls" and "polyphenyls - Santotar". It is assumed that the material was unhydrogenated as there is no mentioning of hydrogenation in the test report. The Registrant(s) have confirmed that there is no additional information available regarding the substance identification of the test substance beside what is stipulated in the test report.

In the test report one significant deviation from the protocol is mentioned: The final time point was taken at 32 weeks rather than the originally planned 30-week time point. In the test report this is not considered to affect the quality of data in any way, as the final time point was to provide data on an extended time frame. However, the dossier submitter notes that according to the OECD TG 307 the rate and pathway studies should normally

not exceed 120 because thereafter a decrease of the soil microbial activity with time would be expected in an artificial laboratory system isolated from natural replenishment (discussed below in 'Assessment of relevance')

Two concentrations of test material (50 ppm and 0.5 ppm) were prepared in methylene chloride. Two soil types were employed: (A) Missouri Bottoms, a sandy soil with 0.5 % organic carbon and moisture content 11.4% at 1/3 bar and (B) Florida muck, a heavy loam containing 32.9 % organic carbon and moisture content 81.9% at 1/3 bar. A microtox screen was employed prior to study start to ensure that no bacterial toxicity would interfere with the main test (described in more detail below). Soil samples were stabilised prior to study start so that they contained a uniform level of microorganisms. Soil samples (25 g) were placed in 125 ml jars and spiked with 250 µl of test solution containing the test material in solvent. Jars were capped and shaken, and then lids removed and restirred manually. After being lightly recapped, the jars containing the test material and soil were incubated in the dark at 25 ºC and 80 % humidity until sampled. The total weight of the jar and soil was recorded and used to readjust the soil moisture content throughout the duration of the test (the number or days of moisture adjustments, or the amount of water added are not indicated). Soil samples containing the test material were extracted and analyzed by HPLC after 0, 0.5, 1, 2, 4, 6, 8, 12, 15 and 32 weeks of the study. Three replicates of each treated group plus a sterile control (spiked at 50 ppm and 0.5 w/w mercury chloride added) per time period per soil type were used. Matrix blanks were also employed, four of the matrix blanks (two per each soil type) were pulled at each time point; two of these served as quality control samples with a nominal soil concentration of 30 ppm. As the bottles were opened during treatment and incubation happened in lightly cappen bottles, losses due to volatilisation cannot be overruled.

Regarding the sterile controls, on week 8 of the test, two of the sterile control jars (one for each soil type) were pulled at random and subsamples were plated onto standard plate count agar to check sterility. Following 8 days of incubation several faint colonies were observed; because of this the procedure was altered. All further moisture adjustments on the sterile controls were accomplished by the addition of saturated mercuric chloride solution rather than deionised, sterile water. The quality control check was repeated at week 24 and no viable colonies were observed. In summary, there was a considerable difference in sterilisation treatment between the 0-9 week period and 9-24 week period. During the 0-9 week period mercuric chloride was added at day 0 only and during the 9-24 week period mercuric chloride was added presumably on week 9 (as the first sterility check result was available on week 9) and after that with every moisture adjustment. However, is not known on which days, and how many times mercuric chloride solution addition was done and what was the amount of the solution added.

Extraction procedure

The analyte was extracted from the soil with methylene chloride in the original 125 ml test container using a sonicator, and dried by pouring through a powder funnel packed with anhydrous sodium sulphate. The dried extract was collected in a concentrator. The test container and powder funnel were rinsed with additional methylene chloride. The combined solvent extract was then concentrated to about 5 mls. After cooling, the extract concentrate was passed through silica gel, which was then rinsed with methylene chloride and the combined solvent was concentrated to less than 1 ml, diluted to 10 ml with n-hexane, and again concentrated to 1.0 ml under nitrogen. The concentrate was then filtered (1 µm filter) and analysed by HPLC.

Preliminary toxicity testing

Preliminary toxicity testing (a standard Microtox™ microbial toxicity test) was performed due to possible toxicity of the test compound and/or the carrier solvent (methylene chloride). The tested samples were 1) 5% ethanol control, 2) an extract of soil, 3) a standard of the test compounds (50 ppm each, total of 150 ppm) made up in methylene

chloride, and 4) a combination sample consisting of an extract of soil B spiked with 150 ppm of the mixed standard in methylene chloride. EC50 values after 15 minutes of exposure to the sample at 15°C were: 0.36%, 0.46%, 0.77%, and 0.46%, for the above mentioned samples 1-4, respectively. It is mentioned that the calculated EC50's for the soil extract alone (0.60%) and the test material/carrier solvent alone (0.77%) were slightly higher (i.e. less toxic), but all the results were effectively the same given 95% confidence limits ranging from 0.3% to 1.9% for the test material solvent standard. The results are included in Appendix A of the test report.

It is noted that there are several weaknesses related to this toxicity test. The test report does not give results on only the solvent methylene chloride. In addition, from the original report it is unclear how the test solutions were prepared and what were the actual concentrations. Ethanol at the tested concentrations and the unspiked soil extract are not expected to be toxic.

The colour of the test samples was not reported and no colour correction was used for soil extracts. Soil extracts in general may hamper the detection of luminescence in Microtox test either due to colour or due to extracted soil components especially if the extract is something else than water.

The results do not indicate any significant differences between spiked and unspiked soil extract samples, and ethanol used for extraction, in their toxicities towards luminescent bacteria. This suggests that in the soil degradation test inhibition by test substance is not expected.

Spike recovery test for validation of analytical method

The original analytical method (Appendix B of test report) was modified slightly for this particular test. The changes (Appendix C of test report) were validated by performing a series of spike recovery analysis covering a range of concentrations to be used in the fate test (Table 17). The method validation was performed on both soils to account for any matrix effects due to the drastically different soil types. According to the test report the results of the method validation study (Appendix D of test report) indicate that the test compounds could be quantitatively recovered from the test soils using this analytical method. It is also mentioned that matrix interferences were encountered in the analysis of terphenyl and quatraphenyl (tetraphenyl) in soil B, resulting in recoveries greater than 100%. Although corrected values are presented with the method validation data, no corrections were made to the actual fate test.

Table 17. Mean spike recoveries (± std.dev.) (Monsanto Company 1989). The values in brackets are results corrected for matrix interference (matrix interference was observed for soil B only). The final results were not corrected for matrix effects.

	Method validation data (nominal concentrations 0.20-50 mg/kg)		Spike recovery controls in final test (nominal conc. 30 ppm)	
	Soil A	Soil B	Soil A	Soil B
terphenyl	113 ± 22	165 (71) ± 100 (32)	86 ± 11	79 ± 12
quatraphenyl	96 ± 17	77 (73) ± 12 (9)	90 ± 28	83 ± 12
polyphenyl	91 ± 26	67 (64) ± 11 (8)	104 ± 24	98 ±26

Spike recovery in final test

Spike recoveries in final test were reported as mean values (Table 17). For each of the studied group of constituents, the spike recovery was lower in soil B than in soil A. It is assumed that these are the mean values (± std.dev.) of all individual samples collected

during the duration of the study. The values for the individual samples are not presented, so it is not possible to evaluate the effect of recovery percentage on an individual sample and neither is it known whether there were any trend in spike recovery during the duration of the study that could affect the results (no such trends are reported).

Kinetic analysis of data

The half-lives presented in the test report are based only on the initial and final concentrations of test substance using a first-order equation. No kinetic modelling was presented in test report. The validity of the first order calculations and consequently the validity of the presented half-lives (Error! Reference source not found.) is not known without a kinetic analysis. Therefore, re-modelling was conducted using KinGUI v 2.1 (© Bayer CropScience 2014) program. Modelling was conducted using a pathway with one compartment and a sink. Default kinetic variables of the program were used.

In the PBT guidance (ECHA 2017a) it is recommended to consult the FOCUS guidance document (EFSA 2014) for in-depth analysis of simulation degradation test results. It is noted that an up-date of the FOCUS guidance (EFSA 2014) has been used for the present assessment (there are only editorial changes in the guidance compared to the earlier version).

The single first-order (SFO) model as well as bi-phasic models (FOMC, HS, DFOP) were used in order to find the best fit for the data. In the case of bi-phasic models, both the overall half-life during the study as well as the half-life during the second phase ('slow phase') of the dissipation curve were reported and considered in the assessment when appropriate. For the HS model the DT_{50} for the whole study period was calculated according to equation (1) (if DT50 ≤ t_b) or equation (2) (if DT50 > t_b) (EFSA 2014).

$$DTx = \frac{\ln \frac{100}{100 - x}}{k_1}$$

equation (1)

$$DTx = t_b + \frac{\ln \frac{100}{100 - x} - k_1 t_b}{k_2}$$

equation (2)

where
k_1 Rate constant until $t = t_b$
k_1 Rate constant from $t = t_b$
t_b = Breakpoint (time at which rate constant changes)

The slow-phase DT50 value was calculated using (LN(2)/k2). In the case of the FOMC model calculation of the slow-phase DT50 is not feasible. Therefore, to take into account the slow-phase degradation in the case of FOMC model, estimated half-lifes were calculated from the non-first order DT90 values by dividing the DT90 by 3.32 (EFSA 2014). The part of the FOCUS guidance that was followed (EFSA 2014, page 111) introduces the DT90/3.32 approach for the FOMC model only and this approach was not used for the HS and DFOP models. Best fit was determined using the statistical parametres and visual assessment.

ECHA guidance R. 11 (ECHA 2017a) mentions: "When the kinetics of transformation are first-order, single-first order (SFO) kinetic models can be used for predicting degradation half-lives. The predicted degradation half-lives should be used for comparison with the

P/vP criteria. Use of bi-phasic kinetic models is recommended to be limited to cases where clear deviations from first-order kinetics occur."

According to the FOCUS guidance, the first steps in modelling should be running SFO model when the purpose is to derive endpoints for use as triggers for additional work, and, running both SFO and FOMC models when the purpose is to derive endpoints for use as modelling inputs. It is noted that the PBT guidance does not specify which one of these two approaches ('triggers for additional work' or 'modelling endpoints') should be used in PBT assessment. In the present assessment, both approaches are used (EFSA 2014, page 111).

Following the "additional work approach" in FOCUS guidance, SFO and FOMC models were compared first. According to the FOCUS guidance, if FOMC is better than SFO, other bi-phasic models should be tested. In the FOCUS guidance, the Chi^2 test is recommended as a tool for model comparison, and as a supplementary tool for assessing the goodness of fit of an individual model (the visual assessment is the main tool for assessing goodness of fit).

Following the "modelling endpoint" approach, if the error at which the the Chi^2 test is passed at the 5% significance level (Chi^2Err percentage) for SFO is <15 and visual fit is acceptable, then the SFO DT50 should be used. If the Chi^2Err% for SFO is >15 and visual fit not acceptable, then bi-phasic models should be run. If 10% of initial concentration is reached in study period, DT50 should be calculated as DT90 FOMC / 3.32. If 10% of initial concentration is not reached in study period, then the longer DT50 of HS or DFOP should be used. It is noted that no specific guidance is given for a situation where Chi^2Err% <15 but visual fit is not acceptable.

Half-lives for biodegradation were estimated by subtracting the rate constant (*k*) (based on SFO model) of the sterile control from that of the active test and by calculating the half-life (DT50=LN(2)/*k*) (Table 20, Table 21). It is noted that the sterility of the sterile controls appears to have been incomplete during the 9 first weeks (described above). Therefore, occurrence of biodegradation in sterile controls cannot be completely ruled out. If biodegradation occurred in sterile controls, the estimated biodegradation rates are underestimated and biodegradation half-lives overestimated.

In addition, as there were differences in the initial concentration between the different tests, the data were plotted as percentages to the initial concentration and ratios in order to make a comparison between the active tests and sterile controls as well as between the different constituents.

Temperature correction of half-lives

For comparing with the P/vP criteria the half-lives in the test report and those obtained from modelling were converted to 12°C in accordance with EFSA (2007) (page 7-32 (Eqn 3)):

$$DT50_{(12oC)} = DT50_{(25oC)} \exp(((65.4/0.008314*((1/285.15)-(1/298.15))))$$

The values used are:

activation energy, E_a: 65.4 kJ/mol
gas constant, R,: 0.008314 (kJ K^{-1} mol^{-1})
temperature 1: 285.15 K
temperature 2; 298.15 K

Results

The half-lives used for comparing to the P/vP criteria are presented in Table 18. The results

based on initial and final concentrations are presented in Table 19. Results from modelling are in Table 20, Table 21, and Table 22. Graphs for the 50 ppm tests are in Figure 1, Figure 2, and Figure 3 and for the 0.5 ppm tests in Figure 4 and Figure 5. It is noted that the test report does not include results for individual replicates but only means and standard deviations.

Accuracy of the analytical method

The detemination limit and accuracy of the analytical method have not been reported. However, in the study report the development of the analytical method is described and it is stated that "*The [method development] results indicate that it should be possible to perform the biodegradation experiment at a nominal concentration of 1 to 10 mg/kg. However, the lower concentrations may be difficult for the polyphenyls and slightly higher concentrations are suggested for this analyte.*" Based on spike recovery results, the accuracy of the method is at best 20 % (e.g. 0.5 ppm ± 0.1 ppm). This would indicate that the limit of quantification would be at best around 0.3 ppm. In the final test, the relative standard deviation (std.dev/mean) between replicate jars (Figures 1-6) was generally higher for the 0.5 ppm test compared to 50 ppm test, also suggesting that the accuracy of the analytical method was not optimal for the 0.5 ppm test. It is also noted that the results for the lower concentration (0.5 ppm) are expressed consistently at 0.10 ppm intervals. The results < 1 ppm are possibly below the determination limit and therefore related with considerable uncertainty.

Terphenyl

Terphenyl appears to be more biodegradable than the quatraphenyls and polyphenyls tested (Table 19; Annex 2, Figure 2, Figure 3). In the active tests, concentration of terphenyl in the test containers began dropping immediately after test initiation with no evident lag period. By comparing the measurements at the beginning and at the end of the study (week 32) the 50 ppm test dropped to 3 ppm in soil A (94% reduction) and 6 ppm in soil B (84% reduction). The 0.5 ppm test dropped to 0.1 ppm (75% reduction) in soil A but remained at 0.3 ppm (0 % reduction) in soil B.

The loss of terphenyl in the sterile controls, down to 16 ppm in soil A (60% reduction) and 20 ppm in soil B (54% reduction) at the end of the study (week 32), indicates that at least some of the losses seen in the non-sterile test systems were due to abiotic causes. According to the test report, for terphenyl, the primary route of removal appears to be biological. The reported removal rate in the active systems is more than double that in the sterile system (in soil A and B, T50 = 57-85 days and 167-199 days in active and sterile systems, respectively). Converted to 12°C these are 147-219 days and 431-513 days for the active and sterile systems, respectively. Terphenyl half-lives for the biotic processes are 222-382 days 12°C (Table 19).

Terphenyl, results from degradation modelling

Regarding terphenyl Soil A 50 ppm active test with all data points included, based on visual assessment, chi^2 test and other statistical parametres, no clear difference can be made between SFO and FOMC models. Chi^{2}Err percentage is 9.1 for SFO and 9.5 for FOMC. The solution according to the "additional work approach" would be to use the SFO model. However, this does not seem appropriate as the SFO model overestimates the concentration at last point. Considering the "modelling endpoint approach", in this case the Chi^{2}Err% is <15 for SFO but visual fit is not acceptable as concentration is overestimated at last point. As 10% of initial concentration is reached in study period, then DT50 should be calculated as DT90 FOMC/3.32. However, this does not seem appropriate either as also the FOMC model overestimates the concentration at last point. The HS model is considered best fit based on visual assessment and Chi^{2}Err% and it also

has the best prediction at the last data point. It is noted that the second-phase half-life is 167 days for HS model. However, it is noted that the HS model recognises a lag phase in this test and it would not be appropriate to neglect the lag phase and to use only the second phase degradation for the conclusion. Therefore, for terphenyl in the Soil A 50 ppm active test, the HS result calculated for the whole study period is used (304 days at 12°C).

Regarding terphenyl Soil A 50 ppm sterile control with all data points included, based on visual assessment, no difference can be made between SFO and FOMC models. Chi2err percentage is 8.3 for SFO and 8.7 for FOMC. Therefore, the solution according to the "additional work approach" would be to use the SFO model. This seems appropriate; however, it is noted that the concentration is overestimated by SFO at last point (although not as much as for the active test). Considering the "modelling endpoint approach", in this case the $Chi^2Err\%$ is <15 but visual fit is not acceptable as concentration is overestimated at last point. As 10% of initial concentration is not reached in study period, then the longer DT50 of HS or DFOP should be used (DFOP predicts a longer DT50 in this case). Based on visual assessment it is difficult to decide between HS and DFOP and although $Chi^2Err\%$ is lower for HS (7.4) than DFOP (9.1) the breakpoint for HS is not statistically significant. However, as the HS is the only one of the models which does not overestimate the concentration at the last point (on the contrary, the concentration is underestimated) it is considered appropriate to use also the HS model. It is noted that the second-phase half-life is 472 days for HS model. As the HS model recognises a lag phase in this test it would not be appropriate to neglect the lag phase and to use only the second phase of the dissipation curve for the conclusion. Thus in the case of the HS model the result calculated for the whole study period is used. Therefore, for terphenyl in Soil A 50 ppm sterile control, the SFO, DFOP, and HS results are used. The half-life (12°C) for SFO is 606 days, for DFOP 606 days (first phase and second phase), and for HS 566 days (for the whole study period).

The calculated half-life (12°C) for biodegradation of terphenyl in Soil A is 604 days (Table 21). The calculation of the biodegradation half-life is based on SFO rate constants corresponding to half-lives of 303 days for the active test and 606 days for the sterile control.

Regarding terphenyl Soil B 50 ppm active test with all data points included, based on visual assessment, chi^2 test and other statistical parametres, no clear difference can be made between SFO and FOMC models. $Chi^2Err\%$ percentage is 11.4 for SFO and 11.9 for FOMC. As it cannot be concluded that FOMC is better than SFO, the solution according to the "additional work approach" would be to use the SFO model. However, it does not seem appropriate to use only the SFO model as it underestimates the concentration at last point. Considering the "modelling endpoint approach", in this case the $Chi^2Err\%$ for SFO is <15 but visual fit is not acceptable as concentration is underestimated at last point. As 10% of initial concentration is not reached in study period, then the longer DT50 of HS or DFOP should be used. $Chi^2Err\%$ is similar for both models (12.4 for DFOP and 12.5 for HS) and both models underestimate the concentration at the last data point; however, for DFOP the error is lowest. As the *p*-values for the DFOP are high, also the SFO model is used. Therefore, for terphenyl in Soil B 50 ppm active test, the SFO and DFOP results are used. The half-life (12°C) for SFO is 225 days and for DFOP 218 days for the first phase and 969 days for the second phase.

Regarding terphenyl Soil B 50 ppm sterile control with all data points includednone of the models were acceptable based on the statistical parametres and visual assessment. However, the concentration was reduced to less than 50% of the initial at weeks 8, 12, and 32 (Figure 1), therefore suggesting that dissipation could be faster. However, there is no consistent trend in the data, for example, in particular on week 15 the concentration was higher than on weeks 8 and 12. Therefore, for terphenyl Soil B 50 ppm sterile control the half-life is estimated to exceed the test duration (224) days.

The calculated half-life (12°C) for biodegradation of terphenyl in Soil B is 336 days (Table 21). However, the value is uncertain as no acceptable kinetic fit was obtained for the

sterile control. The calculation of the biodegradation half-life is based on SFO rate constants corresponding to half-lives of 225 days for the active test and 680 days for the sterile control).

For terphenyl in the 0.5 ppm tests for Soil A and B with all data points included, none of the models were acceptable based on the statistical parametres and visual assessment. Regarding the Soil A 0.5 ppm test, the concentration was ca. 50% of the initial at week 15 and below 30% at week 32 (Figure 4), suggesting that a half-life may have been within the test duration. It is possible that the reason why no acceptable model was obtained is related to the accuracy of the method. Based on the ca. 50% conc. at week 15 it can be roughly estimated that the half-life for terphenyl in Soil A 0.5 ppm test is at least 15 weeks (105 days) at 25°C, which corresponds to 305 days at 12°C. Regarding Soil B, the concentration did not reach 50% during the test and therefore the half-life is estimated to exceed the test duration. The results in the 0.5 ppm test particularly for Soil B may be influenced by the accuracy of the measurement method at this concentration range and due to matrix effects in Soil B as discussed above.

Quaterphenyl

Concentrations of quaterphenyl in the test containers began dropping much more slowly (compared to terphenyl) with relatively little loss through 8 weeks (Figure 2). At the end of the test (week 32), the 50 ppm test showed a 54% reduction in soil A and 42 % reduction in soil B. The 0.5 ppm test showed 50 % reduction in soil A and 33 % reduction in soil B. The loss of quaterphenyl in the sterile controls was similar to that seen in the non-sterile tests. According to the test report this indicates that most of the loss in the non-sterile test systems could be attributed to abiotic losses.

According the test report there appears to be no biological contribution to the removal of quaterphenyl in soil A assuming that no microbial activity occurred in the sterile systems. The half-lives (T50) (in test report) of the active and sterile systems are considered by the authors “virtually the same given the variability in the data” (28.9 weeks (203 days) and 24.6 weeks (173 days)) (converting to 12°C the values are 524 and 446 days). There is a difference in the removal rates in the soil B systems (active T50 = 40.9 weeks (286 days), sterile T50 = 52.9 weeks (370 days)), which is, according to the authors, likely explained by higher biologically mediated degradation in the active system (values at 12°C are 738 and 954 days).

Quaterphenyl, results from degradation modelling

For quaterphenyl for Soils A 50 ppm test and sterile control, none of the models were acceptable based on the statistical parametres and visual assessment. Regarding Soil A active test, the concentration was below 50% of the initial level on weeks 12, 15, and 32. However, there was no consistent decreasing trend in the data (no further decrease in concentration occurs after week 12) (Figure 2). Regarding Soil A sterile control, the concentration was close to or below 50% of the initial level on weeks 12 and 32 and there was no consistent decreasing trend after week 12 (the concentration is higher on week 15). Therefore, for quaterphenyl in active tests as well as sterile controls at 50 ppm for both soils (A and B), the half-life is estimated to exceed the test duration (224) days.

Regarding quaterphenyl Soil A at 0.5 ppm with all data points included, based on visual assessment, SFO and FOMC are equally applicable and based on statistical parametres it cannot be concluded that FOMC is better than SFO. Thus, according to the “additional work approach” SFO model should be used. According to the “modelling endpoint approach”, SFO should be run as a first step. As in this case Chi^2Err%is <15 and visual fit is acceptable, SFO model should be used. Therefore, for quaterphenyl in Soil A 0.5 ppm test, the SFO result is used. The half-life (12°C) for SFO is 700 days. For comparison, the concentration

reached 50% of the initial level on week 32 (Figure 5) and based on these results the half-life can be estimated to be 32 weeks (224 days), which corresponds to 699 days at 12°C, being very close to the value from modelling.

For quaterphenyl Soil B 50 ppm test and sterile control, none of the models were acceptable based on the statistical parametres and visual assessment. Regarding soil B active test and sterile control, the concentration did not decrease to a level of 50% of the initial level, with the exception of week 12 when a clearly lower value was obtained (Figure 2). The week 12 measurement can be treated as an outlier as this data point was considerably below the prediction and the concentrations were higher in the later measurements (weeks 15 and 32). As none of the models resulted in an acceptable fit, the half-life is estimated to exceed the test duration (224) days in the active test as well as in the sterile control. Excluding the outlier increased the half-lives predicted by the models (data not shown) and thus the conclusion regarding P/vP criteria would not be changed.

For quaterphenyl Soil B at 0.5 ppm with all data points included, none of the models were acceptable based on the statistical parametres and visual assessment. In addition, the concentration did not decrease to 50% of the initial level during the test (Figure 5). Therefore, the half-life for quaterphenyl in Soil B at 0.5 ppm is estimated to exceed the test duration (224 days).

The half-life (12°C) for biodegradation for quaterphenyl in soil A was 2661 days (Table 21). However, the calculation is uncertain as acceptable kinetic fits were not obtained for either of the tests (active test and sterile control). The calculation of the biodegradation half-life is based on SFO rate constants corresponding to half-lives of 385 days for the active test and 451 days for the sterile control.

The half-life (12°C) for biodegradation for quaterphenyl in soil B was 1782 days. The calculation of the biodegradation half-life is based on SFO rate constants corresponding to half-lives of 608 days for the active test and 922 days for the sterile control.

Polyphenyl

For polyphenyl in the 50 ppm test, the test report mentions that in the active test all losses in soil A after 32 weeks are due to biodegradation. However, it is also mentioned that there was a “possibly spurious data point” for the soil A sterile control at 32 weeks (Figure 3). As the half-lives in the test report were based only on initial and final concentrations, the influence of one data point was high. The test report also mentions that if the kinetic analysis is repeated using the 15 week data instead of 32 weeks the picture changes and indicated that there may be a small contribution attributable to biodegradation.

In the 0.5 ppm test (Figure 4, Figure 5), some decrease in concentration was observed in both soils based on mean concentrations. However, deviation between replicates was considerably large.

No modelling has been conducted for the polyphenyl data. However, based on the temperature correction of the reported half-lives for polyphenyl, visual assessment of data, and comparison to the terphenyl and quaterphenyl results, polyphenyl is likely to fulfil the P and vP criteria in soil (Table 19)

Assessment of reliability

The study was conducted under GLP and differences in analytical method compared to internal laboratory method (see above) made for this particular study were validated by spike recovery study. One deviation from test protocol was mentioned (the final time point was taken at 32 weeks rather than the originally planned 30-week time point). This

deviation is not considered to affect the reliability of the study. However, there are several issues with reporting and conducting of the test that can decrease the reliability of the study:

-Matrix interferences were observed in the method validation test in soil B for terphenyl and quatraphenyl, resulting in recoveries greater than 100% but the results were not corrected for matrix interference in the final test. In the final test, spike recovery control results are given as mean value and it is therefore not possible to evaluate whether the spike recovery depended on incubation time, or, whether there was any trend in spike recovery that could possily affect the results.

- It is mentioned that the bottles were opened during treatment and that incubation happened in lightly cappen bottles. Therefore, losses due to volatilisation cannot be overruled. The dossier submitter considers that possible losses due to volatilization should be seen in the sterile controls and thus differentiated from degradation. As the results point to persistence rather than non-persistence there is no concern of false positive degradation results due to volatilization. Therefore, the dossier submitter has not evaluated further whether differences in volatilization could explain some of the differences between the results for the different constituents.

-The duration of the test (32 weeks=224 days) was relatively long compared e.g. to OECD 307 test (normally 120 days). According to the OECD 307 TG the rate and pathway studies should normally not exceed 120 days, because thereafter a decrease of the soil microbial activity with time would be expected in an artificial laboratory system isolated from natural replenishment. There are no biomass measurements to estimate the level change in microbial activity in the present study. However, half-lives calculated for the 105-day period also exceed the vP criterion (data not shown). Therefore, the possible decline in microbial activity due to the duration of the test (beyond the normal 120 days) does not influence the conclusion vP.

-The detemination limit and accuracy of the analytical method have not been reported. The dossier submitter considers that results < 1 ppm are possibly below the determination limit and therefore related with considerable uncertainty. Especially in soil B the possible decrease in concentration in the 0.5 ppm test may not have been detectable with the method used. It is also noted that no sterile controls were conducted at 0.5 ppm concentration.

-The sterile control jars were not replicated.

-The sterility of sterile control jars appears to have been incomplete during the first 9 weeks. Therefore, occurrence of biodegradation in sterile controls cannot be completely ruled out.

- The test temperature (25°C) exceeds the recommended temperature according to the OECD TG 307, which is 20 ± 2°C, for all test substances which may reach the soil in temperate climates.

The experimental part of the study is reliable with restrictions (reliability score 2 in the Klimisch scale) due to the reasons mentioned above.

Regarding the calculation of half-lives, the half-lives presented in the test report (and in Table 19) are not necessarily reliable because only the initial and final concentrations are used. The data was therefore remodeled using all the data points. The half-lives derived from modelling are likely to be less sensitive to individual samples.

Assessment of relevance

Compared to standard simulation tests generally preferred for persistence assessment, the study has some differences/deficiencies, including:

Uncertainty of the identity of test substance. The used test material was a mixture of “Quatraphenyls - Santowax”, "terphenyls" and "polyphenyls - Santotar". It is assumed that the material was unhydrogenated as there is no mentioning of hydrogenation in the test report. There is no information on the proportions of the different isomers within each group (terphenyls, quatraphenyls, and polyphenyls) of the test mixture. In addition, there is uncertainty regarding the level of hydrogenation of the test mixture. No additional information is available regarding the substance identification of the test substance beside what is stipulated in the test report.

As only a decrease in concentrations of the studied constituents was analysed there is no exact information on mass balance. There were no measurements of mineralization, metabolites, and non-extractable residues. Spike recovery validation study as well as the spike recovery controls give information on the recovery of test substance in the method (extraction and preparation for analysis); however, this is not as detailed information as can be obtained in a standard OECD 307 test with radiolabelled test substance. For example, it is not known whether the non-recovered part of the spiked substance has been mineralised, volatilised, or included as non-extractable residues. In addition, the exact recovery percentage in individual samples is not known (although the magnitude of deviation can be estimated from method validation data results). Therefore it is not possible to evaluate whether there were changes in spike recovery during the test which might have affected the results. However, as the results point rather to persistence than non-persistence and as sterile controls were used for comparison, the dossier submitter considers that the results can be used as there is no risk that lack of mass balance would cause a false positive degradation result. Considering that a part of the decrease in concentration in the active tests may be due to formation of non-extractable residues (which can be formed from parent substance or metabolites), the real degradation half-lives may be longer than reported here (i.e. the half-lives calculated do not represent a worst-case scenario).

It is noted that the highest test substance concentration used (50 ppm) corresponds to 47 mg/kg dry soil for soil A and 35 mg/kg dry soil for soil B (assuming that WHC is equal to moisture content at 1/3 bar (WHC is not reported) and that moisture content at 1/3 bar is expressed as % of dry weight) which corresponds to application rate of 47 kg/ha and 35 kg/ha for soils A and B (assuming dry bulk density of 1 kg/dm^3 and even distribution in the top 10 cm soil layer). This is relatively high concentration compared to the OECD 307 test conducted for an individual constituent (NOTOX 2009a) of terphenyl, hydronegated (0.40 mg/kg dry soil) and higher compared to PECs (highest PECs e.g. 0.02 mg/kg (local sediment); 0.6 mg/kg (agricultural soil)). The total concentration of the test mixture in the present study was 150 ppm (50 ppm of the three constituent groups). It is noted that the test substance concentration can influence the degradation rate due to bioavailability/mass transfer limitations and potentially by affecting the predominant type of degradation (growth associated degradation vs. co-metabolic degradation, the latter being dependent on the metabolism of substrates available in the test soil).

In the test report it is mentioned that there appears to be no trend in test concentration as the % reduction are similar for both 50 ppm and 0.5 ppm for quatraphenyl and polyphenyl whereas for terphenyl it is mentioned that the results are probably confounded by the matrix interferences identified during the method validation study.

Even though the accuracy of the method was limited for the 0.5 ppm test in particular for Soil B (the possible decrease in concentration in soil B at that concentration level may not have been detectable with the method used), the dossier submitter considers that the results of the 0.5 ppm tests for soil A indicate that concentrations of terphenyl and

quaterphenyl decreased during the test but with a slower rate than in the 50 ppm test. Even though in the Soil A 0.5 ppm tests the models were accepted for half-life determination only in the case of quaterphenyl and not for terphenyl, based on visual assessment and the statistically significant rate constant in SFO model it appears that the method was suitable to detect changes in concentration at this concentration level also for terphenyl. The comparison of the results at 0.5 and 50 ppm tests in Soil A suggest that the relatively high concentration used at the 50 ppm test did not negatively affect the degradation. Therefore, the results at 0.5 ppm support the conclusions that the studied constituents fulfil the P and vP criteria in soil.

The study is regarded relevant for the purpose of persistence assessment despite the above discussed differences compared to standard simulation tests.

Summary

Dissipation half-lives were calculated using results from active and sterile flasks in a soil dissipation study. For the active tests (including both biotic and abiotic processes) at initial concentration of 50 ppm the half-lives for terphenyl at 12°C were ≥218 and 304 days for the two soils whereas for quaterphenyl no acceptable kinetic models could be obtained and the half-lives were estimated to be above test duration, i.e., >224 days. Based on sterile controls a part of the dissipation may be due to abiotic processes. The calculated half-lives for biodegradation (excluding abiotic processes such as losses due to volatilisation) (obtained from SFO model) were 336-604 days for terphenyl and 1782-2661 days for quaterphenyl in the two soils. It should be noted, however, that based on sterility check some biodegradation may have occurred in the sterile control jars, which might theoretically cause an overestimation of biodegradation half-lives. However, this is not considered an issue for the interpretation of the present study for the purpose of PBT assessment as half-lives from the active tests are above the vP criterion and considering that the decrease in concentration may be partly due to other (abiotic) processes than degradation.

For polyphenyl, no modelling was done but based on comparison of the data with terphenyl and quaterphenyl the degradation half-lives for polyphenyl are estimated to be similar or higher than for quaterphenyl.

It is noted that the higher test substance concentration used (50 ppm) was relatively high. The results for the lower test concentration (0.5 ppm) are less accurate; however, they indicate that degradation rate at 0.5 ppm is likely to be similar or slower than at 50 ppm. Therefore, the results indicate that studied constituents (terphenyl, quatraphenyl, and polyphenyl) fulfill the P and vP criteria in soil.

The different isomers of terphenyl, quaterphenyl, of polyphenyl were not differentiated in this study and there is uncertainty regarding the level of hydrogenation.

The study is considered reliable with restrictions and relevant for the purpose of persistence assessment.

Table 18. DT50 values (derived using data presented in Monsanto Company (1989) used for comparing to the P/vP criteria. Please note that the study was conducted at 25°C and if a reliable DT50 was derived, it was converted to 12°C. In most cases the DT50 values estimated for 12°C were longer than the experimental period (224 days). Extrapolation of data is always insecure and thus respective DT50 should be interpreted with care. However, the values presented are considered appropriate for comparing to the P/vP criteria.

	overall DT50 (days) (12°C)	second-phase DT50 (days) (12°C)
terphenyl, soil A, 50 ppm	304 (HS)	not used[a]

terphenyl, soil A, 50 ppm, sterile control	606 (SFO), 566 (HS), 606 (DFOP)	not used (HS)[a], 606 (DFOP)
terphenyl, Soil A, 0.5 ppm	≥305[b]	not applicable
terphenyl, soil B, 50 ppm	225 (SFO), 218 (DFOP)	969 (DFOP)
terphenyl, soil B, 50 ppm, sterile control	>224[c]	not applicable
terphenyl, Soil B, 0.5 ppm	>224[c]	not applicable
quaterphenyl, Soil A, 50 ppm	>224[c]	not applicable
quaterphenyl, Soil A, 50 ppm, sterile control	>224[c]	not applicable
quaterphenyl, Soil A, 0.5 ppm	700 (SFO)	not applicable
quaterphenyl, Soil B, 50 ppm	>224[c]	not applicable
quaterphenyl, Soil B, 50 ppm, sterile control	>224[c]	not applicable
quaterphenyl, Soil B, 0.5 ppm	>224[c]	not applicable

[a]Second-phase half-life is lower than first-phase half-life. Therefore, it is considered not appropriate to use the second-phase half-life for comparing to P/vP criteria as the apparent lag phase would then be ignored.

[b]No acceptable kinetic model was obtained (based on statistical parametres and visual assessment). A half-life at 25°C was approximated to be equal to or longer than the time point when the concentration decreased to ≤50% of the initial concentration. The value presented here was obtained by applying temperature correction (to 12°C) to the estimated half-life at 25°C.

[c]No acceptable kinetic model was obtained (based on statistical parametres and visual assessment) and, either the concentration did not decrease to 50% of initial or, 50% was reached at some measurement day(s) but the data did not indicate a consistent decrease in concentration. The half-life was estimated to be longer than test duration (224 days).

Table 19. Biodegradation of terphenyls, quatraphenyls and polyphenyls in two different soils (Monsanto Company 1989)

	% reduction relative to initial concentration at week 32 (224 days)		T50 (days)[1]		T50 (days) at 12°C (temperature-corrected)	
	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B
Terphenyl						
50 ppm (active)	94	84	57	85	147	219
50 ppm (sterile)	60	54	167	199	431	513
50 ppm active−sterile			86	148	222	382
0.5 ppm[2]	75	0	112	*	289	*
Quatraphenyl						
50 ppm (active)	54	42	203	286	524	738

50 ppm (sterile)	59	34	173	370	446	954
50 ppm active−sterile			**	1258	**	3245
0.5 ppm	50	33	224	383	578	988
Polyphenyl						
50 ppm (active)	45	27	263	499	678	1287
50 ppm (sterile)	0	22	*	612	*	1579
50 ppm (active−sterile)				2699		6963
0.5 ppm[2]	40	50	304	224	784	578

[1]T50 calculated based on first order kinetics (T50 = ln2*T/k_1, k_1 = ln ($conc._{initial}$ / $conc._{final}$), T = 32 weeks (224 days).) (the equation is from the test report). For T50 (active – sterile) k_1 = k_{active} - $k_{sterile}$ (calculated).
[2]For the 0.5 ppm sterile samples no results were reported
* No change in concentration from T=0 to T=32 weeks, no kinetic analysis performed.

**removal rate faster in sterile than in active system

Table 20. DT50 and DT90 values and estimated half-lives obtained from remodelling by the dossier submitter using the data reported in Monsanto Company (1989). No temperature conversion applied. (n.r.=not reported; n.a.=not applicable

	DT50				DT90				estimated half-life (DT90/3.32)*	second-phase DT50 (LN(2)/k2)	
	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP
Results obtained from modelling (KinGUII v 2.1 software)											
Terphenyl, Soil A											
Soil A, 50 ppm	90.9	91.4	90.9	90.9	301.8	207.6	301.8	301.8	90.2	50.0	90.8
Soil A, 50 ppm, sterile	181.9	170.0	181.9	181.9	604.4	499.5	604.2	604.2	54.8	141.9	181.9
Soil A, 0.5 ppm	190,5	185.8	190.5	190.5	633.1	639.9	633.1	633.1	190.7	195.6	190.6
Soil A, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	181.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Terphenyl, Soil B											
Soil B, 50 ppm	67.1	67.5	65.6	67.5	233.9	224.4	287.3	224.4	70.5	64.5	5.77E+10
Soil B, 50 ppm, sterile	345.2	144.6	>7000	204.3	>7000	774.9	>7000	671.7	104.0	271.5	2.13E+14
Soil B, 0.5 ppm	>7000	>7000	>7000	>7000	>7000	>7000	>7000	>7000	301.2	n.d.*	n.d.*
Soil B, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	100.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quaterphenyl, Soil A											
Soil A, 50 ppm	113.5	115.2	106.6	115.7	693.4	350.3	>7000	384.4	208.4	101.2	2.08E+14
Soil A, 50 ppm, sterile	135.3	114.6	135.3	135.3	449.5	289.7	449.5	449.4	135.4	75.4	135.3
Soil A, 0.5 ppm	210.2	208.1	210.1	210.1	699.6	646.6	698.2		210.7	176.7	210.0
Soil A, 50 ppm, biodegradation (calculated)*	n.d.	n.d.	n.d.	799.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quaterphenyl, Soil B											

Soil B, 50 ppm	192.9	182.4	200.8	182.5	2186	584.9	>7000	606.2	658.4	173.4	3.48E+09
Soil B, 50 ppm, sterile	582.6	270.8	>7000	276.9	>7000	1083.6	>7000	919.8	175.5	349.9	1.43E+14
Soil B, 0.5 ppm	225.0	197.7	253.1	206.6	2435	607.9	>7000	686.4	733.5	176.7	1.88E+14
Soil b, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	535.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* no degradation detected; k2=0.000

Table 21. DT50 and DT90 values and estimated half-lives obtained from remodelling by the dossier submitter using the data reported in Monsanto Company (1989). Values converted to 12°C. (n.r.=not reported; n.a.=not applicable)

	DT50				DT90				estimated half-life (DT90/3.32)*	second phase DT50 (LN(2)/k2)	
	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP
Results obtained from modelling (KinGUII v 2.1 software)											
Terphenyl, Soil A											
Soil A, 50 ppm	302.5	304.4	302.5	302.5	1005.0	691.0	1004.7	1004.7	302.7	166.6	302.5
Soil A, 50 ppm, sterile	605.5	565.9	605.5	605.5	2012.3	1662.9	2011.6	2011.6	182.4	472.4	605.5
Soil A, 0.5 ppm	634.9	641.7	634.9	634.4	2107.8	2130.5	2107.5	2107.5	634.9	651.2	634.5
Soil A, 50 ppm, biodegradation (calculated)	n.d.	n.d	n.d	604.4	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Terphenyl, Soil B											
Soil B, 50 ppm	223.6	224.9	218.3	224.9	778.9	747.0	956.5	747.0	234.6	224.9	1.92E+11
Soil B, 50 ppm, sterile	1149	481.3	>7000	680.3	>7000	2580.0	>7000	2236.3	346.2	904.0	7.01E+14
Soil B, 0.5 ppm	>7000	>7000	>7000	>7000	>7000	>7000	>7000	>7000	>301	n.d.*	n.d.*
Soil B, 50 ppm, biodegradation (calculated)	n.d.	n.d	n.d	336.0	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Quaterphenyl, Soil A											
Soil A, 50 ppm	377.8	383.6	355.0	385.3	2308.5	1166.3	>7000	1279.8	695.3	337.0	6.91E+14
Soil A, 50 ppm, sterile	450.5	381.5	450.5	450.5	1496.7	964.4	1496.5	1496.3	450.8	251.0	450.5
Soil A, 0.5 ppm	699.9	692.9	699.7	699.7	2329.3	2152.8	2324.6	2324.6	701.6	588.3	699.3
Soil A, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	2661	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quaterphenyl, Soil B											
Soil B, 50 ppm	642.3	607.3	668.3	607.6	7278.6	1947.5	>7000	2018.4	2192.4	577.2	1.16E+10
Soil B, 50 ppm, sterile	1939.8	901.7	>7000	922.0	>7000	3607.9	>7000	3062.5	584.3	1165	4.77E+14
Soil B, 0.5 ppm	749.1	658.2	842.8	688.0	8108.4	2023.9	>7000	2285.2	2442.3	588.3	6.25E+14
Soil b, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	1782	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* no degradation detected; k2=0.000

Table 22. Statistical parametres for the kinetic modelling of the results by Monsanto Company (1989) and remarks on model applicability. The statistical parametres include error percentage at which the chi^2-test is passed (Chi^{2}Err%) at a significance level of 5%, coefficient of determination (r^2) (measured vs. predicted data), and probabilities (p-values) for t-tests for the different parametres. (n.d.=not determined). The p-value is considered significantly different from zero if the probability is smaller than 0.05.

	Chi^{2}Err%; r^2; (p-values)				Remarks by evaluating MSCA			
	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP	SFO
Terphenyl, Soil A								
Soil A, 50 ppm	9.496; 0.9139; (0.9811; (alpha 0.0002, beta <2e-16)	6.05; 0.9669; (k1 0.0668, k2 0.0009; tb 0.0007)	9.97; 0.9139; (k1 0.0008; k2 0.0001; g <2e-16)	9.091; 0.9139; (k 6.93e-05)	overestimation of concentration at last point	best fit (based on visual assessment, and statistics; however, high p for k1))	overestimation of concentration at last point	overestimation of concentration at last point
Soil A, 50 ppm, sterile control	8.699; 0.7926; (alpha 0.001, beta <2e-16)	7.355; 0.8655; (k1 0.50, k2 0.0035, tb 0.121)	9.133; 0.7926; (k1 0.0003, k2 0.0046, <2e-16)	8.328; 0.7926; (k 0.000577)	overestimation of concentration at last point	lowest Chi^{2}Err% and highest r^2; however, high p-values for k1 and tb and	overestimation of concentration at last point	best fit (however, overestimation of concentration at last point)
Soil A, 0.5 ppm	17.49; 0.4934; (alpha <2e-16, beta <2e-16)	18.22; 0.502; (k1 0.464, k2 0.058, tb 0.474)	18.37; 0.4934; (k1 0.013, k2 0.067, g <2e-16)	16.75; 0.4934; (k 0.025)	not acceptable (overestimation of concentration at last point, high Chi^{2}Err%, low r^2)	not acceptable (overestimation of concentration at last point, high Chi^{2}Err%, low r^2)	not acceptable (overestimation of concentration at last point, high Chi^{2}Err%, low r^2)	best fit (based on lowest Chi^{2}Err%) however, not acceptable due to overestimation of concentration at last point, low r^2
Soil A, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	biodegradation half-life calculated using k values (SFO model) from the active and sterile tests at 50 ppm
Terphenyl, Soil B								
Soil B, 50 ppm	11.92; 0.8891; (alpha 0.463, beta	12.52; 0.8891; (k1 <2e-16, k2	12.41; 0.8907; (k1 0.341, k2	11.42; 0.8891; (k 0.0001)	high p-values, underestimation of	high p-value for tb, underestimati	best fit (based on visual assessment);	best fit (based on statistics); however, underestimation of

	0.464)	0.001, tb 0.500)	0.500, g 0.355)		concentration at last point	on of concentration at last point	however, high p-values for k1, K2, and g and underestimation of concentration at last point)	concentration at last point
Soil B, 50 ppm, sterile control	15.24; 0.5305; (alpha 0.082; beta 0.390)	14.91; 0.5921; (k1 0.0005; k2 0.060; tb 0.009)	16.65; 0.4914; (k1 0.376; 0.500; 0.344)	16.35; 0.4113; (k 0.0275)	not acceptable (high p-values, high $Chi^2Err\%$, low r; overestimation of concentration at last point	not acceptable (high $Chi^2Err\%$; low r^2; underestimation of concentration at last point)	not acceptable (predicts the last point well but does not capture initial concentration; high $Chi^2Err\%$; low r^2)	not acceptable (underestimation of concentration at last point; does not capture initial concentration; high $Chi^2Err\%$; low r^2)
Soil B, 0.5 ppm	19.1; NA; (alpha 0.500; beta <2e-16)	20.04; NA; k1 0.500, k2 0.500, tb <2e-16)	20.02; NA; (k1 0.5, k2 0.5*, g <2e-16)	18.25; 0.001; (k 0.5)	not acceptable based on statistics and visual assessment	not acceptable based on statistics and visual assessment	not acceptable based on statistics and visual assessment	not acceptable based on statistics and visual assessment
Soil B, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	biodegradation half-life calculated using k values (SFO model) from the active and sterile tests at 50 ppm
Quaterphenyl, Soil A								
Soil A, 50 ppm	14.58; 0.7035; (alpha 0.349, beta 0.379)	15.07; 0.7187; (k1 0.500, k2 0.017, tb 0.387)	14.92; 0.7181; (k1 0.413, k2 0.500, g 0.422)	14.31; 0.6912; (k 0.003)	not acceptable (p-value, underestimation of concentration at last point)	not acceptable (statistical parametres, underestimation of concentration at last point	best fit (based on visual assessment) but not acceptable (weak statistical parametres; underestimation of concentration at last point)	not acceptable (underestimation of concentration at last point)
Soil A, 50 ppm, sterile control	15.86; 0.6404; (alpha 0.010, beta <2e-16)	14.11; 0.7522; (k1 0.500, k2 0.022, tb 0.093)	16.65; 0.6404; (k1 0.030, k2 0.004, g <2e-16)	15.19; 0.6404; (k 0.007)	not acceptable (underestimation of concentration at last point; high $Chi^2Err\%$)	not acceptable (underestimation of concentration at last point; high $Chi^2Err\%$;	not acceptable (underestimation of concentration at last point; high $Chi^2Err\%$)	best fit (based on visual assessment and $Chi^2err\%$) but not acceptable (high $Chi^2Err\%$, underestimation of concentration at last point)

						high p-values)		
Soil A, 0.5 ppm	4.953; 0.8988; (alpha 5.36e-05, beta <2e-16)	4.801; 0.0.9139; (k1 0.500, k2 0.0002, tb 0.228)	5.2; 0.8989; (k1 0.0003, k2 5.67e-05, g <2e-16)	4.742; 0.8989; (k 1.90e-05)	acceptable but $Chi^2Err\%$ slightly higher than for SFO	acceptable based on statistical parametres but underestimation of concentration at last point	acceptable but $Chi^2Err\%$ slightly higher than for SFO	best fit (based on statistical parametres and visual assessment)
Soil A, 50 ppm, biodegradation (calculated)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	biodegradation half-life calculated using k values (SFO model) from the active and sterile tests at 50 ppm
Quaterphenyl, Soil B								
Soil B, 50 ppm	12.9; 0.611; (alpha 0.339, beta 0.381)	13.78; 0.5993; (k1 0.500, k2 0.024, tb 0.440)	13.33; 0.6234; (k1 0.443, k2 0.500, g 0.452)	12.69; 0.5903; (k 0.0068)	not acceptable (high p-values, low r^2, underestimation of concentration at last point, one outlier)	not acceptable (high p-values, low r^2, underestimation of concentration at last point, one outlier)	best fit (based on visual assessment) but not acceptable (low r^2, high p-values, one outlier)	not acceptable (low r^2, underestimation of concentration at last point, one outlier)
Soil B, 50 ppm, sterile control	15.51; 0.3878; (alpha 0.249, beta 0.390)	16.91; 0.3407; (k1 0.301, k2 0.140, tb 0.276)	15.9; n.r.; (k1 0.397, k2 0.500)	15.91; 0.2989; (k 0.0503)	not acceptable (poor visual fit and weak statistical parametres, one outlier)	not acceptable (poor visual fit and weak statistical parametres, one outlier)	not acceptable (poor visual fit and weak statistical parametres, one outlier)	not acceptable (poor visual fit and weak statistical parametres, one outlier)
Soil B, 0.5 ppm	12.62; 0.5743; (alpha 0.364, beta 0.397)	13.1; 0.5861; (k1 0.500, k2 0.033; tb 0.378)	13.08; 0.5852; (k1 0.458, k2 0.500; g 0.466)	12.32; 0.5578; (k 0.008)	not acceptable (high p-values, low r^2)	not acceptable (high p-values, low r^2)	not acceptable (high p-values, low r^2)	not acceptable (high p-value, low r^2)
Soil b, 50 ppm, biodegradation	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	biodegradation half-life calculated using k values (SFO model) from the active and

(calculated)								sterile tests at 50 ppm

* Program output includes a note “*Hessian not invertible – NA was calculated for standard deviation, confidence interval and t-test.*”

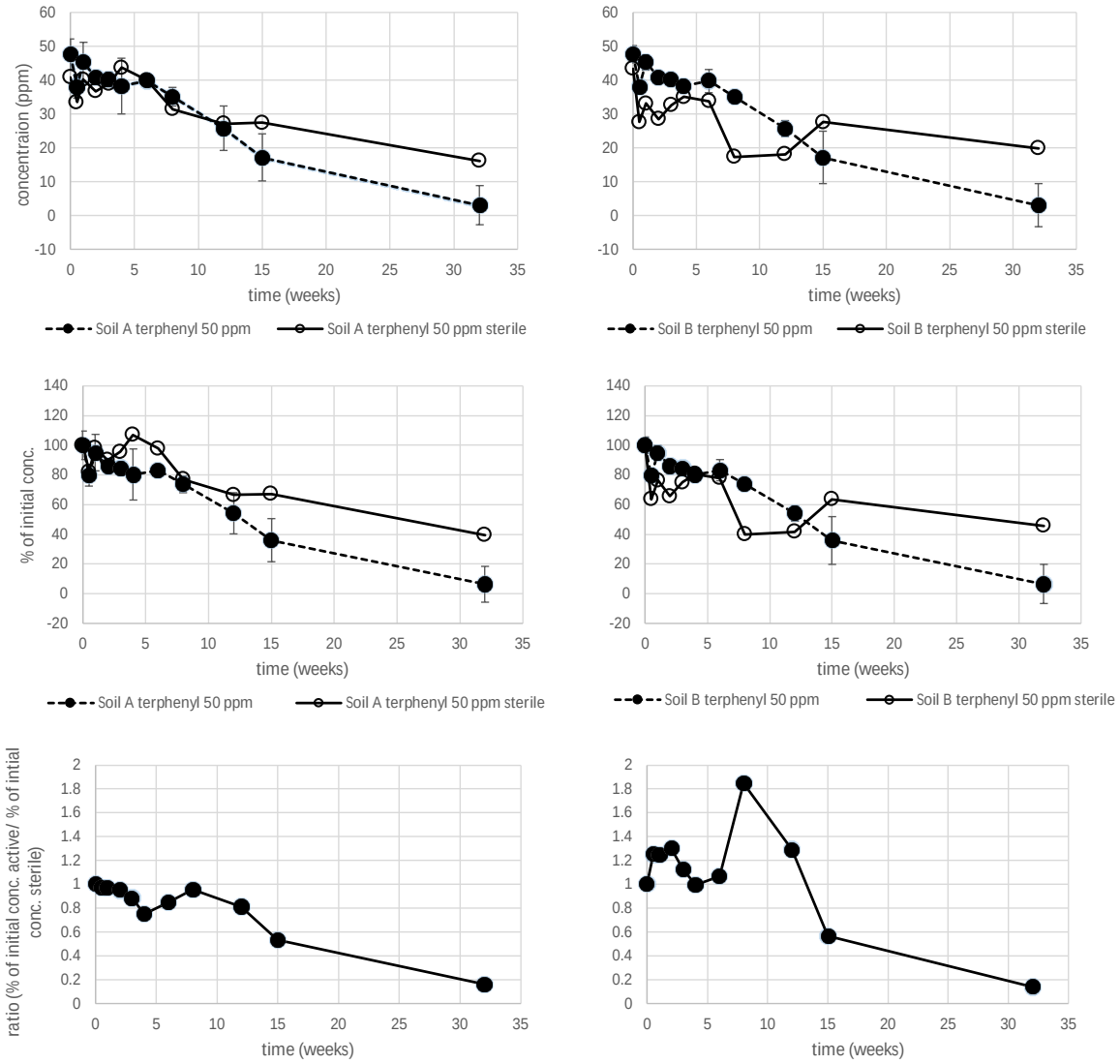


Figure 1. Concentrations of terphenyl in the active test and sterile control (with initial concentration ca. 50 ppm) in ppm (top) and relative to initial concentrations (middle), and the ratio of concentrations relative to initial in the active test and sterile control (low) (data from Monsanto Company 1989). The data for the active samples are means (n=3). Error bars in the upmost graphs represent ± 1 standard deviation. Error bars in the middle graphs represent scaled standard deviations: ± 1 *[(standard deviation/mean)*(% of initial conc.)]*. For data points without error bars, the std.dev. was reported as 0.0 ppm. The data for the sterile samples represent single samples as the sterile samples were not replicated.

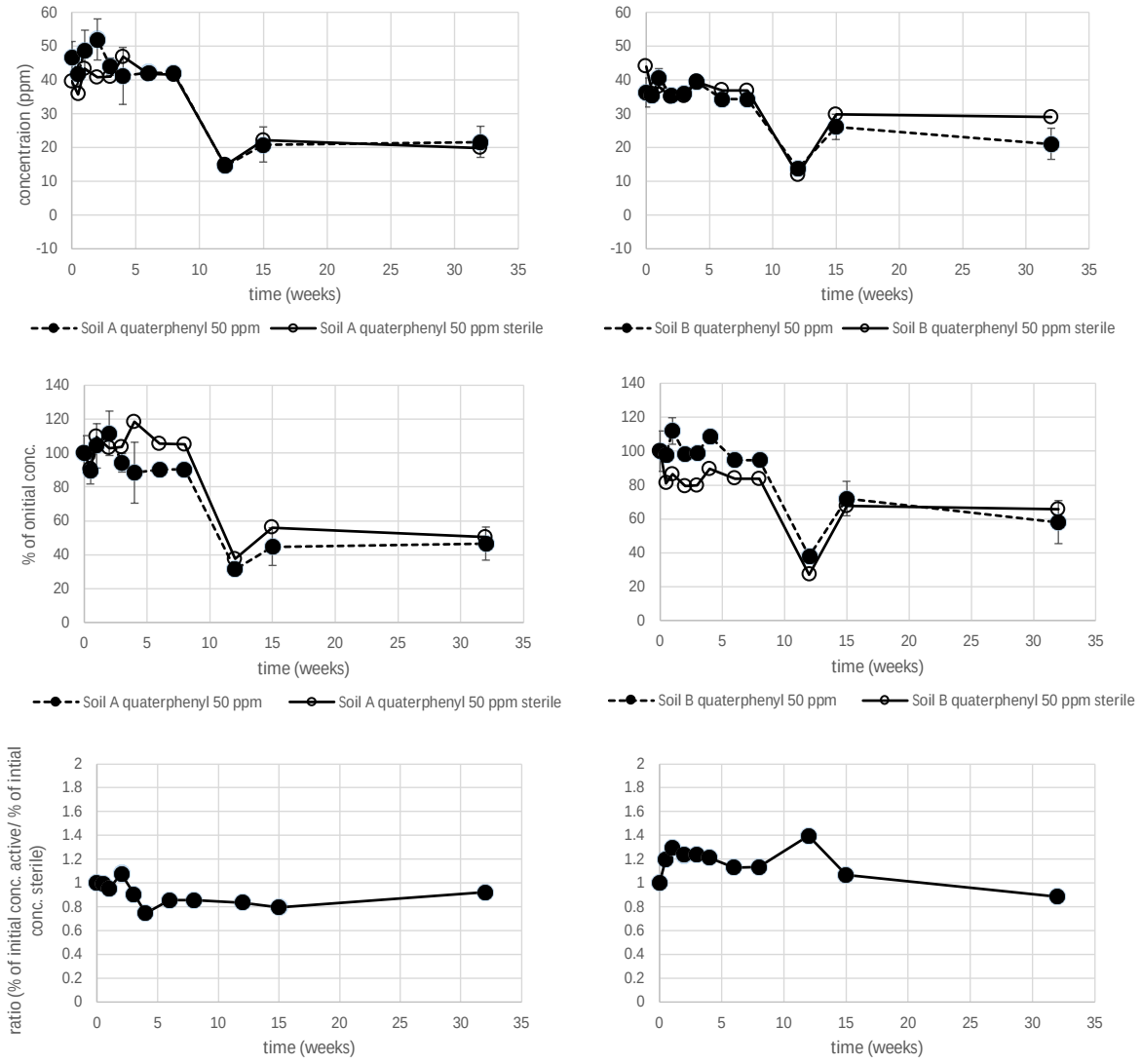


Figure 2. Concentrations of quaterphenyl in the active test and sterile control (with initial concentration ca. 50 ppm) in ppm (top) and relative to initial concentrations (middle), and the ratio of concentrations relative to initial in the active test and sterile control (low) (data from Monsanto Company 1989). The data for the active samples are means (n=3). Error bars in the upmost graphs represent ± 1 standard deviation. Error bars in the middle graphs represent scaled standard deviations: ± 1 *[(standard deviation/mean)*(% of initial conc.)]*. For data points without error bars, the std.dev. was reported as 0.0 ppm. The data for the sterile samples represent single samples as the sterile samples were not replicated.

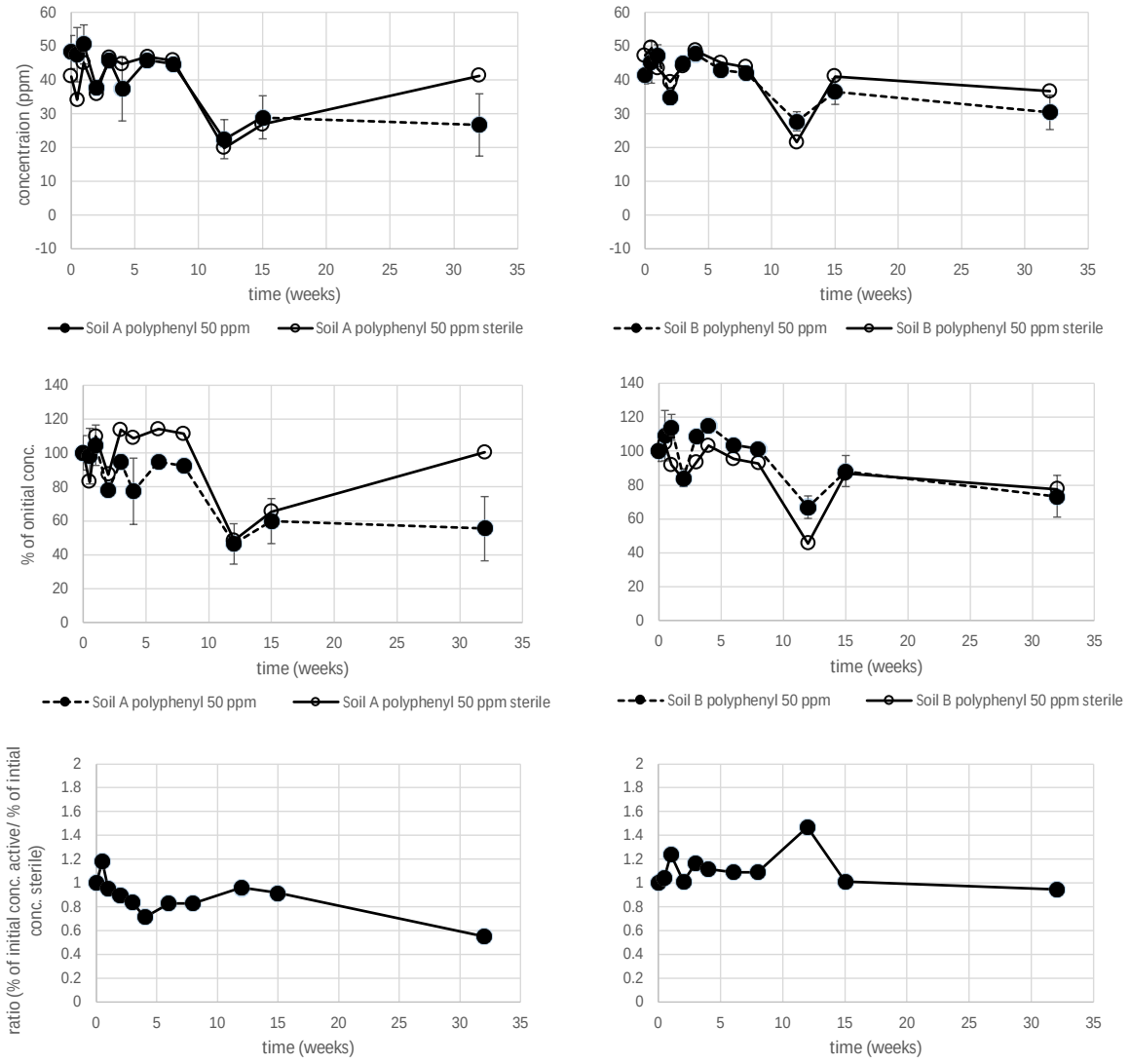


Figure 3. Concentrations of polyphenyl in the active test and sterile control (with initial concentration ca. 50 ppm) in ppm (top) and relative to initial concentrations (middle), and the ratio of concentrations relative to initial in the active test and sterile control (low) (data from Monsanto Company 1989). The data for the active samples are means (n=3). Error bars in the upmost graphs represent ± 1 standard deviation. Error bars in the middle graphs represent scaled standard deviations: ± 1 *[(standard deviation/mean)*(% of initial conc.)]*. For data points without error bars, the std.dev. was reported as 0.0 ppm.The data for the sterile samples represent single samples as the sterile samples were not replicated.

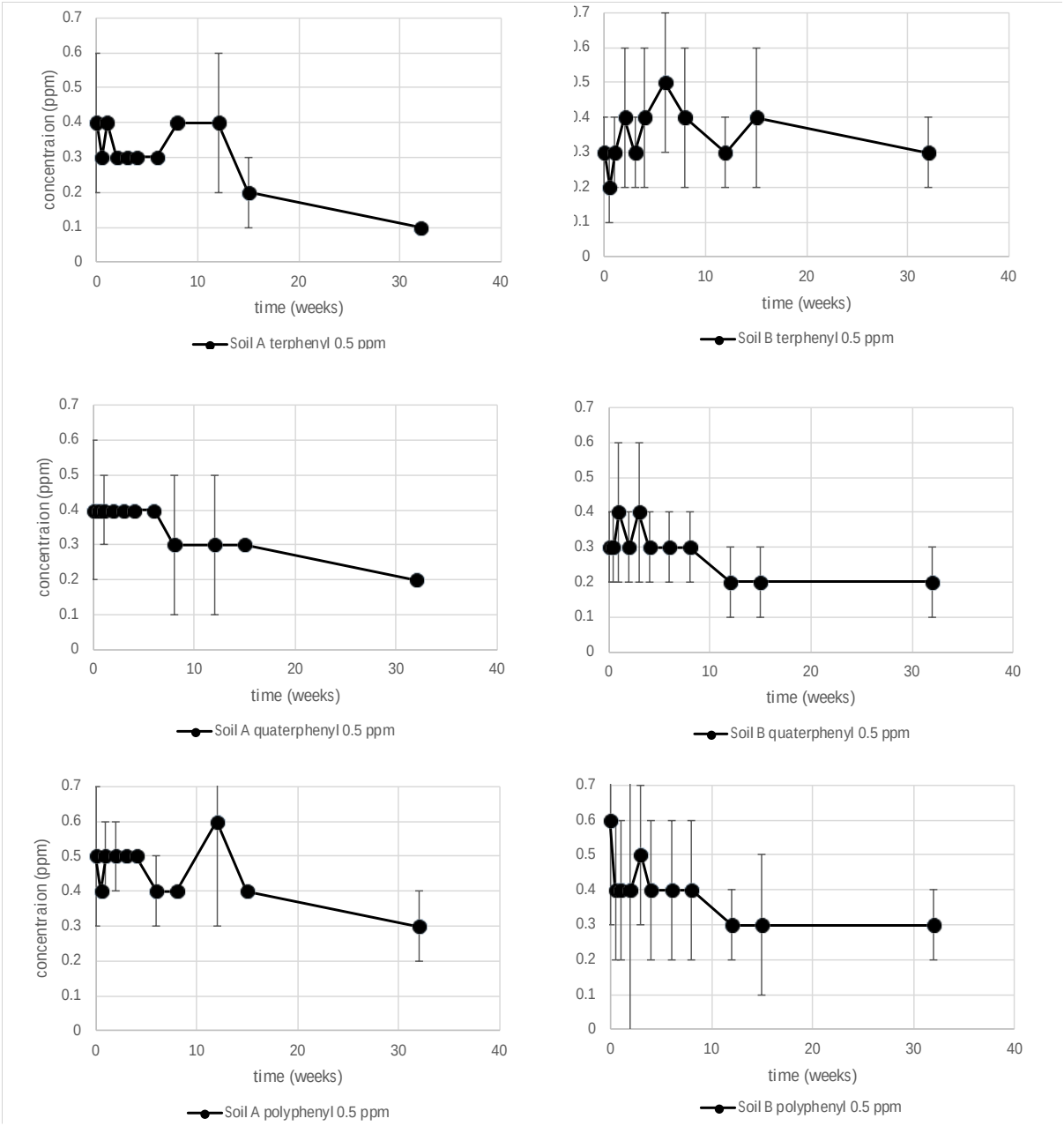


Figure 4. Concentrations of terphenyl (top), quaterphenyl (middle), and polyphenyl (low) in the tests with initial concentration ca. 0.5 ppm (data from Monsanto Company 1989). The data are means (n=3). Error bars represent ± 1 standard deviation. For data points without error bars, the std.dev. was reported as 0.0 ppm.

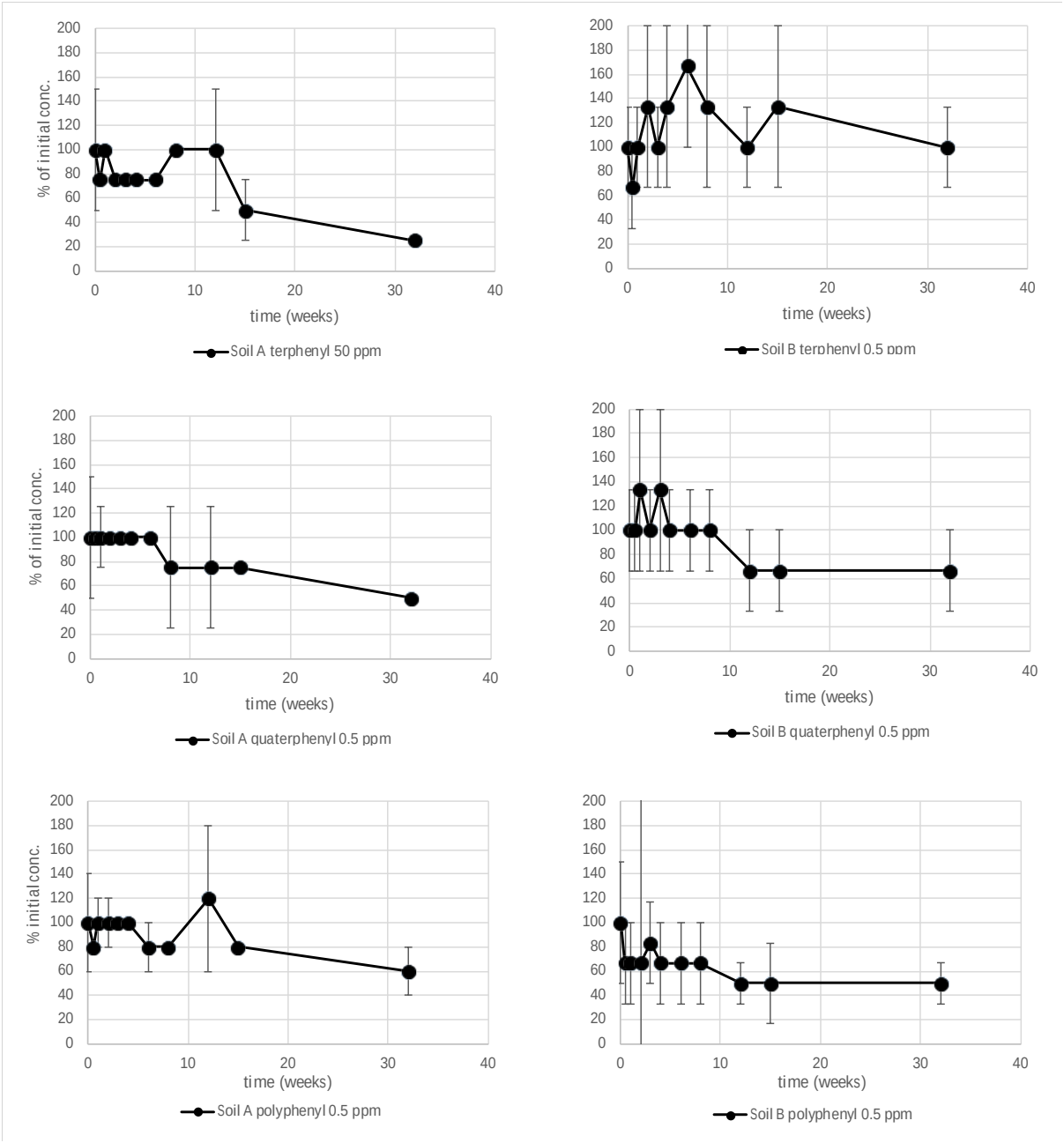


Figure 5. Concentrations of terphenyl (top), quaterphenyl (middle), and polyphenyl (low) in the tests with initial concentration ca. 0.5 ppm relative to initial concentrations (data from Monsanto Company 1989). The data are means (n=3). Error bars represent scaled standard deviations: ± 1 *[(standard deviation/mean)*(% of initial conc.)]*. For data points without error bars, the std.dev. was reported as 0.0 ppm.

NOTOX 2009a. Determination of the aerobic degradation rate and route of (Phenyl-14C(U)) -P-dicyclohexylbenzene in soil.

The degradation of terphenyl, hydrogenated was tested using [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene (belongs to group HT2) as the test substance (Figure 6).

Figure 6. Location of radiolabel of test substance used in the OECD 307 study (NOTOX 2009a).

The test was performed according to OECD Guideline 307 Aerobic and anaerobic transformation in soil (adopted 24 April 2002) and was conducted in compliance with GLP except for missing information on the stability of the labelled test substance under storage conditions and biomass determination. The dissipation and degradation of ^{14}C-labelled 1,4-dicyclohexylbenzene was investigated in three soils Speyer 2.2 (loamy sand), Speyer 2.3 (sandy loam) and Speyer 6S (clay) at 20 ± 2°C in the dark at a moisture content of approximately 40 % of the water holding capacity for a period of 120 days. The substance was applied at a concentration of 0.40 mg/kg dry soil. The soil microbial biomass was determined for all three soils before and after the incubation period and was always > 1 % of the organic carbon content as recommended in the test guideline.

Activity was fractioned into $^{14}CO_2$, organic volatile compounds, extractable residues and non-extractable residues (bound residue).

The concentration of the substance and its metabolites was determined after various incubation periods by HPLC of the soil extracts.

Extraction procedures

Each soil sample was transferred to a 250 ml centrifuge beaker. Extraction was performed with 100 ml methanol on a shaker (200 rpm) for 15 minutes. After centrifugation (765 g, 5 min at 20°C), the supernatant was collected. This was repeated twice. The combined supernatants were weighed and radioactivity was determined by LSC of a weighed 0.2 mL aliquot. Subsequently the methanol was evaporated on a rotary evaporator at 30°C and 10 ml methanol was added. The extract was stored in a vial. The recipient was rinsed once with 5 mL methanol. The rinsate was combined with the extract. The extract was weighed and the radioactivity of a weighed 0.2 ml aliquot was determined. Mean recovery of the concentration step was 89 % (relative standard deviation (RDS) 19%; n-27).

Additional extractions were performed for Speyer 2.3. For Speyer 2.3 soil samples containing bound residues above 10 % of the applied activity, approx. 50g of soil was extracted (plate shaker 200 rpm, 5min) with 50 mL acetonitrile containing 0.1% of concentrated HCl. The soil and extraction liquid was separated by centrifugation (1800 rpm, 5 min at 20°C). The extraction liquid was concentrated with a rotary evaporator till approx. 10 ml. The activity in the sample was determined in a 0.2 ml subsample by LSC. The extracted soil was further extracted (plate shaker 200 rpm, 5 min) with 50 ml n-hexane. The soil and extraction liquid was separated by centrifugation (1800 rpm, 5 min at 20°C). The extraction liquid was concentrated with a rotary evaporator till approx. 10 ml. The activity in the sample was determined in a 0.2 ml subsample by LSC. Finally, the extracted soil was subjected to Soxhlet extraction for 3 hours. As extraction liquid, 200 ml methanol was used. The radioactivity in the methanol was measured in 0.2 ml by LSC. If the extract contained above 5 % of the applied radioactivity the samples were

concentrated till 10 ml and a weighted subsample was analysed on LSC and a sample was subjected to HPLC.

Significant reduction of the non-extractable residue was not achieved with these additional extractions (Table 23). However, it is noted that in the single sample for which the composition of the additional extract was analysed by HPLC, the sample consisted of parent compound. The fact that this sample was at the beginning of the study indicates that a part of the parent substance quickly became extractable only by the additional extraction procedure and not by the standard procedure used for the rest of the samples.

Table 23. Additional extractions of Speyer 2.3 soil (NOTOX 2009a).

Time (d)	% of applied radioactivity		
	acetonitrile + 0.1 % HCl	hexane	Soxhlet (with methanol)
3	8.6*	3.6	2.9
7	1.5	0.4	1.3
14	3.7	1.0	1.0
28	1.4	4.1	1.1
63	1.9	0.2	2.5
120	0.7	0.1	0.8

*analysed on HPLC to consist of parent compound. Original and additional extract were combined and used for the DT50 calculations.

DT_{50} and DT_{90} calculation approach used in the test report (NOTOX 2009a)

In the test report the DT_{50} and DT_{90} values were calculated using the amounts of [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene (relative to nominal applied) as determined by HPLC. The choice of models to which the data were fitted is based on the FOCUS guidance document on estimating persistence and degradation kinetics (European Commission 2006). The *chi*-square test was based on the average results for each time point. Optimisations were performed using the program ModelMaker (AP Benson, Wallingford, Oxfordshire, UK). To obtain endpoints for assessment of persistence, the parent data (not averaged) were fitted to single first order kinetics (SFO) and to the Gustafson and Holden model (FOMC). If the SFO fit was acceptable and better than the FOMC fit (based on visual assessment, r^2 and *chi*-square test; *chi*-square error should not exceed 15%), no further work was done. If the SFO fit was not acceptable or the FOMC fit was better, exclusion of outliers, constraining M0 or weighting was tried provided this could be justified by the data. If the SFO results were not improved by these adjustments, the data were also fitted to the hockey stick model (HS) and the bi-exponential model (DFOP). Degradation of the metabolites were only fitted to SFO kinetics.

DT_{50} and DT_{90} calculation approach used by the dossier submitter

The dossier submitter used KinGUII v 2.1 (Bayer CropScience 2014) program to reproduce and evaluate the modelling for the parent substance presented in the test report as well as to conduct remodelling of data as described below. The DT50 and DT90 calculation approach was the same as that described in the case of the other soil simulation study described in this report (Monsanto Company (1989)). However, additional calculations were performed to consider non-extractable residues (NER) in the assessment as described below. It is noted that the term ‘bound residues’ is used in this report (in accordance with the test report), with the same meaning as ‘NER’. Default kinetic variables of the program were used. The DT50 and DT90 values obtained from remodelling

match relatively well with those reported (
Table 25), indicating that the results reported by NOTOX (2009a) were relatively well reproducible by the KinGUI 2.1 software.

In the case of DFOP and HS models the dossier submitter calculated also the half-lives for the second phase ('slow phase) phase of the dissipation (the test report only include the overall half-lives determined for the whole test duration).

Regarding the comparison of half-lives to P/vP criteria it is noted that the current PBT guidance (ECHA 2017a) has the following paragraph:

"It should be noted that for direct comparison to the P/vP criteria only estimates of degradation half-life are appropriate. When the kinetics of transformation are first-order, single-first order (SFO) kinetic models can be used for predicting degradation half-lives. The predicted degradation half-lives should be used for comparison with the P/vP criteria. Use of bi-phasic kinetic models is recommended to be limited to cases where clear deviations from first-order kinetics occur. When the kinetics of transformation are bi-phasic, the best-fit model (FOMC, DFOP, HS) should be selected and used for predicting a DT_{50}. The DT_{50} predicted from the best-fit bi-phasic model should be used for comparison with the P/vP criteria. When applicable (DFOP or HS), the DT_{50} predicted from the slow phase should be preferred and used for comparison with the P/vP criteria. In case other DT_{50} are used, a justification should be provided with adequate and reliable documentation of the applied method."

Because bound residues were detected but their chemical composition was not characterised, it was necessary to calculate a worst-case half-life on the assumption that bound residues represent the parent compound. For this purpose, the data was remodeled using a sum parameter of parent substance and bound residues.

In addition, remodeling of the parent substance results was done to investigate the effect of the last data point on the results. This was done because an increase in parent substance concentration, or reduced rate of decline, was observed at the last sampling point, together with a decrease in level or rate of production of CO_2, suggesting a possible decline in microbial activity. Therefore, the data were modelled by the evaluating MSCA also by removing the last sampling point, in accordance with guidance (EFSA 2014, p. 114).

Another difference compared to the the assessment regarding the Monsanto Company (1989) study is that the approaches for endpoint derivation in accordance with the FOCUS guidance (EFSA 2014) page 111 (i.e., 'additional work approach' and 'modelling endpoints approach') were not explicitly followed in the assessment of the NOTOX (2009a) study. The reason is that this was considered not relevant at this stage as no definitive conclusion from this study can be drawn in terms of P/vP due to the insufficient information on the composition of NER as described below.

Temperature correction of half-lives

For comparing with the P/vP criteria the results were converted to 12°C in according to EFSA (2007) (page 7-32 (Eqn 3)):

$$DT50_{(12oC)} = DT50_{(20oC)} \exp(((65.4/0.008314*((1/285,15)-(1/293,15))))$$

The values used are:

activation energy, E_a: 65.4 kJ/mol
gas constant, R,: 0.008314 (kJ K^{-1} mol^{-1})

temperature 1: 285.15 K
temperature 2; 293.15 K

DT50 results for the parent compound

The DT50 values and the modelling presented in the test report and calculated are presented in Table 24,
Table 25, Table 26, and Table 27. Distribution of recovered radioactivity is shown in Table 28, Table 29, Table 30, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12, and Figure 13. HPLC results are shown in Table 31, Table 32, and in Table 33.

[Phenyl-14C(U)]-p-dicyclohexylbenzene quickly dissipated in the tested soils, primary biodegradation was observed as well as mineralisation.

According to the test report the SFO model overestimated the decrease of [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene in all three soils at the end of the incubation period. The DT90 of [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene was estimated by the SFO model to be 14.7 days on average. The dossier submitter agrees that the SFO model is not appropriate in this case based on visual assessment and *chi*-square test (Table 22). The SFO results are presented in the Tables but are not discussed any further.

According to the test report [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene dissipation was best described by three different models for the three different soils (DFOP for Speyer 2.2, HS for Speyer 2.3 and FOMC for Speyer 6S). The best fit for the data are shown in (Table 22). The dossier submitter calculated the overall DT50s for the different biphasic models and these range from 2-10 days (at 12°C) for the three soil samples.

The concentration of the parent substance decreased rapidly during approximately the first 20 days and concomitantly there was a rapid increase in non-extractable residues (Figure 7). This suggests that the initial phase was largely influenced by physically or chemically mediated dissipation. Even though the rate of CO_2 production was also relatively fast in the beginning of the study the percentage of applied radioactivity released as CO_2 was clearly lower than that observed in non-extractable residues at least for the first 14 days. Therefore the slow phase of the dissipation curve was considered in the assessment, in addition to the overall dissipation during the test.The estimated half-lives (DT90/3.32) for the FOMC model were 17-21 d at 12°C. The second-phase DT50s for the HS and DFOP models were 38-46 d for the Speyer 6S soil, 185 (HS) for the Speyer 2.2 soil. However, for the Speyer 6S soil, both HS and DFOP models underestimate the concentration at the last point and for the Speyer 2.2 soil HS model, the p-value for k_2 was 0.091; in addition, the half-life for the Speyer 6S soil (when converted to 12°C) is longer than the experimental period. Therefore, the half-lives for the Speyer 6S soil and Speyer 2.2 soils should be used with additional caution. For Speyer 2.2 the k_2 parameter for the DFOP model was high and consequently no reliable half-lifes could derived for the second phase. For the Speyer 2.3 soil, the p-values for the k_2 parameter were high and consequently no reliable half-lives could be derived for the second phase.

Because an increase in parent substance concentration and reduced rate of CO_2 production were observed from day 60 to day 120 the data were modelled also by removing the last sampling point. Excluding the last point from modelling, the overall half-lives for the biphasic models were between 4-18 days (12°C) for the different soil samples and including all the four models. When removing the last sampling point the estimated half-lives obtained (FOMC) (16-18 d at 12°C) were similar for all soil samples. For the Speyer 2.2 and Speyer 6S soils the second-phase DT50s for the HS and DFOP models were relatively similar (58-81 d and 35-38 d for Speyer 2.2. and Speyer 6S, respectively) (however, it should be noted that for Speyer 2.2 DFOP model, the p-value for k_2 was 0.098 and therefore this half-life (81 days) should be used with additional caution). For the HS and DFOP models for the Speyer 2.3. soil when the last point was excluded, the second-phase DT50 values -values for the k_2 parameter were high and consequently no reliable

half-lives could be derived for the second phase.

There are no obvious reasons for preferring the result obtained by removing the last data point. It should also be noted that the mass balance differed between the second last and last sampling points which can complicate the interpretation of the significance of the last data point on the results. Therefore, the conclusion is based on the modelling with all data points.

In conclusion, second-phase half-lives were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k2 parametre was not statistically significant) whereas for one of the soils, no reliable second-phase half-lives could be determined, indicating that the possible dissipation and degradation during that period of the study occurred at rates too low to be determined with the method used.

DT50 results with unextractable residues assumed as parent compound

It should be noted that the above-mentioned DT50 values are based on parent substance concentrations and can be influenced by other dissipation phenomena in addition to degradation. The percentage of unextractable residues is very high from 23.6 to 51.5 %, which makes it difficult to interpret the results. Therefore, the data was modelled using a sum parameter of parent substance and unextractable residues.

The sum of parent substance and bound residues generally decreased during the experiment. Exceptions to this trend were observed in Speyer 6S soil (the sum parameter remained constant from day 3 to day 7 and was increased at day 14) and Speyer 2.2 soil (increase from day 60 to day 120). The statistical parametres and the visual fits for the models were not acceptable for the Speyer 2.2 soil. SFO model was not applicable to any of the soils based on visual assessment and *chi*-square test. The overall half-lives obtained using the biphasic models (FOMC, HS, DFOP) were 18-33 d for Speyer 2.3 soil, and 0-6 d for Speyer 6S soil at 12°C. The DT90 values or the second-phase DT50s for the sum of parent substance and bound residues are not considered relevant for the assessment.

Removing the last data point resulted in overall half-lives 57-64 d for Speyer 2.2 soil, 18-32 d for Speyer 2.3 soil, and 0-6 days for Speyer 6S soil. The SFO model was not applicable based on visual assessment and *chi*-square test.

In conclusion, in one of the three soils bound residues as well as the sum of parent substance and bound residues clearly increased from 60 to 120 days. Removing the last data point (at 120 days) gave a model with improved statistical parametres; however, there is no obvious reason to treat the 120 day measurement as an outlier/measurement error and therefore it is concluded that the dissipation half-life for the sum parameter (parent + bound residues) obtained by removing the last point is not reliable for the Speyer 2.2 soil. Thus the half-life for the decrease of the sum parameter (parent substance+ bound residues) is concluded to be higher than the test duration (120 days) in one of the soils. Therefore, if assuming that bound residues represent unexctracted parent substance, the results for the sum parameter of parent substance and unextractable residues, the half-life of the parent substance would be above 120 days at 20°C.

Mineralisation and formation of metabolites

CO_2 production (32.4-51.0% during 120 days) indicates that a relatively high proportion of the loss of parent substance is due to biodegradation. However, it should be noted that CO_2 production would likely be significantly lower at 12°C.

The metabolite formation/dissipation was described by first order (SFO) kinetics in the test

report. The DT50 values of parent and metabolites are summarised in Table 24. The half-lives reported have not been evaluated.

In Speyer 6S soil three major metabolites (Met 2, Met 3 and Met 7) were formed that were above 10 % of the applied activity (Table 33). In Speyer 2.3 two metabolites (Met 3 and Met 7) were twice above 5 % of the applied radioactivity at two consecutive time points (Table 32). The major metabolites dissipated with reported DT50 values below 40 days (20°C). Other metabolites were only formed in minor amounts (maximum 2.9 % and once 4.6 %). No metabolites could be identified despite efforts with GC-MS analyses.

Assessment of reliability and relevance

In the test report one protocol deviation is listed. The purity of the test substance was not determined in the spike solution in the beginning of the test. The stock and spike solutions were checked during and at the end of the study and the purity was above 99%. Therefore, according to the test report there were no adverse effect on study integrity.

In the test report it is also mentioned that there were no deviations from standard operating conditions that affected the integrity of the study.

Regarding mass balance, according to OECD 307 test guideline recoveries should range from 90% to 110% for labelled chemicals and from 70% to 110% for non-labelled chemicals. In general, mass balances were within the range 90%-110%. Mass balances <90% were determined several times (at three, four, and zero measurement times of the total of seven measurement times for Speyer 2.2., Speyer 2.3., and Speyer 6S soils, respectively), but according to test report these deviations were small and since no trend of decreasing mass balances was observed, the results of the study were accepted.

It is considered that the mass balance deviations in Speyer 2.2. and Speyer 2.3. soils are considerable and need to be taken into account in reliability score.

It is considered that the study is reliable with restrictions (reliability score 2 in the Klimisch scale) due to the relatively low mass balances in Speyer 2.2. and Speyer 2.3. soils at several measurement times.

The modelling of the parent substance presented in the test report is considered generally acceptable. The remodeling gave overall half-lives which match relatively well with those in the test report. However, the parent substance data were interpreted differently for the purpose of P/vP assessment (e.g., the second-phase half-lives from the biphasic models were considered). In addition, remodeling was conducted due to the need for other calculation approaches that were not included in the test report (the sum of parent and unxtracted residues; exclusion of last data point) and to derive statistical parametres not included in the test report.

This study is considered relevant for the purpose of PBT assessment.

Summary

[Phenyl-^{14}C(U)]-p-dicyclohexylbenzene dissipated quickly in the tested soils. Except for formatiofn of the metabolites especially in Speyer 6S soil, other important reaction routes were formation of unextractable residues (max 23.6 - 51.5 % of applied) and mineralisation (32.4 - 51.0 % of applied) in all the tested soils. According to test report the overall DT50 values (for dissipation) for the parent were from 1.8 to 4.6 days (3.8 to 9.8 days when converted to 12°C) and for metabolites from 3 to 36.1 days (6.4 to 76.6 days at 12 °C). Remodelled overall DT50s for the parent were 2-10 days at 12°C.

According to the guidance (ECHA 2017a), "*when applicable (DFOP or HS), the DT_{50} predicted from the slow phase should be preferred and used for comparison with the P/vP criteria.*" Estimated DT50 values (DT90/3.32) (FOMC model) (17-21 d) and second phase

(´slow-phase´) DT50 values (DFOP and HS models) were also calculated. The second phase DT50 values were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k2 parameter was not statistically significant and as the value obtained from temperature conversion is longer than the experimental period) and for one of the soils, no reliable second-phase half-lives could be determined. It is noted that a significant amount of the applied radioactivity was found as non-extractable residues already at a relatively early phase of the test. The formation of NER has a strong influence on shape of the dissipation curve in this dataset. Therefore, the half-life derived from the second phase of the dissipation curve may overestimate the degradation half-life during the whole study period, also considering that there were relatively few measuring points available which affects the statistical parametres obtained particularly for the second phase of dissipation where the change in concentration is small.

The sum of bound residues and parent substance increased in one of the soils at the end of the test (mainly due to increase in bound residues) and therefore it cannot be concluded that the sum of parent substance and bound residues declined. Consequently, no exact half-life can be determined and it is estimated that the half-life for this soil is above test duration, i.e., >120 days (if assuming that bound residues represent unchanged parent substance). According to the ECHA guidance R.11., the non-extractable residues should be regarded, in the absence of systematic methodology, as non-degraded substance, unless, on a case-by-case basis, it can reasonably be justified or analytically demonstrated that a certain part of the residues can be considered to be irreversibly bound. In this case, there are direct measurements on the identity of NER only for one sample indicating that NER consisted of parent substance in that individual sample. Indirect information on degradability of NER can be derived from the present results by comparing changes in NER, extracted residues, and CO_2 during the study (data not shown). Based on this type of analysis it seems that at least in one of the soils, a part of the NER formed may have been mineralised during the study, thus indicating that a part of the NER formed may be degradable. This type of analysis has been done so far only for one of the three soils. Therefore, the dossier submitter considers that it is not possible to draw a definitive conclusion on the interpretation of the NER in terms of P/vP property. A full assessment of the NERs is not considered necessary for the present SVHC proposal as there is data for another constituent indicating vPvB properties and therefore a definitive conclusion on PBT/vPvB status of the HT2 constituents would not have a regulatory consequence in terms of SVHC identification of the registered substance.

In summary, the main observations for [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene are:

1. The constituent showed overall dissipation half-lives of 2-10 days.

2. The second-phase half-lives from bi-phasic models were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k_2 parameter was not statistically significant and as the value obtained from temperature conversion is longer than the experimental period) whereas for one of the soils, no reliable second-phase half-lives could be determined.

3. The constituent formed a significant amount of NER.

4. The second phase half-lives are likely to overestimate the degradation half-life during the study due to the strong influence of NER formation on the shape of the dissipation curve in the beginning of the test.

5. Assuming that all NER is parent substance, the half-life was 6-18 days in two soils and >120 days in one soil.

6. The dossier submitter considers that there is no sufficient information on the NERs in this case to decide on how they should be interpreted in terms of P/vP criteria.

7. It may be possible to derive more information on the NERs from existing data; however, for the purpose of the present SVHC proposal this is not considered necessary.

8. Considering the points above, [Phenyl-^{14}C(U)]-p-dicyclohexylbenzene is regarded as potentially P or vP. Further assessment is needed to define whether the constituent can be considered P or vP, or whether it can be relieved of P/vP concern.

Table 24. Dissipation rates (DT50 and DT90) for parent and metabolites as presented in the test report (NOTOX 2009a). No temperature conversion applied.

Soil		Parent		Met 2		Met 3		Met 7	
		DT_{50} [days]	DT_{90} [days]	DT_{50} [days]	DT_{90} [days]	DT_{50} [days]	DT_{90} [days]	DT_{50} [days]	DT_{90} [days]
Speyer 2.2	loamy sand	4.1	62.1	-	-	-	-	-	-
Speyer 2.3	sandy loam	4.6	60.9	-	-	16.0	53.0	18.3	60.6
Speyer 6S	clay	1.8	32.8	28.0	93.0	36.1	120.0	3.0	9.9

Table 25. DT50 and DT90 values and estimated half-lives reported by NOTOX (2009a) and obtained from remodelling. No temperature conversion applied. (n.r.=not reported; n.a.=not applicable)

	DT50				DT90				estimated half-life (DT90/3.32)	second-phase DT50 (LN(2)/k2)	
	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP
Results presented in test report (Modelmaker software)											
Parent, all data points											
Speyer 2.2 soil	n.r.	n.r.	4.1	4.7	n.r.	n.r.	62.1	15.7	n.a.	n.r.	231.0
Speyer 2.3 soil	n.r.	4.6	n.r.	5.2	n.r.	60.9	n.r.	17.2	n.a.	63.0	n.r.
Speyer 6S soil	1.8	n.r.	n.r.	3.4	32.8	n.r.	n.r.	11.3	9.9	n.r.	n.r.
Results obtained from remodelling (KinGUII v 2.1 software)											
Parent, all data points											
Speyer 2.2 soil	3.7	4.2	4.1	4.7	31.8	63.8	57.7	15.7	9.6	87.2	227.6
Speyer 2.3 soil	4.7	4.6	4.6	5.1	27.1	60.2	33.5	17.1	8.1	65.0	292.8
Speyer 6S soil	1.2	2.3	2.1	3.4	32.3	30.0	32.0	11.3	9.7	17.9	21.6
Parent, last data point excluded											
Speyer 2.2 soil	8.4	4.2	4.0	4.7	27.9	36.9	35.0	15.7	8.4	27.4	38.1
Speyer 2.3 soil	4.8	4.6	4.6	5.1	25.3	44.0	33.8	17.1	7.6	35.6	760.2
Speyer 6S soil	1.9	2.3	2.1	3.4	28.6	28.7	29.0	11.3	8.6	16.7	17.9
Parent+unextracted residues, all data points											
Speyer 2.2 soil	47.9	90.4	34.6	112.9	>1000	735.8	>1000	375.2	>301.2	277.9	2.97.E+13
Speyer 2.3 soil	15.5	8.3	11.1	40.3	>1000	318.1	431.0	133.9	>301.2	140.1	204.6
Speyer 6S soil	1.6	2.6	0.0	132.8	>1000	873.3	873.3	441.0	>301.2	381.1	381.1
Parent+unextracted residues, last data point excluded											
Speyer 2.2 soil	26.6	30.1	27.0	35.5	>1000	137.5	290	117.9	>301.2	46.3	118.2

Speyer 2.3 soil	14.8	8.3	11.3	28.0	>1000	206.8	338	93.06	>301.2	86.5	157.0
Speyer 6S soil	0.12	2.6	0.5	69.3	>1000	684.5	685	230.3	>301.2	297.2	297.2

Table 26. DT50 and DT90 values and estimated half-lives reported by NOTOX (2009a) and obtained from remodelling. Values converted to 12°C. (n.r.=not reported; n.a.=not applicable)

	DT50				DT90				estimated half-life (DT90/3.32)*	second-phase DT50 (LN(2)/k2)	
	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP
Results presented in test report (ModelMaker software)											
Parent, all data points											
Speyer 2.2 soil	n.r.	n.r.	8.7	10.0	n.r.	n.r.	131.8	33.3	n.a.	n.a.	490.5
Speyer 2.3 soil	n.r.	9.8	n.r.	11.0	n.r.	129.3	n.r.	36.5	n.a.	133.8	n.r.
Speyer 6S soil	3.8	n.r.	n.r.	7.2	69.6	n.r.	n.r.	24.0	21.0	n.r.	n.r.
Results obtained from remodelling (KinGUII v 2.1 software)											
Parent, all data points											
Speyer 2.2 soil	7.9	8.9	8.7	10.0	67.5	135.4	122.5	33.3	20.3	185.1	483.1
Speyer 2.3 soil	10.0	9.8	9.8	10.9	57.4	127.8	71.0	36.3	17.3	137.9	621.7
Speyer 6S soil	2.5	4.8	4.5	7.2	68.6	63.8	67.9	23.9	20.7	38.1	45.8
Parent, last data point excluded											
Speyer 2.2 soil	17.8	8.9	8.5	10.0	59.2	78.3	74.3	33.3	17.8	58.2	81.0
Speyer 2.3 soil	10.1	9.8	9.9	10.9	53.8	93.5	71.7	36.3	16.2	75.5	1613.9
Speyer 6S soil	4.1	4.8	4.4	7.2	60.7	60.8	61.5	23.9	18.3	35.4	38.0
Parent+unextracted residues, all data points											
Speyer 2.2 soil	101.6	191.8	73.4	239.7	>2123	1562.1	>2123	796.5	>639.5	590.0	6.3E+13
Speyer 2.3 soil	32.9	17.6	23.7	85.6	>2123	675.3	915.0	284.3	>639.5	297.3	434.3
Speyer 6S soil	3.3	5.5	0.0	281.9	>2123	1854.0	1854.0	936.2	>639.5	809.0	809.0
Parent+unextracted residues, last data point excluded											
Speyer 2.2 soil	56.5	63.8	57.3	75.3	>2123	291.9	614.8	250.3	>639.5	98.2	250.9
Speyer 2.3 soil	31.5	17.6	23.9	59.5	>2123	439.0	718.4	197.6	>639.5	183.7	333.3
Speyer 6S soil	0.25	5.5	1.1	147.2	>2123	1453.2	1453.2	488.9	>639.5	631.0	631.0

Table 27. Statistical parametres for the kinetic modelling of the results by NOTOX (2009a) and remarks on model applicability. The statistical parametres include error percentage at which the chi^2-test is passed (Chi^{2}Err%) at a significance level of 5%, coefficient of determination (r^2) (measured vs. predicted data), and probabilities (p-values) for t-tests for the different parametres. (n.d.=not determined). The p-value is considered significantly different from zero if the probability is smaller than 0.05.

	Chi^{2}Err%; r^2; (p-values)				Remarks by evaluating MSCA			
	FOMC	HS	DFOP	SFO	FOMC	HS	DFOP	SFO
Results presented in test report (Modelmaker software)								
Parent, all data points								
Speyer 2.2 soil	not reported in test report	not reported in test report	7.3; 0.992; (n.r.)	15.4;0.940; (n.r.)	not reported in test report	not reported in test report	best fit according to test report	not acceptable (Chi^{2}Err% >15; visual observation indicates that values at the end of the curve are overestimated)
Speyer 2.3 soil	not reported in test report	19.0; 0.95;(n.r.)	not reported in test report	20.1; 0.906; (n.r.)	not reported in test report	best fit according to test report	not reported in test report	not acceptable (Chi^{2}Err% >15; visual observation indicates that values at the end of the curve are overestimated)
Speyer 6S soil	6.1; 0.989; (n.r.)	not reported in test report	not reported in test report	23.4; 0.908;(n.r.)	best fit according to test report	not reported in test report	not reported in test report	not acceptable (Chi^{2}Err% >15; visual observation indicates that values at the end of the curve are overestimated)
Results obtained from remodelling (KinGUII v 2.1 software)								
Parent, all data								

points								
Speyer 2.2 soil	10.92; 0.9811; (alpha 0.008, beta 0.052)	7.976; 0.9916; (k1 1.75e-05; k2 0.091; tb 0.0002)	7.319; 0.9921; (k1 0.0001; k2 0.28; g 9.10e-07)	15.39; 0.9746; (k 0.0002)	not best fit (poor visual fit at last point; p-value for beta exceeds 0.05)	not acceptable due to high p-value for k2	best fit (good visual fit despite the high p-value for k2)	not acceptable (Chi^2Err% >15; poor visual fit)
Speyer 2.3 soil	21.06; 0.9248; (alpha 0.153, beta 0.232)	18.97; 0.9508; (k1 0.001; k2 0.2445; tb 0.011)	19.66; 0.9445; (k1 0.010; k2 0.440; g 0.0002)	20.13; 0.9319; (k 0.0008)	not best fit (high Chi^2Err% and t values for k1 and k2, overestimation of degradation towards end of test	not best fit (Chi^2Err% and t values for k1 and k2 comparable to DFOP but visual fit slightly better for HS)	best fit (acceptable based on visual fit; despite high Chi^2Err% and p-t values)	not acceptable (Chi^2Err% >15; poor visual fit)
Speyer 6S soil	6.162; 0.9893; (alpha 0.0008; beta 0.039)	10.79; 0.9847; (k1 0.0005; k2 0.024; tb 0.002)	10.51; 0.9843; (k1 0.0152; 0.049; g 0.0002)	23.39; 0.95; (k 0.0006)	best fit (however, degradation overestimated at last point)	not best fit (Chi^2Err% and t values for k1 and k2 acceptable; poor visual fit at end of test)	not best fit (Chi^2Err% and t values for k1 and k2 acceptable; poor visual fit at end of test)	not acceptable (Chi^2Err% >15; poor visual fit)
Parent, last data point excluded								
Speyer 2.2 soil	7.062; 0.9886; (alpha 0.008; beta 0.04)	1.752; 0.9987; (k1 2.63e-06; k2 0.005; tb 8.78e-05)	4.839; 0.9956; (k1 0.001; k2 0.098)	11.81; 0.9751; (k 0.0002)	not best fit	best fit (best visual fit and lowest Chi^2Err%)	not best fit	not acceptable (poor visual fit)

Speyer 2.3 soil	19.54; 0.9222; (alpha 0.199; beta 0.265)	18.78; 0.9484; (k1 0.004; k2 0.274; tb 0.038)	19.85; 0.9395; (k1 0.032; k2 0.491; g 0.003)	18.21; 0.926; (k 0.0015)	not best fit (high Chi^2Err%, and p-values, degradation overestimated at two last points)	best fit (lowest Chi^2Err% of biphasic models, highest r^2; however, high p-value for k2, degradation overestimated at las point)	best fit (high Chi^2Err%r and p-value for k2, degradation underestimated at last point)	not acceptable (Chi^2Err% >15; poor visual fit)
Speyer 6S soil	3.84; 0.9919; (alpha 0.002; beta 0.037)	4.089; 0.9921; (k1 0.0004; k2 0.0114; tb 0.002)	4.104; 0.9922; (k1 0.011; k2 0.021; g 0.0003)	20.94; 0.9477; (k 0.001)	best fit	not best fit (Chi^2Err% and t values for k1 and k2 acceptable; however, degradation overestimated at last point)	best fit (degradation overestimated at last point)	not acceptable (Chi^2Err% >15; poor visual fit)
Parent+unextracted residues, all data points								
Speyer 2.2 soil	11.85; 0.776; (alpha 0.034; beta 0.284)	15.37; 0.6449; (k1 0.179; k2 0.216; tb 0.159)	11.11.; 0.8535; (k1 0.084; k2 0.50; g 0.006)	19.23; 0.3194; (k 0.064)	poor visual fit (especially due to the last data point)	poor visual fit (especially due to the last data point)	poor visual fit (especially due to the last data point);	poor visual fit (especially due to the last data point)
Speyer 2.3 soil	12.11; 0.8832; (alpha 0.018; beta 0.183)	10.2; 0.9368; (k1 0.002; k2 0.070; tb 0.004)	11.45; 0.9137; (k1 0.037; k2 0.204; g 0.001)	21.4; 0.6213; (k 0.022)	not best fit (high p-value for beta)	best fit (good visual fit; lowest Chi^2Err% and p-values,	not best fit (high p-value for k2)	not acceptable (Chi^2Err% >15; poor visual fit)
Speyer 6S soil	Inf; NaN (0.5*)	6.937; 0.949; (k1 9.64e-05; k2 0.097; tb 9.44e-15)	6.937; 0.949; (k1 0.5*; k2 0.097; g 1.84-	25.41; 0.2102; (k 0.128)	not acceptable (high p-value)	best fit (visual fit acceptable despite high p-value for k2)	not acceptable (high p-value)	not acceptable (Chi^2Err% >15; poor visual fit)

			05)					
Parent+unextracted residues, last data point excluded								
Speyer 2.2 soil	4.995; 0.9665; (alpha 0.008; beta 0.080)	6.087; 0.953; (k1 0.034; k2 0.004)	6.416; 0.96; (k1 0.107; k2 0.251; g 0.056)	9.205; 0.8704; (k 0.001)	best fit	not best fit (last two points poorly predicted)	not best fit (high p-values)	not acceptable (poor visual fit)
Speyer 2.3 soil	11.92; 0.8686; (alpha 0.051; beta 0.223)	10.3; 0.9316; (k1 0.005; k2 0.136; tb 0.016)	12.07; 0.8991; (k1 0.091; k2 0.336; g 0.017)	17.47; 0.6701; (k 0.015)	not best fit (high p-values)	best fit (lowest Chi^2Err%, highest r^2, visual fit acceptable despite high p-values for k1 and k2)	not best fit (high p-values)	not acceptable (Chi^2Err% >15; poor visual fit)
Speyer 6S soil	Inf; NaN; (0.5*)	7.419; 0.9466 (k1 0.001; k2 0.210; tb 7.25e-12)	7.419; 0.9466; (k1 <2e-16; k2 0.210; g 0.0002)	24.86; 0.2326; (k 0.130)	not acceptable	best fit (visual fit acceptable despite high p-value for k2)	best fit (visual fit acceptable despite high p-value for k2)	not acceptable (Chi^2Err% >15; low r^2; high p-value for k; poor visual fit)

Table 28. Recovery of radioactivity from Speyer 2.2 (% of applied) (NOTOX 2009a)

Time (days)	Organic volatiles	CO_2	Extractable residues	Bound residues	Mass balance
0	-	-	98.1	3.1	101.2
3	0.7	5.6	75.8	18.3	100.4
7A	0.6	5.3	42.6	44.9	93.4
7B	0.5	19.3	42.8	39.1	101.8
7*(mean)*	0.6	12.3	42.7	42.0	97.6
14	0.8	28.0	25.0	51.2	105.0
28A	0.7	20.8	17.8	35.2	74.5
28B	0.5	13.0	19.6	31.1	69.4
28*(mean)*	0.6	16.9	18.7	33.2	69.4
63	1.0	39.4	12.3.	33.5	86.1
120	0.6	32.4	10.5	51.5	95.0

Table 29. Recovery of radioactivity from Speyer 2.3 (% of applied) (NOTOX 2009a)

Time (days)	Organic volatiles	CO_2	Extractable residues	Bound residues	Mass balance
0	-	-	-	3.8	102.9
3	2.1	1.5	83.9	15.4	103.0
7A	2.1	15.9	47.8	29.1	94.9
7B	2.4	14.4	54.1	24.1	95.0
7*(mean)*	2.2	15.1	51.0	26.6	94.9
14	1.4	21.4	32.0	31.7	86.5
28A	2.5	31.6	23.6	32.0	89.7
28B	1.7	30.2	17.6	32.2	81.7
28*(mean)*	2.1	30.9	20.6	32.1	85.7
63	1.1	46.1	15.5	26.4	89.1
120	1.1	51.0	8.1	23.6	83.8

Table 30. Recovery of radioactivity from Speyer 6S (% of applied) (NOTOX 2009a)

Time (days)	Organic volatiles	CO_2	Extractable residues	Bound residues	Mass balance
0	-	-	98.2	6.55	104.7
3	1.2	0.1	93.1	7.8	102.1

7A	1.5	4.6	69.5	25.3	101.0
7B	1.1	0.5	75.5	21.9	99.0
7*(mean)*	1.3	2.6	72.5	23.6	100.0
14	1.0	13.5	50.3	38.4	103.2
28A	0.9	28.7	26.2	42.5	98.4
28B	1.3	23.9	31.5	43.6	100.3
28*(mean)*	1.2	26.3	28.9	43.1	99.4
63	0.9	46.3	14.4	35.2	96.9
120	1.2	47.7	8.5	33.5	91.0

Table 31. HPLC results Speyer 2.2 soil (% applied)

Time (days)	Parent 15.9-16.8 min	Met 2 2.6-3.1 min	Met 3 3.1-3.4 min	Met 7 7.8-8.0 min
0	98.1	n.d.	n.d.	n.d.
3	61.3	n.d.	2.5	7.4
7A	29.5	1.8	5.2	4.8
7B	32.3	2.2	3.9	4.4
7*(mean)*	30.9	2.0	4.6	4.6
14	17.0	1.4	2.5	2.2
28A	11.9	0.7	2.3	0.9
28B	13.9	1.6	1.4	1.7
28*(mean)*	12.9	1.1	1.8	1.3
63	4.3	1.3	1.2	0.7
120	10.5	n.d.	n.d.	n.d.

n.d. not detected
Percentages based on the sum of radioactivity in the concentrated extracts and/or aqueous residue

Table 32. HPLC results Speyer 2.3 soil (% applied)

Time (days)	Parent 15.9-16.8 min	Met 2 2.6-3.1 min	Met 3 3.1-3.4 min	Met 7 7.8-8.0 min
0	99.2	n.d.	n.d.	n.d.
3	84.2	0.3	1.5	5.2.
7A	30.4	3.6	6.3	5.5
7B	29.5	7.5	8.9	6.8
7*(mean)*	30.0	5.5	7.6	6.2

14	20.2	1.9	6.6	2.9
28A	15.0	2.0	3.8	2.7
28B	11.5	4.4	n.d.	1.8
28*(mean)*	13.2	3.2	1.9	2.2
63	8.6	0.9	3.6	1.2
120	8.1	n.d.	n.d.	n.d.

n.d. not detected
Percentages based on the sum of radioactivity in the concentrated extracts and/or aqueous residue

Table 33. HPLC results Speyer 6S soil (% applied) (NOTOX 2009a)

Time (days)	Parent 15.9-16.8 min	Met 2 2.6-3.1 min	Met 3 3.1-3.4 min	Met 7 7.8-8.0 min
0	98.2	n.d.	n.d.	n.d.
3	39.5	12.5	9.3	28.9
7A	21.6	12.6	24.3	9.6
7B	26.7	24.6	10.1	12.5
7*(mean)*	24.2	18.6	17.2	11.1
14	19.1	13.6	12.4	3.0
28A	12.5	5.3	8.5	0.0
28B	5.3	12.4	11.6	2.2
28*(mean)*	8.9	8.9	10.0	1.1
63	5.2	3.5	3.6	n.d.
120	8.5	n.d.	n.d.	n.d.

n.d. not detected
Percentages based on the sum of radioactivity in the concentrated extracts and/or aqueous residue

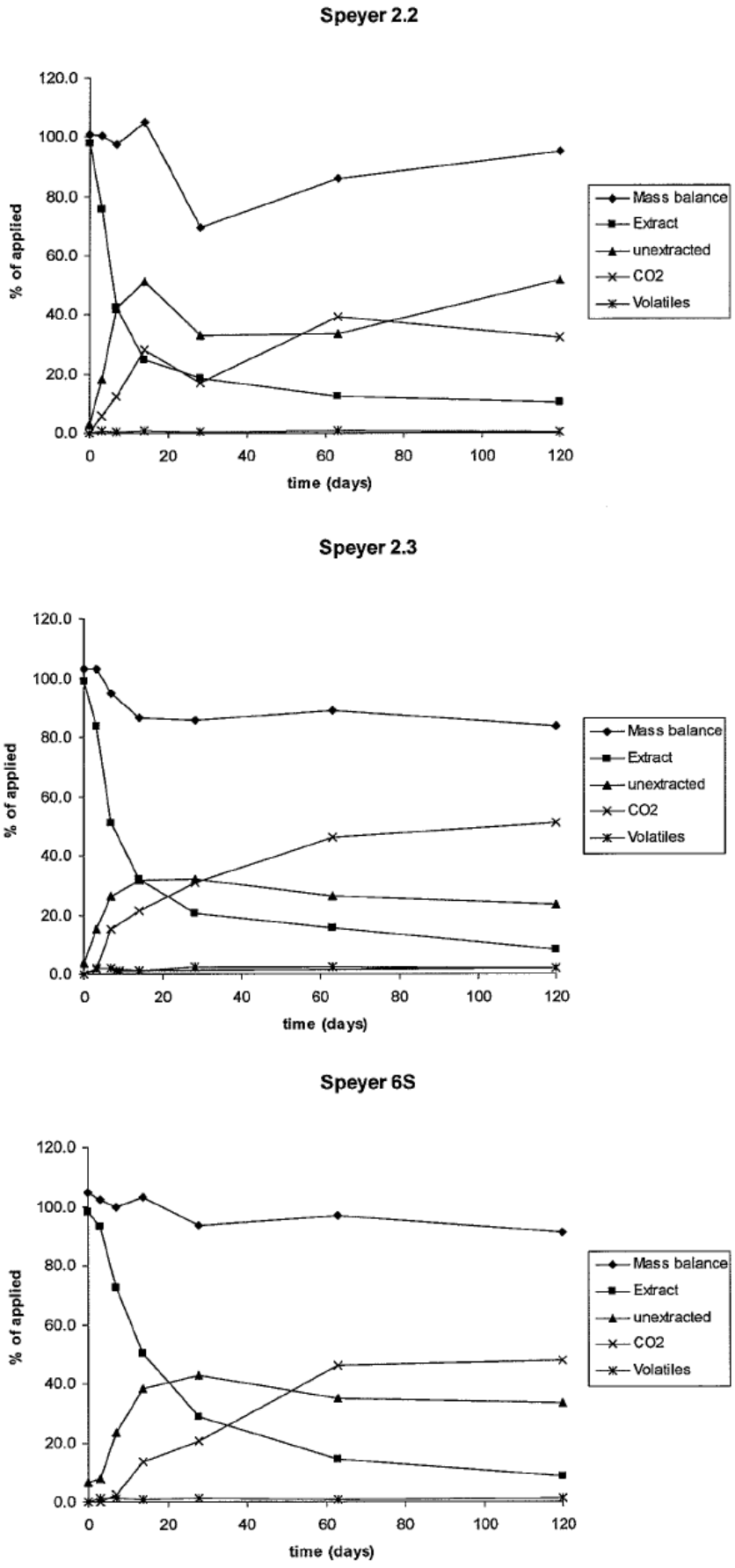


Figure 7. Distribution of radioactivity (reproduced from NOTOX 2009a).

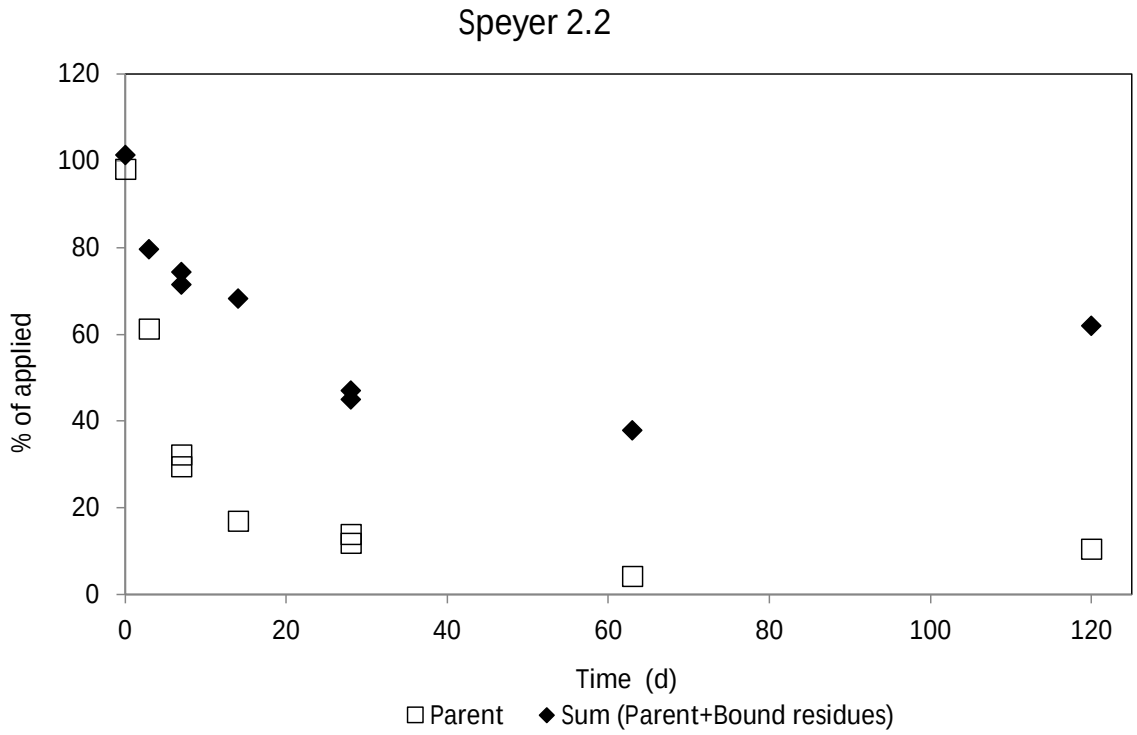


Figure 8. Percentages of applied radioactivity for parent substance and for the sum of parent+bound residue in Speyer 2.2 soil (NOTOX 2009a). Replicate samples, where available (days 7 and 28, two per each day), are shown separately.'

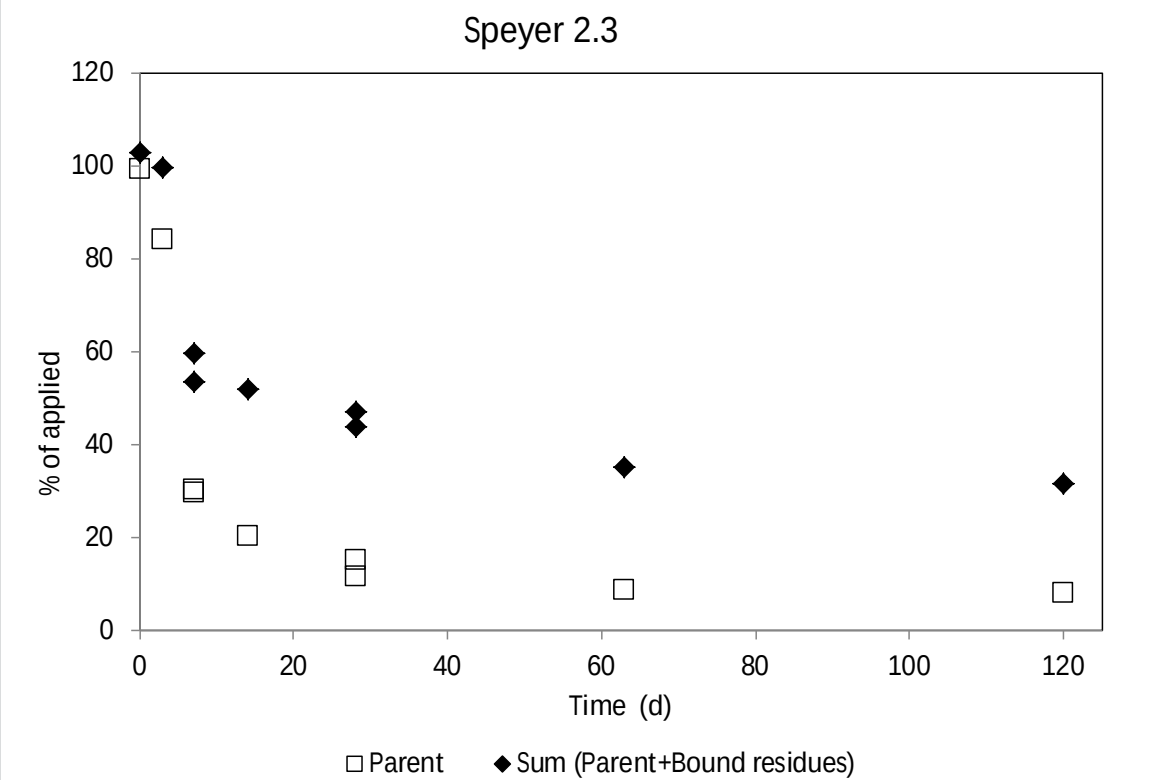


Figure 9. Percentages of applied radioactivity for parent substance and for the sum of parent+bound residue in Speyer 2.3 soil (NOTOX 2009a). Replicate samples, where available (days 7 and 28, two per each day), are shown separately.

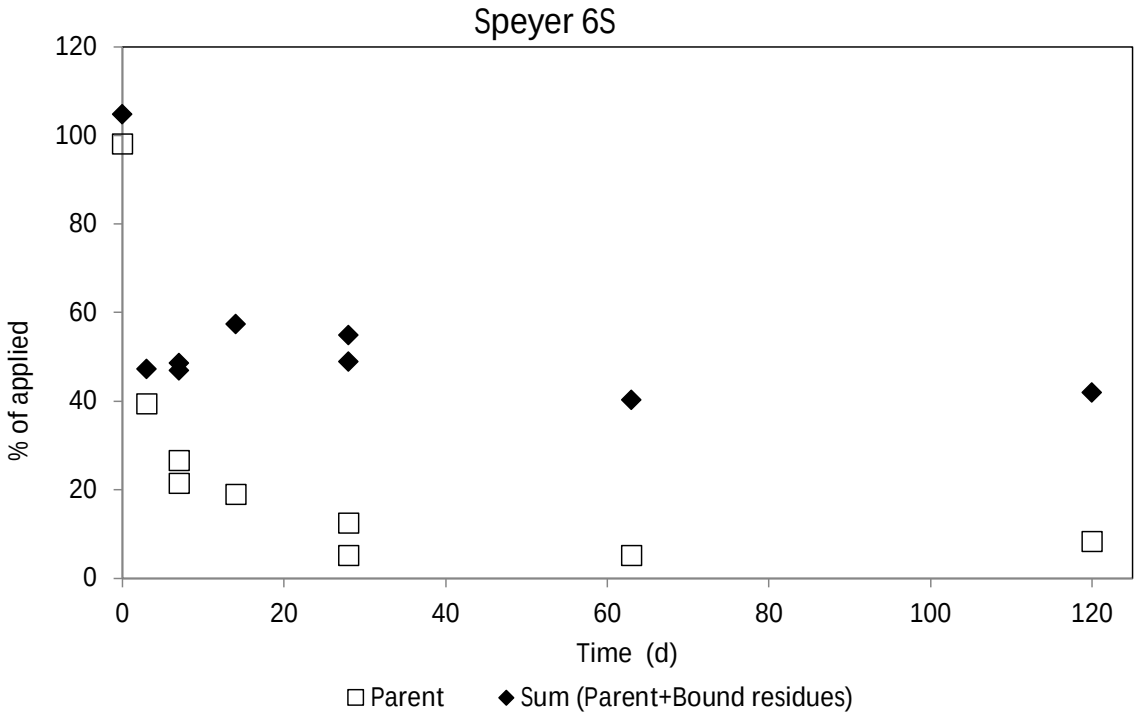


Figure 10. Percentages of applied radioactivity for parent substance and for the sum of parent+bound residue in Speyer 6S soil (NOTOX 2009a). Replicate samples, where available (days 7 and 28, two per each day), are shown separately.

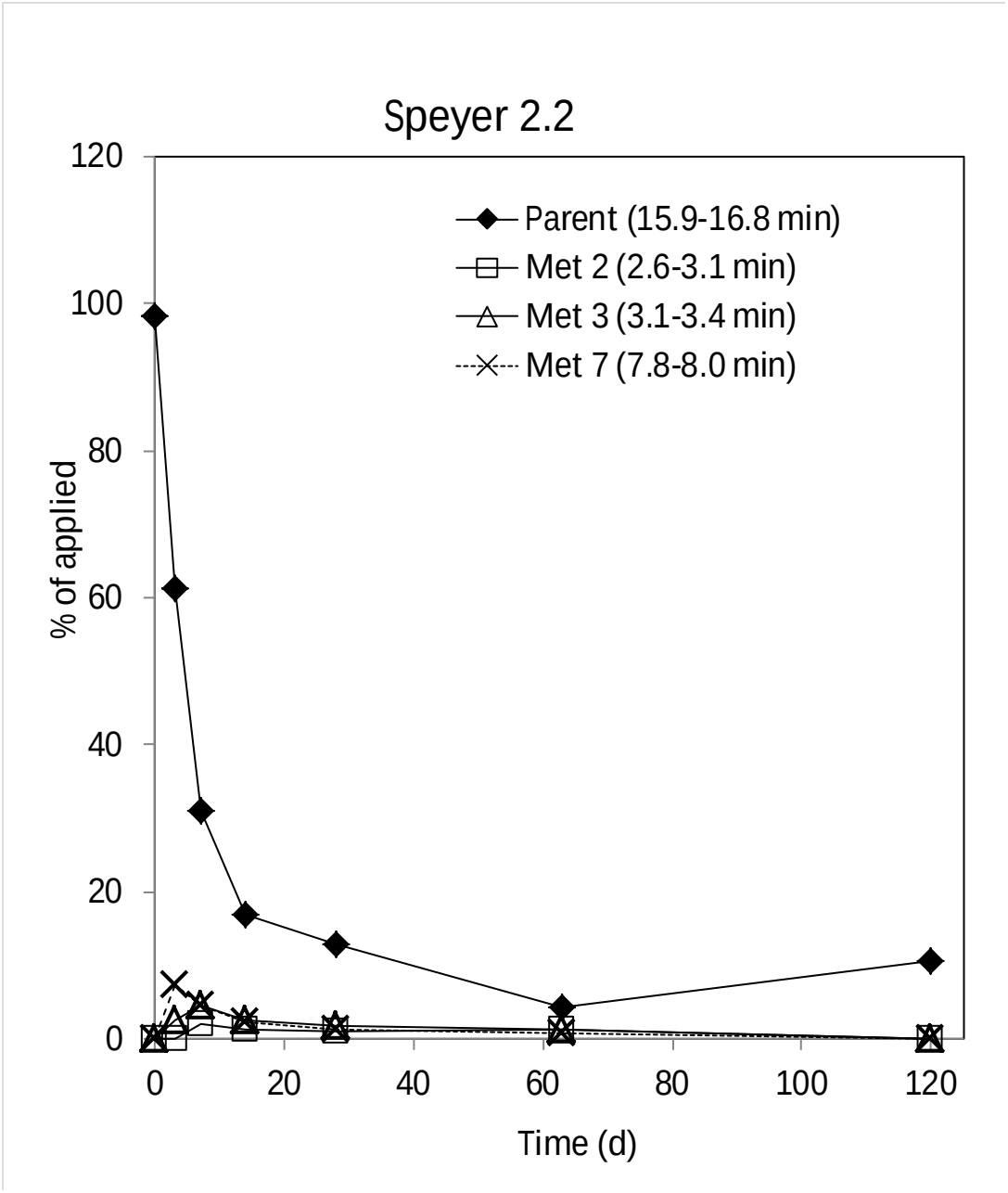


Figure 11. Percentages of applied radioactivity for parent substance and for metabolites for Speyer 2.2 soil (NOTOX 2009a). Replicate samples were taken on days 7 and 28 (two per each day) and are averaged.

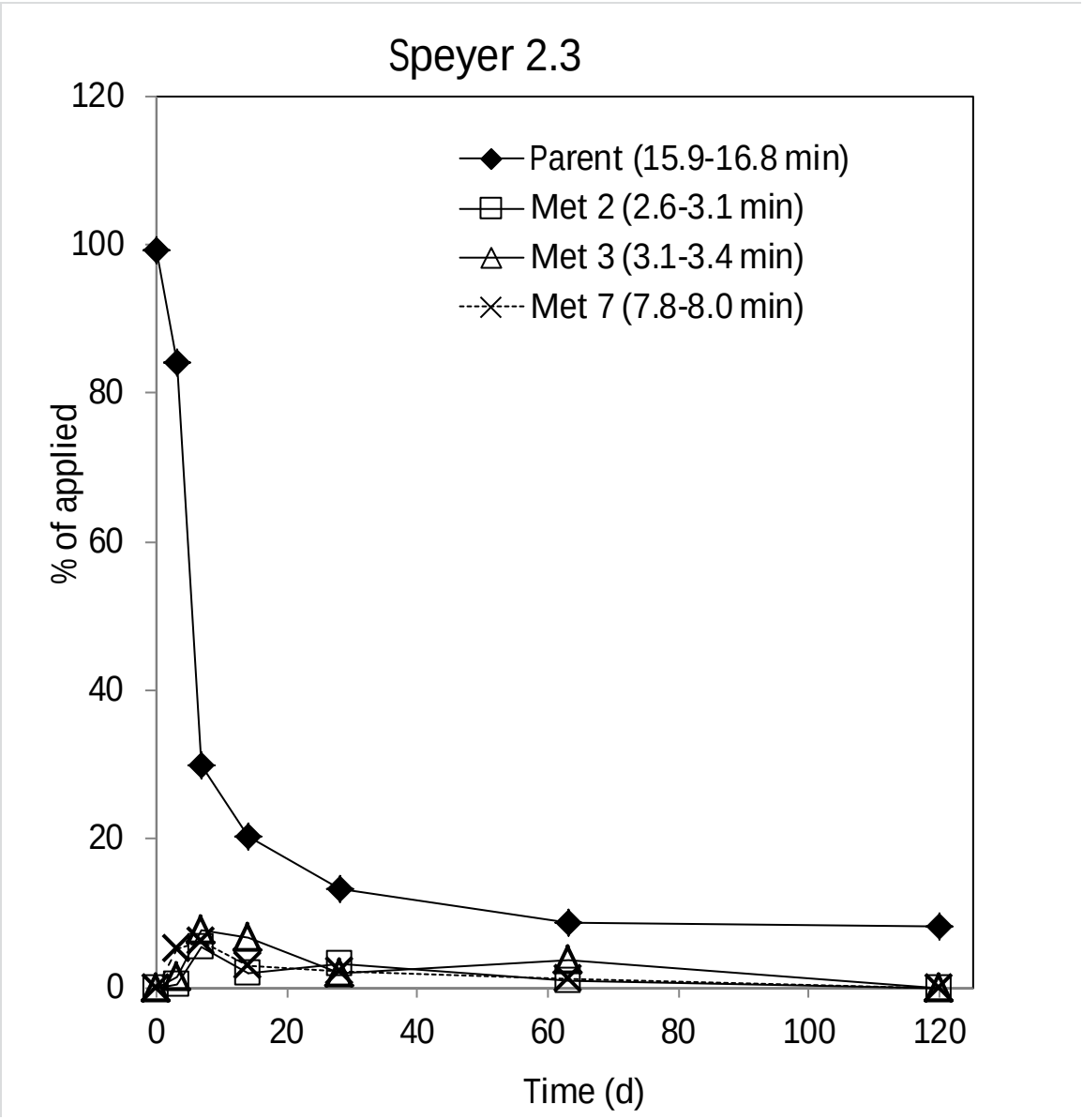


Figure 12. Percentages of applied radioactivity for parent substance and for metabolites for Speyer 2.3 soil (NOTOX 2009a). Replicate samples were taken on days 7 and 28 (two per each day) and are averaged.

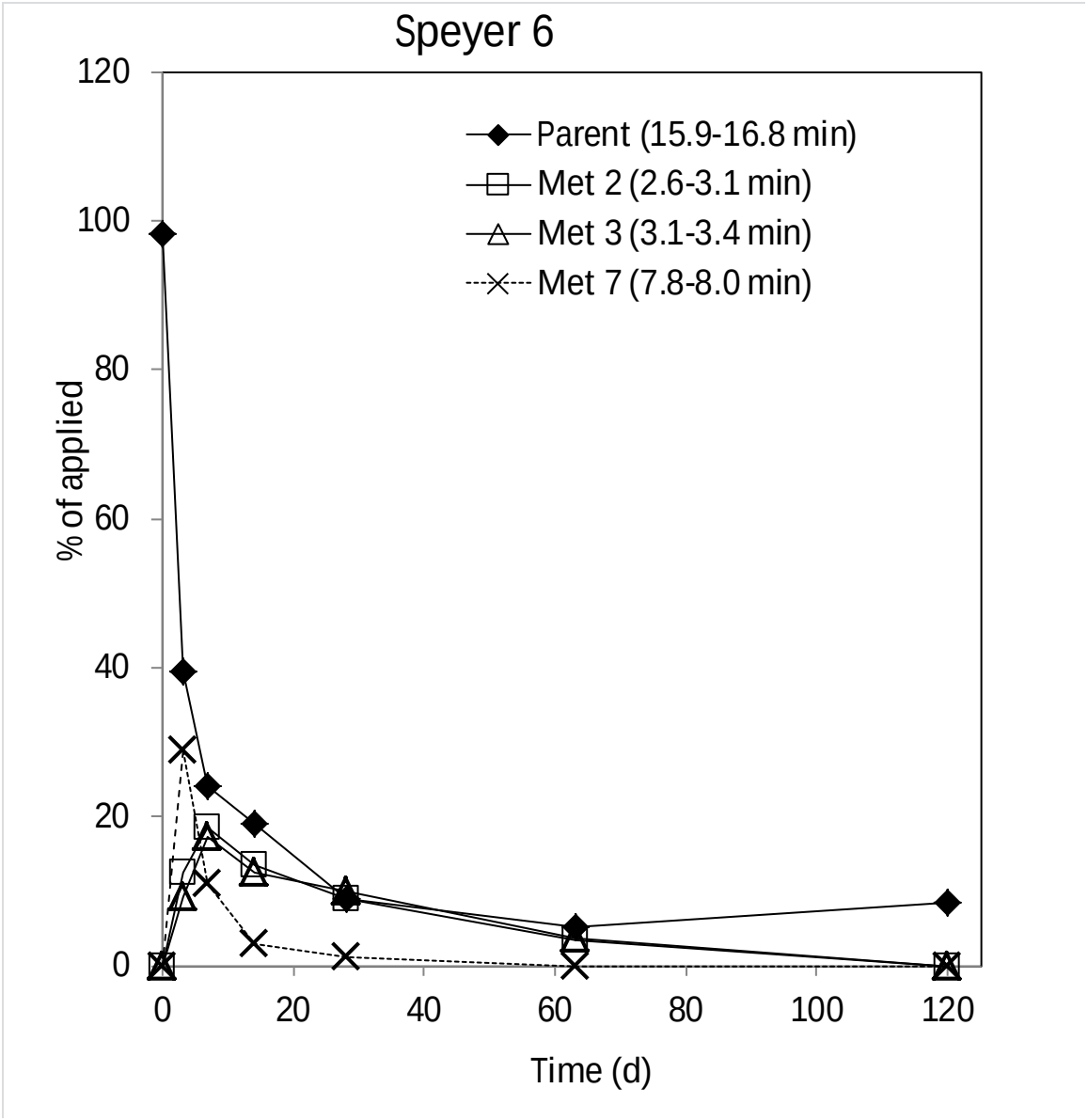


Figure 13. Percentages of applied radioactivity for parent substance and for metabolites for Speyer 6S soil (NOTOX 2009a). Replicate samples were taken on days 7 and 28 (two per each day) and are averaged.

3.1.2.3 Other data on biodegradation

Ohmori et al. 1973

This is a microbial culture study where 258 strains of microorganisms were isolated from 526 samples (soil, leaf, river water). The isolation was carried out by repeating liquid enrichment culture techniques in the medium containg biphenyl, diphenylmethane, diphenylethane or terphenyl, as the sole source of carbon. Most of the isolated microorganisms were short rod motile bacteria. Among those from diphenylethane medium, fungi were also found. Yeast could not be found.

Of the four hydrocarbons used for enrichment, enrichment on terphenyl resulted in clearly lowest amount of isolated strains. Growth on terphenyl was detected for 14 of the 522 samples, growth on biphenyl in 39 of 426 samples, growth on diphenylmethane on 40 of 424 samples, and growth on diphenylethane on 165 of 395 samples.

After the isolation procedure, the assimilation of the hydrocarbons by the strains obtained was tested by observing their growth on agar slant medium containing each hydrocarbon. The hydrocarbons tested were *n*-paraffin, biphenyl, diphenylmethane, diphenylethane, *o*-, *m*-, *p*-terphenyl (*o*-, *m*-, *p*-terphenyl were mixed to be 1:1:1 of weight ratio), *trans*-stilbene and *p*-Cl-biphenyl.

The four microbial strains isolated from terphenyl medium and used for further testing were all able to grow on *n*-paraffin and on terphenyl. Two of them grew on *trans*-stilbene, one on diphenylmethane, whereas none of them grew on biphenyl, diphenylethane, or p-Cl-biphenyl. Regarding the strains isolated on other hydrocarbons, only a low proportion were able to grow on terphenyl (1 out of the 10 strains isolated on biphenyl, 0 out of the 4 strains isolated on diphenylmethane, and 1 out of the 5 strains isolated on diphenylethane) but their ability to grow on biphenyl, diphenylmethane, and diphenylethane was more frequent compared to the terphenyl-isolated strains. Growth on *n*-paraffin was less frequent among strains obtained with other hydrocarbons than those obtained on terphenyl.

The low proportion of strains isolated on terphenyl medium suggests that terphenyl is a less favourable growth substrate compared to the other hydrocarbons used for isolation. This is also supported by the fact that among the strains isolated on other hydrocarbons, the ability to grow on terphenyl was less frequent compared to other tested hydrocarbons.

The lower ability of the terphenyl-isolated strains to grow on biphenyl, diphenylmethane, and dimethylethane, compared to strains isolated on other hydrocarbons, suggests that strains capable of growing on terphenyl may be more specialised in their capability to utilise other hydrocarbons.

In summary, the amounts and properties of microbial strains isolated from environmental samples using terphenyl or other hydrocarbons as a sole carbon source suggest that terphenyl is a less favourable growth substrate compared to other hydrocarbons tested (*n*-paraffin, biphenyl, diphenylmethane, diphenylethane, *trans*-stilbene) and therefore the ultimate degradability of terphenyl in the environment may be limited. The P or vP status for the other tested substances is not known; however, it is noted that for biphenyl, testing for P/vP property has been requested in substance evaluation (ECHA 2018).

Reliability: This study is considered reliable with restrictions (reliability score = 2) as it describes investigations which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable.

Relevance: This study is considered relevant for P/vP assessment of *o*-, *m*-, and *p*-terphenyl as supporting information. The most important findings include the comparison between terphenyl and the other tested hydrocarbons as microbial growth substrates, as well as the differences in substrate utilization between the strains isolated on terphenyl compared to those isolated on other hydrocarbons. It is noted that the isomer composition of the terphenyl is explicitly mentioned in the case of the growth experiments done with the isolated strains, but not for terphenyl used in the isolation procedure.

3.1.4 Summary and discussion of degradation

For the persistence assessment of terphenyl, hydrogenated, most weight is given to half-lives measured in standard biodegradation simulation tests, or simulation tests considered comparable to standard tests in terms of reliability and test conditions. Results from simulation tests with conditions differing from standard tests or lack of details in documentation, screening tests, microbial culture studies, and QSAR predictions are used as supporting information.

Table 34 summarises the availability of information on the different constituents and the

weighting assigned in the WoE for P/vP assessment. The observations included in the WoE are listed below:

- Based on available information, abiotic degradation is expected to occur at such a low rate that it is not considered a relevant route of degradation for P/vP assessment. Reaction with hydroxyl radicals in the atmosphere is relatively fast based on QSAR predictions (half-lives 3-14 days).
- In a soil simulation test, dissipation half-lives in soil of ≥218 days (temperature-corrected to 12°C) were determined for terphenyl and >224 days for quaterphenyl (Monsanto Company 1989). Only the decrease of concentrations was determined in this study and the proportion metabolised or bound to the soil matrix is not known. As a part of the substance may have formed non-extractable residues, these half-lives should be considered 'best case' values and the real half-lives are likely to be higher. These half-lives were determined for a mixture of terphenyls, quaterphenyls, and polyphenyls (the proportions of the different isomers are not reported). Of these terphenyls and quaterphenyls are relevant constituents of the UVCB substance. A read-across using the mixture of terphenyls as a source substance and o-T and p-T as target substances is considered appropriate, taking into account that in a river-die away tests (Mic 1983a) o-T and p-T showed similar degradability whereas m-T was more degradable. A read-across using the mixture of quaterphenyls as a source substance and p-Q as a target substance is considered appropriate, taking into account that in a shake-flask CO_2 evolution test with hydrocarbon adapted inoculum (Mic 1983b) p-Q did not show a higher degradability than o-T and p-T.
- In a seawater simulation test with hydrocarbon mixtures (ExxonMobil Biomedical Science, Inc., 2009) primary degradation half-live (temperature-corrected to 12°C) of >182 days was reported for o-terphenyl and half-lives of 32 d and 108 d for m-terphenyl.
- In an OECD 307 soil simulation test (NOTOX, 2009) a dissipation half-life was 2-10 days for p-dicyclohexylbenzene (HT2) when the half-lifes are calculated for the whole test duration using bi-phasic models. Assuming that all NER are parent substance, the half-life (calculated for the whole test duration) was 6-18 days in two soils whereas for one soil no exact half-life could be determined and it is estimated that the half-life for this soil is above test duration, i.e., >120 days. When the second phase ('slow phase') from bi-phasic models is used the half-lives were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k_2 parameter was not statistically significant and as the half-life obtained from temperature conversion is longer than the experimental period) whereas for one of the soils, no reliable second-phase half-lives could be determined. In this study a significant part of applied radioactivity partitioned to soil and was recovered as NER. The formation of NER has a strong influence on the shape of the dissipation curve, causing uncertainty for the determination of the degradation half-life. NER was not characterised, except for one sample which indicated that NER consisted of parent substance. A definitive P/vP conclusion has not been drawn in this assessment as there are still open questions regarding the interpretation of NER as well as the choice of kinetic model and half-life to be used for comparison with the criteria.
- In non-standard ultimate biodegradation tests (Monsanto report ES-80-SS34, Monsanto 1977a), degradation of UVCB substances (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) based on CO_2 evolution was at the most 14 % within 35 days, suggesting that the tested substances are not readily biodegradable.
- In a river die-away test (MONSANTO 1970a) a 68% decrease in concentration in 21 days was obtained for a UVCB substance (expected to contain same or

structurally similar constituents as terphenyl, hydrogenated), whereas a 13% decrease was obtained for distilled water control, suggesting that significant primary degradation of UVCB constituents occurred.

- In a semi-continuous activated sludge (SCAS) study (MONSANTO 1970b) the removal of a UVCB substance (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) was 19.1-55% at different sampling periods, suggesting that primary degration occurred in a test system considered to be favourable for microbial adaptation.

- In a die-away procedure with an adapted inoculum (MONSANTO 1972a) the removal of a a UVCB substance (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) was 95-100% at different sampling periods, suggesting that primary degration occurred.

- In an SCAS study (MONSANTO 1972b) the removal of a UVCB substance (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) was 68.1-65.5% at different sampling periods and doses, suggesting that primary degration occurred in a test system considered to be favourable for microbial adaptation.

- In an SCAS study (Monsanto report 1970) a 49% primary degradation rate of a UVCB substance (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) was reported after acclimation period in a test system considered to be favourable for microbial adaptation.

- In a river die-away procedure (Monsanto report 1970) the decrease in a level of a UVCB substance (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) was 80% after 50 days, suggesting that primary degration occurred

- A river die-away test (Mic 1983a), when tested separately, o- and p-terphenyl showed no or neglible degradation during 28 days whereas m-terphenyl started to degrade after 16 days. When tested in a mixture with m-, o-, and p- terphenyls, o- terphenyl and m-terphenyl started to biodegrade after 30 days. A HT3 constituent showed no degradation in 30 days whereas HT1 and HT2 constituents were more degradable.

- A shake-flask carbon dioxide evolution test with a hydrocarbon-adapted inoculum (Mic 1983b) showed relatively low (9-38%) mineralisation for o-T, m-T, p-T, p-HT2, p-HT3, and p-Q) in 55 days. For p-HT1 a higher mineralization (63%) was observed.

- In an SCAS study (Monsanto 1973) the mean disappearance of hydrogenated quaterphenyls (HQ) was 16% at the end of the SCAS study (with negligible volatilization), in a test system considered to be favourable for microbial adaptation. The presence of a detectable amoung of HQ at the end of the following die-away procedure is in line with the results of the SCAS study. The test substance (HQ40) was a mixture of approximately 80 % quaterphenyls with a degree of 40 % hydrogenation (the residual 20 % consists of terphenyl and higher (> 5-ring) phenyl structures).

- P-terphenyl persisted in an SCAS test system (Monsanto 1974) despite the possible adaptation during the test and in a die-away procedure conducted with an inoculum from the SCAS system. Test substance was a mixture containing mainly o-, m-, and p-terphenyls. M- and p-terphenyl showed higher decrease than p-terphenyl; however, no conclusions on relative degradabilities of the isomers can be done due to different concentrations of the isomers in the test substance and possible abiotic losses.

- In a shake-flask carbon dioxide evolution test (Monsanto 1991) with an inoculum pre-exposed to p-terphenyl, p-terphenyl showed no significant mineralisation or primary degradation in 42 days. The CO_2 production after 42 days was 9-8% in the active test and 7% in sterile control. The mean residue recovery after 42 days was 78.0-81.1% of initial level in the active test and 82.1 in sterile control).

- In a microbial culture study (Ohmori et al 1973) the amounts and properties of microbial strains isolated from environmental samples using terphenyl or other hydrocarbons as a sole carbon source suggest that terphenyl is a less favourable growth substrate compared to other hydrocarbons tested (*n*-paraffin, biphenyl, diphenylmethane, diphenylethane, *trans*-stilbene) and therefore the ultimate degradability of terphenyl in the environment may be limited. The results indicate presence of terphenyl utilising microorganisms but also suggest that microorganisms able to utilise other hydrocarbons are not necessarily able to utilise terphenyl.

- BIOWIN models 2 and 6 in combination indicate that o-T, m-T, P-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4, are Potentially P or vP. , as the P/vP screening criteria for this model combination are fulfilled. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BIOWIN models 2 and 3 in combination indicate that o-T, m-T, P-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4 do not screen as P or vP. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BioHCwin model predicts half-lives of 315 days for HT1, 470 days for HT2, 69 days for HT3, 68 days for HQ1, 809 days for HQ2, 305 days for HQ3, and 7-8 days for o-T, m-T, p-T, and p-Q.

Table 34: The available information on the different constituents and the weighting assigned in the WoE for P/vP assessment

Type of information	Conclusion	Reliability	Weighting assigned in persistence assessment[a] (none/low/moderate/high)	Reference(s) and remarks
Screening information for P and vP[b]				
BIOWIN 2 (non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	o-T, m-T, p-T, HT1, HT2, Q, HQ1, HQ2, and HQ3: screening criteria not fulfilled HT3: not applicable	2 (with the exception of HT3, for which the score is 3)	moderate (o-T, m-T, p-T, HT1, HT2, Q, HQ1, HQ2, HQ3); none (HT3)	
or				
Biowin 6 (MITI non-linear model prediction)	o-T, m-T, p-T, HT1, HT2, Q, HQ1, HQ2, and HQ3:	2 (with the excep-	moderate (o-T, m-T, p-T, HT1, HT2, Q, HQ1, HQ2, HQ3); none (HT3)	

and Biowin 3 (ultimate biodegradation time)	Potentially P or vP HT3: not applicable	tion of HT3, for which the score is 3)		
or				
other models: BIOHCWIN	HT1, HT2, and HT3 Potentially P or vP (half-lifes 315 days for HT1, 470 days for HT2, and 69 days for HT3)	2	moderate (HT1, HT2, and HT3); low (other constituents)	The BIOHCWIN model is partially based on mixture studies and may be influenced by co-metabolism. Therefore, these predictions are not used to support “not P/vP” (ECHA 2017b).
Ready biodegradability test (including modifications allowed in the respective TGs)	not available		not applicable	Ready biodegradability tests according to OECD guideline or equivalent are not available (equivalent ISO and OPPTS tests listed in Appendix R.7.9-1 of ECHA (2017b)
Enhanced screening tests	not available		not applicable	
Specified tests on inherent biodegradability: Zahn-Wellens (OECD TG 302B, MITI II test (OECD TG 302C	not available		not applicable	
Non-standard screening tests	UVCB substance: Potentially P or vP Individual constituents: no conclusion	2	moderate (UVCB substance); none (individual constituents)	Monsanto report ES-80-SS34, Monsanto 1977a*; It is not known which constituents were degraded. *this refers to the tests measuring CO_2 in Monsanto 1977a (Monsanto 1977a also includes primary degradation measurements)
	No conclusion	2	none	Monsanto 1970a; only primary degradation

				reported
	No conclusion	2	none	Monsanto 1970b; only primary degradation measured, test system favours adaptation
	No conclusion	2	none	Monsanto 1972a; adapted inoculum
	No conclusion	2	none	Monsanto 1972b; test system favours adaptation
	No conclusion	4	none	Monsanto report 1970; SCAS study; only primary degradation reported, test system favours adaptation
	No conclusion	4	none	Monsanto report 1970; river die-away study; only primary degradation reported
	o-T, p-T, and HT3: Potentially P or vP m-T,HT1 and HT2: no conclusion	2	moderate (o-T, p-T, HT3, m-T, HT1, and HT2)	Mic 1983a The results are used for 1) in WoE for P/vP property of each constituent and 2) estimating differences in relative biodegradabilities of the constituents.
	o-T, m-T, p-T, p-HT2, p-HT3, and p-Q: Potentially P or vP HT1: no conclusion	2	low (o-T, m-T, p-T, p-HT2, p-HT3, and p-Q); none (HT1)	Mic 1983b The results are used for 1) in WoE for P/vP property of each constituent and 2) estimating differences in relative biodegradabilities of the constituents (with reservation related to adaptation of inoculum).
	HQ: Potentially P or vP	2	moderate (HQ)	Monsanto 1973 The results are used for WoE for P/vP estimation of HQ.
	p-T: Potentially P or vP m-T, o-T: no	2	moderate (p-T); none (m-T, o-T)	Monsanto 1974 The results are used for WoE for P/vP estimation of p-T

	conclusion			but not for estimating differences between the constituents (constituents tested in different concentrations in a mixture; test system favours adaptation)
	p-T: Potentially P or vP	2	low (p-T)	Monsanto 1991 The results are used for WoE for P/vP estimation of p-T.
Hydrocarbon utilization study on microbial cultures	o-T, m-T, p-T: Potentially P or vP	2	low (o-T, m-T, p-T)	Ohmori et al. 1973
Monitoring data	not available		not applicable	
Assessment information[c]				
Abiotic oxidation	not available		not applicable	no information available on abiotic oxidation in conditions and compartments relevant for P/vP assessment
Hydrolysis	all constituents: Potentially P or vP	not applicable	moderate (all constituents)	no functional groups suspectible to hydrolysis:
Phototrans-formation in water	o-T, m-T, p-T: Potentially P or vP	4	low (o-T, m-T, p-T)	o-T, m-T, and p-T: phototransformation in water study available showing that this is not a significant pathway for transformation
Phototrans-formation in soil	o-T, m-T, p-T: Potentially P or vP	not applicable	low (o-T, m-T, p-T)	o-T, m-T, and p-T: conclusion based on phototransformation in water study
Simulation test data in soil	HT2: Potentially P or vP	2	high (HT2)	NOTOX 2009a
	T: P and vP Q: P and vP	2	high (T, Q)	Monsanto Company 1989
Simulation test data in seawater	o-T, m-T, and HT1: P or vP HT2 and HT3: no conclusion	4	moderate (o-T, m-T, HT1); none (HT2, HT3)	Exxonmobil Biomedical Science, Inc., 2009 The study cannot be used for a definitive

	other constituents: no data available			P/vP conclusion in seawater due to lack of information e.g. on kinetic analysis. Therefore, a lower weighting is assigined compared to the soil simulation studies. Test substance was a mixture of hydrocarbons and thus the results may be influenced on co-metabolism; therefore, it is not used to support “not P/vP”.
In situ/field degradation study results	not available		not applicable	
Monitoring data	not available		not applicable	

[a]Determined based on reliability and relevance considerations
[b]Table R.11-4 in Guidance on information requirements and chemical safety assessment Chapter R.11 (ECHA 2017a)
[c]Based on Figure R.11-3 in ECHA (2017a) and Annex XIII to REACH regulation.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

The assessed substances are expected to be adsorptive based on the predicted Log Koc values- The Log Koc values based on KOCWIN are 5.3-6.8 (MCI method) and 4.8-8.8 (Kow method) for selected constituents (Table 37).

Table 35. Soil adsorption coefficients predicted using KOCWIN v2.00 (EPI Suite v4.11) for selected constituents.

Group	Compound	Koc (MCI method)	Log Koc (MCI method)	Koc (Kow method)	Log Koc (Kow method)
o-T	ortho-terphenyl	1.841E+005	5.265	6.172E+004	4.790
m-T	meta-terphenyl	1.805e+005	5.2564	6.172e+004	4.7904
p-T	para-terphenyl	1.805e+005	5.2564	1.71e+005	5.2330
p-HT1	4-cyclohexylbiphenyl	1.805e+005	5.2564	5.032e+005	5.7017
p-HT2	1,4-dicyclohexylbenzene (CAS 1087-02-1)	1.805e+005	5.2564	4.185e+006	6.6217
p-HT3	p-tercyclohexyl	1.805e+005	5.2564	2.631e+007	7.4201
p-Q	para-quaterphenyl	6.349e+006	6.8027	2.079e+006	6.3179
p-HQ1	4-cyclohexylterphenyl	6.349e+006	6.8027	1.729e+007	7.2379

p-HQ2	dicyclohexylbiphenyl	6.349e+006	6.8027	1.087e+008	8.0364
p-HQ3	tercyclohexylbenzene	6.349e+00	6.8027	6.836e+008	8.8348

3.2.2 Volatilisation

Information on volatilisation is included in 1.5.

3.2.3 Distribution modelling

Environmental distribution of selected constituents of terphenyl, hydrogenated was predicted by Level III fugacity model in EPI Suite v.4.11 (Table 36). The model predicts the relative distribution of a compound in the model environment at steady state (but no equilibrium conditions). When assuming that all constituents fulfil the P and vP criteria (Scenario 1) the constituents of terphenyl, hydrogenated, showed a partitioning of ≤0.13% to the air compartment, 1.7-34% to water, 32-85% to soil, and 7.5-60% to sediment. For those constituents (m-T, p-HT1, p-HT2, p-HT3, P-HQ1, p-HQ2, p-HQ3) for which no definitive conclusion on P or vP has been drawn, distribution modelling was performed also assuming a lower degradation half-life (Scenario 2). This resulted in a partitioning of ≤0.20% to the air compartment, 2.9-37% to water, 38-76% to soil, and 8.8-62% to sediment.

Table 36. Environmental distribution of selected constituents of terphenyl, hydrogenated as predicted by Level III fugacity model in EPI Suite v.4.11. The environmental half-lives used for the modelling are included in the footnotes. Other input parameters were according to the default settings of the software (including the emission values air 1000 kg/hr, water 1000 kg/hr, soil 1000 kg/hr)

		Air	Water	Soil	Sediment
Group	Compound				
Scenario 1: Calculation based on water, soil, and sediment half-lives exceeding the P and vP criteria)[a]					
o-T	ortho-terphenyl	0.134	2.91	52.4	44.5
m-T	meta-terphenyl	0.101	2.93	52.8	44.2
p-T	para-terphenyl	0.112	2.82	55.7	41.4
p-HT1	2-cyclohexylbiphenyl	0.0973	2.77	60.2	36.9
p-HT2	4-cyclohexylbiphenyl	0.0855	4.32	71	24.6
p-HT3	1,4-dicyclohexylbenzene	0.53	33.5	31.7	34.2
p-Q	para-quaterphenyl	0.0391	0.987	39.7	59.6
p-HQ1	4-cyclohexylterphenyl	0.0325	1.72	42.5	55.7
p-HQ2	dicyclohexylbiphenyl	0.0394	4.79	62.5	32.7
p-HQ3	tercyclohexylbenzene	0.0441	8.25	84.3	7.46
Scenario 2: Calculation based on water, soil, and sediment half-lives based on the assumption that biodegradation rates would correspond to 'readily biodegradable, but failing 10-d window'[b]					
m-T	meta-terphenyl	0.201	5.04	44	50.7
p-HT1	2-cyclohexylbiphenyl	0.192	4.95	50.8	44.1
p-HT2	4-cyclohexylbiphenyl	0.172	7.82	62.2	29.8
p-HT3	1,4-dicyclohexylbenzene	0.667	36.7	37.7	25
p-HQ1	4-cyclohexylterphenyl	0.0658	2.93	34.6	62.4
p-HQ2	dicyclohexylbiphenyl	0.0845	8.36	53.9	37.7
p-HQ3	tercyclohexylbenzene	0.0898	14.8	76.2	8.84

[a]For air, half-lives used were those listed in Table 7. For the other compartments, the half-lives used for the three-ring structures (o-T, m-T, p-T, p-HT1, p-HT2, p-HT3) were 5239 hours for soil (based on the lowest half-life of 218.3 days (at 12°C) observed for terphenyl based on Monsanto Company (1989)), 2620 hours for water (obtained by using a default water:soil half-life conversion factor of 1:2 used in EPI Suite Level III Fugacity model), and 23576 hours for sediment (obtained by using a default water:sediment half-life conversion factor of 1:9 used in EPI Suite Level III Fugacity model). For the four-ring structures (p-Q, p-HQ1, p-HQ2, p-HQ3) the half-lives were 2688 hours for soil (224 days, which is the estimated miminum half-life for quaterphenyl based on the assessment of the Monsanto Company (1989) study) whereas half-lives 5376 hours for water and 24192 hours for sediment were obtained using the conversion factors.
[b]This calculation was done only for those constituents for which no definitive conclusion on P or vP is drawn in the present SVHC proposal. The half-lives used for air were those listed inTable 7. For the other compartments, the half-lives used were 50 days (1200 hours) for surface water, 2160 hours for soil (corresponding to 'readily, but failing 10-d window according to Table R.16-5 and Table R16-6 in ECHA (2012)), and 10800 hours for sediment. The half-life for sediment is based on a default sediment:water half-life conversion factor of 9:1 used in EPI Suite Level III Fugacity model.

3.2.4 Field data

Environmental occurrence and behaviour of halogenated terphenyls and quaterphenyls have been investigated in several studies (Braune and Simon, 2004, Pagano, J. 1999, Fernandez et al. 1998, Gallagher et al. 1993). Less information is available, however, on the unhalogenated forms.

Partially hydrogenated terphenyls, which appeared to have a petrogenic origin, have been detected in the sediments of Bridgewater Bay (Severn estuary U.K.)(Killops and Howell 1988).

Terphenyls and quaterphenyls can be formed during mechano-chemical milling processes of dioxins (Nomura et. al. 2005), or during pyrolysis of benzene (You, et al. 1995, De Stefanis et al. 1994). Elevated levels of quaterphenyls have been identified in air particles affected by an e-waste recycling plant. Quaterphenyl containing particles were formed from the burning of hard plastic blocks, but not for the burning of wires/cables. (Gu et al. 2010).

Hydrogenated terphenyls have been used in colour formers for carbonless copy paper (CCP) and have been detected in food packaging made of recycled CCP and in food (Sturaro et. al. 1995).

3.3 Data indicating potential for long-range transport

Not assessed.

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

ESTIMATED DATA

Based on BCF estimations in fish, bioaccumulation is predicted to be significant for all assessed constituents with the exception of three ring hydrogenated quaterphenyls HQ2 and HQ3 Partially hydrogenated terphenyls (HT1) appear to have most bioaccumulation potential. (Table 37). An evaluation of the applicability and validity of the estimation models is included in Annex 2. Where reliable measured data on BCF-values and/or BMF-values are available, these are given more weight over predictions. The available QSAR tools to estimate logKow, biotranformation rate and BCF-values are not able to discern between ortho-, meta- and para-isomers of the constituents.

Table 37. Estimated data on log Kow and BCF values (EPIsuite BCF BAF v.3.01)

	logKow (KOW WIN)	BCF Episuite (Regression	BCF Arnot-Gobas, upper trophic (5 % lipids)	BCF Arnot-Gobas, upper trophic (5 % lipids), zero biotransformation	Biotrans-formation half-life normalised to 10 g fish (days)(Arnot-Gobas)
o-T (9.23 Å)	5.52	2041	1146	7425	6.44
m-T (11.5 Å)	5.52	2041	1035	7425	6.44
p-T (13.3 Å)	5.52 6.03*	2037 4422	1034 1301	7416 9776	6.44 9.23
o-HT1	6.57	10 100	1863	8313	21.7
m-HT1	6.57	10 100	1863	8313	21.7
p-HT1	6.57	10 100	1863	8313	21.7
o-HT2	7.63	6559	289	2571	16.4
m-HT2	7.63	6559	289	2571	16.4
p-HT2	7.63	6559	289	2571	16.4
o-HT3	8.55	2315	148	751	71
m-HT3	8.55	2315	148	751	71
p-HT3	8.55	2315	751	751	71
p-Q	7.28	9646	1499	3942	62.2
p-HQ1	8.34	2941	296	1036	99.4
p-HQ2	9.26	1038	(74)	(198)	230
p-HQ3	10.18	366	(6)	(26)	120

*) experimental

() value in bracket not considered reliable (for details see Annex II)

EXPERIMENTAL DATA ON BIOCONCENTRATION FACTORS

Schlechtriem et al. 2016 Fish Bioconcentration Studies with Column-Generated Analyte Concentrations of Slightly Hydrophobic Organic Chemicals

Schlechtriem et al. (2016) developed a solid-phase desorption dosing system, in order to generate stable concentrations of hydrophobic organic chemicals without using solubilizing agents for bioconcentration testing. The system was tested with hexachlorobenzene (HCB), o-terphenyl (o-T), polychlorinated biphenyl (PCB) 153 and dibenz[a,h]anthracene (DBA) in a flow-through fish tests with rainbow trout following the OECD 305 test guideline. Bioconcentration of o-terphenyl was tested simultaneously with HCB. The exposure concentration of o-terphenyl was 0.453 µg/l. A lipid and growth corrected kinetic BCF value of 12 993 was determined.

Table 38. Test design

Test material purity	99 % o-terphenyl,
Test tank	2 x 100 L filled with 70 L test medium (or water for controls)
Water supply	continuous flow 22 L/h
Temperature	14.6 – 15.8 °C
Concentration of dissolved oxygen	5.8 – 8.9 mg/l (> 60 % saturation level (5 mg/l) at 15 °C).
Lighting period	12 h : 12 h
Fish	Juvenile rainbow trout (*O. mykiss*) At start: Weight 3.3 ± 0.4 g Lipids: 5.0 ± 1.1 % End of uptake: weight: 10.6 ±2.2 g lipids: 5.8 ± 0.8 % At end of depuration: weight: 16.9 ± 2.8 g lipids: 5.8 ± 1.3 % Fish per tank: 70 (fish loading 3.3 g/l at beginning) Feeding. 1.5 % of body weight daily (Inicio Plus 0.8mm; Biomar)
Uptake/depuration period	56 / 28
Stock solution	Column-Generated Analyte Concentration 181 µg/l o-T,
Sampling	4 fish sampled at days 7, 14, 21, 28, 35, 42 49 and 56 (uptake) and 1, 2, 4, 8, 16 and 28 (depuration)

	At end of uptake and depuration periods 4 additional fish were sampled for lipid analysis.
Analysis	Water analysis: liquid-liquid extraction + GC-MS. Internal standard: o-T-d_{14}. DL = 0.1 µg/l. Fish analysis: accelerated solvent extraction with acetone: dichloromethane (1:1); GC-MS.

Results

The time-weighted average (TWA) exposure concentration of o-T in water was 0.453 µg/l and the concentrations were maintained within ± 20% limits (Figure 14). Uptake and depuration was measured (Figure 15) and kinetic BCF values were determined applying growth correction and normalising the results to 5 % lipids (Table 39). Tissue concentrations of o-terphenyl showed high deviation and decreased toward the end of the uptake period. According to the study authors, this might be explainded by the adaption of the biotransformation activity in fish following extended exposure, an effect observed in previous studies (Kleinow et al. 1987). Apparent steady state was achieved already after approximately 2 weeks exposure.

BCF values determined for HCB and PCB 153 were comparable to previous studies. DBA did not bioconcentrate in fish. Only minor concentrations or concentrations below the limit of quatification were observed in fish during uptake, which immediately disappeared at the onset of the elimination period. The low accumulation of DBA is explained by the authors by the efficient metabolism of PAHs in fish, which has been described in previous studies.

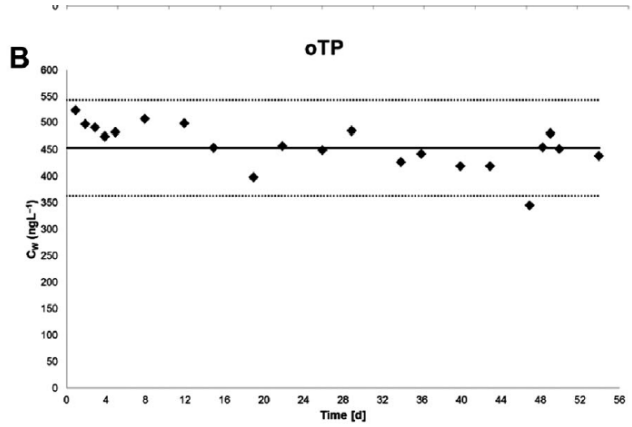


Figure 14. Time-weighted average exposure concentrations (solid line) ± 20 % (dotted lines) of o-terphenyl.

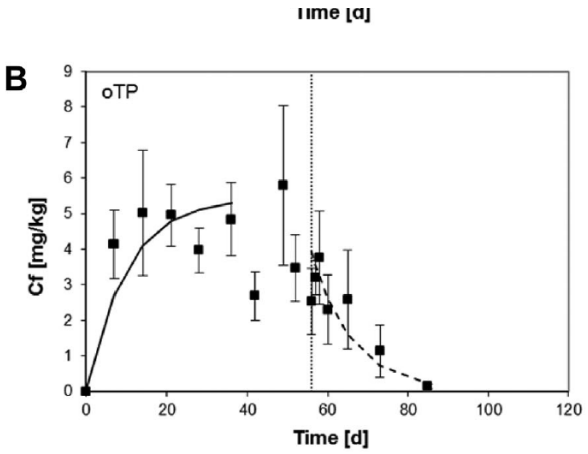


Figure 15. Uptake and depuration of o-terphenyl in rainbow trout. Each data point represents the mean concentration ± 1 standard error of all fish collected at each sampling interval. c_f=concentration in fish (mg/kg).

Test design

Table 39. Parameters for bioconcentration and growth data (based on wwt wt data)

Bioconcentration parameter	o-terphenyl	HCB	PCB 153
k_g (growth rate constant; d^{-1})	0.0201	0.0201	0.0226
k1 (uptake rate constant; Lkg^{-1} d^{-1})	1202	1210	443
k2 (depuration rate constant; d^{-1})	0.0999	0.0340	0.0239
$k2_g$ (growth corrected depuration rate constant; d^{-1}	0.0798	0.0139	0.0013
Cw (concentration in water (TWA); uptake phase. ngL^{-1})	453	390	23.0
Ln (lipid normalization factor; Lkg^{-1})	0.0580	0.0584	0.0740
BCF_k (kinetic bioconcentration factor; Lkg^{-1})	12 040	35 589	18 539
BCF_{kg} (growth corrected kinetic bioconcentration factor; Lkg^{-1})	15 072	87 051	340 825
BCF_{kL} (lipid normalised kinetic bioconcentration	10 379	30 467	12 526

factor; Lkg^{-1})			
BCF_{kgL} (lipid normalised, growth corrected kinetic bioconcentration factor; Lkg^{-1})	12 993	74 524	230 287
$t_{1/2}$ (half-life; d)	11.4	20.1	26.5
$t_{95\%}$ (time required to reach 95 % depuration; d)	34.4	87.9	

Reliability and relevance

The test is considered reliable (Klimish 1), as the OECD 305 test guideline validity criteria are fulfilled,

- The water temperature variation was less than ± 2°C
- The concentration of dissolved oxygen did not fall below 60% saturation;
- The concentration of the test substance in the chambers was maintained within ± 20% of the mean of the measured values during the uptake phase;
- The concentration of the test substance is below its limit of solubility in water
- Fish were observed throughout the test period

and in addition,

- Fish weight and lipid content are reported at start of up-take, end-of uptake and end of depuration and the kinetic BCF values are corrected for growth dilution and normalised to 5 % lipids
- In the study BCFs of substances with known bioaccumulation potential (HCB, PCB 153) and a PAH known not to bioconcentrate are determined with comparable results to previous studies.
- The analytical method is well described and test substance concentrations were above the limit of detection of the analytical method.

Although the study is aimed at testing a novel exposure technique, the study is essentially conducted in accordance with OECD 305 test guideline and is considered relevant.

Some minor deviation from the OECD 305 test guideline were as follows:

- The fish loading (3.3. g/l) was higher that recommended (0.1 – 1.0 g/l). However, as the concentration of test substance was maintained within ± 20% limits, and the concentration of dissolved oxygen did not fall below 60% saturation, this is not considered to compromise the results.
- The fish grew significantly during the test. According to the test guideline, the feeding rate should be selected such that fast growth and large increase of lipid content are avoided. (For example for rainbow trout between approximately 1 to 2 % of body weight per day). Nevertheless, as the results have been corrected

for growth dilution, this is not considered to compromise the results.

NITE 2012. Bioconcentration of o-terphenyl by Carp

In a flow-through Carp (*Cyprinus carpio)* test on 1,1':2',1''-terphenyl (CAS 84-15-1, ortho-terphenyl (o-T)) steady-state whole fish bioconcentration factors (BCFs) determined at 1 µg/l (level 1) and 0.1 µg/l (level 2) were 2300 ± 400 and 1400 ± 300, respectively. Lipid normalised (5 %) BCFs are 1900 ± 300 and 1100 ± 200, respectively. The depuration DT50 values were 1.9 days and 1.6 days, respectively. In addition, tissue BCF values (skin, head, innards, edible part) have been reported (Table 44).

Test design

Table 40. Test design

Test material purity	99.9 % (GC). Structure was verified by means of mass spectrometry and infrared spectroscopy.
Test tank	70L glass water tank
Water supply	Acclimatisation period: 0.04mL/min stock solution and 1600 mL/min testing water were supplied to the testing water tank at 2304 L/day. Excretion period: 1600 mL/min testing water.
Temperature	24.8 – 25.0 ºC
Concentration of dissolved oxygen	7.3 – 8.0 mg/l
Lighting period	14 hours light / 10 hours dark
Fish	Yearling carp juveniles (6.7 – 11.0 cm lengh) Lipid content: 4.34 – 7.42 % Feeding: appr. 3 % of body weight per day Feed composition: ≥ 43 % protein; ≥ 3 % lipids Fish were observed twice a day (once a day during holidays) Number of fish: levels 1 & 2: 48 (at beginning) controls: 12 (at beginning)
uptake/depuration period	uptake 60 days / depuration 5 days
Stock solution	dispersants: HC-40 and methoxyethanol level 1: test material conc. 40.0 mg/l level 2: test material conc. 4.00 mg/l
Sampling and analysis	Analytical method: GC-MS Fish analysis 5 sampling times during exposure; 4 fish per sampling; divided into two groups (two fish per group) 4 sampling times during excretion; 4 fish per sampling; divided into two groups (two fish per group) In the last consecutive 3 analyses, the lipid content was measured in the test fish of level 1 and 2. Controls: 4 fish divided into two groups (two fish per group) at beginning and end of test. In addition, two fish

	for lipid determination. For tissues analysis, sampling was performed once (2 fish).

Results/Conclusions

The measured concentrations in the test medium varied within ± 20 % window of the mean concentrations at both target concentrations (Table 41).

Steady-state was reached as the variation of the BCFs (corrected for lipids) measured on days 36, 48 and 60 was within the 20 % range. BCF-values determined as average values of two replicates from days 36, 48 and 60 were 2300 ± 400 (level 1) and 1400 ± 300 (level 2) (

Table 43). Lipid normalised (5 %) BCFs are 1900 ± 300 and 1100 ± 200, respectively.

Before the beginning of the test the lipid content in fish was 4.40 % and after completion of the test the lipid content was 5.10 %. During the exposure period the lipid content in fish increased upp to approximately 6 – 7 % (Table 42). Therefore, it seems possible that the fish grew during the exposure period, and the determined BCF values might underestimate actual values due to growth dilution. However, as the determined values are steady-state BCF values it is not possible to correct the results for growth dilution. Furthermore, there is no information on fish weights available.

BCF-values measured in tissues show high bioconcentration especially in internal organs (Table 44).

During the depuration period test material concentrations in fish were measured and the residual rate was calculated by setting the average steady state test concentration in fish at 100 % (Table 48). The depuration DT50 thus determined were 1.9 days (level 1) and 1.6 days (level 2).

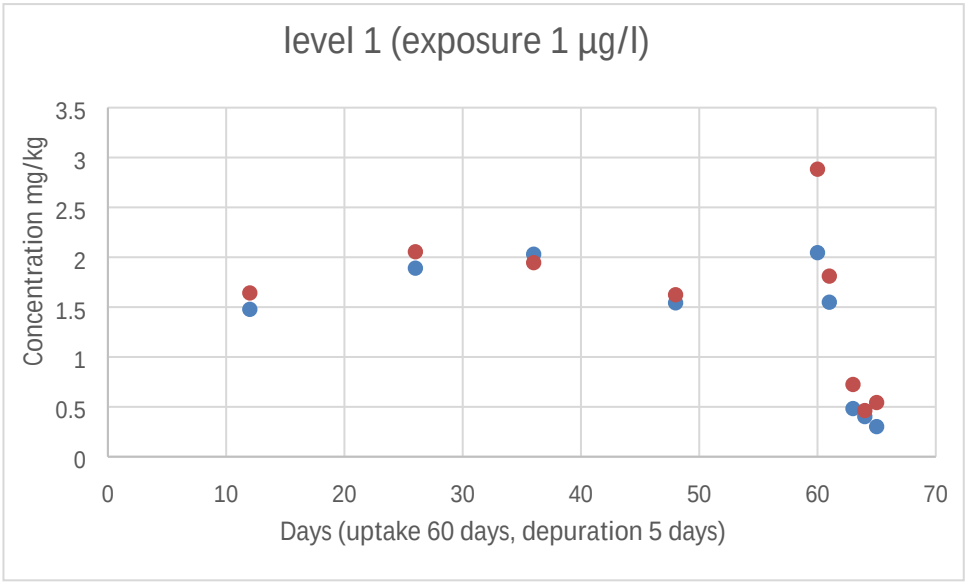
Reliability and relevance

The test is considered reliable with restrictions (Klimish 2), as the OECD 305 test guideline validity criteria are fulfilled,

- The water temperature variation was less than ± 2°C
- The concentration of dissolved oxygen did not fall below 60% saturation;
- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase;
- The concentration of the test substance is below its limit of solubility in water
- Fish were observed and recorded twice a day. No abnormalities were detected.

However,

- Concentrations in fish have not been reported.
- No information on fish weights is available. Therefore, the BCF values might have been affected by growth dilution.



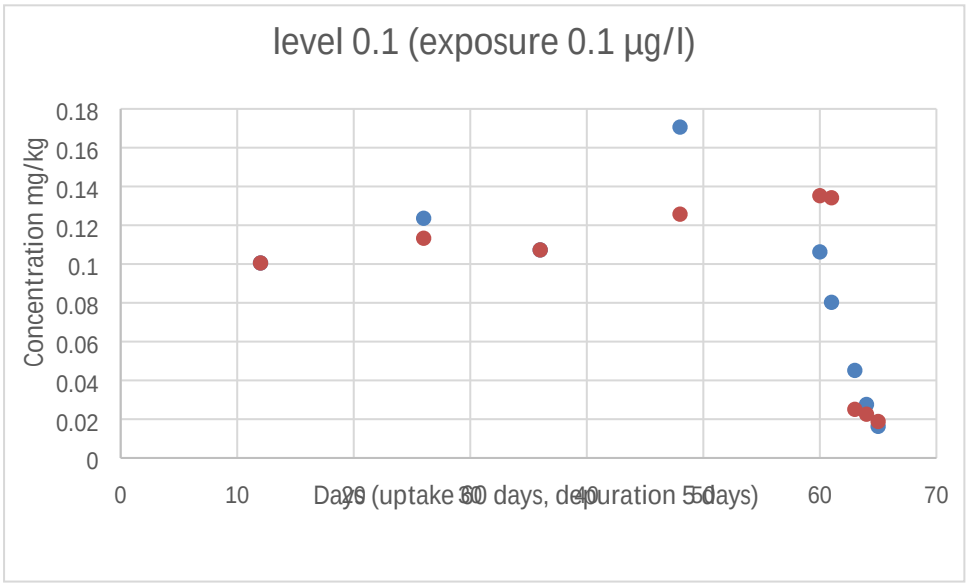


Figure 16. Concentrations of o-terphenyl in fish (mg/kg) during exposure and depuration back calculated from BCF values and depuration rate, respectively.

Table 41. Measured concentrations in test medium (µg/l)

Level	Day 8	Day 12	Day 26	Day 36	Day 48	Day 60	arit. mean	std
1. (1 µg/l)	0.821	0.822	0.846	0.812	0.93	0.887	0.853	0.046
2. (0.1 µg/l)	0.0859	0.0914	0.103	0.0894	0.0898	0.0966	0.093	0.006

Table 42. Lipid content (%) in fish.

Level	Day 36	Day 48	Day 60	Average
1. (1 µg/l)	5.74 5.28	6.04 5.84	7.32 7.04	6.21

2. (0.1 µg/l)	4.34 5.12	7.39 6.59	5.56 7.42	6.07

Table 43. BCF values during exposure period (whole fish, no lipid correction). Average values in brackets.

Level	Day 12	Day 26	Day 36	Day 48	Day 60
1. (1 µg/l)	1800 2000 (1900)	2300 2500 (2400)	2400 2300 (2300)	1900 2000 (2000)	2200 3100 (2700)
2. (0.1 µg/l)	1100 1100 (1100)	1200 1100 (1200)	1200 1200 (1200)	1900 1400 (1700)	1100 1400 (1200)

Table 44. Bioconcentration factors (BCFs) in individual parts.

Level	Part	BCF
1. (1 µg/l)	Integument	2200
		1400
	Head	2700
		2500
	Internal organs	6400
		6000
	Edible part	990
		1200
2. (0.1 µg/l)	Integument	1300
		1300
	Head	1500
		1700
	Internal organs	3100
		3800
	Edible part	610
		790

Table 45. Residual rate in excretion test (% steady-state fish concentration).

Level	Day 1	Day 3	Day 4	Day 5
1. (1 µg/l)	77 90	24 36	20 23	15 27
2. (0.1 µg/l)	64 107	36 20	22 18	13 15

NOTOX 2009b. Bioconcentration of m, m-quaterphenyl (Q) by Carp

A flow-through test was carried out with carp according to OECD 305 test guideline and Good Laboratory Practise (GLP). Fish were exposed to both m,m-quaterphenyl and radiolabelled [Ring-A-^{14}C] m,m-quaterphenyl at two concentrations (0.2 and 2 µg/l). The steady-state BCF values for m,m-quaterphenyl at target concentrations of 0.2 and 2 µg/l were 2727 ± 329 and 3837 ± 85, respectively. The kinetic BCF values of m,m-quaterphenyl at target concentrations of 0.2 and 2 µg/l were 3064 and 3911, respectively. The lipid concentration of the tested fish was 6 % at the end of exposure). The lipid normalised (to 5 % lipid concentration assuming 6 % actual lipid content) BCF values, calculated for the present assessment, are 2273 ± 274 and 3198 ± 71 (steady state), 2553 and 3259 (kinetic), respectively.

Test design

According to information from the sponsor, the solubility of the test substance in water was 4 µg/l (calculated value, ADC labs) and of mixed quaterphenyls 2 µg/l.

Table 46. Test desing

Test material purity	not provided (for unlabelled test material)
Test tank	stainless steel with removable perspex plate (64 L)
Water supply	medium was supplied at a flow rate 13 L/h.
Temperature	20.4 – 22.0 °C
Concentration of dissolved oxygen	4.0 – 8.9 mg/l (7.4 ± 1.25 mg/l) (The oxygen content dropped below 60 % of saturation on day 6. Aeration was introduced after six days of exposure and maintained for one day. During all other measurement days (30 in total) oxyen level was above 60 % saturation).
Lighting period	16 hours photoperiod daily
Fish	Carp Initial length: 3.5 ± 0.1 cm Initial weight 1.43 ± 0.16 g Lipid content: 9 % in the beginning; 7 % (in control) and 6 % in high and low exposure concentrations at day 30 (end of exposure period). Feeding: daily with pelleted food (Cyprico Crumble Excellent (300 – 500 um)) 54 fish per concentration 42 fish in control maximum loading 0.20 g/L/day (In total 150 fish)
uptake/depuration period	uptake 30 days / depuration 28 days

Stock solution	Stock solutions (2 and 20 mg/l) were prepared in acetone and dosed via computer-controlled system into a mixing flask separately from the medium supply.
Sampling and analysis	6 sampling times during exposure, 4 sampling times during depuration: 4 fish per concentration and 2 fish per control. For lipid analysis 10 fish (control) at beginning and 3 x 10 fish (control, low and high concentrations) at end of exposure period were sampled. Treatment of fish samples for quantitative analysis: fish tissues in each replicate were dissolved overnight in Solvable at app. 60°C. Thereafter triplicate samples corresponding with ca. 200 mg fish were transferred to liquid scintillation (LSC) vials and bleached with 30 % H_2O_2. Radiochemically labelled substance (purity 98.5 % by HPLC) was analyzed from water and fish samples. The lower test concentration consisted entirely of the labelled substance. The higher test concentration consisted 10 % of the labelled substance 90 % unlabelled) Recovery in water samples 88 – 110 %.

Results

The mean concentrations of m,m-quaterphenyl (i.e. total radioactivity) in medium were 0.16 ± 0.032 and 1.6 ± 0.23 µg/l at target concentrations of 0.2 and 2 µg/l, respectively. Based on HPLC results, the mean concentrations of m,m-quaterphenyl were 0.13 ± 0.023 µg/l and 1.3 ± 0.22 µg/l. The measured concentrations varied within ± 20 % window of the mean concentrations in fish at both target concentrations. No degradation products exceeding >10 % of applied radioactivity were detected in the test water.

Samples were taken 6 times from water and fish during uptake phase and 4 times from fish during depuration phase. The steady state concentration in fish was reached after 7 days of exposure for the 0.2 µg/l target concentration and after 14 days of exposure for the 2 µg/l target concentration. During steady-state the mean concentration of m,m-quaterphenyl in fish was 0.40 ± 0.034 mg/kg and 5.6 ± 0.38 mg/kg for target concentrations of 0.2 and 2 µg/l, respectively (Figure 17, Figure 18). Depuration was relatively rapid based on DT50 values: DT50 values at target concentration of 0.2 and 2 µg/l were 3.6 and 2.8 days, respectively.

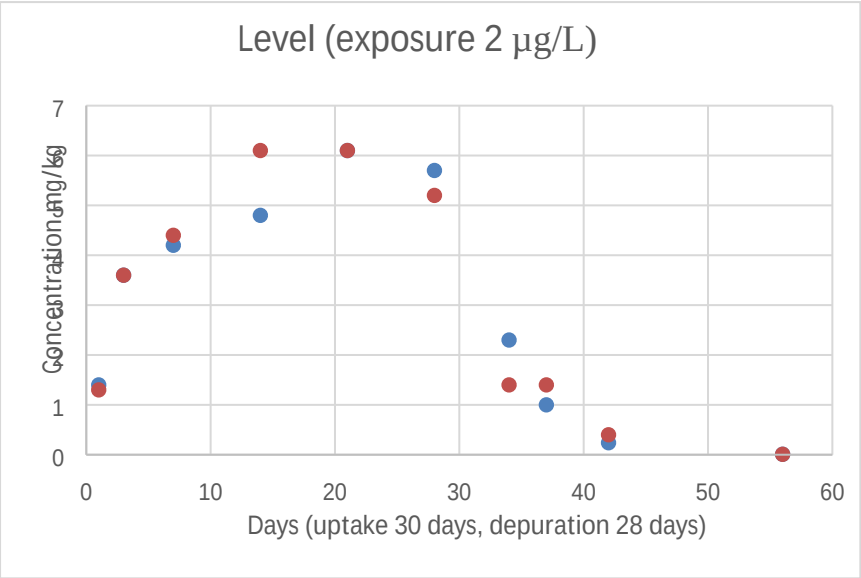


Figure 17. Concentration of m,m-quaterphenyl in fish (mg/kg) vs. time (days)

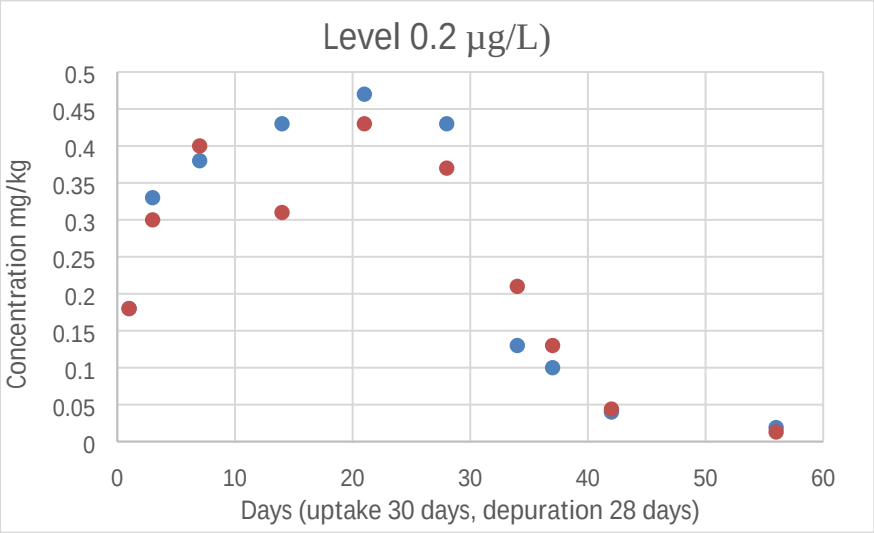


Figure 18. Concentration of m,m-quaterphenyl in fish (mg/kg) vs. time (days)

Reliability and relevance

The test is considered reliable with restrictions (Klimish 2) as the validity criteria of OECD 305 test guideline are generally fulfilled,

- The water temperature variation was less than ± 2°C
- The concentration of dissolved oxygen did not fall below 60 % saturation with one exception on the sixth day of uptake. Fish were not observed to be affected by this temporal lower oxygen level.

- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase;

- The concentration of the test substance is below its limit of solubility in water

- No mortality or other adverse effects were observed in control or treated fish during the test.

Although the purity of the unlabeled test material is not known, the lower test

concentration consisted entirely of the radiochemically labelled substance (purity 98.5 % by HPLC).

The results could not be corrected for possible growth effects, as fish weights at the end of the test were not reported. Neither was the feeding rate reported. Based on the decrease of lipid content in the fish (from 9 % at the beginning to 6 % at end of exposure), it seems possible that the fish lost weight during the test. Therefore, the BCFs might overestimate the actual BCFs to some extent.

NITE 2004. Bioconcentration of terphenyls, hydrogenated (HT1, HT2) by Carp

In an GLP OECD 305 flow-through bioconcentration test on Carp (*Cyprinus carpio),* the fish were exposed to terphenyls, hydrogenated at two exposure levels, 1.99 µg/l (level 1) and 0.199 µg/l (level 2). BCF values were determined for two analytical groups representing 1) ortho-,meta- and/or para-cyclohexyl biphenyls (HT1), and 2) ortho-, meta- and/or para-dicyclohexyl benzenes (HT2). The steady state BCFs ranged between 1551 – 12 436. Depuration half-lives after exposure were in the range of 1.9 – 3.5 day.

Test design

Table 47. Test design

Test material purity	91.5 % (o-terphenyl (5.2 %), m-terphenyl (2.7 %) and p-terphenyl (0.7 %) are reported as impurities). The structure of the tested material was identified using infrared and mass spectrometry.
Test tank	100 L glass tanks
Water supply	2000 mL/min testing water at 2880 L/day (+ 40 µL/min stock solution during exposure period)
Temperature	24.2 – 25.3 ^{O}C
Concentration of dissolved oxygen	6.0 – 8.1 mg/l
Lighting period	14 hours light / 10 hours dark
Fish	*Cyprinus caprio* yearlings (length: 7.6 – 11.5 cm, lipid content at start 3.59 %; during steady state 6.48 % and after completion of test 5.87 %) 54 fish per level 12 fish per control Feeding: 2 % of fish body weight per day Feed composition: (≥ 43 % protein; ≥ 3 % lipids) Fish were observed twice a day (once a day during holidays).
Uptake/depuration period	60 days / 5 days (level 1), 8 days (level 2)
Stock solution	Dispersants: HCO-40 and 2-methoxyethanol
Sampling and analysis	GC-MS Recovery rate: 98.1 – 105 % (water); 90.1 – 85.4 % (fish)

Results

BCF values were determined for seven analytical GC-MS peaks (Table 45). The peaks A-G relate to ortho-, meta-and para-dicyclohexyl benzenes (HT2) (MW 242.4 g/mol) and cyclohexyl biphenyls (HT1) (MW 236.4 g/mol). (The completely hydrogenated terphenyl has a molecular weight of 248.46 g/mol.) The o, m- and p-isomers have slightly different retention times resulting in separate peaks.

Based on the variation of the BCF values, it was determined that steady state was reached at day 39. Steady state BCFs ranged between 1551 – 12 436 (Table 51). The results suggest that there are significant differences in bioaccumulation between the isomers. However, as the identity of the peaks is based only on molecular weight, it is not possible to discern between the ortho-, meta- and para-isomers.

All water solubility estimates for the above-mentioned constituents, (see Table 5), are above the exposure concentrations (seeTable 45) of the test. It can be hence expected that the tests have been carried out below the solubility limits of the substances in the test conditions. The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase.

Reliability and relevance

The test is considered valid with restrictions (Klimisch 2) as the OECD 305 validation criteria are fulfilled,

- The water temperature variation was less than ± 2°C
- The concentration of dissolved oxygen did not fall below 60% saturation

- The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase;

- The concentration of the test substance is below its limit of solubility in water

- The condition of fish was observed twice a day. No abnormalities were detected.

However,

- Concentrations in fish were not reported. Fish weight at end of test are not reported. Therefore, the BCF values might have been affected by growth dilution.

In addition, it is noted, that the BCF values determined at the higher exposure concentration (level 1) are significantly higher than those determined at the lower (level 2). According to OECD 2017, “Differences in BCF values between two exposure concentrations might arise where the (organic) chemical in question requires metabolisation before it can be eliminated. Saturation of the metabolic mechanisms in the fish could result in dramatic increases in the BCF value when the exposure concentration is increased (conversely, BCF values at intermediate concentrations might decrease if a certain body burden is required before relevant metabolic pathways start to operate). Whether such effects have occurred in this study is not known. It is also possible that higher bioconcentration at higher exposure happened due to other reasons such as if the fish were stressed and therefore increased their respiration rate at the higher concentration.

Table 48. BCF values (average lipid content 6.5 %)

Peak (MW)	Level	Average conc. in water µg/l (SD)	BCF				
			After 14 days	After 28 days	After 39 days	After 46 days	After 60 days

		Average	BCF				
A (242.40)	1	1.96 (0.066)	3700 3600	3800 3800	4700 4600	5400 4300	4700 4600
	2	0.190 (0.0020)	1300 1600	1800 1800	2700 3200	1800 1700	1900 1900
B (242.40)	1	1.95 (0.066)	4400 4400	4600 4700	6200 6200	6900 6000	6300 6500
	2	0.189 (0.0056)	1700 1800	2200 2000	3300 3700	2200 2200	2500 2400
C (242.40)	1	1.90 (0.076)	3800 4100	4700 4900	5000 5100	6000 4000	4200 4400
	2	0.186 (0.0028)	1900 1900	2400 2300	3500 3900	2300 2400	2500 2400
D (236.35)	1	1.98 (0.080)	11000 12000	15000 15000	16000 16000	18000 15000	16000 16000
	2	0.194 (0.0052)	4300 5500	5200 5200	8600 12000	6500 5700	6700 6900
E (242.40)	1	2.02 (0.058)	6100 6000	7400 6900	9200 9000	9800 8700	9600 9500
	2	0.197 (0.0043)	1800 1900	1900 2000	3000 3400	2400 2100	2300 2400
F (236.35)	1	1.94 (0.065)	3300 3200	3100 3200	3600 3600	4500 3500	3800 3700
	2	0.193 (0.0028)	1500 1800	1900 1800	2700 3400	2000 1800	2100 2100
G (236.35)	1	1.95 (0.050)	3500 3700	4200 4300	4500 4700	5200 4400	4700 4900
	2	0.192 (0.0061)	1100 1200	1400 1500	2300 2900	1600 1500	1900 1900

Table 49. Mean BCF values at steady-state (corrected for 5 % lipids)

	SS-BCF		SS-BCF	
	mean	std	mean	std
LEVEL 1			LEVEL 2	
A	3628	281	1692	467
B	4885	242	2090	485

C	3679	568	2179	528
D	12436	756	5949	1767
E	7154	315	2000	380
F	2910	281	1808	458
G	3641	221	1551	396

Table 50. Depuration half-lives.

Peak	Level 1	Level 2
A	1.9 days	3.2 days
B	2.0 days	3.3 days
C	2.4 days	3.5 days
D	2.1 days	3.6 days
E	1.9 days	3.6 days
F	2.1 days	2.4 days
G	1.9 days	2.3 days

Table 51. BCF values at steady state (average of days 39 – 60) normalised to 5 % lipids

	level 1 1.99 µg/l	level 2 0.199 µg/l
ortho-, meta-and/or para-dicyclohexyl benzenes (HT2); peaks with MW of 242.40)(peaks A, B, C, E)	– 3628 - 7154	– 1692 – 2179
ortho-, meta- and/or para-cyclohexyl biphenyls (HT1); peaks with MW 236.4 g/mol)(peaks D, F, G)	2910 - 12 436	1551 - 5949

MONSANTO 1983. Bioconcentration of MXP-2020 by Bluegill (Lepomis macrochirus).

Bluegill fish (*Lepomis macrochirus*) were exposed to a mean concentration of 32 µg/l of MXP-2020 (nominal concentration 50 µg/l) in a flow through system for 42 days, after which a depuration phase of 42 days took place. BCF values were determined for three analytical groups representing 1) o-terphenyl (o-T) and 1-ring saturated terphenyls (HT1), 2) mixture of terphenyls with one (HT1) and two rings (HT2) saturated and 3) quaterphenyls (Q) with one (HQ1) and two rings (HQ2) saturated.

Test design

Table 52. Test design

Test material purity	See “Test material and solutions”
Test tank	57 L
Water supply	A continuous flow diluter system (70L water/hour to the test chamber and 35L water /hour to the control chamber). 1.20 mL of MXP-2020 stock per hour to the mixing chamber. Treated city water was used as dilution water.
Temperature	22°C (± 1°C)
Concentration of dissolved oxygen	6.14 mg/l (> 60 % of the saturation level at the test temperature. During the test on a limited number of occasions the dissolved oxygen level fell below 60 % saturation.)
Lighting period	
Fish	130 fish / tank (app. 20 g/ L exceeding the OECD 305 recommendation significantly.) *Lepomis macrochirus* (= bluegill) - Age at study initiation: 8 months - Weight at study initiation: 11.5 ± 1.855 g - Lenght at study initiation: 71.8 ± 4.08 mm - Lipid content: no info - Health status: healthy at initiation of the study - Feeding during test: 2 % of their body weight
Uptake/depuration period	42 days uptake / 42 days depuration
Stock solution	2.916 g/l in dimethylformamide (DMF). Max. solvent concentarion in test solutions 0.02 ml/L.
Sampling and analysis	9 sampling times during uptake and 7 during depuration (4 fish / sampling) (Additional sampling for muscle tissue.) Controls: 3 sampling times (4 fish / sampling) Water samples were collected and analysed each time fish were sampled except on day 38 (no water samle). Analysis: “See Fish analysis and Water analysis”

TEST MATERIAL AND SOLUTIONS

MXP-2020 is a mixture of at least thirty components. Examination of the product by GC/FID and GC/MS indicated that more than 72 % of the product appeared to be terphenyl (T) and terphenyl with one (HT1) or two (HT2) saturated rings. Slightly over 27 % appeared to be quaterphenyls with two saturated rings (HQ2).

A sample of MXP-2020 was characterised and it was found that the gas chromatograms could easily be separated into three groups:

Group I: 5.8 % of total product, has three peaks and probably contains o-terphenyl and some terphenyl with one ring saturated

Group II: 66.8% of the total, has six to eight peaks and contains a mixture of terphenyls with one and two rings saturated

Group III: 27.4 % of the total MXP-2020 and has ten to twenty peaks which appear to be mostly quaterphenyls with one and two rings saturated

FISH ANALYSIS

Fish samples were extracted with hexane and alumina column and analysed with Gas Chromatography – Flame Ionisation Detector (GC-FID). Based on spiked samples, the mean percent recovery during method development were 110% for group I, 103% for group II and 111% for group III. The mean percent recoveries, based on spiked samples, during the exposure period were 120, 109 and 58 % for groups I, II and III, respectively. It is noted that the variation in recovery is quite high, especially for group III, increasing uncertainty in the results.

Because of incomplete sample clean-up during residue analysis, some interpherences were present in the chromatograms and thus were calculated as concentrations of MXP-2020. For groups I and II, the levels of interferences were similar to those observed during method development. For group III the interpherence was 3 times higher in the experimental controls than observed during method development. The authors conclude that “Group III analysis may overestimate the amount of total residue by a factor of three”. And further “Because of analytical limitations, results for Group III should be carefully scrutinised. The product may not have reached equilibrium; however, there is some question as to exactly what was being measured by the fish tissue analytical method for this group.”

The varying interpherence in the control samples (“high background noise”), raises the level of detection/determination of the analytical method, but the possible systematic error should have been corrected by blank extractions. Based on the study report, it is not clear whether blank values (determined during the study) were extracted from measured values. The standard deviation of blanks determined during the study were 0.35, 2.18 and 2.21 ppm for Groups I, II and III respectively. This is quite high, especially for Group III, in comparison to the reported “lowest level of validation” (meaning presumably quantitative or qualitative determination limit). The lowest level of validation for groups I, II and III were 0.5, 6 and 2 ppm, respectively.

WATER ANALYSES

MXP-2020 was extracted from city water with methylene chloride and was concentrated with a Kuderna-Danish evaporative concentrator. MXP-2020 was measured GC-FID. Results were calculated for total MXP-2020 and for the three groups. Based on spiked samples, the mean percent recoveries during method development were 90, 91, 86 and 129 % for MXP-2020 total, group I, group II and group III, respectively. The mean percent recoveries from spiked samples during exposure period were 85, 86, 82 and 91 % MXP-2020 total, group I, group II and group III, respectively. The lowest level of validation was 5 ppb.

Results

Based on graphical interpretation of a plot of the whole fish tissue concentration versus time (Figures 1 – 3 and Table 53) and one-way Analysis of Variance (ANOVA) in conjunction with Dunnett’s t-test, it was determined by the study authors that apparent steady-state was reached by day 2 for group I and by day 5 for group II. Time to equilibrium could not be determined for group III. BCF values for Group III were determined from the beginning of the exposure period (day 1).

The BCF for the total component residue was calculated by dividing the mean fish concentration for each day during equilibruim (5 - 42 days) by the mean water concentration (32 µg/L std. 3.62 µg/L) and averaging these values to obtain a mean BCF for the entire equilibrium period (Table 53).

Water concentrations used for the calculations of BCFs for the different groups:

Group I: for days 5 - 42 at a mean exposure concentration of 1.5 µg/L (std 0.6 µg/L ~ 40 %)

Group II: for days 5 - 42 at a mean exposure concentration of 19 µg/L (std 3.5 µg/L ~ 18 %)

Group III: for days 5 - 42 at a mean exposure concentration of 11 µg/L (std 2.8 µg/L ~ 26 %)

It is noted that for Group I the mean measured exposure concentration is below the "lowest level of validation" (5 µg/L) and therefore related with considerable uncertainty.

Based on the GC/MS chromatograms in water vs. fish, it is apparent that the ratio of constituents changed during the study. According to the study authors, "chemicals were concentrated, they changed in ratio to one another, as compared to the parent mixture. This indicates that there is some selective adsorption and/or metabolism."

Table 53. Mean concentrations (n = 4) in whole fish during steady-state and steady-state BCFs determined for MXP-2020 and its analytical/constituent groups. For BCF calculations the mean water concentrations of 1.5, 19 and 11 µg/l were used for Groups I, II and III respectively.

Day	MXP-2020 total residue		Group I		Group II		Group III	
	mg/kg	BCF	mg/kg	BCF	mg/kg	BCF	mg/kg	BCF
1							5.2	460
2			2.2	1500			7.0	620
5	37	1100	4.6	3200	26	1400	6.2	550
7	75	2300	9.2	6300	55	2900	11	990
14	66	2100	8.2	5600	46	2500	12	1100
21	73	2300	8.9	6100	52	2800	12	1100
28	72	2200	11	7200	54	2900	8.1	720
35	66	2000	11	7400	48	2500	7.2	640
42	53	1600	6.8	4600	38	2000	8.7	770
mean		2000		5200		2400		770
std		430		2000		550		230

RATE CONSTANTS

Rate constants were estimated based on a plot of the tissue concentration versus time. K2 (the depuration rate constant) was derived by taking the negative slope (-S) of the plot of the depuration data. The uptake rate constant (K1) as calculated using the following equation;

K1 = (CfiK2)/(Cw(1-exp(-K2ti)));

where Cfi = concentration in the fish at time I; Cw = mean exposure concentration; ti = time interval during uptake

The time for clearance of half the material from the fish (T1/2) is predicted by the equation T1/2 = ln2/K2.

Time to 90 % of steady state was determined by calculating 90 % of the mean

concentration of the residues in fish tissue during equilibrium and determining where that value first appeared on the graph. For muscle tissue, the time to 90 % steady state could not be determined because samples were only taken at equilibrium.

Table 54. Rate constants determined for MXP-2020 and its' analytical/constituent groups

	BCFkin	k1 (L/kg/day)	k2 (1/day)	T1/2 days	90 % SS days
MXP-2020 total	4801	48.06	0.01	69	6.5
Group I	8148	162.95	0.02	35	6
Group II	3548	70.95	0.02	35	6.5
Group III	4980	19.92	0.004	69	6

DOW BIOFAC

The Dow Biofac program was used to calculate kinetic BCFs, rate constants, time to 90 % of equilibrium and T1/2s (Table 55). According to the study authors "It is difficult to say which calculations are "more correct". Biofac uses a more sophisticated statistical package to determine its' rate constants. It then uses rate constants to make BCF, T1/2 and 90 % of equilibrium calculations." It is noted that regarding the kinetic BCFs, the two different methods give reasonably similar results.

Table 55. Kinetic BCF-values and rate constants determined with Dow Biofac model.

	BCF	k1 (L/kg/day)	k2 (1/day)	T1/2 days	90 % SS days
MXP-2020 total	3000	192.16	0.06	11	36
Group I	9100	611.74	0.07	10	34
Group II	3700	249.14	0.07	10	34
Group III	2700	35.09	0.01	53	175

Figure 2. MXP-2020 Group I concentration in wholefish tissue over time as plotted by the Biofac model. Each X represents the mean concentration of Group I observed for that day.

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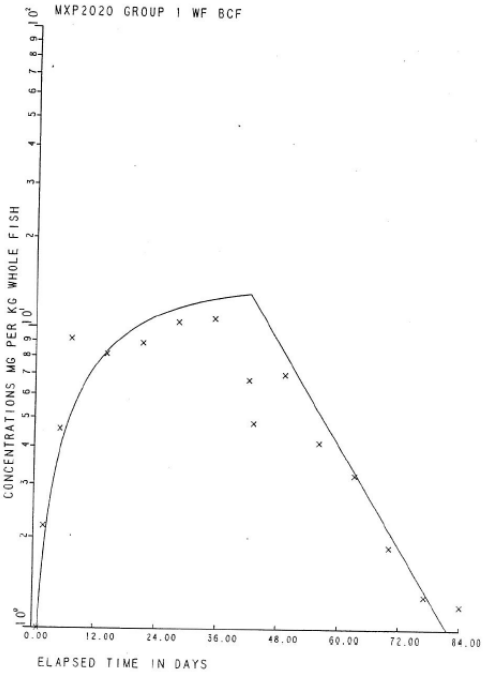

Figure 3. MXP-2020 Group II concentration in wholefish tissue over time as plotted by the Biofac model. Each X represents the mean concentration of Group II observed for that day.

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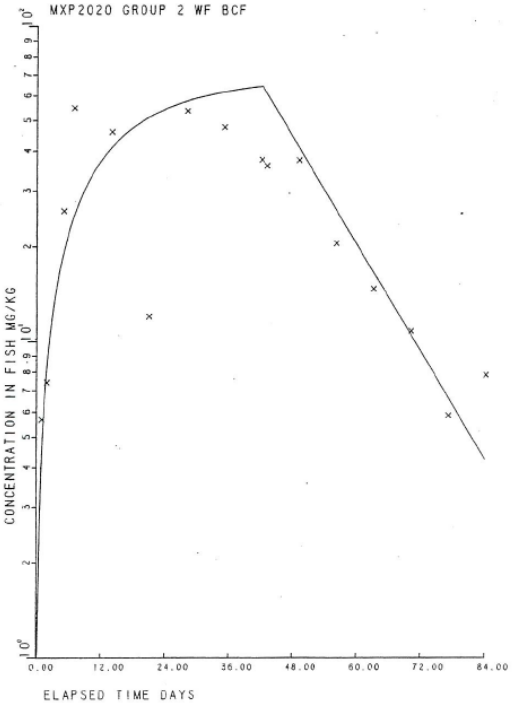

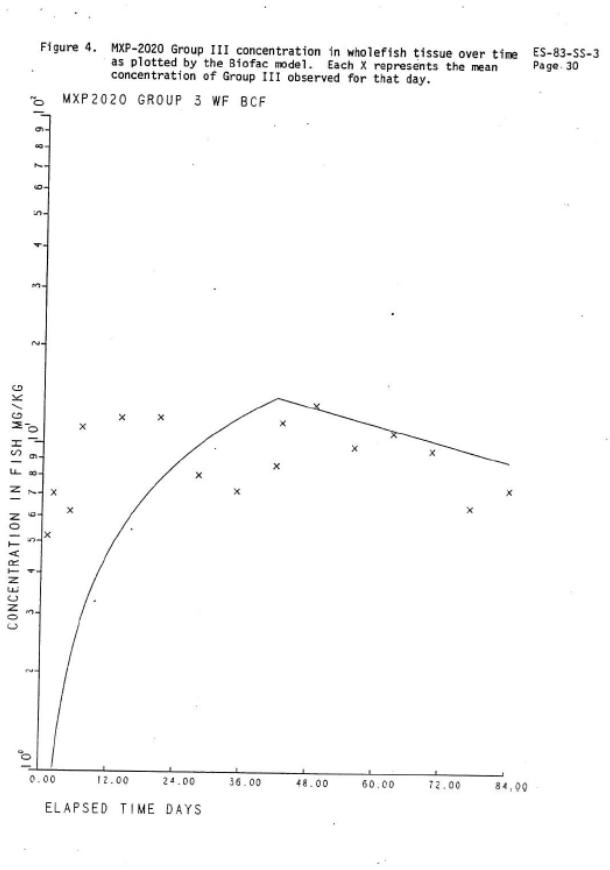

Figure 19. Concentration in whole fish over time as plotted by the Biofac model for Groups I, II and III.

Table 56. BCF-values (whole fish). Values not considered reliable are in brackets.

GC-MS group	Constituents	% in MXP-2020	Exposure conc. µg/l	BCF (steady-state)	BCF (kinetic)	BCF Dow biofac (kinetic)
I	o-T and HT1	5.8	1.46	(5200)	8148	9100
II	HT1 and HT2	66.8	18.76	2400	3548	3700
III	HQ1 and HQ2	27.4	11.27	(770)	(4980)	(2700)

Reliability and relevance

The following OECD 305 validity criteria are fulfilled,

- The water temperature variation was less than ± 2°C
- Fish mortality < 10 % (Six mortalities were observed during the study, one in the exposure tanks and five in the control aquaria (< 5 %). All six fish appeared to have fungus growth. All other fish looked healthy.)
- The exposure concentration of test substance in the chambers was maintained within ± 20% of the mean of the measured values during the uptake phase for Group II.
- The exposure concentration of Group I was probably below its limit of solubility in

water.

The following OECD 305 validity criteria were compromised,

- During the test on a limited number of occasions the dissolved oxygen level fell below 60% saturation. (The occasional low level of oxygen might reflect the fact that the fish loading was significantly higher than recommended.)

- The exposure concentration of test substance in the chambers was not maintained within ± 20% of the mean of the measured values during the uptake phase for groups I and III. In addition, for Group I the exposure concentration was below the "lowest level of validation".

- For Group II, and especially for Group III, the exposure concentration might have exceeded the water solubility. It is, however, difficult to estimate whether concentrations were below or above the water solubility, as estimates for water solubility vary significantly (Table 5) and the exact composition of the test substances is not known.

Therefore, as the test substance concentrations in water varied approximately 40 % for Group I the steady state BCFs are not considered reliable for Group I. Since the kinetic BCFs are not so sensitive to measured exposure concentrations in water, the kinetic BCF values may be considered valid with restrictions for Group I.

For Group II both steady-state and kinetic BCF values can be considered valid with restrictions.

With regard to Group III, the results are not considered reliable (Klimish 3), because, in addition to the uncertainty in measured water and fish concentrations, steady state was not achieved during the exposure period. This might be due to the fact that the concentrations in fish were around and possible below the method determination limit.

In addition, it is noted that

- fish weight at end of test were not reported and the results were not corrected for growth dilution.
- fish lipid content was not reported and the results could not be normalized for 5 % lipids.

NITE 1978 Bioconcentration of terphenyl by Carp

In NITE 1978 (J-CHECK) a flow-through BCF test on (unhydrogenated) terphenyl (CAS 26140-60-3) is reported. The BCFs determined (at week 2, 4, 6 and 8) in Carp (*Cyprinus carpio)* at 0.25 and 0.025 ppm (mg/l) ranged between 15 to 80 and 21 to 129, respectively (Table 58).

Study design

Test material purity	"Terphenyl isomers, main component contains diphenyls and polyphenyls." The ratio of o-T: m-T: pT was determined as 14.6:58.8:26.6.
Test tank	Glass tank, 100 L tanks
Water supply	Flow-through system (water flow 582 L/day).Source dilution water 4 ml:400 ml.
Temperature	25 ± 2 ºC
Concentration of dissolved oxygen	information not available

Lighting period	
Fish	*Cyprinus caprio* (average weight 22 g, average body length 11 cm)
uptake/depuration period	8 weeks (56 days)
Stock solution	1 g of test material dissolved in hydrogenated castor oil and diluted with water to 1000 ppm (w/v)
Sampling and analysis	Fish sample treatment: after body weight measurement fish were chopped, treated with heat dissolution in N-potassium hydroxide ethyl alcohol and extracted with hexane. HPLC-LQ (detection limit app. 0.5 ppm in water, 0.25 ppm in fish; water samples were concentrated in chloroform)

Results

The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake (Table 57). Exposure level 1 test concentrations might have been above the water solubility of the substance (Table 5). The variation between BCFs values measured at weeks 4-6 is quite high (> 20%) and therefore it is not clear whether steady-state was achieved during the test.

In addition to the BCF values determined by Liquid Chromatography (LC), BCFs were measured also by Gas Chromatography and the results were compared (Table 59). Based on these results, the study authors conclude that o-terphenyl is selectively bioconcentrated. It is difficult to interpret the results as part of the information is illegible/missing. E.g. in the study report Figures 28, 29 and Tables 12-14 are referred to but they are missing.

Table 57. Average concentrations used for bioconcentration factor calculations (mg/l)

level	2 week	4 weeks	6 weeks	8 weeks
level 1 (0.25 mg/l)	0.22	0.21	0.22	0.21
level 2 (0.025 mg/l)	0.016	0.017	0.0196	0.019

Table 58. BCFs values determined by LC.

level 1	2w	4w	6w	8w
0.25 ppm	50	18	44	20
0.25 ppm	59	80	74	15
average	55	49	59	17.5
level 2				
0.025 ppm	129	29	62	71
0.025 ppm	45	21	38	38
average	87	25	50	55

Table 59. Fish (n=4) after 8 weeks of rearing were used for measurements with GC and

LC and the results were compared

Fish 8 weeks	BCF according to GC ortho (?)	meta (?)	para (?)		BCF according to LC
a	418.7	15.6	29.8	80.1	41.3
b	460.7	10.1	38.7	86.3	44.0
c	322.1	illegible	-----	48.9	31.1
d	180.5	13.6	18.5	37.8	30.8

Reliability and relevance

The reliability of the test is considered as not assignable (Klimish 4) because,

- The figures on dissoleved oxygen levels are missing from the report – therefore, the OECD 305 validity criterion on dissolved oxygen level (> 60% saturation) is not possible to verify.
- There is no information on the condition of the fish
- Tables 3, 4, 6, 12-14 are missing from the full study report and some figures are illegible
- It is not clear whether steady-state was achieved during the test.
- Lipid concentrations in fish are not measured.
- Fish weight at end of test not measured.

The following OECD 305 validity criteria are fulfilled:

- The water temperature variation was less than ± 2°C
- The concentration of test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase.

The relevance of the study is therefore considered low and it is not used in this assessment.

EXPERIMENTAL DATA ON BIOMAGNIFICATION FACTORS

Inoue et. al. 2012 Comparison of Bioconcentration and Biomagnification Factors for Poorly Water-Soluble Chemicals Using Common Carp (*Cyprinus carpio* L.)

Inoue *et. al* 2012 determined BMF values for nine poorly water soluble chemicals, including o-terphenyl in order to investigate the correlation between BMF and BCF values. For *ortho*-terphenyl a lipid and growth corrected elimination factor K_2 of 0.348 ± 0.051 was measured and a BMF value of 0.0912 was determined from this.

Test design

The test was conducted according to a protocol similar to OECD 305 TG. Hexachlorobenzene was used as a reference substance to check whether the diet-spiking technique was adequate to ensure maximum homogeneity. Fish were exposed to a mixture of o-T, musk xylene and methoxychlor in diet.

There were no mortalities of any exposed or control fish.

Table 60. test design

Test material purity	o-terphenyl > 99.0 %
Test tank	100 L glass

Water supply	flow-through 1 600 ml/min
Temperature	24.7 – 25.2 °C
Concentration of dissolved oxygen	7.6 – 8.0 mg/l
Lighting period	16:8 (light:darkness)
Fish	Yearling carp cultivated according to OECD 305 lipid content at beginning: 3.89 ± 0.89 % weight at beginning: 3.23 ±0.66 g Feed: 3 % by weight
Uptake/depuration period	10 – 13 / 14 - 38
Test diet	Test substances were mixed in corn oil and spiked into fish feed pellets. Nominal concentration of o-T was 50 µg/g. Measured test substance concentrations in diet were maintained at 88.2 to 114 % of nominal. Variation was within 10 % throughout uptake phase. Lipid content in feed: 16.6 ± 0.3 %
Sampling and analysis	GC-MS (extraction with acetone) During depuration fish were samples at 5 points (10 fish per sampling analysed in two groups (5 per group).

Results

A lipid and growth corrected elimination factor (K_2) of 0.348 ± 0.051 was measured for o-terphenyl and a BMF value of 0.0912 was determined from this.

An up-take constant (k_1) can be derived using methods published in Crookes and Brooke (2011), and a mean BCF value (BCF = k_1/k_2) of 1482 ±549 can thus be estimated.

Table 61. BCF values for o-terphenyl derived from a dietary test

k_1	k_2	logKow	BCF	estimation method for k1[1]	reference
619	0.348	5.52	1778	log k1 = 0.147*logKow*+ 1.98	Spacie and Hamelink 1982 in Crookes and Brooke (2011)
734	0.348	5.52	2108	log k1 = 0.122*logKow*+ 2.192	Tolls and Sijm 1995 in Crookes and Brooke (2011)
353	0.348	5.52	1015	k1 (l/kg/day) = W(exp. -0,197)*445)	Barber 2003 in Crookes and Brooke (2011)
357	0.348	5.52	1027	k1 (l/kg/day)= 520*W exp. -0.32	Sijm et al 1995 in Crookes and Brooke (2011)
mean			1482 ±549		

[1]Methods based on fish weight at the end of the test could not be used as fish weights at end of the test were not reported.

Reliability and relevance

The test is considered valid with restrictions (Klimish 2) as the following OECD 305 validity criteria are met,

- Water temperature variation is less than ± 2 ºC in treatment or control groups
- Concentration of dissolved oxygen does not fall below 60 % of the air saturation value

- The concentration of the test substance in fish food before and at the end of the uptake phase is within a range of ± 20% (based on at least three samples at both time points)

- A high degree of homogeneity of substance in food should be demonstrated in preliminary analytical work on the spiked diet; at least three sample concentrations for the substance taken at test start should not vary more than ± 15% from the mean

- Mortality or other adverse effects/disease in both control and test group fish should be ≤10% at the end of the test; if the test is extended for any reason, adverse effects in both groups are ≤ 5% per month, and ≤ 30% cumulatively. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical.

The fulfilment of the following OECD 305 validity criterion is not known, as concentrations of test substance in control fish and/or un-spiked feed are not reported.

- Concentrations of test substance are not detected, or are present only at typical trace levels, in un-spiked food or control fish tissues relative to treated samples

OECD 2012. Validation report of a ring test for the OECD 305 dietary exposure bioaccumulation fish test

A total of 10 laboratories participated in a ring test of the OECD 305 dietary exposure bioaccumulation test. Ten studies were conducted on Rainbow trout and one on Carp with five substances including o-terphenyl (o-T). The mean BMF value for o-terphenyl in trout measured in the different laboratories was 0.5 (std. 20 %) (BMF normalised using mean fish lipid content over the entire experimental period).

In one laboratory BMF values were determined for both carp and trout. For *ortho*-terphenyl, the growth and lipid corrected measured BMF values ranged between 0.12 - 0.25 for carp and was 0.59 for trout. BCF values were calculated from the measured BMF data with different methods. The mean of the calculated BCF values was 1575 ± 420 for Carp and 6219 ± 1647 for trout.

ExxonMobil 2010a. Fish, Dietary Bioaccumulation Study

In a dietary bioaccumation study the dietary Biomagnification factor (BMF), dietary assimilation efficiency and growth corrected, whole body half-life for 13 test substances, including o-terphenyl, were determined using rainbow trout (*Oncorhynchus mykiss*). A single treatment was prepared containing the 13 test substances and was administered to the test system via the diet. A (lipid corrected) BMF value of 0.2 and a depuration half life of 8.1 days was determined for o-terphenyl. This corresponds to estimated BCF values of 7241 or 8587.

Study design

Test substances	Anthracene Benzo(b)fluorene, aka (2,3-BenzoFluorene) Benzo(k)fluoranthene 2,7-Diisopropylnaphthalene 4-Ethyl-1,1'-Biphenyl (4-Ethylbiphenyl, 99%) Fluoranthene 2,2,4,4,6,8,8-Heptamethylnonane

	Hexachlorobenzene 7-Methylbenz(a)anthracene 2 - Isopropyl Decalin n-octyl benzene (1-Phenyloctane, 99%) o-terphenyl Triphenylene
Test tank	40 L glass
Water supply	flow-through system; at least seven volume replacements of water per day through each test chamber.
Temperature	13.5°C to 14.5°C
Concentration of dissolved oxygen	8.7 – 9.4 mg/l
Lighting period	16 hour light, 8 hour dark light
Fish	*Oncorhynchus mykiss*, app. 75 days old 45 fish / tank (control + 1 treatment) Lipid content. 5.58 % (mean) There were no mortalities in either treatment. All fish were observed to be normal throughout the study, no abnormal behavioral or appearance was noted. No substantial difference in growth rate, or lipid content between the treatment and the control for the study.
Uptake/depuration period	10 days / 21 days
Test diet	3 % of fish wet weight per day Uptake: target feed conc. of o-terphenyl 100 µg/g, actual measured concentrations (mean of three replicates) 73.6 µg/g (pre-study); 72.7 µg/g (Day-13 uptake). Depuration: uncontaminated
Sampling and analysis	Fish samples: after day 10 of uptake; and onn day 1, 4, 7, 14 and 21 of depuration. At each sampling 5 fish from each tank. Analysis: HS-SPME-GC-MSD(SIM) Sampling for lipid analyses: Day 1 and beginning and end of depuration.

Results

A (lipid corrected) BMF value of 0.2 and a depuration half life of 8.1 days was determined for o-terphenyl (Table 62). The corresponding BCF values can be estimated by methods described in Crookes and Brooke (2011) and range between 3513 – 7694 (lipid normalised for 5 % lipids)(mean 4887, standard deviation 1611).

k1	k2	BCF	BCF (lipid corrected)	Method for k1 estimation
619	0.085	7241	6489	log k1 = 0.147*logKow*+ 1.98[1]
734	0.085	8587	7694	log k1 = 0.122*logKow*+ 2.192[2]
375	0.085	4390	3934	k1 (l/kg/day) = W(exp. -0,197)*445); W = fish weight (g) at start of test[3]
339	0.085	3973	3560	k1 (l/kg/day) = W(exp. -0,197)*445); W = fish weight (g) at end of test[3]
394	0.085	4611	4132	k1 (l/kg/day)= 520*W exp. -0.32; W = fish weight (g) at start of test[4]

335	0.085	3921	3513	k1 (l/kg/day)= 520*W exp. -0.32; W = fish weight (g) at end of test[4]

[1]Spacie and Hamelink 1982 in Crookes and Brooke (2011)(Table 3.3.)
[2]Tolls and Sijm 1995 in Crookes and Brooke (2011)(Table 3.3.)
[3]Barber 2003 in Crookes and Brooke (2011)(Table 3.3.)
[4]Sijm et al 1995 in Crookes and Brooke (2011)(Table 3.3.)

The test is considered valid with restrictions (Klimish 2) as the following OECD 305 validity criteria are met,

• Concentration of dissolved oxygen did not fall below 60% of the air saturation value

• Concentrations of test substance was not detected in un-spiked food or control fish tissues relative to treated samples

• No mortality or other adverse effects/disease in both control and test group fish was observed

• According to the study authors, the substances were stable in the feed matrix. However, only mean concentrations of three replicates are reported.

However,
• homogeneity of the substance in food was not sufficiently demonstrated.

Table 62. Biomagnification factor (BMF), dietary assimilation efficiency (E) and growth corrected, whole body half-life ($t_{1/2}$).

Test Substance	BMF_{lipid}	E (%)	$t_{1/2}$ (days)
Anthracene	0.027	49	0.44
Benzo(b)fluorene, aka **(2,3-BenzoFluorene)**	0.041	93	0.37
Benzo(k)fluoranthene	0.012	16	0.60
2,7-Diisopropylnaphthalene	0.043	77	0.46
4-Ethyl-1,1'-Biphenyl **(4-Ethylbiphenyl, 99%)**	0.031	70	0.36
Fluoranthene	0.032	60	0.44
2,2,4,4,6,8,8-Heptamethylnonane	0.17	2.8	51
Hexachlorobenzene	1.3	42	25
7-Methylbenz(a)anthracene	0.010	15	0.60
2 - Isopropyl Decalin	0.056	15	3.1
n-Octyl Benzene **(1-Phenyloctane, 99%)**	0.034	27	1.0
o-Terphenyl	0.20	20	8.1
Triphenylene	0.026	42	0.50

BMF_{lipid} - Lipid corrected Biomagnification Factor
E - Assimilation Efficiency
$t_{1/2}$ - whole body half life, growth corrected
Note: 1-Tridecanol was included in the test feed, however, it was not recovered in fish spikes during analytical analysis and was not analyzed in the fish tissue.

ExxonMobil 2010b Fish, Dietary Bioaccumulation Study

In a dietary bioaccumation study the dietary Biomagnification factor (BMF), dietary assimilation efficiency (EF) and growth corrected, whole body half-life for 10 test substances, including m-terphenyl, were determined using rainbow trout (*Oncorhynchus mykiss*). A single treatment was prepared containing the ten test substances and was administered to the test system via the diet. A (lipid corrected) BMF value of 0.045 and a depuration half life of 0.52 days was determined for m-terphenyl. The corresponding BCF values can be estimated by methods described in Crookes and Brooke (2011) and range between 469 – 984 (lipid normalised for 5 % lipids)(mean 636; standard deviation 199).

k1	k2	BCF	BCF (lipid corrected)	Method for k1 estimation
619	1.331	465	830	log k1 = 0.147*logKow*+ 1.98
734	1.331	551	984	log k1 = 0.122*logKow*+ 2.192
383	1.331	288	514	k1 (l/kg/day) = W(exp. -0,197)*445); W = fish weight (g) at start of test
350	1.331	263	469	k1 (l/kg/day) = W(exp. -0,197)*445); W = fish weight (g) at end of test
407	1.331	306	546	k1 (l/kg/day)= 520*W exp. -0.32; W = fish weight (g) at start of test
352	1.331	264	472	k1 (l/kg/day)= 520*W exp. -0.32; W = fish weight (g) at end of test

[1]Spacie and Hamelink 1982 in Crookes and Brooke (2011)(Table 3.3.)
[2]Tolls and Sijm 1995 in Crookes and Brooke (2011)(Table 3.3.)
[3]Barber 2003 in Crookes and Brooke (2011)(Table 3.3.)
[4]Sijm et al 1995 in Crookes and Brooke (2011)(Table 3.3.)

Study design

Test substances	1-Heptadecyne 1,1':3',1" - Tercyclohexane Dicyclohexylbenzene Cyclohexylbiphenyl m-Terphenyl Dodecahydrochrysene Hexahydrochrysene Octahydrochrysene Chrysene Benzo(C)chrysene
Test tank	40 L glass
Water supply	flow-through system; at least five volume replacements of water per day through each test chamber.
Temperature	13.5°C to 16.5°C
Concentration of dissolved oxygen	8.5 – 9.7 mg/l
Lighting period	16 hour light, 8 hour dark light
Fish	*Oncorhynchus mykiss*, app. 81 days old 45 fish / tank (control + 1 treatment) Lipid content. 3.6 % (mean) There were no mortalities in either treatment. All fish were observed to be normal throughout the study, no abnormal behavioral or appearance was noted. No substantial difference in growth rate, or lipid content between the treatment and the control for the study.

Uptake/depuration period	10 days / 21 days
Test diet	3 % of fish wet weight per day Uptake: target feed conc. of m-terphenyl 70 µg/g, actual measured concentration (mean of three replicates) 47.6 µg/g (pre-treatment), 42.8 µg/g (Day 13 uptake). (Measured feed concentrations were lower than the protocol targets due to difficulty keeping the corn oil/test substance suspension homogeneous during preparation and sampling. Nevertheless, according to the authors, The concentrations were well within acceptable limits for the purposes of the study and the substances were stable in the feed matrix.) Depuration: uncontaminated
Sampling and analysis	Fish samples: after day 10 of uptake; and one day 1, 3, 7, 14 and 21 of depuration. At each sampling 5 -6 fish from each tank. Analysis: SPME-GC-MSD(SIM) Sampling for lipid analyses: Day 1 and beginning and end of depuration.

Results

A (lipid corrected) BMF value of 0.045 and a depuration half life of 0.52 days was determined for o-terphenyl (Table 62). The corresponding BCF values can be estimated by methods described in Crookes and Brooke (2011) and are 465 (using log k1 = 0.147*logKow*+ 1.98) and 551 (log k1 = 0.122*logKow*+ 2.192).

The test is considered valid with restrictions (Klimish 2) as the following OECD 305 validity criteria are met,

• Concentration of dissolved oxygen did not fall below 60% of the air saturation value

• Concentrations of test substance was not detected in un-spiked food or control fish tissues relative to treated samples

• No mortality or other adverse effects/disease in both control and test group fish was observed

• According to the study authors, the the substances were stable in the feed matrix. However, only mean concentrations of three replicates are reported.

However,
• homogeneity of the substance in food was not sufficiently demonstrated. On the contrary, the study authors state that measured feed concentrations were lower than the protocol targets due to difficulty of keeping the corn oil/test substance suspension homogeneous during preparation and sampling.

Table 63. Biomagnification factor (BMF), dietary assimilation efficiency (E) and growth corrected, whole body half-life ($t_{1/2}$).

Test Substance	BMF_{lipid}	E (%)	$t_{1/2}$ (days)
1-Heptadecyne	0.82	22	20
1,1':3',1" - Tercyclohexane	0.44	12	19
Dicyclohexylbenzene	0.068	6.6	5.6
Cyclohexylbiphenyl	0.056	37	0.83
m-Terphenyl	0.045	47	0.52
Dodecahydrochrysene	0.17	18	5.0
Hexahydrochrysene	0.046	49	0.51
Octahydrochrysene	0.052	55	0.51
Chrysene	0.034	18	1.0
Benzo(c)chrysene	0.053	54	0.54

BMF_{lipid} - Lipid corrected Biomagnification Factor
E - Assimilation Efficiency
$t_{1/2}$ - whole body half life, growth corrected

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

No information available.

3.4.3 Field data

No information available.

3.4.4 Summary and discussion of bioaccumulation

A summary of relevant information available on bioaccumulation is given in Table 64. The measured and estimated BCF values vary significantly between, but also within, constituent groups.

Based on QSAR results, all assessed constituents have a (predicted) logKow above 4.5 raising concern for bioaccumulation potential. LogKow values increase with both increasing molecular weight (MW) and increasing degree of hydrogenation. This is partially reflected in the BCF-values predicted by QSAR-models (regression based and Arnot-Gobas methods in EPIsuite BCF BAF v.3.01). Highest BCF-values are predicted for the one-ring hydrogenated terphenyls. For the completely hydrogenated 4-ring structure (HQ3), the predicted logKow exceeds 10 and the predicted BCF values drop to below 500.

BCF and BMF values available for o-terphenyl both in Rainbow trout and Carp indicate that bioaccumulation is more pronounced by Rainbow trout compared to Carp (Table 64). This can explain partly the large variation between BCF values for the same constituent groups. Another explanation for the variation in measured values can be the difficulty in determining accurately exposure concentrations. As the constituents are adsorptive, a part of the test substance is likely to be adsorbed (e.g. to feed or feces) and therefore not bioavailable. As the commonly used liquid-liquid extractions are expected to extract both the adsorbed and bioavailable fraction to the organic solvent, the measured concentrations can overestimate the actual bioavailable dissolved concentrations. This would result in underestimation of BCF values in studies where adsorption was significant.

Two dietary bioaccumulation studies (ExxonMobil 2010a and b) indicate that bioaccumulation differs significantly between o-terphenyl and m-terphenyl. Whereas o-terphenyl depurates slowly from rainbow trout $T_{1/2}$ = 8.1 days), m-terphenyl seems to depurate quite fast (0.52 days). In addition, the results from NITE 2004 suggest that there can be significant differences in bioaccumulation between the isomers. However, as the analytical method used was not able to discern between the ortho-, meta- and para-isomers, it is not possible to address the varying BCF-values to specific isomers in this study. Neither are the available QSAR tools able to discern between the isomers (Table 37).

Table 64. Summary of estimated and experimental information on bioaccumulation[1].

Constituent (log Kow)	BCFs (BCFs estimated or derived from BMFs in brackets)	Experimental BMFs	Fish	Reference (Klimish code)
o-T (5.52)	12 993		RT	Schlechtriem 2016 (1)
	1900 ± 300 1100 ± 200		C C	NITE 2012 (2)
	(1575 ± 420) (6219 ± 1647)	0.12 – 0.25 0.59	C RT	OECD 2012
	(1482 ±549)	0.0912 ± 0.0134	C	Inoue et al. 2012 (2)
	4887 ± 1611	0.2	RT	ExxonMobil 2010a (2)
	2041			Regression (2)
	1146			Arnot-Gobas (2)
m-T (5.52)	(636 ± 199)	0.045	RT	ExxonMobil 2010b (2)
	2041			Regression (2)
	1035			Arnot-Gobas (2)
p-T 5.52 6.03*	2037 4422*			Regression (2)
	1034 1301*			Arnot-Gobas (2)
HT1 (6.57)	1551 – 12 436		C	NITE 2004 (2)
	10 100			Regression (2)
	1863			Arnot-Gobas (2)
HT2 (7.63)	1692 - 7154		C	NITE 2004 (2)
	3700		B	Monsanto 1983 (2)
	6559			Regression (2)
	289			Arnot-Gobas (2)
HT3 (8.55)	2315			Regression (2)
	148			Arnot-Gobas (2)
T/HTI/HT2	2400 - 9100		B	Monsanto 1983 (2)
Q (7.28)	2273 - 3259		C	NOTOX 2009b (2)
	9646			Regression (2)
	1499			Arnot-Gobas (2)
HQ1 (8.34)	2941			Regression (2)

	296			Arnot-Gobas (2)
HQ2 (9.26)	1038			Regression (2)
HQ3(10.18)	366			Regression (2)

[1]C = Carp, RT = Rainbow trout, B = Bluegill. All values normalised to 5 % lipids except in Monsanto (1983). BCFs in brackets have been estimated form measured BMFs. Only studies with Klimish reliability 1 or 2 are included. Regression = EPIsuite BCF BAF v.3.01 regression based. Arnot Gobas = EPIsuite BCF BAF v.3.01 Arnot-Gonas, upper trophic, including biotransformation. *depending on used logKow 5.52 / 6.03). In general, the BCF values have not been growth corrected due to lack of missing data on fish weights with the exception of Schlechtriem 2016, ExxonMobil 2010a and b.

4. Human health hazard assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 points (e) of REACH. Information related to the T criterion of Article 57 (d) of REACH is presented in Annex IV as additional information.

5. Environmental hazard assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 points (e) of REACH. Information related to the T criterion of Article 57 (d) of REACH is presented in Annex V as additional information.

6. Conclusions on the SVHC Properties

6.1 CMR assessment

Not relevant for the proposal.

6.2 PBT and vPvB assessment

6.2.1 Assessment of PBT/vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to assess the PBT/vPvB properties of the substance. All available information (such as the results of standard tests, modelling and (Q)SAR results) was considered together in a weight-of-evidence approach.

According to the ECHA guidance (ECHA 2017a, R.11), the *Weight-of-Evidence* determination by expert judgement enables the use of all (screening and assessment) information types listed in Section 3 of Annex XIII to the REACH Regulation in the PBT/vPvB assessment for comparing with the criteria, although not all of these information types can be directly (numerically) compared with the criteria.

6.2.1.2 Persistence

A substance fulfils the persistence criterion (P) in any of the following situations:
(a) the degradation half-life in marine water is higher than 60 days;
(b) the degradation half-life in fresh or estuarine water is higher than 40 days;
(c) the degradation half-life in marine sediment is higher than 180 days;
(d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days;

(e) the degradation half-life in soil is higher than 120 days.

A substance fulfils the “very persistent” criterion (vP) in any of the following situations:
(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days;
(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days;
(c) the degradation half-life in soil is higher than 180 days.

For the persistence assessment of terphenyl, hydrogenated, most weight is given to half-lives measured in standard simulation tests or simulation tests which are considered comparable to standard tests in terms of reliability and test conditions. Half-lives from such tests can be directly compared with the P/vP criteria. Results from simulation tests with conditions differing from standard tests (or with insufficient documentation), screening tests, QSAR predictions, and microbial culture studies, are used as supporting information.

Based on the weight-of-evidence assessment of available relevant information, terphenyl, hydrogenated fulfils the P and vP criteria. The relevant findings are summarised below:

- Based on available information, abiotic degradation is expected to occur at such a low rate that it is not considered a relevant route of degradation for P/vP assessment
- In a soil simulation test, dissipation half-lives in soil of ≥218 days (temperature-corrected to 12°C) were determined for terphenyl and >224 days quaterphenyl (Monsanto Company 1989) thus fulfilling the P and vP criteria. These half-lives were determined for a mixture of terphenyls, quaterphenyls, and polyphenyls (the proportions of the different isomers are not known). Quaterphenyls and terphenyls are relevant constituents of the UVCB substance.
- In a seawater simulation test with hydrocarbon mixtures (ExxonMobil Biomedical Science, Inc., 2009) primary degradation half-live (temperature-corrected to 12°C) of >182 days was reported for o-terphenyl and half-lives of 32 d and 108 d for m-terphenyl, suggesting that o-terphenyl and m-terphenyl fulfil the P/vP criterion in marine water.
- In an OECD 307 soil simulation test a dissipation half-life of 2-10 days (NOTOX 2009a) for p-dicyclohexylbenzene (HT2) was detected during the test when the half-lifes are calculated for the whole test duration using bi-phasic models. Assuming that all non-extractable residues (NER) are parent substance, the half-life is 6-18 days in two soils whereas for one soil no exact half-life can be determined and it is estimated that the half-life for this soil is above test duration, i.e., >120 days. When the second phase (‘slow phase’) from bi-phasic models is used the half-lives were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k_2 parameter was not statistically significant and as the half-life obtained from temperature conversion is longer than the experimental period) whereas for one of the soils, no reliable second-phase half-lives could be determined. In this study a significant part of applied radioactivity partitioned to soil and was quantified as NER, which has a strong influence on the shape of the dissipation curve, which causes uncertainty for the determination of the degradation half-life. The results indicate that p-dicyclohexylbenzene (HT2) is potentially P or vP. Definitive P/vP conclusion has not been drawn in this assessment due to limited data on NER.
- In non-standard biodegradation ultimate biodegradation tests (Monsanto report ES-80-SS34, Monsanto 1977a), degradation of UVCB substances (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) based on CO_2 evolution was at the most 14 % within 35 days, suggesting that the tested substances are not readily biodegradable and therefore potentially P or vP.

- In a river die-away test, when tested separately, o- and p-terphenyl showed no or neglible degradation during 28 days whereas m-terphenyl started to degrade after 16 days. When tested in a mixture of m-, o-, and p-terphenyls, o- terphenyl and m-terphenyl started to biodegrade after 30 days. A HT3 constituent showed no degradation in 30 days whereas HT1 and HT2 constituents were more degradable (Mic 1983a). The results suggest that the tested o-T, p-T, and HT3 constituents are potentially P or vP whereas for the constituents with higher degradation, m-T, HT1, and HT2, no conclusion can be drawn as only primary degradation was measured and, in the case of m-T, as the results were different when tested in mixture or as individual compound.
- A shake-flask carbon dioxide evolution test with a hydrocarbon-adapted inoculum (Mic 1983b) showed relatively low (9-38%) mineralization for o-T, m-T, p-T, p-HT2, p-HT3, and p-Q) in 55 days, suggesting that o-T, m-T, p-T, p-HT2, p-HT3, and p-Q are potentially P or vP. No conclusion can be drawn from this study for p-HT1 as its higher degradation (63%) may be explained by the adapted inoculum.
- In a semi-continuous activated sludge (SCAS) study (Monsanto 1973) the mean disappearance of hydrogenated quaterphenyls (HQ) was 16% at the end of the SCAS study (with negligible volatilization), in a test system considered to be favourable for microbial adaptation. The presence of a detectable amoung of HQ at the end of the following die-away procedure is in line with the results of the SCAS study. The test substance (HQ40) was a mixture of approximately 80 % quaterphenyls with a degree of 40 % hydrogenation (the residual 20 % consists of terphenyl and higher (> 5-ring) phenyl structures). The results suggest that HQ is potentially P or vP.
- P-terphenyl persisted in an SCAS test system (Monsanto 1974) despite the possible adaptation during the test and in a die-away procedure conducted with an inoculum from the SCAS system. Test substance was a mixture containing mainly o-, m-, and p-terphenyls. The results suggest that p-terphenyl is potentially P or vP whereas for m- and o-terphenyl no conclusions can be done due to different concentrations of the isomers in the test substance and possible abiotic losses.
- In a shake-flask carbon dioxide evolution test (Monsanto 1991) with an inoculum pre-exposed to p-terphenyl, p-terphenyl showed no significant mineralisation or primary degradation in 42 days. The CO_2 production after 42 days was 8-9% in the active test and 7% in sterile control. The mean residue recovery after 42 days was 78.0-81.1% of initial level in the active test and 82.1 in sterile control. The results suggest that p-terphenyl is potentially P or vP.
- In a microbial culture study (Ohmori et al 1973) the amounts and properties of microbial strains isolated from environmental samples using terphenyl or other hydrocarbons as a sole carbon source suggest that terphenyl is a less favourable growth substrate compared to other hydrocarbons tested (*n*-paraffin, biphenyl, diphenylmethane, diphenylethane, *trans*-stilbene) and therefore the ultimate degradability of terphenyl in the environment may be limited. The results indicate presence of terphenyl utilizing microorganisms but also suggest that microorganisms able to utilise other hydrocarbons are not necessarily able to utilise terphenyl. The results suggest that o-, m-, and p-terphenyl are potentially P or vP.
- BIOWIN models 3 and 6 in combination indicate that o-T, m-T, p-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4, are potentially P or vP, as the P/vP screening criteria for this model combination are fulfilled. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BIOWIN models 2 and 3 in combination indicate that o-T, m-T, P-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4 do not screen as P or vP. Regarding

HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.

- BioHCwin model predicts primary degradation half-lives of 315 days for HT1, 470 days for HT2, 69 days for HT3, 68 days for HQ1, 809 days for HQ2, and 305 days for HQ3, exceeding the P and vP criteria in water (HT1, HT2, HT3, HQ1, HQ2, and HQ3) and in soil and sediment (HT1, HT2, HQ2, HQ3). No conclusion could be done for o-T, m-T, p-T, and Q (for which half-lives were 7-8 days and thus below the P and vP criteria) because BioHCwin model gives a primary biodegradation half-life estimate and because data obtained with mixtures has been used in its training set. Half-lives used to derive the BioHCwin model include results obtained from water, soil, and sediment studies.

Table below summarises the conclusions on P/vP for the selected constituents of terphenyl, hydrogenated.

P/vP conclusion of selected constituents of terphenyl, hydrogenated

	Persistence
o-T	P and vP
m-T	potentially P or vP
p-T	P and vP
p-HT1	potentially P or vP
p-HT2	potentially P or vP
p-HT3	potentially P or vP
p-Q	P and vP
p-HQ1	potentially P or vP
p-HQ2	potentially P or vP
p-HQ3	potentially P or vP

6.2.1.2 Bioaccumulation

A substance fulfils the B criterion when the bioconcentration factor in aquatic species is higher than 2000, and the vB criterion when the bioconcentration factor in aquatic species is higher than 5000. A weight-of-evidence determination using expert judgement is applied by comparing all relevant and available information. For the bioaccumulation assessment of terphenyl, hydrogenated most weight is given to valid measured BCF-values, because these are directly comparable with the criteria. Measured BMF-values and BCF-values derived from these are used as supporting information as well as QSAR predictions.

Based on the weight-of-evidence assessment of available relevant information, terphenyl, hydrogenated fulfils the B and vB criteria because:

- A measured BCF value in Rainbow trout above the vB criterion, 12 993, is determined for o-terphenyl (o-T), a relevant constituent of the UVCB substance (Schlechtriem 2016). This study result is supported by measured BMF values in Rainbow trout, 0.59 (OECD 2012) and 0.2 (ExxonMobil 2010a), which predict BCF-values of 6219 ±1647 and 4887 ± 1611, respectively. Based on these data, it is concluded that this constituent is B and vB.

- Measured BCF values for o-terphenyl (o-T) in Carp, 1900 ± 300 and 1100 ± 200, (NITE 2012) are close to the B criterion. (It is noted that these BCF values might be underestimations due to growth dilution.) They are supported by measured BMF values of 0.09 – 0.25, (OECD 2012, Inoue et al. 2012) leading to estimated BCF values of 1575 ± 420 and 1482 ± 549. Based on these data, it is concluded that this constituent is B.

- Partially hydrogenated terphenyls (HT1, HT2) show high measured BCF-values (1551 – 12 436) in Carp and Bluegill (NITE 2004, Monsanto 1983) exceeding the vB criterion. Based on these data, it is concluded that these constituents are B and vB.

- Based on the BCF values measured for m,m-quaterphenyl (Q), 2273 – 3259, (NOTOX 2009b) in carp, it can be concluded that this constituent fulfils the B criterion but not the vB criterion. QSAR predictions are 9646 (regression model) and 1499 (Arnot-Gobas), thus supporting this conclusion. Based on these data, it is concluded that this constituent is B.

For some constituents (m-T, p-T, HT3, HQ1, HQ2, HQ3) a definitive conclusion is not possible due to lacking or contradictory data,

- For p-T, HT3, HQ1, HQ2, HQ3 no experimental data on bioaccumulation is available.

- Based on log Kow values (> 4.5), it is concluded that p-T, HT3, HQ1, HQ2 are potentially B andvB.

- For HQ3 the predicted logKow exceeds 10 and the predicted BCF values drop below 500. According to ECHA guidance (ECHA 2014), the aquatic BCF of a substance is probably lower than 2000 if the calculated Log K_{ow} is higher than 10. Therefore, it is concluded that the constituent in probably not B or vB.
- For m-terphenyl QSAR predictions and the logKow value indicate that the substance is potentially B. A dietary biomagnification study, on the other hand, shows rapid depuration in rainbow trout ($T_{1/2}$ = 0.52), which corresponds to estimated BCF-values of 636 ± 199. As the information is scarce and contradictory, it is not possible to conclude.

Table 65. B/vB conclusion of selected constituents of terphenyl, hydrogenated

	Bioaccumulation
o-T	B and vB
m-T	not possible to conclude
p-T	potentially B and vB
p-HT1	B and vB
p-HT2	B and vB
p-HT3	potentially B
p-Q	B
p-HQ1	potentially B and vB
p-HQ2	potentially B and vB

p-HQ3	probably not B or vB

6.2.1.3 Toxicity

6.2.1.3.1 Fulfilment of the T criterion based on human health classification

The T criterion is met based on toxicity testing, if the substance meets the criteria for classification as carcinogenic (cat. 1A/B), germ cell mutagenic (cat. 1A/B) or toxic for reproduction (cat. 1 A/B or 2), or if there is other evidence on chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE cat. 1 or 2) according to CLP-regulation.

Based on the available information, the T criterion for toxicity is not fulfilled at present, although it is acknowledged that there is a data gap concerning developmental toxicity. Therefore, a definitive conclusion on T is not possible (for details see Annex IV).

6.2.1.3.2 Fulfilment of the T criterion based on ecotoxicity data

A substance fulfils the T criterion based on ecotoxicity testing if the long term NOEC or EC10 values is less than 10 µg/l. Substances that have EC50 values below 0.1 mg/l are considered as screening T (ECHA 2017a). A weight-of-evidence determination using expert judgement is applied by comparing all relevant and available information (see Annex IV for details).

Based on the weight-of-evidence assessment of available relevant information, it is not possible to conclude on T because,

- Many of the available studies have been conducted with water accommodated fractions of commercial products of the UVCB substance (terphenyl,hydrogenated) using nominal concentrations. Documentation on the test substance identity in the commercial products is scarce or non-existent. As the constituents of terphenyl, hydrogenated are scarcely water soluble and quite volatile from water solutions, use of nominal concentrations without information on measured concentrations is not considered reliable. Therefore, it is not possible to make conclusions based on these tests.
- Ecotoxicity test results are available for the constituents o-, m- and p-terphenyl, but the level of detail on most of the acute ecotoxicity tests, and all of the long-term ecotoxicity tests, is low and crucial information is missing. Therefore, the reliability of most of the studies cannot be rated (Klimish 4 - not assignable).
- The Monsanto 1993 study is considered as reliable with restriction (Klimish 2). The acute *Daphnia magna* EC50 values determined in this test are below the T screening criterion (0.1 mg/l) (0.045 mg/l for o-terphenyl and 0.022 mg/l for m-terphenyl.) A definitive conclusion cannot, however, be made based on the screening criterion.
- Although a definitive conclusion cannot be drawn based on available information, the ECOSAR predictions and testing results give rather consistent results for o-terphenyl and indicate that NOECs for fish and Daphnids (11 – 25 µg/l) are close to the T criterion. Also EC50 values for o-terphenyl are close to the T screening criterion (0.025 – 0.13 mg/l) with one exception (EC50 0.52 mg/l).

Table 66. T conclusion of selected constituents of terphenyl, hydrogenated

	Toxicity Human health	Toxicity Aquatic Environment
o-T	-	potentially T / close to T
m-T	-	potentially T /close to T
p-T	-	no experimental data; not possible to conclude
p-HT1	-	no experimental data; not possible to conclude
p-HT2	-	no experimental data; not possible to conclude
p-HT3	-	no experimental data; not possible to conclude
p-Q	-	no experimental data; not possible to conclude
p-HQ1	-	no experimental data; not possible to conclude
p-HQ2	-	no experimental data; not possible to conclude
p-HQ3	-	no experimental data; not possible to conclude

6.2.2 Summary and overall conclusions on the vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to assess the PBT/vPvB properties of the substance. All available information (such as the results of standard tests, modelling and (Q)SAR results) was considered together in a weight-of-evidence approach.

According to the ECHA guidance (ECHA 2017a, R.11), the *Weight-of-Evidence* determination by expert judgement enables the use of all (screening and assessment) information types listed in Section 3 of Annex XIII to the REACH Regulation in the PBT/vPvB assessment for comparing with the criteria, although not all of these information types can be directly (numerically) compared with the criteria.

Persistence

A substance fulfils the persistence criterion (P) in any of the following situations:
(a) the degradation half-life in marine water is higher than 60 days;
(b) the degradation half-life in fresh or estuarine water is higher than 40 days;
(c) the degradation half-life in marine sediment is higher than 180 days;
(d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days;
(e) the degradation half-life in soil is higher than 120 days.

A substance fulfils the “very persistent” criterion (vP) in any of the following situations:
(a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days;
(b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days;
(c) the degradation half-life in soil is higher than 180 days.

For the persistence assessment of terphenyl, hydrogenated, most weight is given to half-lives measured in standard simulation tests or simulation tests which are considered comparable to standard tests in terms of reliability and test conditions. Half-lives from

such tests can be directly compared with the P/vP criteria. Results from simulation tests with conditions differing from standard tests (or with insufficient documentation), screening tests, QSAR predictions, and microbial culture studies, are used as supporting information.

Based on the weight-of-evidence assessment of available relevant information, terphenyl, hydrogenated fulfils the P and vP criteria. The relevant findings are summarised below:

- Based on available information, abiotic degradation is expected to occur at such a low rate that it is not considered a relevant route of degradation for P/vP assessment
- In a soil simulation test, dissipation half-lives in soil of ≥218 days (temperature-corrected to 12°C) were determined for terphenyl and >224 days quaterphenyl (Monsanto Company 1989) thus fulfilling the P and vP criteria. These half-lives were determined for a mixture of terphenyls, quaterphenyls, and polyphenyls (the proportions of the different isomers are not known). Quaterphenyls and terphenyls are relevant constituents of the UVCB substance.
- In a seawater simulation test with hydrocarbon mixtures (ExxonMobil Biomedical Science, Inc., 2009) primary degradation half-live (temperature-corrected to 12°C) of >182 days was reported for o-terphenyl and half-lives of 32 d and 108 d for m-terphenyl, suggesting that o-terphenyl and m-terphenyl fulfil the P/vP criterion in marine water.
- In an OECD 307 soil simulation test a dissipation half-life of 2-10 days (NOTOX 2009a) for p-dicyclohexylbenzene (HT2) was detected during the test when the half-lifes are calculated for the whole test duration using bi-phasic models. Assuming that all non-extractable residues (NER) are parent substance, the half-life is 6-18 days in two soils whereas for one soil no exact half-life can be determined and it is estimated that the half-life for this soil is above test duration, i.e., >120 days. When the second phase ('slow phase') from bi-phasic models is used the half-lives were 38-46 days in one soil (with possible underestimation as the kinetic fit was not optimal), 185 days in one soil (with uncertainty as the k_2 parameter was not statistically significant and as the half-life obtained from temperature conversion is longer than the experimental period) whereas for one of the soils, no reliable second-phase half-lives could be determined. In this study a significant part of applied radioactivity partitioned to soil and was quantified as NER, which has a strong influence on the shape of the dissipation curve, which causes uncertainty for the determination of the degradation half-life. The results indicate that p-dicyclohexylbenzene (HT2) is potentially P or vP. Definitive P/vP conclusion has not been drawn in this assessment due to limited data on NER.
- In non-standard biodegradation ultimate biodegradation tests (Monsanto report ES-80-SS34, Monsanto 1977a), degradation of UVCB substances (expected to contain same or structurally similar constituents as terphenyl, hydrogenated) based on CO_2 evolution was at the most 14 % within 35 days, suggesting that the tested substances are not readily biodegradable and therefore potentially P or vP.
- In a river die-away test, when tested separately, o- and p-terphenyl showed no or neglible degradation during 28 days whereas m-terphenyl started to degrade after 16 days. When tested in a mixture of m-, o-, and p-terphenyls, o- terphenyl and m-terphenyl started to biodegrade after 30 days. A HT3 constituent showed no degradation in 30 days whereas HT1 and HT2 constituents were more degradable (Mic 1983a). The results suggest that the tested o-T, p-T, and HT3 constituents are potentially P or vP whereas for the constituents with higher degradation, m-T, HT1, and HT2, no conclusion can be drawn as only primary degradation was measured and, in the case of m-T, as the results were different when tested in mixture or as individual compound.

- A shake-flask carbon dioxide evolution test with a hydrocarbon-adapted inoculum (Mic 1983b) showed relatively low (9-38%) mineralization for o-T, m-T, p-T, p-HT2, p-HT3, and p-Q) in 55 days, suggesting that o-T, m-T, p-T, p-HT2, p-HT3, and p-Q are potentially P or vP. No conclusion can be drawn from this study for p-HT1 as its higher degradation (63%) may be explained by the adapted inoculum.
- In a semi-continuous activated sludge (SCAS) study (Monsanto 1973) the mean disappearance of hydrogenated quaterphenyls (HQ) was 16% at the end of the SCAS study (with negligible volatilization), in a test system considered to be favourable for microbial adaptation. The presence of a detectable amoung of HQ at the end of the following die-away procedure is in line with the results of the SCAS study. The test substance (HQ40) was a mixture of approximately 80 % quaterphenyls with a degree of 40 % hydrogenation (the residual 20 % consists of terphenyl and higher (> 5-ring) phenyl structures). The results suggest that HQ is potentially P or vP.
- P-terphenyl persisted in an SCAS test system (Monsanto 1974) despite the possible adaptation during the test and in a die-away procedure conducted with an inoculum from the SCAS system. Test substance was a mixture containing mainly o-, m-, and p-terphenyls. The results suggest that p-terphenyl is potentially P or vP whereas for m- and o-terphenyl no conclusions can be done due to different concentrations of the isomers in the test substance and possible abiotic losses.
- In a shake-flask carbon dioxide evolution test (Monsanto 1991) with an inoculum pre-exposed to p-terphenyl, p-terphenyl showed no significant mineralisation or primary degradation in 42 days. The CO_2 production after 42 days was 8-9% in the active test and 7% in sterile control. The mean residue recovery after 42 days was 78.0-81.1% of initial level in the active test and 82.1 in sterile control. The results suggest that p-terphenyl is potentially P or vP.
- In a microbial culture study (Ohmori et al 1973) the amounts and properties of microbial strains isolated from environmental samples using terphenyl or other hydrocarbons as a sole carbon source suggest that terphenyl is a less favourable growth substrate compared to other hydrocarbons tested (*n*-paraffin, biphenyl, diphenylmethane, diphenylethane, *trans*-stilbene) and therefore the ultimate degradability of terphenyl in the environment may be limited. The results indicate presence of terphenyl utilizing microorganisms but also suggest that microorganisms able to utilise other hydrocarbons are not necessarily able to utilise terphenyl. The results suggest that o-, m-, and p-terphenyl are potentially P or vP.
- BIOWIN models 3 and 6 in combination indicate that o-T, m-T, p-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4, are potentially P or vP, as the P/vP screening criteria for this model combination are fulfilled. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BIOWIN models 2 and 3 in combination indicate that o-T, m-T, P-T, p-HT1, p-HT2, p-Q, p-HQ1, p-HQ2, p-HQ3, and p-HQ4 do not screen as P or vP. Regarding HT3 no conclusion can be done as the BIOWIN 3 model is not applicable.
- BioHCwin model predicts primary degradation half-lives of 315 days for HT1, 470 days for HT2, 69 days for HT3, 68 days for HQ1, 809 days for HQ2, and 305 days for HQ3, exceeding the P and vP criteria in water (HT1, HT2, HT3, HQ1, HQ2, and HQ3) and in soil and sediment (HT1, HT2, HQ2, HQ3). No conclusion could be done for o-T, m-T, p-T, and Q (for which half-lives were 7-8 days and thus below the P and vP criteria) because BioHCwin model gives a primary biodegradation half-life estimate and because data obtained with mixtures has been used in its training set. Half-lives used to derive the BioHCwin model include results obtained from water, soil, and sediment studies.

Table below summarises the conclusions on P/vP for the selected constituents of terphenyl, hydrogenated.

P/vP conclusion of selected constituents of terphenyl, hydrogenated

	Persistence
o-T	P and vP
m-T	potentially P or vP
p-T	P and vP
p-HT1	potentially P or vP
p-HT2	potentially P or vP
p-HT3	potentially P or vP
p-Q	P and vP
p-HQ1	potentially P or vP
p-HQ2	potentially P or vP
p-HQ3	potentially P or vP

Bioaccumulation

A substance fulfils the B criterion when the bioconcentration factor in aquatic species is higher than 2000, and the vB criterion when the bioconcentration factor in aquatic species is higher than 5000. A weight-of-evidence determination using expert judgement is applied by comparing all relevant and available information. For the bioaccumulation assessment of terphenyl, hydrogenated most weight is given to valid measured BCF-values, because these are directly comparable with the criteria. Measured BMF-values and BCF-values derived from these are used as supporting information as well as QSAR predictions.

Based on the weight-of-evidence assessment of available relevant information, terphenyl, hydrogenated fulfils the B and vB criteria because:

- A measured BCF value in Rainbow trout above the vB criterion, 12 993, is determined for o-terphenyl (o-T), a relevant constituent of the UVCB substance (Schlechtriem 2016). This study result is supported by measured BMF values in Rainbow trout, 0.59 (OECD 2012) and 0.2 (ExxonMobil 2010a), which predict BCF-values of 6219 ±1647 and4887 ± 1611, respectively. Based on these data, it is concluded that this constituent is B and vB.
- Measured BCF values for o-terphenyl (o-T) in Carp, 1900 ± 300 and 1100 ± 200, (NITE 2012) are close to the B criterion. (It is noted that these BCF values might be underestimations due to growth dilution.) They are supported by measured BMF values of 0.09 – 0.25, (OECD 2012, Inoue et al. 2012) leading to estimated BCF values of 1575 ± 420 and 1482 ± 549. Based on these data, it is concluded that this constituent is B.
- Partially hydrogenated terphenyls (HT1, HT2) show high measured BCF-values (1551 – 12 436) in Carp and Bluegill (NITE 2004, Monsanto 1983) exceeding the vB criterion. Based on these data, it is concluded that these constituents are B and vB.

- Based on the BCF values measured for m,m-quaterphenyl (Q), 2273 – 3259, (NOTOX 2009b), it can be concluded that this constituent fulfils the B criterion but not the vB criterion. QSAR predictions are 9646 (regression model) and 1499 (Arnot-Gobas), thus supporting this conclusion. Based on these data, it is concluded that this constituent is B.

For some constituents (m-T, p-T, HT3, HQ1, HQ2, HQ3 a definitive conclusion is not possible due to lacking or contradictory data,

- For p-T, HT3, HQ1, HQ2, HQ3 no experimental data on bioaccumulation is available.

- Based on log Kow values (> 4.5), it is concluded that p-T, HT3, HQ1, HQ2 are potentially B and vB.

- For HQ3 the predicted logKow exceeds 10 and the predicted BCF values drop below 500. According to ECHA guidance (ECHA 2014), the aquatic BCF of a substance is probably lower than 2000 if the calculated Log K_{ow} is higher than 10. Therefore, it is concluded that the constituent in probably not B or vB.
- For m-terphenyl QSAR predictions and logKow value indicate that the substance is potentially B. A dietary biomagnification study, on the other hand, shows rapid depuration in rainbow trout ($T_{1/2}$ = 0.52), which corresponds to estimated BCF-values of 636 ± 199. As the information is scarce and contradictory, it is not possible to conclude.

B/vB conclusion of selected constituents of terphenyl, hydrogenated

	Bioaccumulation
o-T	B and vB
m-T	not possible to conclude
p-T	potentially B and vB
p-HT1	B and vB
p-HT2	B and vB
p-HT3	potentially B
p-Q	B
p-HQ1	potentially B and vB
p-HQ2	potentially B and vB
p-HQ3	probably not B or vB

Conclusion: It can be definitively concluded that at least o-terphenyl fulfils both vP and vB criteria. As o-terphenyl occurs in significant concentrations in the UVCB substance (> 0.1 % w/w), terphenyl, hydrogenated is considered to fulfil the vPvB criteria.

In conclusion, terphenyl, hydrogenated meets the criteria for a vPvB substance according to Article 57 (e) of REACH.

Overall conclusion:

In conclusion, terphenyl, hydrogenated meets the criteria for a vPvB substance according to Article 57 (e) of REACH by comparing all relevant and available information according to Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

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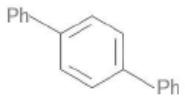
Annex I – Composition[1]

Terphenyl, hydrogenated

Type:	boundary composition of the substance
State Form:	liquid

Constituent 1



Reference substance name:	Terphenyl
EC Number:	247-477-3
EC Name:	Terphenyl
CAS Number:	26140-60-3
Molecular formula:	C18H14
IUPAC Name:	terphenyl

Constituent 2

Reference substance name:	Terphenyl, hydrogenated
EC Number:	262-967-7
EC Name:	Terphenyl, hydrogenated
CAS Number:	61788-32-7
Molecular formula:	C18Hn (n >18-36)

Constituent 3

Reference substance name:	Quaterphenyls, Pentaphenyls and hexahydropentaphenyls, their isomers and other hydrocarbons
IUPAC Name:	Quaterphenyls, Pentaphenyls and hexahydropentaphenyls, their isomers and other hydrocarbons

[1] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15941/1 (retrieved 20.2.2018)

Annex II. Estimation of the applicability and validity of QSAR predictions related to Bioaccumulation

Estimation of the applicability of KOWWIN predictions

According to EPI Suite help, "The KOWWIN model is built on a training set of 2447 compounds. The model has been tested on an external validation dataset of 10 946 compounds (compounds not included in the training set). The validation set includes a diverse selection of chemical structures that rigorously test the predictive accuracy of any model. It contains many chemicals that are similar in structure to chemicals in the training set, but also many chemicals that are different from and structurally more complex than chemicals in the training set. The average molecular weight of compounds in the validation set is 258.98 versus 199.98 for the training set. "The correlation between the experimental and predicted logKow values in the validation set is r^2 = 0.943, std = 0.479." "Currently there is no universally accepted definition of model domain. However, users may wish to consider the possibility that log Kow estimates are less accurate for compounds outside the MW range of the training set compounds (18.02 – 719.92), and/or that have more instances of a given fragment than the maximum for all training set compounds. It is also possible that a compound may have a functional group(s) or other structural features not represented in the training set, and for which no fragment coefficient was developed. These points should be taken into consideration when interpreting model results."
In general, the correlation between the experimental and predicted log Kow values in the validation set is considered quite good (r^2 = 0.943, std = 0.479).
The assessed terphenyl and quaterphenyl constituents fit the MW range of the model. The model identifies the relevant fragments (aromatic and aliphatic carbons). The number of re instances of a given fragment does not exceed the maximum for all training set compounds.
In conclusion, it can be stated that the applicability and validity of the KOWWIN estimates for the assessed terphenyl/quaterphenyl constituents seem reasonable.

Fragment / factor	*Maximum number of instances in any substance in the training set**	*KOWWIN Number of fragments in analyzed constituents (in bold when exceeding the maximum)*				
		T	*HT1*	*HT3*	*Q*	*HQ3*
Aromatic Carbon Equation Constant	*24*	*18*	*12*		*24*	*6*
-CH2- aliphatic carbon	*18*		*5*	*14*		*13*
-CH- aliphatic carbon	*16*		*1*	*4*		*5*

**See Episuite Help (Accuracy and Domain and Appendix D)*

Estimation of the applicability of EPI Suite BCFBAF regression model
The BCFBAF regression model uses the non-ionic regression models to predict BCF values for the relevant terphenyl and quaterphenyl constituents. The non-ionic training dataset includes 466 compounds. The dataset is divided into three groups based on log Kow values (log Kow < 1.0, log Kow 1.0 to 7.0 and log Kow > 7.0). For each group a "best-fit" straight line has been derived by common statistical regression methodology. The regression methodology includes derivation of correction factors based on specific structural features (ketone, phosphate ester, multi-halogenated biphenyl/PAH, aromatic ring-CH-OH, aromatic sym-triazine ring, ter-butyl ortho-phenol type, phenanthrene ring, cyclopropyl-C(=O)-O-ester, alkyl chains, disulfide, multihalogenated phenol).

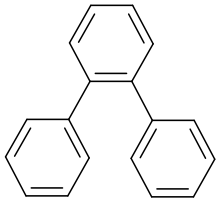
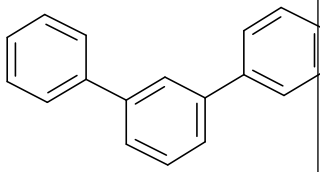
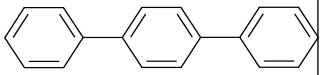
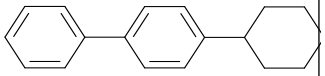
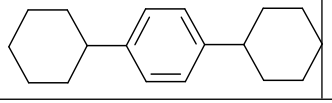
The model predictions are based on the regression equations without structure related correction factors. The following equations are used to predict the BCF-values:
Log BCF = 0.6598 log Kow - 0.333 (1 < log Kow < 7; no applicable correction factor)
Log BCF = -0.49 log Kow + 7.554 (log Kow > 7.0, no applicable correction factor)
According to EPI Suite help, "there is currently no universally accepted definition of model domain. However, users may wish to consider the possibility that bioconcentration factor estimates are less accurate for compounds outside the MW and logKow ranges of the training set compounds, and/or that have more instances of a given correction factor than the maximum for all training set compounds. It is also possible that a compound may have a functional group(s) or other structural features not represented in the training set, and for which no fragment coefficient was developed; and that a compound has none of the fragments in the model's fragment library. In the latter case, predictions are based on molecular weight alone. These points should be taken into consideration when interpreting model results."
The minimum and maximum values for molecular weight and log Kow for the training set are listed below:
Molecular Weight:
Minimum MW: 68.08 (Furan)
Maximum MW: 959.17 (Benzene, 1,1 -oxybis[2,3,4,5,6-pentabromo-)
Average MW: 244.00

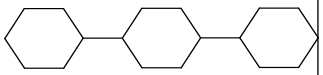
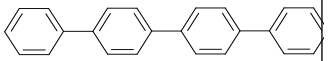
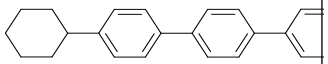
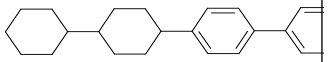
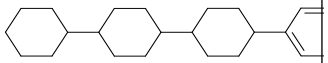
Log Kow:
Minimum Log Kow: -1.37 (1,3,5-Triazine-2,4,6-triamine)
Maximum Log Kow: 11.26 (Benzenamine, ar-octyl-N-(octylphenyl)-)

The terphenyl and quaterphenyl constituents are within the set boundaries for molecular weight and log Kow values.

Estimation of the applicability of EPI Suite BCFBAF Arnot-Gobas model to terphenyl/quaterphenyl constituents
According to EPI Suite help, "the Arnot-Gobas model estimates steady-state bioconcentration factor (BCF; L/kg) and bioaccumulation factor (BAF; L/kg) values for non-ionic organic chemicals in three general trophic levels of fish (i.e., lower, middle and upper) in temperate environments. The model calculations represent general trophic levels (i.e., not for a particular fish species) and are derived for "representative" environmental conditions (e.g., dissolved and particulate organic carbon content in the water column, water temperature). Thus, it provides general estimates for these conditions in absence of site-specific measurements or estimates. The default temperature for the BCF and BAF calculations is 10°C."
There is no specific description of model applicability or accuracy. However, "model predictions may be highly uncertain for chemicals that have estimated log K_{OW} values > 9." It is noted, that for HQ2 and HQ3, the predicted logKow eatimates exceed 9 and therefore the Arnot-Gobas estimations are not considered reliable for these.
It is further noted, that the Arnot-Gobas model assumes default lipid contents of 10.7%, 6.85% and 5.98% for the upper, middle and lower trophic levels. Since the laboratory studies from which most data in the measured BCF database were derived typically used fish with 3-5% lipid content, this may help explain why the regression-based BCF model typically yields estimated BCF values lower than from the Arnot-Gobas model. Therefore the values predicted for this assessment have been normalised to 5 % lipids.

Annex III Evaluation of the performance of the BIOWIN 1-6 models based on comparison of the investigated constituents with the chemicals used to derive the models

		Remarks on model applicability	
Group	Representative structure	Biowin 1-4 [a-d]	Biowin 5-6 [e,f]
o-T		The chemicals used to derive the models do not include three-ring aromatics.	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings or with C-C bond between aromatic rings.
m-T		The chemicals used to derive the models do not include three-ring aromatics.	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings or with C-C bond between aromatic rings.
p-T		The chemicals used to derive the models do not include three-ring aromatics.	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings or with C-C bond between aromatic rings.
HTI		The chemicals used to derive the Biowin models 1-2 and 3-4 include relatively few chemicals with two or more aromatic rings, with non-aromatic ring structures, or with a combination of aromatic and non-aromatic rings,	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings, with non-aromatic ring structures, with a combination of aromatic and non-aromatic rings, or with C-C bond between aromatic rings.
HT2		The chemicals used to derive the Biowin models 1-2 and 3-4 include relatively few chemicals with two or more aromatic rings, with non-aromatic ring structures, or with a combination of aromatic and non-aromatic rings. Chemicals with two or	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with a combination of aromatic and non-aromatic rings, with C-C bond between aromatic rings, or with non-aromatic ring structures. Chemicals with two or more

		more non-aromatic rings are not included.	non-aromatic rings are not included.
HT3		The chemicals used to derive the Biowin models 1-2 and 3-4 do not include chemicals with two or more non-aromatic rings.	The chemicals used to derive the Biowin models 5-6 do not include chemicals with two or more non-aromatic rings.
Q		The chemicals used to derive the Biowin 1-2 and 3-4 include relatively few chemicals with aromatic compounds with two or more rings.	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings.
HQ1		The chemicals used to derive the Biowin models 1-2 and 3-4 include relatively few chemicals with two or more aromatic rings, with non-aromatic ring structures, or with a combination of aromatic and non-aromatic rings.	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings, with a combination of aromatic and non-aromatic rings, with C-C bond between aromatic rings, or with non-aromatic ring structures.
HQ2		The chemicals used to derive the Biowin models 1-2 and 3-4 include relatively few chemicals with two or more aromatic rings, with non-aromatic ring structures, or with a combination of aromatic and non-aromatic rings. Chemicals with two or more non-aromatic rings are not included	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings, with a combination of aromatic and non-aromatic rings, with C-C bond between aromatic rings, or with non-aromatic ring structures. Chemicals with two or more non-aromatic rings are not include.
HQ3		The chemicals used to derive the Biowin models 1-2 and 3-4 include relatively few chemicals with with non-aromatic ring structures, or with a combination of aromatic and non-aromatic rings. Chemicals with two or more non-aromatic rings are not included.	The chemicals used to derive the Biowin models 5-6 include relatively few chemicals with two or more aromatic rings, with a combination of aromatic and non-aromatic rings, with C-C bond between aromatic rings, or with non-aromatic ring structures. Chemicals with two or more non-aromatic rings are not included.

The performance of the models in prediction of biodegradation of the representative structures was evaluated by considering relevant chemicals in the list of chemicals used to derive the models. It should be noted however that all relevant structures have not necessarily been considered.

[a]Performance of Biowin 1 and 2 in prediction of biodegradation of aromatic structures. The chemicals considered include aromatic structures with one ring (1-naphthol, cumene, phenol), two rings (2-phenylphenol, 1,1´-biphenyl, naphthalene), four rings (benz(a)anthracene), and five rings (dibenz(a,h)anthracene). Of these, the one- or two-ring compounds were evaluated as “biodegrades fast” and the four- and five-ring compounds as “does not biodegrade fast”. In addition, a compound with both aromatic and non-aromatic ring structures is included (acenaphthalene). The predictions match with the evaluations. It is noted that of the mentioned compounds only one (1,1’-biphenyl) includes the C-C bond between the aromatic rings which is found in many of the studied terphenyl constituents; for 1,1’-biphenyl the prediction by Biowin 1 and 2 is “biodegrades fast”. In addition, no three-ring aromatic compounds are included. As there is a difference between degradability of the two-ring and four/five-ring aromatics among the mentioned chemicals, and considering that no three-ring aromatic compounds were included in the model derivation, predicting the degradation of

three-ring aromatics can be questionable. In addition, the Biowin models 1-4 recognise only two unsubstituted phenyl groups in 1,1'-biphenyl, terphenyl (o-, m-, or p-), and quatraphenyl and therefore the differences in predictions for these compounds are solely due to molecular weight. This can possibly explain a part of the differences between the Biowin 1-4 and Biowin 5-6 results.

[b]Performance of Biowin 3 and 4 in prediction of biodegradation of aromatic structures. The chemicals considered include aromatic structures with one ring (n-hexylbenzene, 2-phenylethanol), two rings (bibenzyl, diphenyl ether, o-phenylphenol), and four rings (benzanthracene). No three-ring aromatic compounds are included. In addition, a compound with both aromatic and non-aromatic ring structures is included (acenaphthalene). Of these, the predictions by Biowin 3 and 4 match with the observed values reasonably well as the difference between evaluated and predicted value is at maximum 0.51 and 0.35 for Biowin 3 and 4, respectively (the scale of values is 0-5). The difference in the assigned degradation category based on predicted and evaluated values would be at maximum one category (e.g., "weeks" vs. "days to weeks" when the results are 2.65 and 2.8161 and therefore on different sides of the range cut-off value of 2.75 (Biowin help Chapter 7.2.2.). Compared to the Biowin 3 cut-off value of 2.25 used in the ECHA P/vP screening criteria, there would be no difference between the predicted and evaluated values for these compounds as they exceed the cut-off value of 2.25 and therefore would not screen as P based on Biowin 3, with the exceptions of the four-ring compound, benzanthracene (evaluated 1.76, predicted 1.8953) and the evaluated value for acenaphthene (evaluated 2.2, predicted 2.71). It is noted that of the mentioned compounds only one (o-Phenylphenol) includes the C-C bond between the aromatic rings which is found in many of the studied terphenyl constituents. For o-Phenylphenol the evaluated and predicted values (for Biowin 3, 3.08 and 2.9014 and for Biowin 4, 3.64 and 3.6467) would result in the same degradation category ("weeks" for Biowin 3 and "days to weeks" for Biowin 4). As there seems to be a difference between degradability of the two-ring and four-ring aromatics among the mentioned chemicals, and considering that no three-ring aromatic compounds were included in the model derivation, predicting the degradation of three-ring aromatics can be questionable. In addition, The Biowin models 1-4 recognise only two unsubstituted phenyl groups in 1,1'-biphenyl, terphenyl (o-, m-, or p-), and quatraphenyl and therefore the differences in predictions for these compounds are solely due to molecular weight. This can possibly explain a part of the differences between the Biowin 1-4 and Biowin 5-6 results.

[c]Performance of Biowin 1 and 2 in prediction of biodegradation of non-aromatic cyclic structures. The lists of chemicals used to derive the Biowin 1 and 2 include two non-aromatic cyclic structures containing only C, O, and H: cyclohexanone and cis-cyclopentanetetracarboxylic acid. For cyclohexanone, the predictions match the evaluation whereas for cis-cyclopentanetetracarboxylic acid the prediction "biodegrades fast" is conflicting with the evaluation. Therefore, predicting the degradation of non-aromatic cyclic hydrocarbon structures can be questionable.

[d]Performance of Biowin 3 and 4 in prediction of biodegradation of non-aromatic cyclic structures. The lists of chemicals used to derive the Biowin 3 and 4 include only one cyclic structure containing only C,O, and O: E-caprolactone. The evaluated and predicted values (for Biowin 3, 3.7 and 3.0871 and for Biowin 4, 4.13 and 3.912) would differ by one category ("days to weeks" and "weeks") for Biowin 3 or result in the same category ("days") for Biowin 4. Therefore, predicting the degradation of non-aromatic cyclic hydrocarbon structures can be questionable.

[e]Performance of Biowin 5 and 6 in prediction of biodegradation of aromatic cyclic structures. The lists of chemicals used to derive the Biowin 5 and 6 include one-ring (benzene), two-ring (1,1'-biphenyl; phenol, 4-(phenylmethyl), and three-ring (anthracene) aromatic structures. In addition, a structure including both non-aromatic and aromatic ring (naphthalene, 1,2,3,4-tetrahydro) is included. For these compounds the prediction matches with observation for the other compounds except 1,1'-biphenyl. It is noted that of the mentioned compounds only one (1,1'-biphenyl) includes the C-C bond between the aromatic rings which is found in many of the studied terphenyl constituents but which is not recognised by the Biowin 5 and 6 models. For 1,1'-biphenyl the prediction by Biowin 5 and 6 is "not readily biodegradable" although it was observed to be readily biodegradable in the MITI test. Therefore, predicting the degradation of three-ring aromatics or compounds containing the C-C bond between the aromatic rings can be questionable.

[f]Performance of Biowin 5 and 6 in prediction of biodegradation of non-aromatic cyclic structures. The lists of chemicals used to derive the Biowin 5 and 6 do not include non-aromatic structures with two or more rings. Some non-aromatic structures with C-C bonds are included (cyclohexanol, cyclohexane, cyclododecane, cyclohexanone). For these compounds the prediction matches with evaluation in three (Biowin 5) and two (Biowin 6) cases. Looking closer at cyclohexane and cyclododecane, which consist of the same fragments as HT3, indicates that there is no match between prediction and evaluation (with the exception of Biowin 6 for cyclododecane). Therefore, predicting the degradation of non-aromatic cyclic hydrocarbon structures can be questionable.

Annex IV Human health hazard assessment

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

A toxicokinetic study (Monsanto Company, Environmental Health Laboratory 1990) was performed with radioactive 3H Therminol 66 to determine the disposition and localization of the substance in rats as a function of dose and time, and to determine the effects on liver and kidney microsomal drug-metabolizing enzymes (i.e. ethoxycoumarin-o-deethylase, hydrocarbon hydroxylase) following oral and inhalation administration. Therminol 66 was administered to male Sprague Dawley rats as either a single oral dose by gavage at 0,100, or 300 mg/kg body weight, or as a single 6 hour inhalation exposure of 0 or 350 mg/m3, or in the diet at concentrations of 0, 100, 500 or 5000 ppm, or as a repeated inhalation exposure for 6 hours/day for 14 days at 0, 25, 250, at 1200 mg/m3. The study was conducted in general accordance with GLP but several definicies were reported (e.g. the stability of the test substance, neat and after mixing with carrier, was not determined; characterization of the test substance was not conducted according to the standards and characterization and stability data for reference substances were not developed according to the standards).

Results from the disposition study indicated that Therminol 66 did not appear to be extensively absorbed after a single oral dose of 300 mg/kg and did not appear to accumulate in the body tissues. Absorption was not determined in this study. However, the minimum amount absorbed, calculated as the amount of radioactivity excreted in the urine plus the amount of radioactivity in the organs and carcass, was approximately 31.9% (normalised data) of the administered dose 8 hours after gavage. The greatest amount of radioactivity was found in the intestinal contents (followed by the carcass and liver) 8 hours after an oral dose of Therminol 66 at 300 mg/kg, with the faces representing the major route of elimination. Very little radiolabel was evident in the kidney and liver 48 hours after gavage. After 48 hours the amount detected in the feces had increased to approximately 75% of the administered dose and at 168 hours greater than 85% of the dose had been excreted by fecal elimination. Approximately 11 % of the administered dose was excreted in the urine over the 168 hour observation period. The half-lives for elimination via the urine and feces were estimated to be 23.0 and 13.0 hours, respectively. The whole body elimination reflected that of the feces and the half-life was estimated to be 14.0 hours.

In a supporting study (Adamson et al. 1974) mice (male) were exposed by inhalation for 4 or 7 hours to radioactive (partial) hydrogenated terphenyl (HB40) at 10µCi/mL. Clearance of the radiolabel from the respiratory tract was complete within 24 hours. Radioactivity in the gut, which was significantly increased immediately after inhalation, was reportedly equivalent to control values within 24 hours of compound administration. No accumulation was noted in the gut, kidney, and liver since radioactivity levels 24 hours after the final exposure were similar for mice exposed once compared with mice exposed for five consecutive days. Mice were also exposed by oral administration to radioactive (partial) hydrogenated terphenyl at 100µCi/mL and demonstrated radioactivity in the gut, liver, and kidney. The radioactivity was maximal at 4 to 5 hours after administration and steadily disappeared to background levels within 7 days.

4.1.3 Conclusion on toxicokinetics (and bioaccumulation in humans)

The evaluation is based on summarised information from the registration dossier (ECHA 2017c). Several definicies related to the test substance stability and characterisation of the test substance were reported in the study summaries. In addition, it is mentioned in the study summaries that radiolabelled Therminol 66 was used in the toxicokinetic tests, but no detailed information is available on which compounds were radiolabelled and how

the radiolabeling was conducted. The limited information makes it difficult to interpret available results. Because of limited information available in the registration dossier, no final conclusion can be draw on accumulation potential of therphenyl, hydrogenated in mammals.

The available information in rats seems to suggest that approximately 30% of an oral dose of Therminol 66 was absorbed from the gastrointestinal tract, there was little accumulation in tissues, and the whole body half-life was less than 1 day. In mice, after inhalation administration no accumulation was noted. The available information suggests no indication of bioaccumulation in rats and mice. It is, further noted that the repeated dose toxicity studies included in the dossier indicate that Therminol 66 is systemically available at least to some extend via oral and inhalation routes.

4.3 Irritation

Not relevant.

4.4 Corrosivity

Not relevant.

4.5 Sensitisation

Not relevant.

4.6 Repeated dose toxicity

Terphenyl, hydrogenated has neither a harmonised classification nor self-classifications for specific target organ toxicity–repeated exposure (STOT RE) in CLP Regulation. The repeated dose toxicity of terphenyl hydrogenated has been studied via oral, inhalation and dermal routes in various species including rats, rabbits and mice. The studies are considered adequate. The studies mainly revealed slight changes in clinical chemistry and haematology parameters and changes in organ weights. Liver, kidney, spleen and skin were identified as target organs. No significant toxic effects indicative of organ dysfunction (below guidance values of ECHA guidance on the application of the CLP criteria) potentially warranting classification were reported.

A repeated dose toxicity via oral route (Bio dynamics Inc. 1984) was conducted according to EPA and GLP guidelines with minor deviations from OECD TG 408. Sprague-Dawley CD rats (12/sex/group) received Therminol 66 at dose levels of 50, 200 and 2000 ppm in the diet for a period of approximately 14 weeks, corresponding with nominal doses of 3, 12 and 120 mg/kg bw/day. The NOAEL was determined to be 200 ppm in the diet for both sexes, corresponding with 12 mg/kg bw/day. The body weights of the high dose (120 mg/kg bw/day) females were slightly lower (3-7%) than controls throughout the treatment and food consumption of high dose animals was slightly lower than controls during the first week of the study. Slight but statistically significant and consistent changes in hematological and clinical chemistry parameters were observed in high dose animals. In males these included decreased hemoglobin, hematocrit and erythrocyte counts and increased platelet counts, cholesterol and albumin levels. The high dose females had slightly reduced glucose levels. The absolute and relative liver weights were significantly increased in high dose males and females (47% and 21% increases in absolute liver weights compared to controls in males and females, respectively). Kidney weights were increased in high dose males and females and adrenal weights were significantly increased in high dose females. There were no remarkable gross or histological findings correlating

with organ weight changes. However, there was an increased incidence (but not increased severity) of a spontaneously occurring renal tubular lesion in high dose males (incidences 4/12, 5/12, 5/12 and 10/12 at 0, 3, 12 and 120 mg/kg, respectively). The lesion was characterised by single or multiple small foci (young regenerative cells) of proximal tubule epithelial cell hypertrophy and basophilia. Females were essentially free of this lesion. The cause and the toxicological significance of the lesion remained obscure.

In a preceding dose range finding study Sprague-Dawley CD rats (5/sex/dose) received Therminol 66 in a diet at 0, 1.000, 5.000, 10.000, 20.000 ppm corresponding to 60, 300, 600, 1.200 mg/kg bw/day nominal (Bio dynamics Inc. 1985a). The NOAEL was determined to be 60 mg/kg bw/day. Increased liver weights were observed at all doses (only slightly at 60 mg/kg bw/day) accompanied with liver enlargement, surface irregulations and discoloration at doses ≥300 mg/kg bw/day. Decreased body weights and increased spleen weights were observed in females dosed with ≥300 mg/kg bw/day. In males receiving ≥600 mg/kg bw/day decreased body weights and increased food consumption were observed. The high dose (1200 mg/kg bw/day) animals had decreased kidney weights. One high dose male died during the study but the cause of death was not determined.

In an open literature study mice were exposed via oral route for 112 days (1 or 2 doses/week) at doses 20 to 2.000 mg/kg bw/day (Adamson and Weeks 1973). Effects observed were kidney histopathogical findings in males at 600 (only slight) and 1200 mg/kg bw/day. NOAEL was 600 mg/kg body weight/day.

The repeated dose toxicity of terphenyl hydrogenated via dermal route has been studied in a GLP compliant study (International Research and Development Corporation, 1981). HB-40 was administered by dermal application to groups of 10 male and 10 female New Zealand White rabbits, one-half with intact skin and one-half with abraded skin, five days/week 6h/day for 21 days at doses 0, 125, 500 and 2000 mg/kg bw. There were no major signs of systemic toxicity. Thus the NOAEL was determined to be 2000 mg/kg bw. All dosed groups exhibited signs of dermal irritation and gross and microscopic skin changes including blanching (125 and 2000 mg/kg), subcutaneous hemorrhaging (125 and 500 mg/kg), thickening and crust formation of the skin, epithelial acanthosis, epidermal hyperkeratosis, inflammatory cell infiltrates and microabscesses (2000 mg/kg). The severity of the skin changes were generally more pronounced in high dose animals. Thus these findings were considered to be related to the dermal application of HB-40.

A repeated dose toxicity study via inhalation was conducted according to OECD 413 and GLP guidelines, and is considered reliable (Bio dynamics Inc. 1986a). Therminol-66 was administered by whole-body inhalation exposure as an aerosol to 90 CD (Sprague-Dawley derived) rats (15/sex/group) for 6h/day, 5 days/week for thirteen weeks at target concentrations of 0, 10, 100 and 500 mg /m3. The NOAEL was determined to be 100 mg/m^3 corresponding to 0.1 mg/L. There were few deaths but these were not considered to be treatment-related. Increased chromodacryorrhea, excess lacrimation and rough coat were observed in all dosed male groups and increased incidences of dried brown material around the facial area in all treated groups of females. The body weights of high dose (500 mg/m^3 corresponding to 0.5 mg/L) males decreased approximately by 8% compared to controls. The serum glutamic oxaloacetic transaminase and glucose levels were slightly decreased in high dose females (0.5 mg/L) and the mean total protein, albumin and calcium levels were increased in mid dose (100 mg/m3, 0.1 mg/L) and high dose females compared to controls. These findings appeared to be treatment related but were not considered toxicologically significant in the absence of supporting microscopic or organ weight findings. The mean blood urea nitrogen level was increased in high dose males at week 14 compared to control males. However, no renal pathology was seen and thus the finding was not considered toxicologically significant. The absolute and relative liver weights were significantly increased for all dose male groups compared to control males. There were no remarkable histological findings.

In an open literature inhalation study, mice were exposed to terphenyl, hydrogenated as an aerosol for up to 8 days at a concentration of 0.5 mg /L air, followed by a 56-day recovery period (Adamson, et al 1969). Histopathology of the lungs showed change of mitochondria in alveolar type 2 cells, however this change was reversible 42 days after final exposure. A 30-day pilot aerosol inhalation toxicity study in rats with SANTOSOL®340 at target chamber concentrations 0, 10, 50 and 250 mg/m3 air showed some hypoactivity only noted in the high dose group and only during the exposure period. The NOAEL was between 0.05 and 0.025 mg/L air (Industrial Bio-test lab. 1976).

4.7 Mutagenicity

Mutagenicity of terphenyl, hydrogenated, has been assessed based on study summaries of experimental *in vitro* and *in vivo* studies. There are five *in vitro* studies available: two bacterial reverse mutation assay (MRC Dayton 1978, Clark et al. 1979), one mammalian cell gene mutation assay (Pharmakon Research International 1985), and two unscheduled DNA synthesis in mammalian cells (Monsanto 1982, SRI International 1985). In addition to these, one *in vivo* chromosome aberration assay is avaiblable (Monsato 1986). There is no human information available on mutagenicity. All results from *in vitro* and *in vivo* studies indicate consistently that terphenyl, hydrogenated, does not cause genotoxicity under test conditions in the reported studies. Thus, there are no indications of terphenyl, hydrogenated-induced genotoxicity.

Mutagenicity in four *Salmonella typhimurium* strains (TA 1535, TA 1537, TA 98 and TA 100) was tested in a bacterial reverse mutation assay with six concentrations of terphenyl, hydrogenated (0.01, 0.04, 0.2, 1.0, 3.0, 10 µl/plate) with S9 mix (metabolic activation) and without it (MRC-Dayton 1978). No mutagenicity was observed in any of the strains tested under test conditions. Cytotoxicity was also tested in TA 100 strain with six concentrations (100.0, 30.0, 10.0, 1.0, 0.3, 0.1 µl/plate) and no toxicity was observed at any concentrations studied with or without metabolic activation.

In another reverse mutation assay (Clark et al. 1979), mutagenicity in five *Salmonella typhimurium* strains (TA 1535, TA 1537, TA 1538, TA 98 and TA 100) was tested with and without metabolic activation. No detailed information on the tested dose levels is available. Also, information on metabolic activation system is lacking. In the study summary, it is reported that all strains tested were negative for mutagenicity under test conditions up to 10 000 µg terphenyl, hydrogenated/plate with and without metabolic activation. There were no signs of cytotoxicity.

Mammalian cell gene mutation assay (Pharmakon Research International1985) was conducted equivalent to OECD 476 guideline and according to the GLP guidelines. In this study, Chinese hamster ovary cells were treated with five doses of the Therminol 66, i.e. terphenyl, hydrogenated, (25, 50, 75, 100 and 300 µg/ml) with and without metabolic activation. Under test conditions, tested doses of the test substance in the presence or absence of S-9 (metabolic activation), did not statistically significantly increase the frequency of mutations compared to negative controls. No cytotoxicity was observed when test substance was tested up to concentration of 1000 µg/ml. However, precipitation of terphenyl, hydrogenated was observed in the treatment media at all doses above 100 µg/ml.

Unscheduled DNA synthesis (UDS) study (SRI International1985) for DNA damage and repair was conducted under GLP according to SRI International method. In this study primary rat hepatocytes were treated with various concentrations of Therminol 66 (terphenyl, hydrogenated), i.e. 0.1, 0.5, 1, 5, 10, 50, 100, 250, 500 and 1000 µg/ml, with and without metabolic activation. No information is provided on the metabolic activation system. Under the test conditions, test results were negative. Cytotoxicity was not observed up to concentration of 5000 µg/ml.

In another UDS study (Monsanto 1982), rat primary hepatocytes were treated with the terphenyl, hydrogenated (NBP2087922). However, there is no information on doses and whether study was carried out both with and without metabolic activation. The test results are reported to be negative.

A GLP compliant chromosome aberration assay (micronucleus test) according to SRI International method (similar to OECD 475 guideline;Mammalian Bone Marrow Chromosome Aberration Test)) (Monsanto 1986) was performed in male and female rats (Fischer 344). Rats were exposed by a single intraperitoneal injection to terphenyl, hydrogenated (Therminol 66) at dose levels of 250, 1250 and 2500 mg/kg bw. Rats were sacrificed 6, 12, and 24 hours after the treatment. Positive control groups received 0.2 mg triethylenemelamine/kg bw intraperitoneally and were sacrificed 24 hours after the treatment. Cells from control rats and rats treated with the test substance (2500 mg/kg bw), and positive control rats were microscopically evaluated for mitotic index and chromosomal abnormalities. As a conclusion, Therminol 66 does not induce chromosomal damage in male or female Fischer 344 rats under the conditions used in this study.

4.8 Carcinogenicity

A dermal carcinogenicity study published in open scientific literature is available (Henderson 1973). HB40 was dermally administered at a dose of 50 mg/week for 37 weeks in mice under different conditions (once or more times a week; followed by croton oil administration) and in different strains (Balb/c and PLA). No indications of neoplastic effects were observed. The NOAEL for carcinogenicity was therefore 50 mg/week, corresponding with 280 mg/kg bw/day. The in vitro and in vivo studies for mutagenicity and clastogenicity reported in the dossier revealed no indication for genotoxicity. Moreover, there were no evidence of hyperplasia and/or pre-neoplastic lesions following sub chronic repeated dose toxicity exposures. Based on this information it is concluded that terphenyl hydrogenated is not expected to be carcinogenic.

4.9 Toxicity for reproduction

Reproductive toxicity was assessed in three studies: in a two-generation reproductive toxicity study, in a dose range-finding study for developmental toxicity in rat and in a prenatal developmental toxicity study in rat. The main findings from the studies can be summarised as follows:

In a two-generation reproductive toxicity study (Monsanto Agricultural Company, Environmental Health Laboratory, 1991) male and female Sprague-Dawley rats (30 adults/sex/dose) were continuously fed Therminol 66 through two generations at target levels of 0, 30, 100, 300 and 1000 ppm in their diet. The study was reported to be conducted according to OECD 416 and GLP guidelines. The study was conducted prior to revision of the original OECD test guideline 416 (from 1986) in 2001.

Analyses to verify the stability of the test material both neat (presumably pure) and when mixed with the diet, the diet homogeneity and concentrations of the test material in the diet were reported and were all performed with satisfactory results. Overall study averages for consumption of test material (mg Therminol 66/kilogram body weight/day), based on the target concentrations, were for the F0 animals as follows: 1.8, 6.1, 18.5 and 62.0 for males and 2.5, 8.3, 24.4 and 81.2 for females and for F1a animals 1.9, 6.1, 18.2 and 63.1 for males and 2.4, 8.1, 24.3 and 80.6 for females, respectively. At the start of the study animals were approximately 5 weeks of age. Information on the length of premating exposure time is not available in the study summary. F0 and F1 animals were treated until day 21 postpartum (F1A and F2A litter) and F2A animals were treated until day 21 postpartum (duration of test 275 days, 7 days/week).
No adverse reproductive effects were reported in any of the measured parameter or indices in adult rats or their offspring. In adult rats minor decrease in group mean body weights

of high dose level F0 males (1000 ppm) near the end of the study (last three weeks) were seen, and slightly decreased body weights of F1a dams during gestation. Maternal weight gain was slightly reduced (bw approximately 93% of the control value) during the gestation day 0 to 21 period, and was increased (regained) during the lactation day 0-7 period (bw 1546% of controls). The increase in maternal weight gain was approximately 15 g (15.9 g at the highest dose vs. 1.0 g in the controls) during the lactation period. There were no differences in the final maternal body weights at the lactation day 21.

On the basis of the above findings, the 1000 ppm was considered the no-observed-adverse-effect-level (NOAEL) for reproductive effects (corresponding with 62-81 mg/kg bw/day for F0 and F1 males and females), and the 300 ppm was considered the NOAEL for parental toxicity in this study (corresponding with 18-24 mg/kg bw/day).

In a dose range finding study (Monsanto 1985b) Therminol 66 was administered orally (gavage) to female Sprague-Dawley rats (5/group) at 0, 125, 250, 500, 1000, 2000 mg/kg bw/day from day 6-15 of pregnancy. Females were sacrificed on Day 20 of gestation and uterine implantation data were evaluated. Fetuses recovered at this time were weighed, sexed and evaluated for external malformations. The maternal NOAEL was 250 mg/kg bw/day. At 500 and 1000 mg/kg bw/day, maternal food consumption was decreased. There were no offspring effects at 1000 mg/kg bw/day. At 2000 mg/kg bw/day, embryonic death and decreased fetal weights were observed. The developmental NOAEL was 1000 mg/kg bw/day.

In a developmental toxicity study (Bio dynamics Inc., 1986b) Therminol 66 was aministered orally (gavage) to female Sprague-Dawley rats (24/group) at 125, 500 and 1500 mg/kg bw/day from day 6 to 15 of gestation. Oral doses given were based upon a preceding dose range finding study (Monsanto, 1985). The study was reported to be conducted according to OECD 414 and GLP guidelines. Females were sacrificed on Day 20 of gestation and subjected to post mortem examinations. Ovaries and uterine content was examined and foetuses were examined for morphological changes (external, soft tissue, skeletal and head). The study was conducted prior to revision of the original OECD test guideline 414 (from 1981) in 2001.

No mortality occurred in the control, low- or mid-dose groups. At the high-dose level, a total of 4 females died, however, the death of one female was reported to be attributed to an intubation error. Excluding this one female, the mortality rate in the high-dose group was 12.5%. No adverse effect of treatment was evident in pregnancy rate data. The pregnancy rate was 100% in the control, mid- and high-dose groups and, 91.7% (22/24) in the low-dose group.

At the low- and mid-dose levels, mean body weight and mean weight gain data during the treatment or post-treatment periods were not considered adversely affected by treatment. At the high-dose level, mean body weights were significantly lower than control on Days 9, 12, 15 and 20 of gestation and mean weight gain during the Day 6-15 gestation interval was significantly lower than control for this same group. No effect on food consumption data was evident at the low-dose level. In the mid-dose group, mean food consumption was significantly lower than control during the Day 8-9 and 9-12 gestation Intervals and in the high-dose group, mean food consumption was significantly lower than control only during the Day 8-9 gestation interval. A statistically significant increase in mean food consumption for the high-dose group during the Day 15-20 was observed.

At the low-dose level, no adverse effect of treatment was evident from the physical evaluations. At the mid-dose level, the incidence of females with areas of alopecia was notably increased at Days 16 and 20 of gestation; no other adverse effects of treatment at the mid-dose level were evident from the physical in-life observation data. At the high-dose level, the incidence of females with staining of the fur in the ano-genital area and/or soft stool was increased during the treatment interval of gestation. Additionally, several

high-dose females were noted early in gestation (Day 9) as emaciated with red material about the snout.

No adverse effect of treatment at the low- or mid-dose level was evident from uterine implantation data. An increase in both the mean number of resorption sites and the mean ratio of resorptions to implants was seen at the high-dose level. These resorption data did not differ statistically from control data and the mean number of resorptions at the high-dose level was reported to be within the range of historical control data for this laboratory. Thus, authors concluded that it is not clear if the increase in resorption data seen at the high-dose level represents an adverse effect of treatment.

No adverse effect of treatment was evident in foetal weight data or foetal sex distribution data at the low- and mid-dose levels. In the high-dose group, mean foetal weight was significantly lower than control. Foetal sex distribution data were not adversely affected at this same dose level.

No increase in the incidence of malformations was seen during the external, visceral or skeletal evaluations of foetuses recovered from females treated at the low- or mid-dose levels. At the high-dose level, no increase in malformation rate was seen during foetal external evaluations, the incidence of external malformations was 0.4 % (1/245). At the high dose level one foetus had abnormal elevation of the snout and a cleft palate. No other external malformations were seen in the remaining 244 high-dose foetuses (20 litters). The incidence of foetuses with a glassy (shiny) appearance (16/245, foetus incidence 6.5 %), considered to be an external variation observation, was significantly increased. Foetuses noted as having a glassy (shiny) appearance were usually the smaller foetuses within the litters and the observation was considered related to retarded foetal development within this group.

The only visceral malformation seen among treated groups was distended renal pelvis (graded as slight). The only visceral variation seen during the study was the distended and/or tortuous condition of the uterus. These incidences were considered be comparable between the control group.

At the high dose the incidence of foetuses with skeletal malformations was statistically significantly higher than control. Skeletal malformations were seen in seven high-dose foetuses (7/119, an incidence of 5.9%) in respect to the control incidence of (1/116, an incidence of 0.6%). The incidences of litters containing foetuses with skeletal malformations for the control, low-, mid-, and high –dose groups were 4.2 % (1/24 litters), 0 % (0/22 litters), 4.2 % (1/24) and 20.0 % (4/20 litters) and the difference was not statistically significant. The incidences of foetuses with one or more ossification variation observations for the control, low-, mid- and high dose groups were 95.7 (154/161 foetuses), 99.3 (146/147), 98 % (159/161) and 100% (119/119).

Two of the seven high-dose foetuses (one foetus from each of two litters) had dissimilar, relatively minor malformations (wavy ribs seen in one foetus and seven lumbar vertebrae seen in one foetus) which were not considered related to treatment. Five high-dose foetuses (four foetuses from one litter and one foetus from a second litter) had one or more skeletal malformations that involved misshapen the exoccipital bones, fusion of the exoccipital bones to the transverse processes of the first cervical vertebra, fused ribs, misaligned thoracic vertebral centre and reduction in number of cervical vertebrae. The two females whose litters contained foetuses with one or more skeletal malformations were reported to be stressed during the treatment period. Both females experienced weight loss during the Day 6-9 gestation interval and at Day 9, and were noted with marked staining of the fur in the ano-genital area and extreme soft stool.

The authors suggested that the skeletal malformations seen in the high-dose foetuses were secondary to maternal toxicity. In conclusion, NOAEL of 125 mg/kg bw/day for

maternal toxicity was derived and was; NOAEL of 500 mg/kg bw/day for foetotoxicity.

Conclusion

The results from available studies do not show adverse effects that could potentially warrant classification for fertility.

In the developmental toxicity study in rats, the incidence of high-dose foetuses (in two different litters) with skeletal malformations was statistically higher than control (i.e misshapen the exoccipital bones, fusion of the exoccipital bones, fused ribs, misaligned thoracic vertebral centre and reduction in number of cervical vertebrae). The litter incidence was not statistically different. In addition, at the high dose level one foetus had visceral malformations i.e. abnormal elevation of the snout and a cleft palate. The visceral and skeletal malformations observed were suggested to be secondary to maternal toxicity at the highest dose level. Based on this study, there is indication of developmental toxicity in the rat study which cause concern and further evaluation is needed.

The registration dossier (ECHA 2017c) does not include developmental toxicity study in second species (i.e rabbit) or adaptation statements to fulfil the standard information requirement. Prenatal developmental toxicity test on a second species is a standard information requirement under REACH for substances manufactured or imported at 1 000 or more tonnes per year. No final conclusion could be drawn on developmental toxicity based on the available information, before this data gap has been fulfilled.

4.10 Summary and discussion of human health hazard assessment

Terphenyl, hydrogenated has neither harmonised classification nor self-classifications for specific target organ toxicity–repeated exposure (STOT RE), carcinogenicity, germ cell mutagenicity or reproductive toxicity in the CLP Regulation.

A toxicokinetic study with radiolabelled Therminol 66 suggests low systemic availability (appr.30%) of therphenyl, hydrogenated via oral route. Only little accumulation in tissues was indicated and the whole body half-life was less than 1 day. After inhalation administration no accumulation was noted. Because of limited information available, no final conclusion can be draw on accumulation potential of therphenyl, hydrogenated. It is noted, that the repeated dose toxicity studies indicate at least some systemic availability of Therminol 66 via oral and inhalation routes.

The repeated dose toxicity of terphenyl hydrogenated has been studied via oral, inhalation and dermal routes in various species including rats, rabbits and mice. The studies mainly revealed slight changes in clinical chemistry and haematology parameters and changes in organ weights. Liver, kidney, spleen and skin were identified as target organs. No significant toxic effects indicative of organ dysfunction potentially warranting classification were reported.

There are two bacterial reverse mutation assays available, one mammalian cell gene mutation assay, two unscheduled DNA synthesis in mammalian cells and one *in vivo* chromosome aberration assay. There were no indications of terphenyl, hydrogenated-induced genotoxicity in these studies. There are no guideline compliant studies available on carcinogenicity of terphenyl, hydrogenated. In a dermal carcinogenicity study published in open scientific literature no indications of neoplastic effects were observed (Henderson 1973). Moreover, there were no evidence of hyperplasia and/or pre-neoplastic lesions following sub chronic repeated dose toxicity exposures. Based on this information including the negative findings on genotoxicity, it is concluded that terphenyl, hydrogenated is not expected to be carcinogenic.

The registration dossier includes three rat studies on reproductive toxicity: a two-generation reproductive toxicity study, a developmental toxicity dose range-finding study, and a prenatal developmental toxicity study. In a two generation reproductive toxicity study no adverse reproductive effects were reported in the parental animals or in the offspring.

In the prenatal developmental toxicity study the incidence of high-dose foetuses (1500 mg/kg/day) with skeletal malformations was statistically significantly higher than the controls. The observed skeletal malformations included misshapen exoccipital bones, fusion of the exoccipital bones, fused ribs, misaligned thoracic vertebral centre and reduction in number of cervical vertebrae. In addition, one high dose foetus had visceral malformations i.e. abnormal elevation of the snout and a cleft palate.
It is concluded that the results from available studies do not show adverse effects that could potentially warrant classification for fertility. However, the rat developmental toxicity study indicates concern for teratogenicity and further evaluation is needed. The registration dossier (ECHA 2017c) does not include a developmental toxicity study in second species (i.e rabbit) or adaptation statements to fulfil the standard information requirement. Based on currently available information no final conclusion can thus be draw on developmental toxicity, before this data gap has been fulfilled.

Annex V Environmental hazard assessment

The environmental hazard evaluation is based on the robust study summaries available in the registration dossier of terphenyl, hydrogenated (ECHA 2017c). In addition, full study reports considered crucial for T assessment were evaluated in detail.

5.1 Aquatic compartment (including sediment)

5.1.1 Fish

For ECOSAR predictions on fish toxicity see Table 83.

5.1.1.1 Short-term toxicity to fish

There are 14 short term toxicity tests on fish available (see Table 1). Most of the studies (10) are on the UVCB substance and based on nominal concentrations. LC50 values based on measured concentrations have been determined in four acute tests for o- or m-terphenyl. These are assessed in detail below.

NITE 5B431G Prolonged Toxicity Test of o-terphenyl with *Oryzias latipes*

A 21-day toxicity test of o-terphenyl with *Oryzias latipes* was conducted in compliance with OECD TG 204. It is noted that the OECD 204 test guideline was deleted 2.4.2014. According to ECHA guidance R.7b (ECHA 2017b), only studies in which sensitive life-stages (juveniles, eggs, larvae) are exposed, can be regarded as long-term fish test. Thus, tests performed according to OECD 204 cannot be considered suitable long-term tests. They are, in effect, prolonged acute studies with fish mortality as the major endpoint examined.

Table 67. Test design

Test material (water solubility (WS) under test conditions)	o-terphenyl WS = -
Stock solution	-
Test organisms	*Oryzias latipes* (medaka)
Feeding	-
Test media	Hardness: - Dissolved oxygen: - Temperature: 24 ± 1 °C pH: -
Quantity of testing liquid / Test vessels	5.0 L glass beaker
Test system	1 container (replicate) / concentration and controls 20 fish / container flow-through (a continuous dilution device with a metering pump), 35 L/day (24.45 ml/min)
Test duration	21 days
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.0051; 0.0102; 0.0225; 0.0450; 0.0941; 0.2045 mg/l + control + auxiliary control (8.2 mg/l HCO-30 / acetone used)

Sampling and analysis	HPLC method

Results

Because variation of measured concentrations exceeded in some cases 20 %, the results presented below were based on measured concentrations. It is noted, that the reported 21-day LC50 value (0.025 mg/l) is lower than the reported 21-day LOEC value (0.041 mg/l). The meaning of the reported LOEC value (0.041 mg/l) is therefore unclear. Neither is the meaning of the reported MLD value clear. Therefore, the LOEC and MLD values are not used in the assessment. Because the test is an prolonged acute test, where apparently only moratality has been observed, only LC50 values are used for the T assessment.

	mg/l	95 % confidence interval mg/l
7-day LC50	0.12	0.098 – 0.14
14-day LC50	0.066	0.053 – 0.084
21-day LC50	0.025	0.020 – 0.033
(21-day MLD)	(0.0087)	-
(21-day LOEC)	(0.041)	-
(21-day NOEC)	(0.0048)	-

Reliability and relevance:

The reliability is evaluated with respect to the OECD 204 “Fish, prolonged toxicity, 14-day study” test guideline although it is noted that the TG was deleted 2.4.2014. The OECD 204 validity criteria are:

- there must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably is should be at least 80 per cent of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20 per cent, results should be based on the measured concentration.
- the mortality in the controls(s) should not exceed 10 per cent at the end of the test
- the dissolved oxygen concentration must have at least 60 % per cent of the air saturation values throughout the test

As crucial information is missing from the study report, it is not possible to evaluate the reliability of the study. There is no information available on:

- measured oxygen levels in the test
- condition of fish in the control groups
- condition of fish in the test groups
- performance (such as sensitivity and accuracy) of the analytical method used to ensure maintenance of the test substance
- measured concentrations of the test substance are not available

Therefore, it is not possible to assess whether the OECD 204 validity criteria are fulfilled or not.

In addition,

- there is no information on the feeding of the fish,

- the meaning of the reported LOEC and MLD values is unclear.

The study is rated Klimish 4 (not assignable).

NITE 5B437G Prolonged Toxicity Test of m-terphenyl with *Oryzias latipes*

A 21-day toxicity test of m-terphenyl with *Oryzias latipes* was conducted in compliance with OECD TG 204. It is noted that the OECD 204 test guideline was deleted 2.4.2014. According to ECHA guidance R.7b (ECHA 2017b), only studies in which sensitive life-stages (juveniles, eggs, larvae) are exposed, can be regarded as long-term fish test. Thus, tests performed according to OECD 204 cannot be considered suitable long-term tests. They are, in effect, prolonged acute studies with fish mortality as the major endpoint examined.

Test design

Test material (water solubility (WS) under test conditions)	m-terphenyl WS = -
Stock solution	-
Test organisms	*Oryzias latipes* (medaka)
Feeding	-
Test media	Hardness: - Dissolved oxygen: - Temperature: 24 ± 1 °C pH: -
Quantity of testing liquid / vessel	5.0 L glass beaker
Test system	1 container (replicate)/ concentration and controls 20 fish / container flow-through (a continuous dilution device with a metering pump), 35 L/day (24.45 ml/min)
Test duration	21 days
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.041; 0.092; 0.204; 0.479; 1.100; 2.546 mg/l + control + auxiliary control (99.3 mg/l HCO-30 / tetrahydrofurane used)
Sampling and analysis	HPLC method

Results

Because variation of measured concentrations exceeded in some cases 20 %, the results presented below were based on arithmetic averages of measured concentrations. The reported 21-day LOEC value is within the 95 % confidence interval of the 21-day LC50 value. Because the test is an prolonged acute test, where apparently only moratality has been observed, only LC50 values are used for the T assessment.

	mg/l	95 % confidence interval mg/l
7-day LC50	>2.4	
14-day LC50	>2.4	
21-day LC50	2.4	1.1 – 2.4

(21-day MLD)	(0.49)	
(21-day LOEC	(1.1)	
(21-day NOEC)	(0.18)	

Reliability and relevance:

The reliability is evaluated with respect to the OECD 204 “Fish, prolonged toxicity, 14-day study” test guideline although it is noted that the TG was deleted 2.4.2014. The OECD 204 validity criteria are:

- there must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably is should be at least 80 per cent of the nominal concentration throughout the test. If the deviation from the nominal concentration is greater than 20 per cent, results should be based on the measured concentration.
- the mortality in the controls(s) should not exceed 10 per cent at the end of the test
- the dissolved oxygen concentration must have at least 60 % per cent of the air saturation values throughout the test

It is not possible to evaluate the reliability of the study as crucial information is missing from the study report. There is no information available on:

- measured oxygen levels in the test
- condition of fish in the control groups
- condition of fish in the test groups
- performance (such as sensitivity and accuracy) of the analytical method used to ensure maintenance of the test substance
- measured concentrations of the test substance are not available.

Therefore, it is not possible to assess whether the OECD 204 validity criteria are fulfilled or not.

In addition,

- there is no information on the feeding of the fish,

The study is rated Klimish 4 (not assignable).

NITE – 5B432G Acute toxicity test of o-terphenyl with *Oryzias latipes*

An 96h acute toxicity test of o-terphenyl with Oryzias latipes was conducted in compliance with OECD 203 TG (1992).

Test design

Test material (water solubility (WS) under test conditions)	o-terphenyl WS = -
Stock solution	-
Test organisms	*Oryzias latipes* (medaka)

Test media	Hardness: - Dissolved oxygen: - Temperature: 24 ± 1 °C pH: -
Quantity of testing liquid	3.0 L
Test system	1 container (replicate) / concentration and controls 10 fish / container semi-static (entire quantity of testing liquid replaced every 24 h)
Test duration	96 h
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.10; 1.20; 0.40; 0.80 and 1.60 mg/l + control + auxiliary control (28.8 mg/l HCO-30 / acetone used)
Sampling and analysis	HPLC method

Results

Because variation of measured concentrations exceeded in some cases 20 %, the results were based on measured concentrations. The 96-hour LC50 values was determined as 0.12 mg/l (95 % confidence interval: 0.053 – 0.27 mg/l).

Reliability and relevance:

It is not possible to evaluate the reliability of the study as crucial information is missing form the study report. There is no information available on:

- measured oxygen levels in the test
- condition of fish in the control groups
- condition of fish in the test groups
- performance (such as sensitivity and accuracy) of the analytical method used to ensure maintenance of the test substance
- measured concentrations of the test substance are not available.

Therefore, it is not possible to assess whether the OECD 203 validity criteria are fulfilled or not.

The study is rated Klimish 4 (not assignable).

NITE – 5B438G Acute toxicity test of m-terphenyl with *Oryzias latipes*

An 96h acute toxicity test of m-terphenyl with Oryzias latipes was conducted in compliance with OECD 203 TG (1992).

Table 68. Test design

Test material (water solubility (WS) under test conditions)	m-terphenyl WS = -
Stock solution	-
Test organisms	*Oryzias latipes* (medaka)

Test media	Hardness: - Dissolved oxygen: - Temperature: 24 ± 1 °C pH: -
Quantity of testing liquid	3.0 L
Test system	1 container (replicate) / concentration and controls 10 fish / container semi-static (entire quantity of testing liquid replaced every 24 h)
Test duration	96 h
Lighting	16 hours light / 8 hours dark
Tested concentrations	1.0; 1.8; 3.2; 5.6 and 10.0 mg/l + control + auxiliary control (100 mg/l HCO-30 / acetone used)
Sampling and analysis	HPLC method

Results

Because variation of measured concentrations was within 20 %, the results were based on set (nominal) concentrations. The 96-hour LC50 values was determined as 3.1 mg/l (95 % confidence interval: 2.1 – 4.8 mg/l).

Reliability and relevance:

It is not possible to evaluate the reliability of the study as crucial information is missing from the study report. There is no information available on:

- measured oxygen levels in the test
- condition of fish in the control groups
- condition of fish in the test groups
- performance (such as sensitivity and accuracy) of the analytical method used to ensure maintenance of the test substance
- measured concentrations of the test substance are not available.

Therefore, it is not possible to assess whether the OECD 203 validity criteria are fulfilled or not.

The study is rated Klimish 4 (not assignable).

Table 1. Short-term effects on fish.

Method	*Results*	*Remarks*	*Reference*
Oryzias latipes *flow-through* *OECD Guideline 204 (Fish, Prolonged Toxicity Test: 14-day Study, prolonged to 21 d)*	*LC50 (14d): 0.066 mg/l (95 % CI 0.053 – 0.084 mg/l)* *LC 50 (21 d): 0.025 mg/l (95 % CI 0.020 – 0.033 mg/l)* *(meas. (arithm. mean)*	*Reliability: 4 (not assignable)* *Test material (o-terphenyl; CAS 84-15-1)* See Chapter 5.1.1.1 for details.	*National Institute of Technology and Evaluation (c) (5B431G)* *J-CHECK database:*

Method	*Results*	*Remarks*	*Reference*
			http://www.safe.nite.go.jp/jcheck/template.action?ano=10858&mno=4-0017&cno=84-15-1&request_locale=en
Oryzias latipes *flow-through* *OECD Guideline 204 (Fish, Prolonged Toxicity Test: 14-day Study, prolonged to 21 d)*	*LC50 (14 d): 2.4 mg/l (meas. arithm. mean)* *LC50 (21 d): 0.49 mg/l l*	*Reliability: 4 (not assignable)* See Chapter 5.1.1.1 for details. *Test material (m-terphenyl; CAS 92-06-8)*	*National Institute of Technology and Evaluation (e) (5B437G)* *J-CHECK database: http://www.safe.nite.go.jp/jcheck/template.action?ano=10975&mno=4-0017&cno=92-06-8&request_locale=en*
Oryzias latipes *semi-static* *OECD Guideline 203 (Fish, Acute Toxicity Test)*	*LC50 (96 h): 0.12 mg/L (meas.) (95 % CL: 0.053 mg/L - 0.27 mg/L*	*Reliability: 4 (not assignable)* See Chapter 5.1.1.1 for details. *Test material (o-Terphenyl; CAS 84-15-1)*	*National Institute of Technology and Evaluation (a) (5B432G)* *National Institute of Technology and Evaluation* *http://www.safe.nite.go.jp/jcheck/template.action?ano=10855&mno=4-0017&cno=84-15-1&request_locale=en*
Oryzias latipes *semi-static* *OECD Guideline 203 (Fish, Acute Toxicity Test)*	*LC50 (96 h): 3.1 mg/L test mat. (nominal) (95 % CL: 2.1 mg/L - 4.8 mg/L*	*Reliability: 4 (not assignable)* See Chapter 5.1.1.1 for details. *Test material (m-terphenyl; CAS 92-06-8)*	*National Institute of Technology and Evaluation (b) (5B438G)* *J-CHECK database: http://www.safe.nite.go.jp/jcheck/TemplateAction?ano=10973&*

Method	Results	Remarks	Reference
			mno=4-0017&cno=92-06-8&request_locale=en
Salmo gairdneri (new name: Oncorhynchus mykiss) freshwater static EPA-660/3-75-009 Methods for acute toxicity tests with fish Macroinvertebrates and Amphibians.	LC50 (96 h): > 1000 mg/L based on: mortality	Reliability: not assessed. Test material (Common name): WCM Form: viscous	Unnamed. (1979a)(Www.echa.europa.eu)
Pimephales promelas freshwater static EPA-660/3-75-009 Methods for acute toxicity tests with fish Macroinvertebrates and Amphibians.	LC50 (96 h): > 1000 mg/L based on: mortality	Reliability: not assessed Test material (Common name): WCM Form: viscous	Unnamed (1979b) (Www.echa.europa.eu)
Salmo gairdneri (new name: Oncorhynchus mykiss) freshwater static EPA-660/3-75-009; Methods for acute toxicity tests with fish, macroinvertebrates and amphibians standard methods for examination of water and wastewater	LC50 (24 h): > 1000 mg/L test mat. (nominal) based on: mortality (median) LC50 (48 h): > 1000 mg/L test mat. (nominal) based on: mortality (median) LC50 (96 h): > 1000 mg/L test mat. (nominal) based on: mortality (median)	Reliability: not assessed Test material (EC name): Terphenyl, hydrogenated (Therminol 66)	Unnamed (1979) (Www.echa.europa.eu)
Pimephales promelas freshwater static EPA-660/3-75-009; Methods for acute toxicity tests with fish, macroinvertebrates and amphibians standard methods for examination of water and wastewater	LC50 (24 h): > 1000 mg/L test mat. (nominal) based on: mortality (median) LC50 (48 h): > 1000 mg/L test mat. (nominal) based on: mortality (median) LC50 (96 h): > 1000 mg/L test mat. (nominal) based on: mortality (median)	Test material (EC name): Terphenyl, hydrogenated (Therminol 66)	Unnamed (1979) (Www.echa.europa.eu)
Salmo gairdneri (new name: Oncorhynchus mykiss) freshwater It is not possible to evaluate whether the test was performed according to any guidelines since there is no access or availability to the study report.	LC50 (96 h): > 100 ppm test mat. (nominal) based on: mortality	Reliability; not assessed. Test material (Common name): Hydrogenated Terphenyls (Therminol 66)	Unnamed (1972) (Www.echa.europa.eu)
Lepomis macrochirus freshwater It is not possible to evaluate whether the test was performed according to any guidelines since	LC50 (96 h): > 100 ppm test mat. (nominal) based on: mortality	Reliability; not assessed. Test material (Common name): Hydrogenated	Unnamed (1972) (Www.echa.europa.eu)

Method	Results	Remarks	Reference
there is no access or availability to the study report.		Terphenyls (Therminol 66)	
Salmo gairdneri (new name: Oncorhynchus mykiss) freshwater It is not possible to evaluate whether the test was performed according to any guidelines since there is no access or availability to the study report.	LC50 (96 h): > 10 — < 100 ppm test mat. (nominal) based on: mortality	Reliability; not assessed. Test material (Common name): not published	Unnamed (1972a) (Www.echa.europa.eu)
Lepomis macrochirus freshwater It is not possible to evaluate whether the test was performed according to any guidelines since there is no access or availability to the study report.	LC50 (96 h): > 10 — < 100 ppm test mat. (nominal) based on: mortality	Reliability; not assessed. Test material (Common name): Santosol 300	Unnamed (1972b) (Www.echa.europa.eu)
Oryzias latipes semi-static OECD Guideline 203 (Fish, Acute Toxicity Test)	LC50 (96 h): 0.12 mg/L test mat. (meas. (not specified)) (95 % CL: 0.053 mg/L - 0.27 mg/L (Calculation of result was based on measured concentration because percentages of measured concentrations compared to nominal areas. exceeded +/- 20 % in some concentration areas)	Reliability: 4 (not assignable) supporting study Study on constituent of UVCB substance. Test material (o-Terphenyl; CAS 84-15-1)	National Institute of Technology and Evaluation (a) (5B432G) National Institute of Technology and Evaluation http://www.safe.nite.go.jp/jcheck/template.action?ano=10855&mno=4-0017&cno=84-15-1&request_locale=en
Oryzias latipes semi-static OECD Guideline 203 (Fish, Acute Toxicity Test)	LC50 (96 h): 3.1 mg/L test mat. (nominal) (95 % CL: 2.1 mg/L - 4.8 mg/L (Percentages of measurement concentrations for nominal concentrations were within ±20 % in all concentration areas. Accordingly, the result was calculated based on nominal concentrations.)	Reliability: 4 (not assignable) supporting study Study on constituent of UVCB substance. Test material (m-terphenyl; CAS 92-06-8)	National Institute of Technology and Evaluation (b) (5B438G) J-CHECK database: http://www.safe.nite.go.jp/jcheck/TemplateAction?ano=10973&mno=4-0017&cno=92-06-8&request_locale=en
Salmo gairdneri (new name: Oncorhynchus mykiss) no guideline mentioned	LC50 (96 h): > 1000 mg/L	Reliability; not assessed. Test material (Common	Unnamed (1979) (Www.echa.europa.eu)

Method	*Results*	*Remarks*	*Reference*
		name): Therminol 66	
Fathead minnows no guideline mentioned	*LC50 (96 h): > 1000 mg/L*	*Reliability; not assessed. Test material (Common name): Therminol 66*	*Unnamed (1979) (Www.echa.europa.eu)*

5.1.1.2 Long-term toxicity to fish

There is one long term toxicity tests on fish available for o-terphenyl. The tests is based on measured concentrations and is assessed in detail below.

NITE 0115EEL Early life stage toxicity test of o-terphenyl with Oryzias Latipes

An Early life stage toxicity test of o-terphenyl with *Oryzias Latipes* was conducted in compliance with OECD 210 test guideline (1992). It is noted that the guideline has been updated in 2013.

According to the OECD 210 (1992) test guideline, "*early-life stages of fish are exposed to, at least, five concentrations of the test substance dissolved in water, preferably under flow-through conditions, or where appropriate, semi-static conditions. The test starts with placing fertilised eggs (at least 60) in the test chambers and continues at least until all the control fishes are free-feeding. Lethal and sub-lethal effects are assessed and compared with control values to determine the lowest observed effect concentration and the no observed effect concentration. The study report should include measurement of the concentrations of the test substance in water at regular intervals (five at least), the dissolved oxygen, pH, total hardness and salinity, fish weight and length, as well as the observations of abnormal appearance, abnormal behaviour, hatching and survival.*

For a test to be valid the following conditions applied:
- the dissolved oxygen concentration must be between 60 and 100 per cent of the air saturation value throughout the test;
- the water temperature must not differ by more than + 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species (Annexes 3 and 6);
- evidence must be available to demonstrate that the concentrations of the test substance in solution have been satisfactorily maintained within ± 20% of the mean measured values;
- overall survival of fertilised eggs in the controls and, where relevant, in the solvent-only controls must be greater than or equal to the limits defined in Annexes 3 and 6;
- when a solubilising agent is used it must have no significant effect on survival nor produce any other adverse effects on the early-life stages as revealed by a solvent-only control.
Food and feeding are critical, and it is essential that the correct food for each stage should be supplied from an appropriate time and at a level sufficient to support normal growth. Feeding should be ad libitum whilst minimising the surplus."

Table 69. Test design

Test material (water solubility (WS) under test	o-terphenyl WS = -

conditions)	
Stock solution	-
Test organisms	*Oryzias latipes* (medaka)
Feeding	-
Test media	Hardness: - Dissolved oxygen: - Temperature: 24 ± 1 °C pH: -
Quantity of testing liquid	2.0 L
Test system	60 fish / test group (20 fish / container x 3 replicates) 3 containers / test group flow-through (a continuous dilution device with a metering pump), 35 L/day (24.45 ml/min) no aeration
Test duration	41 days
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.0046; 0.010; 0.022; 0.046; 0.10 mg/l + control + auxiliary control (100 µg/l DMF)
Sampling and analysis	HPLC method

Results

Because variation of measured concentrations exceeded in some cases 20 %, the results (presented below) were based on averages of measured concentrations.

41-day LOEC: 0.023 mg/l

41-day NOEC: 0.011 mg/l

Reliability and relevance:

It is not possible to evaluate the reliability of the study as crucial information is missing from the study report. There is no information available on:

- measured oxygen levels in the test
- hatching and post-hatch success in the control groups
- hatching and post-hatch success in the test groups
- feeding of the fish
- performance (such as sensitivity and accuracy) of the analytical method used to

ensure maintenance of the test substance

- measured concentrations of the test substance

Therefore, it is not possible to assess whether the OECD 210 validity criteria are fulfilled or not.

The study is rated Klimish 4 (not assignable).

5.1.2 Aquatic invertebrates

For ECOSAR predictions on toxicity to aquatic invertebrates see Table 83.

5.1.2.1 Short-term toxicity to aquatic invertebrates

There are 18 short term toxicity tests available on aquatic invertebrates (table 2). Most of the studies are on the UVCB substance and based on nominal concentrations. One study on the UVCB substance (MONSANTO 1993) as well as two studies on o-, m- and p-terphenyl (NITE studies) are based on measured concentrations and are assessed in detail below. In addition, two studies on individual constituents (Monsanto 1983a and 1983c) were assessed in detail as, although they are based on nominal concentration, they report clear acute effects.

The reliability of the assessed tests is assigned with respect to the OECD 202 test method guideline. For a OECD 202 test to be valid, the following performance criteria apply:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids should have been immobilised (or show other signs of disease or stress, for example, discoloration or unusual behaviour such as trapping

- The dissolved oxygen concentration at the end of the test should be 3 mg/l in control and test vessels.

Monsanto 1993 Acute toxicity of Santotar 9 to *Daphnia Magna*

A flow-through acute toxicity test on *Daphnia magna* was conducted following the ABC Protocol no. 8101-PMN and in accordance with EPA-TSCA, 40 CFR, Part 797, Guideline 797.1300. The test substance was Santotar 9 Polyphenyl. Based on a SDS attached to the study report, the tested material consisted of unhydrogenated quaterphenyl isomers and higher polyphenyls. An EC50 value > 0.069 mg/l is reported.

Table 70. Test design

Test material	Santotar 9 Polyphenyl (brown chunky solid)
Stock solution	950 mg/L in DMF. 0.1 ml aliquot of the stock injected into the 950 ml mixing box.
Test organisms	*Daphnia magna* (In-house ABC Laboratories daphnid culture; first-instar (< 24 hours old)
Test media	Hardness: 134 – 146 mg/l ($CaCO_3$) Dissolved oxygen: 7.4 – 8.0 mg/l Temperature: 20 ± 1 °C pH: 8.2 – 8.4 Flow rate: 3.7 mL/min All solutions were clear throughout the study.

Test system	2 replicates of 5 concentrations (0.006, 0.012, 0.025, 0.050 and 0.10 mg/l), control, vehicle blank 10 daphinds per test vessel (loading 1 daphnid per 100 ml)
Test duration	48 h
Test vessels	1 L
Lighting	16-hour daylight (46 - 62 footcandles), 30 min dusk and dawn.
Sampling and analysis	Water samples were collected from each replicate at 0 and 48 hours and sent to Monsanto Company for analysis. No further details available.

Results

Immobilisation occurred at 0.016 mg/l and higher concentrations (Table 71. Immobility and Behavioral observations (NNo dose-response occurred and therefore, no EC50 values can be determined. According to the study authors, The EC50 values could not be calculated, but were shown to be greater than the water solubility limits of the test material. EC50 was reported as > 0.069 mg/l. NOEC of 0.008 mg/l was reported based on the lack of immobility at this concentration. As there was no dose-response and only 1-2 out of 10 Daphnids were immobilised, it is not meaningful to derive a NOEC from the study.

Table 71. Immobility and Behavioral observations (N = normal, SUR = surfacing, CLR = clear solution).

Measured test Conc. (mg/l)	No. placed in test vessel	24 h		48 h	
		Imm.	Observations	Imm.	Observations
Control A	10	0	10 N; CLR	0	10 N; CLR
Control B	10	1	9 N; CLR	1	9 N; CLR
Veh. Blank A	10	0	10 N; CLR	0	10 N; CLR
Veh. Blank B	10	0	10 N; CLR	0	10 N; CLR
0.004 A	10	0	10 N; CLR	0	10 N; CLR
0.004 B	10	0	10; CLR	0	10 N; CLR
0.008 A	10	0	10 N; CLR	0	10 N; CLR
0.008 B	10	0	8 SUR; 2 N; CLR	0	10 N; CLR
0.016 A	10	0	5 SUR; 5 N, CLR	2	8 N; CLR
0.016 B	11	1	10 N; CLR	1	10 N; CLR
0.031 A	10	1	9 N; CLR	2	8 N; CLR
0.031 B	10	0	10 N; CLR	0	10 N; CLR
0.069 A	10	0	10 N; CLR	0	10 N; CLR
0.069 B	10	1	1 SUR; 8 N; CLR	1	9 N; CLR

Reliability and relevance

The results are considered reliable with restictions (Klimisch 2). No significant deviations from the OECD 202 TG were detected. The OECD 202 validity criteria were fulfilled:
- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids were immobilised (nor other signs of disease or stress, for example, discoloration or unusual behaviour such as trapping at surface of water.)

- The dissolved oxygen concentration at the end of the test was above 3 mg/l in control and test vessels.

However, although the results are based on measured concentrations, there are no details on the used analytical test method. In addition, information on the test substance identity is limited.

It is noted that no dose-response was identified in the study. A NOEC value derived from a short-term acute toxicity test is not considered relevant.

NITE 5B434G Acute Immobilisation Test of o-terphenyl with *Daphnia magna*

An Acute Immobilisation Test of o-terphenyl with *Daphnia magna* was conducted in accordance with OECD test guideline 202 (1984).

Test design

Table 72. Test design

Test material (water solubility (WS) under test conditions)	o-terphenyl WS = -
Stock solution	-
Test organisms	*Daphnia magna*
Test media	Hardness: - Dissolved oxygen: - Temperature: 20 ± 1 °C pH: -
Quantity of testing liquid	100 ml
Test system	4 container / level 20 organism / level (5 organism per 1 replicate, 20 organism per 1 level) semi-static (entire quantity of testing liquid replaced every 24 h)
Test duration	48 h
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.010; 0.027; 0.072; 0.19; 0.52; 1.4; 3.7; 10 mg/l + control + suplementary control (100 mg/l HCO-30 / acetone used)
Sampling and analysis	HPLC method

Results

Because all measured concentrations (measured at the beginning of exposure and after 24h) were within 20 % of the set (nominal) value, the results were based on set (nominal) concentrations. Based on immomilization, the following effect concentrations were reported.

	24 h (mg/l)(95 % CI)	48 h (mg/l) (95 % CI)
EC50	6.2 (4.2 – 11)	0.52 (0.37 – 0.74)
(NOEC)	(0.52)	(0.072)
EC100	>10	> 3.7

Reliability and relevance

It is not possible to evaluate the reliability of the study as crucial information is missing form the study report. There is no information available on:

- measured oxygen levels in the test
- the number and percentage of daphnids that were immobilised or showed any adverse effects (including abnormal behaviour) in the controls and in each treatment group,
- performance of the analytical method (such as sensitivity and accuracy)
- actual measured concentrations

The study is rated Klimish 4 (not assignable).

NOEC values from an acute toxicity test are not considered relevant.

NITE 5B440G Acute Immobilisation Test of m-Tterphenyl with *Daphnia magna*

An Acute Immobilisation Test of m-terphenyl with *Daphnia magna* was conducted in accordance with OECD test guideline 202 (1984).

Test design

Table 73. Test design

Test material (water solubility (WS) under test conditions)	m-terphenyl WS = -
Stock solution	-
Test organisms	*Daphnia magna*
Test media	Hardness: - Dissolved oxygen: - Temperature: 20 ± 1 ºC pH: -
Quantity of testing liquid	100 ml
Test system	4 container / level 20 organism / level (5 organism per 1

	replicate, 20 organism per 1 level) semi-static (entire quantity of testing liquid replaced every 24 h)
Test duration	48 h
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.027; 0.072; 0.19; 0.52; 1.4; 3.7; 10 mg/l + control + auxiliary control (100 mg/l HCO-30 / acetone used)
Sampling and analysis	HPLC method

Results

Because all measured concentrations (measured at the beginning of exposure and after 24h) were within 20 % of the set (nominal) value, the results were based on set (nominal) concentrations. Based on immobilisation, the following effect concentrations were reported,

	24 h (mg/l)(95 % CI)	48 h (mg/l) (95 % CI)
EC50	>10	0.65 (0.47 – 0.86)
(NOEC)	(0.52)	(0.19)
EC100	>10	> 10

Reliability and relevance

It is not possible to evaluate the reliability of the study as crucial information is missing form the study report. There is no information available on:

- measured oxygen levels in the test
- the number and percentage of daphnids that were immobilised or showed any adverse effects (including abnormal behaviour) in the controls and in each treatment group,
- performance of the analytical method (such assensitivity and accuracy)
- actual measured concentrations

The study is rated Klimish 4 (not assignable).

NOEC values from an acute toxicity test are not considered relevant as it is not possible to verify whether a dose-response was observed in the test.

Monsanto 1983c. Acute toxicity of o-terphenyl to *Daphnia magna*.

An Acute Immobilisation Test of m-terphenyl with *Daphnia magna* was conducted in closed vessels according to MIC assessment method and US EPA 1975 Method for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.

Test design

Table 74. Test design

Test material (water solubility (WS) under test conditions)	o-terphenyl WS = -
Stock solution	dimethylformamide (DMF) solution (max. amount of solvent in test 0.5 ml DMF/l)
Test organisms	*Daphnia magna (< 24 h old)*
Test media	Hardness: 180 – 206 mg/l. Dissolved oxygen: 7.5 – 8.4 mg/l Temperature: 20.9 ºC pH: 7.8 – 8.3
Quantity of testing liquid	200 ml
Test system	Static. Closed vessels. 10 Daphnids per vessel. 30 Daphnids per concentration (in three replicates).
Test duration	48 h
Lighting	
Tested concentrations (nominal)	0.022; 0.036; 0.06; 00.1, 0.167 mg/l; control and solvent control
Sampling and analysis	-

Results

Based on the results a 48-hour EC50 value of 0.045 (0.036 – 0.060) mg/l was determined.

Nominal concentration (mg/l)	% Immobilisation for combined replicates 24 h	% Immobilisation for combined replicates 48 h
Control	0	0
Solvent Control	0	3
0.022	0	0
0.036	0	3
0.06	13	100
0.1	27	100
0.167	60	100

Reliability and relevance

The test is rated reliable with restrictions (Klimish 2). The OECD 202 validity criteria were fulfilled:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids were immobilised
- The dissolved oxygen concentration at the end of the test was above 3 mg/l in control and test vessels.

The test is based nominal concentrations and there is no measured data on the test substance or its concentrations. Nevertheless, the test is performed in closed vessels to prevent losses by volatilisation. As clear effects were observed compared to controls, it can be concluded that the EC50 values is 0.045 mg/l or lower (if test substance was lost during the test).

Monsanto 1983a. Acute toxicity of m-terphenyl to *Daphnia magna*.

An Acute Immobilisation Test of m-terphenyl with *Daphnia magna* was conducted in closed vessels according to MIC assessment method and US EPA 1975 Method for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.

Test design

Table 75. Test design

Test material (water solubility (WS) under test conditions)	m-terphenyl WS = -
Stock solution	dimethylformamide (DMF) solution (max. amount of solvent in test 0.5 ml DMF/l)
Test organisms	*Daphnia magna (< 24 h old)*
Test media	Hardness: 180 – 204 mg/l. Dissolved oxygen: 6.0 – 8.3 mg/l Temperature: 21.2 °C pH: 7.8 – 8.2
Quantity of testing liquid	200 ml
Test system	Static. Closed vessels. 10 Daphnids per vessel. 30 Daphnids per concentration (in three replicates).
Test duration	48 h
Lighting	-
Tested concentrations (nominal)	0.011; 0.018; 0,03; 0.05; 0.083 mg/l; control and solvent control
Sampling and analysis	-

Results

Based on the results a 48-hour EC50 value of 0.022 (0.019 – 0.025) mg/l was determined.

Nominal concentration (mg/l)	% Immobilisation for combined replicates 24 h	% Immobilisation for combined replicates 48 h
Control	0	0
Solvent Control	3	3
0.011	0	7
0.018	10	37
0.03	13	73
0.05	7	93
0.083	37	100

Reliability and relevance

The test is rated reliable with restrictions (Klimish 2). The OECD 202 validity criteria were fulfilled:

- In the control, including the control containing the solubilising agent, not more that 10 percent of the daphnids were immobilised
- The dissolved oxygen concentration at the end of the test was above 3 mg/l in control and test vessels.

The test is based on nominal concentrations and there is no measured data on the test substance or its concentrations. Nevertheless, the test is performed in closed vessels to prevent losses by volatilisation. As clear effects were observed compared to controls, it can be concluded that the EC50 values is 0.045 mg/l or lower (if test substance was lost during the test).

Table 2. Short-term effects on aquatic invertebrates.

Method	*Results*	*Remarks*	*Reference*
Daphnia magna *freshwater* *static* *MIC Environmental Assessment Method for Conducting Acute Toxicty Tests with Daphnia magna (Grueber and Adams, 1980) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US EPA 1975)*	*EC50 (24 h): ca. 0.144 mg/L test mat. (nominal) based on: mobility (range: 0.117-0.235 mg/l)* *EC50 (48 h): ca. 0.045 mg/L test mat. (nominal) based on: mobility (range: 0.036-0.060 mg/l)*	*Reliability: 2 (reliable with restriction)* *Test material (EC name): o-terphenyl*	*Monsanto 1983c* *(www.echa.europa.eu)*
Daphnia magna *freshwater* *static*	*EC50 (24 h): ca. 168 µg/L test mat. (nominal) based on: mobility (95% CL 93-1201 µg/l)*	*Reliability: 2 (reliable with restriction)*	*Monsanto 1983a (www.echa.europa.eu)*

Method	*Results*	*Remarks*	*Reference*
MIC Environmental Assessment Method for Conducting Acute Toxicity Tests with Daphnia magna (Grueber and Adams, 1980) and Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US EPA 1975)	*EC50 (48 h): ca. 22 µg/L test mat. (nominal) based on: mobility (95% CL 19 – 25 µg/l)*	*Test material (EC name): m-terphenyl*	
Daphnia magna *freshwater* *static* *MIC Environmental Assessment Method for Conducting Acute Toxicity Tests with Daphnia magna (Grueber and Adams, 1980) and Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US EPA 1975)*	*EC50 (24 h): > 5.5 mg/L test mat. (nominal) based on: mobility* *EC50 (48 h): > 5.5 mg/L test mat. (nominal) based on: mobility*	*Reliability: not assessed. Test material (EC name): p-terphenyl*	*Unnamed (1983c) (www.echa.europa.eu)*
Daphnia magna *freshwater* *semi-static* *OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)*	*EC50 (24 h): 6.2 mg/L test mat. (nominal) based on: mobility (95 % CL: 4.2 - 11 mg/L)* *EC50 (48 h): 0.52 mg/L test mat. (nominal) based on: mobility (95 % CL: 0.37 - 0.74 mg/L)*	*Reliability: 4 (not assignable)* *Test material (o-terphenyl, CAS 84-15-1)*	*National Institute of Technology and Evaluation (f)*(5B434G) *J-CHECK database: http://www.safe.nite.go.jp/jcheck/template.action?ano=10861&mno=4-0017&cno=84-15-1&request_locale=en*
Daphnia magna *freshwater* *semi-static* *OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)*	*EC50 (24 h): > 10 mg/L test mat. (nominal) based on: mobility* *EC50 (48 h): 0.65 mg/L test mat. (nominal) based on: mobility (95 % CL: 0.47 - 0.86 mg/L)*	*Reliability: 4 (not assignable)* *Test material (m-terphenyl, CAS 92-06-8)*	*National Institute of Technology and Evaluation (g) (5B440G)* *J-CHECK database: http://www.safe.nite.go.jp/jcheck/template.action?ano=10977&mno=4-0017&cno=92-06-8&request_locale=en*
Daphnia magna *freshwater* *flow-through* *EPA OTS 797.1300 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids)*	*EC50 (48 h): > 0.5 mg/L test mat. (nominal) based on: mobility*	Reliability: not assessed. *Test material (Common name): HQ-40* *Form: viscous*	*Unnamed (1992) (www.echa.europa.eu)*

Method	Results	Remarks	Reference
Daphnia magna freshwater static Standard Methods for Examination of Water and Wastewater Methods of acute toxicity tests with fish, macroinvertebrates and amphibians	LC50 (24 h): 21 mg/L test mat. (nominal) based on: mortality (95% CI (16-28)) LC50 (48 h): 13 mg/L test mat. (nominal) based on: mortality (95%CI (11-15))	Reliability: not assessed. Test material (Common name): WCM Form: viscous	Unnamed (1979) (www.echa.europa.eu)
Daphnia magna freshwater flow-through EPA OTS 797.1300 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids)	EC50 (48 h): > 0.069 mg/L test mat. (meas. (arithm. mean)) based on: mobility	Reliability: 2 (reliable with restrictions) . Test material (Common name): Santotar 9 (Quaterphenyl isomers and higher polyphenyls, no hydrogenation based on chemical formula) Form: brown chunky solid	Monsanto (1993) (www.echa.europa.eu)
Daphnia magna freshwater flow-through EPA OTS 797.1300 (Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids)	EC50 (48 h): > 0.068 mg/L test mat. (meas. (arithm. mean)) based on: mobility	Reliability: not assessed. Test material (Common name): Santowax Q Form: tan chunks	Unnamed (1991) (www.echa.europa.eu)
Daphnia magna freshwater static No 7806 Static Bioassay Procedure for Determining Toxicity of Chemical Substances to Daphnia magna	LC50 (24 h): ca. 0.7 mg/L test mat. (nominal) based on: mortality (0.49 - 1.0) LC50 (48 h): ca. 0.1 mg/L test mat. (nominal) based on: mortality (0.075 - 0.13)	Reliability: not assessed.Test material (Common name): Therminol 66	Unnamed (1979) (www.echa.europa.eu)
Daphnia magna freshwater static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): > 1.34 mg/L test mat. (meas. (initial)) based on: mobility	Reliability: not assessed. Test material (EC name): Terphenyl, hydrogenated (Therminol 66)	Unnamed (1996) (www.echa.europa.eu)
Gammarus fasciatus freshwater static equivalent or similar to The procedures used in this acute toxicity study followed those described in the protocol entitled "Bionomics protocol for static acute toxicity tests with the scud (Gammarus fasciatus), December 1983, and modifications dated 26th of March 1984.	LC50 (3 h): > 1 mg/L test mat. (nominal) based on: mortality LC50 (6 h): > 1 mg/L test mat. (nominal) based on: mortality LC50 (24 h): ca. 0.9 mg/L test mat. (nominal) based on: mortality (0.69 - 1.5) LC50 (48 h): ca. 0.54 mg/L test mat.	Reliability: not assessed. Test material (Common name): Therminol 66	Unnamed (1984a) (www.echa.europa.eu)

Method	Results	Remarks	Reference
	(nominal) based on: mortality (0.42 - 0.75)		
Paratanytarsus parthenogenica freshwater static Protocol cited: Bionomics Protocol for Static Acute Toxicity Tests with Midge Larvae (Paratanytarsus parthenogenica) (December, 1983) and modifications dated 26 March 1984	LC50 (48 h): > 1.5 mg/L test mat. (nominal) based on: mortality LC50 (24 h): > 1.5 mg/L test mat. (nominal) based on: mortality	Reliability: not assessed. Test material (Common name): Therminol 66	Unnamed (1984b) (www.echa.europa.eu)
Daphnia magna freshwater No specific guideline mentioned	EC50 (48 h): 0.1 mg/L (CI = 0.08-0.13)	Reliability: not assessed. Test material (EC name): Terphenyl, hydrogenated (Therminol 66)	Unnamed (1979) (www.echa.europa.eu)
Daphnia magna freshwater static - the MIC Environmental Assessment Method for Conducting Acute Toxicity Tests with Daphnia magna (Grueber and Adams, 1980) - Methods for Acute Toxicity tests with Fish, Macroinvertebrates and Amphibians (US EPA, 1975)	EC50 (48 h): 0.011 mg/L (nominal) (95% CI (0.008 - 0.014))	Reliability: not assessed. Test material (Common name): XA2020	Monsanto report MO-92-9046 (1980) (www.echa.europa.eu)
Chironomus tentans freshwater static EPA. Methods for Acute Toxicity Tests with Fish,Macroinvertebrates, and Amphibians. EPA-660/3-75-009. MIC environmental Assessment Method for Conducting Acute Toxicty Tests with Chironomus tentans (Mosher and Adams, 1982)	LC50 (24 h): 0.95 mg/L (nominal) based on: mortality (95% CI = 0.72-1.40) LC50 (48 h): 0.52 mg/L (nominal) based on: mortality (95% CI = 0.34-0.75)	Reliability: not assessed. Test material (Common name): XA 2020	Unnamed (1982) (www.echa.europa.eu)
Mysidopsis bahia (new name: Americamysis bahia) saltwater static equivalent or similar to ASTM Committee E-35 on pesticides, 1980. Standard Practice E729-80, Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Philadelphia, PA 25 pages.	LC50 (24 h): > 1000 µg/L test mat. (nominal) based on: mortality LC50 (48 h): ca. 410 µg/L test mat. (nominal) based on: mortality (300 - 480) LC50 (72 h): ca. 340 µg/L test mat. (nominal) based on: mortality (290-460) LC50 (96 h): ca. 310 µg/L test mat. (nominal) based on: mortality (280-440)	Reliability: not assessed. Test material (Common name): Therminol 66	Unnamed (1984) (www.echa.europa.eu)
Daphnia magna OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48 h): 0.05 mg/L	Reliability: not assessed.	Unnamed (1984)

Method	*Results*	*Remarks*	*Reference*
		Test material (Common name): XA2020	*(www.echa.europa.eu)*

5.1.2.2 Long-term toxicity to aquatic invertebrates

There are three long term toxicity tests on aquatic invertebrates available. In addition to the tests from the Japanese database (J-CHECK) on o- and m-terphenyl, which result in NOEC values of 0.025 and 0.01 mg/l, respectively, there is a *Daphnia magna* study from 2014 on the UVCB substance (WIL 2014).

The reliability of the tests is evaluated with respect to the OECD 211 test method guideline. For an OECD 211 test to be valid, the following performance criteria should be met in the controls:
- the mortality of the parent animals (female *Daphnia*) does not exceed 20% at the end of the test;
- the mean number of living offspring produced per parent animal surviving at the end of the test is > 60.

WIL 2014. *Daphia magna*, reproduction test with Therminol 66

A 21 day *Daphnia magna* reproduction test on Therminol 66 (UVCB substance) was conducted according to OECD 211 test guideline under GLP conditions. The test was performed on Water Accommodated Fractions (WAFs) of Therminol 66 with loading rates of 1.0 (WAF 1) and 5.0 mg/l (WAF 2).

Analysis of the WAF-solutions

During identification of the constituents 78 peaks were detected in the GC-MS chromatogram of the test substance. Two individual peaks (o-terphenyl, m-phenyl cyclohexyl benzene) were selected for method validation. These constituents were chosen as o-terphenyl is the most soluble constituent and represents a significant portion of the test substance and m-phenyl cyclohexyl benzene is the most abundant hydrogenated terphenyl constituent, representative for other hydrogenated terphenyl constituents and still sufficiently soluble to be monitored in the WAFs.

Concentrations of o-terphenyl (o-T) and m-Phenyl cyclohexyl benzene (PCHB)(HT2) were analysed in WAF-solutions renewed and incubated similarly as the test solutions, but containing no daphnids. Samples for analysis were taken from additional vessels containing no daphnids, because the volume of sample needed for analysis was 1000 ml. The analyses were intended to confirm repeatability of the preparation procedure and differences between applied doses.

It is noted that in the loading rate 1 mg/l WAF-solutions the measured concentrations are significantly higher during days 17 – 19 compared to days 0 -3 and 10 – 12 (Table 41). The reason for this is not known. The variation within the refreshment periods (0-3, 10-12 and 17 – 19 days) is not reported.

Table 76. Measured mean concentrations of o-terphenyl and PCHB in WAF-solutions.

Therminol 66 loading rate (mg/l)	mean concentration[1] (mg/l)	Average (mg/l)

o-Terphenyl				
	Day 0-3	day 10 - 12	day 17 - 19	
1.0	0.0037	0.0039	0.011	0.0057
5.0	0.021	0.017	0.019	0.019
PCHB				
1.0	0.00077	0.00058	0.0031	0.0014
5.0	0.0074	0.0041	0.011	0.0076

[1]Geometric mean calculated over the refreshment period

Results

Every workday the condition of the parental daphnids was recorded, during the reproduction phase the number of living offspring, immobile young and appearance of unhatched (aborted) eggs was recorded. At the end of the test the lengths of the surviving parental daphnids were measured.

No mortality was observed in the control during the entire exposure period. In the lower loading rate one daphnid was found dead at day 18 of exposure. In the highest loading rate only one daphnia survived to the end of exposure. Clear reduction in reproduction is seen at both loading rates (Table 77). Also growth of parental daphnids was reduced in the test systems (Table 78).

o-terphenyl (T) and/or m-phenyl cyclohexyl benzene (HT2) are expected to be the main components of the WAF-solution based on the water solubility and the composition of the tested material. This is supported by the GC-MS chromatograms of the WAF-solutions, where these components have the largest peaks (Figure 20, Figure 21) compared to other peaks. The observed effects on mortality and reproduction can, however, also results from other constituents present in the solution. In the chromatograms there appears to be around 6 other peaks above background noise with sufficient scaling. All of these are smaller than the o-terphenyl and m-phenyl cyclohexyl benzene peaks. The GC-MS peak areas are not presented in the report and therefore this comparison is based only on visual observation of the graph. Assuming that the sensitivity of MS detector is similar to these constituents, the total concentrations in the WAF 1 mg/l test solution is estimated to be around 10 – 20 µg/l based on the peak height. As all the constituents present in Therminol 66 (see confidential Annex) are expected to have the same toxic mode of action (non-specific mode of toxic action resulting in narcosis) it is reasonable to estimate that effects are probable at concentrations around 10 – 20 µg/l, possibly even at lower concentrations.

However, it is noted that variation in measured WAF solutions is considerable and therefore this estimation is related with significant uncertainty and merely gives a rough idea of the expected concentration range of soluble constituents in the WAF solution. Furthermore, it is noted that concentrations in the actual test solutions were not measured. Therefore, the results are reported based on loading rates:

NOELR for reproduction < 1.0 mg/l

NOELR for mortality 1.0 mg/l

NOELF for growth < 1.0 mg/l

Table 77. Results from *Daphnia magna* 21 d reproduction study

	WAF 1 (1 mg/l loading)	WAF 2 (5 mg/l loading)
Survival of parents at end of test	9 / 10	1 / 10
% reduction in juveniles/introduced parent compared to control	32	93
% reduction in juveniles/surviving parent compared to control	30	34

Table 78. Group mean body lengths (mm) and reduction of growth of parental daphnids at the end of the test

Therminol 66 WAF prep. at (mg/l)	Mean (mm)	Std. Dev.	n	%Reduction
Control	4.42	0.218	20	0.0
1.0	4.26	0.235	9	3.6*
5.0	3.93	n.d.	1	11*

* Statistically significant ($p<0.05$)
n.d. not determined

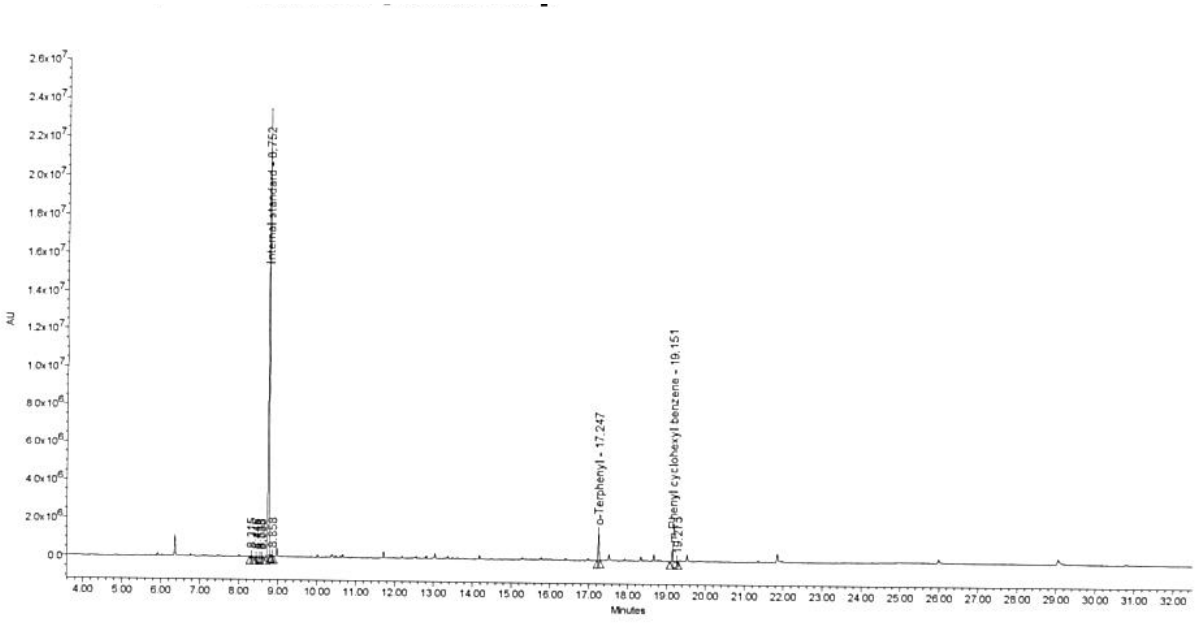


Figure 20. GC-MS chromatogram of a WAF prepared at a loading rate 1 mg TS/l from a preliminary test 2.

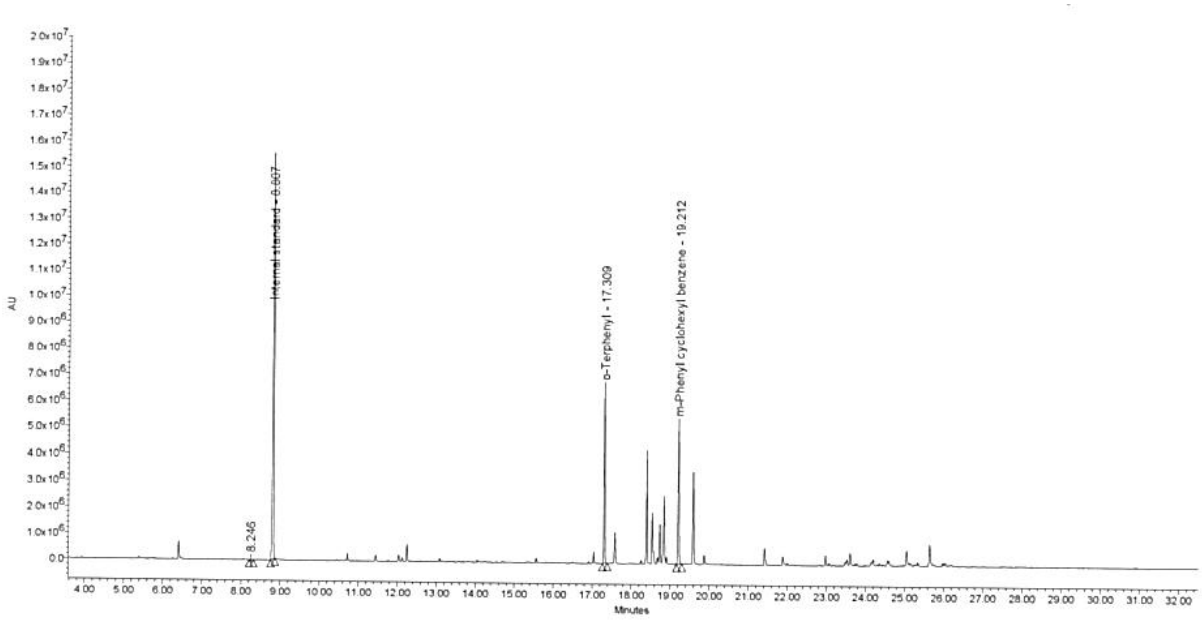


Figure 21. GC-MS chromatogram of a WAF prepared at a loading rate 50 mg TS/l from a preliminary test 1.

Reliability and relevance

The WAF I loading can be considered to fulfil the OECD 211 validity criteria as,

- the mortality of the parent animals (female *Daphnia*) did not exceed 20% at the end of the test;

- the mean number of living offspring produced per parent animal surviving at the end of the test was > 60.

The WAF 2 loading cannot be considered reliable for assessing chronic toxicity (and NOEC derivation) as most (9/10) parent animals did not survive.

According to OECD 211, at least five test concentrations should normally be tested. In this study only two WAF loadings were tested.

According to the OECD Guidance Document nro. 23 on the Testing of Difficult Substances, chemical specific analysis is required to demonstrate attainment of equilibrium in WAF-preparations and stability during the test. Concentration of expected main components were measured during the test from WAF-solutions prepared similarly to test solutions. Significant variation of these constituents was observed during the test. Therefore, the relevance and reliability of the test results is compromised by the fact that the actual composition and concentrations of the test substance is not known.

Due to these deviations from the OECD 211 test guideline and OECD guidance Document 23 (lack of sufficient test concentrations and lack of information on WAF-solution stability) the study is rated Klimish 4 (not assignable).

NITE 5B433G Reproduction Inhibition Test of o-terphenyl with *Daphnia magna*

The test was conducted in compliance with OECD TG 202 (1984). The OECD 202 TG from 1984 included, in addition to the acute test protocol, a (at least 14-day) reproduction test protocol.

Test design

Table 79. test design

Test material (water solubility (WS) under test conditions)	o-terphenyl WS = -
Stock solution	-
Test organisms	*Daphnia magna*
Feeding	-
Test media	Hardness: - Dissolved oxygen: - Temperature: 20 ± 1 °C pH: -
Test vessels	800 ml
Test system	4 containers / level 40 organism / level (10 organism per 1 replicate, 40 organism per 1 level) semi-static (entire quantity of testing liquid replaced three times a week)
Test duration	21 d
Lighting	16 hours light / 8 hours dark
Tested concentrations	0.010, 0.025, 0.060, 0.16, 0.40 mg/l + control + supplementary control (8 mg/l HCO-30 / acetone used)
Sampling and analysis	HPLC method

Results

All measured concentrations of the tested material, which were measured during the exposure period, when the testing liquids were prepared and before water was replaced, were within ± 20 % of the the set values. The set (nominal) values were used, therefore, for the calculation of each effect concentration,

	mg/l (95 % CI)
LC50	0.088 (0.060 – 0.16)
Er50	0.054
NOECr	0.025
LOECr	0.060

Reliability and relevance

The test was conducted in compliance with OECD TG 202 (1984). The OECD 202 (1984) included, in addition to the acute test protocol, a (at least 14-day) reproduction test protocol.

It is not possible to evaluate the reliability of the study as crucial information is missing form the study report. There is no information on,

- the mortality of the parent animals in the controls,

- the mean number of living offspring produced per parent animal surviving at the end of the test in controls.

The study is rated Klimish 4 (not assignable).

NITE 5B439G Reproduction Inhibition Test of m-terphenyl with *Daphnia magna*

The test was conducted in compliance with OECD TG 202 (1984). The OECD 202 (1984) included, in addition to the acute test protocol, a (at least 14-day) reproduction test protocol.

Test design

Table 80. Test design.

Test material (water solubility (WS) under test conditions)	m-terphenyl WS = -
Stock solution	-
Test organisms	*Daphnia magna*
Feeding	-
Test media	Hardness: - Dissolved oxygen: - Temperature: 20 ± 1 °C pH: -
Test vessels	800 ml
Test system	4 containers (replicates) / level 40 organism / level (10 organism per 1 replicate, 40 organism per 1 level) semi-static (entire quantity of testing liquid replaced three times a week)
Test duration	21 d

Lighting	16 hours light / 8 hours dark
Tested concentrations	0.010, 0.025, 0.060, 0.16, 0.40 mg/l + control + supplementary control (8 mg/l HCO-30 / acetone used)
Sampling and analysis	HPLC method

Results

All measured concentrations of the tested material, which were measured during the exposure period, when the testing liquid were prepared and before water was replaced, were within ± 20 % of the the set values. The set (nominal) values were used, therefore, for the calculation of each effect concentration,

	mg/l (95 % CI)
LC50	0.033 (0.027 – 0.041)
Er50	0.061
NOECr	0.010
LOECr	0.025

Reliability and relevance

The test was conducted in compliance with OECD TG 202 (1984). The OECD 202 (1984) included, in addition to the acute test protocol, a (at least 14-day) reproduction test protocol.

It is not possible to evaluate the reliability of the study as crucial information is missing form the study report. There is no information on,

- the mortality of the parent animals in the controls,

- the mean number of living offspring produced per parent animal surviving at the end of the test in controls.

The study is rated Klimish 4 (not assignable).

5.1.3 Algae and aquatic plants

For ECOSAR predictions on toxicity to algae see Table 83.

In total, there are five tests on algae (table 3). In addition to three tests on the UVCB-substance, which are based on nominal concentrations, there are two tests based on measured concentrations available from the Japanese database (J-CHECK) for o- and m-terphenyl (NOECs 1.4 and 0.23 mg/l, respectively).

The tests with measured concentrations are evaluated in detail. The reliability of the tests is evaluated respective to the OECD 201 test guideline and its' validity criteria. According to OECD 201, for the test to be valid, the following performance criteria should be met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day-1. For the most frequently used species the growth rate is usually substantially higher (see Annex 2). This criterion may not be met when species that grow slower than those listed in Annex 2 are used. In this case, the test period

should be extended to obtain at least a 16-fold growth in control cultures, while the growth has to be exponential throughout the test period. The test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached.

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures (See Annex 1 under ―coefficient of variation‖) must not exceed 35 %. See paragraph 49 for the calculation of section-by-section specific growth rate. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures.

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%.

NITE 5B435G Growth Inhibition Test of o-terphenyl with *Selenastrum capricornutum* (new name *Pseudokirchnerella subcapita*)

The test was conducted in compliance with OECD 201 test guideline.

Test design

Table 81. Test design

Test material (water solubility (WS) under test conditions)	o-terphenyl WS = -
Stock solution	-
Test organisms	*Selenastrum capricornutum* (new name *Pseudokirchnerella subcapita*)(NIES-35)
Test media	“OECD medium” Hardness: - Dissolved oxygen: - Temperature: 23 ± 2 °C pH: -
Test vessels / quantity of liquid	100 ml
Test system	3 containers (replicates) / level static, culture shaking (100 rpm)
Initial cell concentration	1 x 10^4 calls/mL
Test duration	72 hours
Lighting	4000 lux (continuous lighting)
Tested concentrations	1.0, 1.8, 3.2, 5.6 and 10 mg/l + control + supplementary control (100 mg/l acetone and HCO-30 used)

Sampling and analysis	HPLC method

Results

Since the initial concentration in each testing liquid was within ±20 % of the set (nominal) values, the set values were used for the following calculations of the growth inhibiting concentration.

	0 – 72 h (mg/l)	24 – 48 h (mg/l)	24 – 72 (mg/l)
$E_{bC}50$	4.7		
E_rC50	> 8.3	>10	>10
$NOEC_b$	1.8		
$NOEC_r$	1.4	1.8	1.8

E_bC50 / $NOEC_b$ = EC50/NOEC based on biomass

E_rC50 /$NOEC_r$ = EC50/NOEC based on growth rate. E_rC50 /$NOEC_r$ values are as reported on the J-CHECK site (recalculated from the original data).

Reliability and relevance

It is not possible to evaluate the reliability of the study as crucial information is missing from the study report. There is no information available on:

- The growth of biomass and variation in the growth rate in the control cultures
- performance of the analytical method (such as sensitivity and accuracy) used to ensure maintenance of the test substance

The test is ranked Klimish 4 (not assignable).

NITE 5B441G Growth Inhibition Test of m-Terphenyl with *Selenastrum capricornutum* (new name *Pseudokirchnerella subcapita*)

The test was conducted in compliance with OECD 201 test guideline.

Test design

Table 82. Test design

Test material (water solubility (WS) under test conditions)	m-terphenyl WS = -
Stock solution	-
Test organisms	*Selenastrum capricornutum* (new name *Pseudolirchnerella subcapita*)(NIES-35)

Test media	"OECD medium" Hardness: - Dissolved oxygen: - Temperature: 23 ± 2 °C pH: -
Test vessels / quantity of liquid	100 ml
Test system	3 containers (replicates) / level static, culture shaking (100 rpm)
Initial cell concentration	1 x 10^4 calls/mL
Test duration	72 hours
Lighting	4000 lux (continuous lighting)
Tested concentrations	0.30, 0.53, 0.95, 1.7 and 3.0 mg/l + control + auxiliary control (90 mg/l, acetone and HCO-30 used)
Sampling and analysis	HPLC method

Results

Since the initial concentration in each testing liquid was within ±20 % of the set (nominal) values, the set values were used for the following calculations of the growth inhibiting concentration.

	0 – 72 h (mg/l)	24 – 48 h (mg/l)	24 – 72 (mg/l)
$E_{bC}50$	1.6		
E_rC50	> 2.4	>3.0	>3.0
$NOEC_b$	0.30		
$NOEC_r$	0.23	0.95	0.53

E_bC50 / $NOEC_b$ = EC50/NOEC based on biomass

E_rC50 /$NOEC_r$ = EC50/NOEC based on growth rate. E_rC50 /$NOEC_r$ values are as reported on the J-CHECK site (recalculated from the original data).

Reliability and relevance

It is not possible to evaluate the reliability of the study as crucial information is missing from the study report. There is no information available on:

- The growth of biomass and variation in the growth rate in the control cultures
- performance of the analytical method (such as sensitivity and accuracy) used to ensure maintenance of the test substance

The test is ranked Klimish 4 (not assignable).

Table 3. Effects on algae and aquatic plants[5]

Method	*Results*	*Remarks*	*Reference*
Pseudokirchnerella subcapitata (algae) *freshwater* *static* *OECD Guideline 201 (Alga, Growth Inhibition Test)*	*The presence of water soluble fractions of Therminol 66 could be measured in freshly prepared WAF using HPLC. This soluble fraction however disappeared during the exposure time both in filtered and unfiltered WAFs. It is not clear from the results how fast the test substance disappears. The initial presence of these soluble compounds in WAFs does not interfere with normal algal growth rate for WAFs produced with nominal concentrations up to 100 mg/L Therminol 66. As no effects were seen in the test range no effect values can be defined.*	Reliability: not assessed. *Test material (Common name): Therminol 66*	*Unnamed (2010) (www.echa.europa.eu)*
Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae) *freshwater* *equivalent or similar to 'Culture and test procedures followed those of U.S. Environmental Protection Agency (1971)*	*EC50 (24 h): > 320 mg/L based on: chlorophyll a* *EC50 (48 h): > 320 mg/L based on: chlorophyll a* *EC50 (72 h): > 100 — < 320 mg/L based on: chlorophyll a* *EC50 (96 h): ca. 44 mg/L based on: chlorophyll a (1-1586)* *EC50 (96 h): 56 mg/L based on: cell number (4-773)*	Reliability: not assessed. *Test material (Common name): Therminol 66*	*Unnamed (1979) (www.echa.europa.eu)*
Selenastrum capricornutum (new name: Pseudokirchnerella subcapitata) (algae) *freshwater* *static* *EPA 1971. Algal Assay procedure: Bottle test. National Eutrophication program, Pacific Northwest Water Labortatory, Corvallis, OR. 82p.*	*EC50 (96 h): > 1000 mg/L test mat. (nominal) based on: cell number* *EC50 (96 h): > 1000 mg/L test mat. (nominal) based on: decrease of in vivo Chlorophyll a*	Reliability: not assessed. *Test material (Common name): WCM* *Form: viscous*	*Unnamed (1979) (www.echa.europa.eu)*
algae: no species mentioned (algae) *No guideline mentioned in summary sheet.*	*EC50 (72 h): > 8.3 mg/L based on: growth rate* *EC50 (72 h): 4.7 mg/L based on: areas under the growth curves*	*Reliability: 4 (not assignable)* *Test material (o-terphenyl, CAS 84-15-1)*	*National Institute of Technology and Evaluation (1995a) (5B435G)*

Method	*Results*	*Remarks*	*Reference*
	NOEC (72 h): 1.4 mg/L based on: growth rate *NOEC (72 h): 1.8 mg/L based on: areas under the growth curves*		*J-CHECK database: http://www.safe.nite.go.jp/jcheck/template.action?ano=10865&mno=4-0017&cno=84-15-1&request_locale=en*
algae: no species mentioned (algae) *No guideline mentioned in summary sheet.*	*EC50 (72 h): > 2.4 mg/L based on: growth rate* *EC50 (72 h): 1.6 mg/L based on: areas under the growth curves* *NOEC (72 h): 0.23 mg/L based on: growth rate* *NOEC (72 h): 0.3 mg/L based on: areas under the growth curves*	*Reliability: 4 4 (not assignable)* *Test material (m-terphenyl, CAS 92-06-8)*	*National Institute of Technology and Evaluation (NITE) (1995b) (5B441G)* *J-CHECK database: http://www.safe.nite.go.jp/jcheck/template.action?ano=10982&mno=4-0017&cno=92-06-8&request_locale=en*

5.2 Terrestrial compartment

No relevant information available.

5.3 Atmospheric compartment

No relevant information available.

5.4 Microbiological activity in sewage treatment systems

In an Activated Sludge Respiration Inhibition Test following the OECD 209 test guideline no effects of Therminol 66 were observed (VITO 2010). A NOEC value of 103 mg/l (nominal) was determined. The test solutions were prepared as water accommodated fractions (WAF). The actual concentration of Therminol 66 was 25 ± 5 µg/l all tested solutions.

5.5 Toxicity to birds

No relevant information available.

5.7 Summary and discussion of the environmental hazard assessment

Estimated data

ECOSAR predicts chronic (ChV)[6] values (below 10 µg/l) for all of the constituents with the exception of o-, m- and p-terphenyl for which ECOSAR predicts ChV-values around 20 µg/l (Table 83)It is noted that most of the ECOSAR results are outside the applicability domain of the model. Based on chronic toxicity values, fish seem to be the most sensitive species, followed by Daphnids, then green algae. The ChV-values for o-terphenyl, HT1 and HT2 are within the applicability domain of the model.

Experimental data

Short and long term ecotoxicity test results are available for algae, invertebrate (mostly *Daphnia magna*) and fish (see Annex 3). Many of the studies are quite old (1970s, 1980s) and have been conducted with water accommodated fractions of commercial products of terphenyl, hydrogenated using nominal concentrations. Documentation on the test substance identity in the commercial products is scarce or non-existent. In addition, there are ecotoxicity test results available for the constituents o-, m- and p-terphenyl. As the constituents of terphenyl, hydrogenated are quite volatile and scarcely water soluble, use of nominal concentrations without information on measured concentrations is not considered reliable. Therefore, the focus of the assessment of the ecotoxicity studies is on those studies where information on actual (measured) test substance concentrations was available. Those studies were evaluated in detail. In addition, two studies on individual constituents (Monsanto 1983a and 1983c) were assessed in detail as, although they are based on nominal concentration, they report clear acute effects.

The best data availability is for o-terphenyl for which ECOSAR predictions and experimental test results give a rather consistent picture (Figure 22, Figure 23).

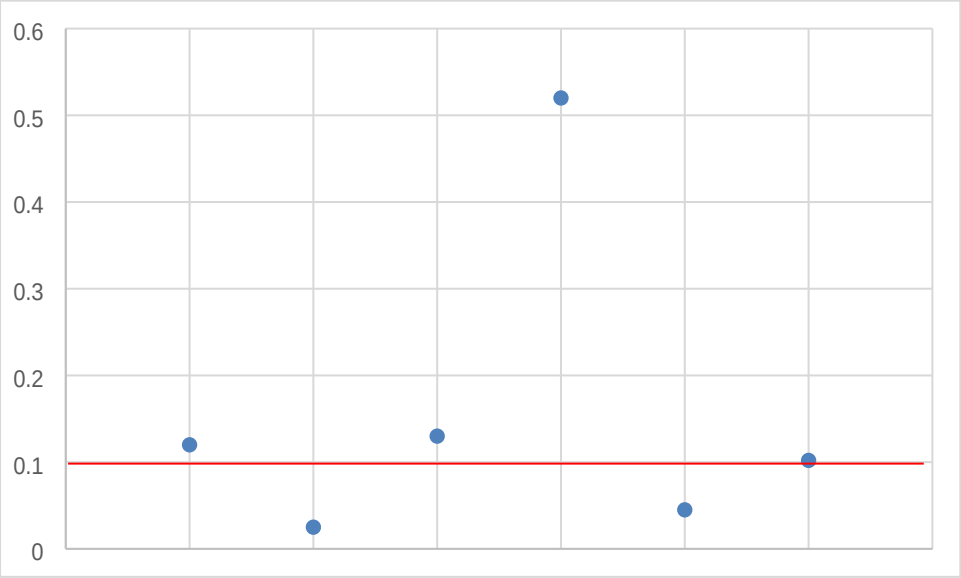


Figure 22. Measured and estimated (ECOSAR) EC50 values for o-terphenyl. The red line marks the T screening criterion 0.1 mg/l.

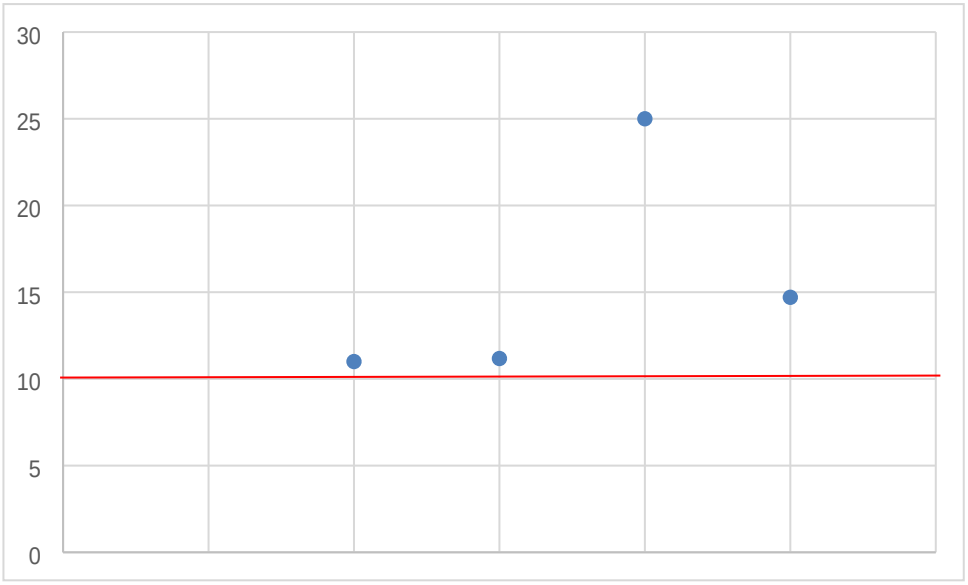


Figure 23. Measured and estimated (ECOSAR) NOEC values for o-terphenyl. The red line marks the T criterion 10 µg/l.

Table 83. ECOSAR (Neutral Organics) predictions. Values outside the applicability domain of the model are in brackets.

Constituent	Smiles	logKow	Water Solubility µg/l	Fish µg/l		Daphnid µg/l		Green Algae µg/l	
				LC50 (96h)	ChV (ChV/1.7 ~NOEC) [4]	LC50	ChV (ChV/1.7 ~NOEC) [4]	EC50	ChV[4]
o-terphenyl[1]	c(c(c(cccc1)c1)ccc2)(c(cccc3)c3)c2	5.52	60 - 1240	(130)	19 (11)	(102)	25 (25)	292	158
HT1[1,2]	C1CCC(CC1)c2ccc(cc2)c3ccccc3	6.57	63 - 68	(150)	2 (1.2)	(13)	4 (2.4)	(56)	38
HT2[1,2]	C1(c2ccc(C3CCCCC3)cc2)CCCCC1	7.63	8 - 70	(1.76)	0.318 (0.19)	(1.68)	0.696 (0.41)	(11 *)	9 *
HT3[1,2,3]	C1(C2CCC(C3CCCCC3)CC2)CCCCC1	8.55	0.35 – 2.5	(0.267)	(5.34e-002)	(0.278)	(0.146)	(3 *)	(3 *)
Q[1,2,3]	c4ccccc4c1ccc(c2ccc(c3ccccc3)cc2)cc1	7.28	0.28 – 6.8	(5*)	(0.784) *	(4 *)	(1.58 *)	(23 *)	(18 *)
HQ1[1,2,3]	C4CCCCC4c1ccc(c2ccc(c3ccccc3)cc2)cc1	8.34	0.31 – 0.79	(0.522)	(0.102)	(0.532)	(0.264)	(4 *)	(4 *)
HQ2[1,2,3]	C4CCCCC4C1CCC(c2ccc(c3ccccc3)cc2)CC1	9.26	0.11 – 0.35	(7.88e-002)	(1.7e-002)	(8.75e-002)	(5.51e-002)	(1.04 *)	(1.21*)
HQ3[1,2,3]	C4CCCCC4C1CCC(C2CCC(c3ccccc3)CC2)CC1	10.18	0.018 – 0.4	(1.19e-002)	(2.84e-003)	(1.44e-002)	(1.15e-002)	(0.244*)	(0.344*)

* = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation are reported.

[1]no acute effects for fish, Daphnids at saturation are expected as logKow > 5.0

[2]no acute effects for algae at saturation are expected as logKow > 6.4

[3]no chronic effects at saturation are expected as logKow > 8

[4] The ChV, or Chronic Value, is defined as the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). This can be mathematically represented as: ChV = 10^([log (LOEC x NOEC)]/2).As the spacing factor for tested concentrations usually is in the order of a factor 3, the NOEC should be around a factor of 1.7 (SQRT(3)) lower than ChV.

Table 84. Short term toxicity tests that were evaluated in detail

Test substance	Test	Species	EC50 (95 % CI) mg/l	Reliability (Klimish code)	Reference
o-terphenyl	21 day test – "OECD 204 Fish prolonged toxicity test: 14-day study"	*Oryzias latipes (medaka)*	LC50 = 0.025 (0.020 – 0.033)	4	NITE 5B431G
m-terphenyl	21 day test – "OECD 204 Fish prolonged toxicity test: 14-day study"	*Oryzias latipes (medaka)*	LC50 = 2.4 (1.1 – 2.4)	4	NITE 5B437G
o-terphenyl	96h "OECD 203 (1992) Fish, Acute Toxicity Test"	*Oryzias latipes (medaka)*	EC50 = 0.12 (0.053–0.27)	4	NITE 5B432G
m-terphenyl	96h "OECD 203 (1992) Fish, Acute Toxicity Test"	*Oryzias latipes (medaka)*	EC50 = 3.1 (2.1 – 4.8)	4	NITE 5B438G
Santotar 9 Polyphenyl	48h "U.S: EPA-TSCA, 40 CFR, 797.1300 Daphnid acute toxicity test"	*Daphnia magna* *(water flea)*	EC50 > 0.069	2	MONSANTO 1993
o-terphenyl	48h "OECD 202 (1984) *Daphnia sp.* Acute Immobilisation Study"	*Daphnia magna* *(water flea)*	EC50 = 0.52 (0.37 – 0.74)	4	NITE 5B434G
m-terphenyl	48h "OECD 202 (1984) *Daphnia sp.* Acute Immobilisation Study"	*Daphnia magna* *(water flea)*	EC50 = 0.65 (0.47 – 0.86)	4	NITE 5B440G
o-terphenyl	48h *Daphnia sp.* Acute immobilisation Study	*Daphnia magna* *(water flea)*	0.045 (0.036 – 0.060)	2	Monsanto 1983c
m-terphenyl	48h *Daphnia sp.* Acute immobilisation Study	*Daphnia magna* *(water flea)*	0.022 (0.019 – 0.025)	2	Monsanto 1983a
o-terphenyl	72 hours – "OECD 201 (1984) Freshwater Alga and Cyanobacteria,	*Selenastrum capricornutum* (new name	EC_r50 > 8.3	4	NITE 5B435G

	Growth Inhibition Test"	*Pseudokirchnerella subcapita*)	$NOEC_r$ = 1.4		
m-terphenyl	72 hours – "OECD 201 (1984) Freshwater Alga and Cyanobacteria, Growth Inhibition Test"	*Selenastrum capricornutum* (new name *Pseudokirchnerella subcapita*)	EC_r50 > 2.4 $NOEC_r$ = 0.23	4	NITE 5B441G

Table 85. Long term toxicity tests that were evaluated in detail (studies with information on actual (measured) test substance concentrations). (MLD = minimum lethal concentration)

Test substance	Test	Species	NOEC / LOEC (95 % CI) µg/l	Reliability (Klimish code)	Reference
o-terphenyl	41 day test - "OECD 210 Fish, Early-life stage toxicity test (1992)"	*Oryzias latipes (medaka)*	NOEC: 11 LOEC: 23	4	NITE 0115EEL
Therminol 66	21 day – "OECD 211 (2012) *Daphnia magna* Reproduction Test"	*Daphnia magna* *(water flea)*	NOEC < 1000 (WAF)	4	WIL 2014
o-terphenyl	21 day - OECD TG 202 (1984) *Daphnia sp.* Acute Immobilisation Study"	*Daphnia magna* *(water flea)*	NOEC = 25 LOEC = 60 LC50 = 88 (60 – 160) EC50 = 54	4	NITE 5B433G
m-terphenyl	21 day - OECD TG 202 (1984) *Daphnia sp.* Acute Immobilisation Study"	*Daphnia magna* *(water flea)*	NOEC = 10 LOEC = 25 LC50 = 33 (27 – 41) EC50 = 61	4	NITE 5B439G