Substance Name: 2,4,6-tri-*tert*-butylphenol

EC Number: 211-989-5

CAS Number: 732-26-3

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF

2,4,6-TRI-*TERT*-BUTYLPHENOL

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS TOXIC FOR REPRODUCTION (ARTICLE 57(C)) AND PBT (ARTICLE 57(D)) PROPERTIES

Adopted on 29 November 2023

This document has been prepared according to template: TEM-0049.04

CONTENTS

TABLES

ABBREVIATIONS

BCFBAF: bioconcentration - bioaccumulation factor
BCF: bioconcentration factor
BOD: Biochemical oxygen demand
CLP: classification, labelling and packaging
DW: dry weight
E_a: *activation energy*
ECHA: European Chemicals Agency
GC-MS: gas chromatography/ mass spectrometry
GLP: good laboratory practice
HCO: hydrogenated castor oil
HP: high performance
HPLC: high performance liquid chromatography
K_{oa}: *octanol-air partition coefficient*
K_{oc} : *organic carbon – water coefficient*
K_{ow}: *octanol-water partition coefficient*
M/e: mass/charge ratio
PBT: persistent bioaccumulative and toxic
POCIS: *polar organic chemical integrative sampler*
POP: persistent organic pollutant
ppm: parts per million
(Q)SAR: (quantitative) structure activity relationship
REACH: Registration, Evaluation and Authorisation of Chemicals
STOT RE: specific target organ toxicity repeated exposure
SPM: suspended particulate matter
SVHC: substance of very high concern
TG: test guideline
ThOD: theoretical oxygen demand
vPvB: very persistent and very bioaccumulative
w/v: weight/volume

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

Substance name: 2,4,6-tri-*tert*-butylphenol (2,4,6-TTBP)

EC number: 211-989-5

CAS number: 732-26-3

- The substance is identified as a substance meeting the criteria of Article 57 (c) of Regulation (EC) No 1907/2006 (REACH) owing to its classification in the hazard class toxic for reproduction category 1B[1].

- the substance is identified as persistent, bioaccumulative and toxic (PBT) according to Article 57 (d) of Regulation (EC) No 1907/2006 (REACH).

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

2,4,6-tri-*tert*-butylphenol is covered by index number 604-097-00-6 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) and it is classified in the hazard class toxic for reproduction category 1B (H360D: May damage the unborn child)[2].

Therefore, this classification of the Substance in Regulation (EC) No 1272/2008 shows that it meets the criteria for classification in the hazard class:

- Toxic for reproduction category 1B in accordance with Article 57 (c) of REACH.

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as PBT. All available relevant information (such as the results of standard tests, non-standard tests and (Q)SAR results) were considered together in a weight-of-evidence approach.

<u>Persistence</u>

No hydrolysis study is available for 2,4,6-TTBP. However, due to its low water solubility and high log K_{oc}, 2,4,6-TTBP is expected to sorb to particles and to mainly distribute to sediment in the aquatic environment. Hydrolysis is expected to be hindered by adsorption potential of 2,4,6-TTBP onto sediment and particulate matter. Therefore, hydrolysis is not considered to be a relevant degradation mechanism for 2,4,6-TTBP. Moreover, due to a lack of hydrolysable functional groups, 2,4,6-TTBP is not expected to hydrolyse.

2,4,6-TTBP is not readily biodegradable according to QSAR estimations (EPIWEB v4.1;

[1] Classification in accordance with section 3.7 of Annex I to Regulation (EC) No 1272/2008.

[2] The harmonised classification has been included in the Commission Delegated Regulation (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Part 3 of Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (the 18th ATP to CLP). Pursuant to the second paragraph of Article 2 of this Regulation, this new harmonised classification applies from 23 November 2023. However, pursuant to the third paragraph of that provision substances and mixtures may already be classified, labelled and packaged in accordance with this classification.

BIOWIN v4.10 and CATALOGIC v.5.12.1; Klimisch score 2), and thus it is concluded that the substance is potentially P/vP.

In a standard seawater-based BOD test (Klimisch score 4), according to OECD TG 306 no ultimate biodegradation (<1%) was measured after 64 days of incubation at 20 °C. This study is merely used as supporting information due to limited information.

2,4,6-TTBP is not readily biodegradable based on the results of an OECD TG 301C (Ready biodegradability; MITI Test (I); Klimisch score 4) test. In this study not only BOD, but also parent compound was followed by HPLC. It appeared that after 28 days, biodegradation as percentage of ThOD was 0% and disappearance of the parent compound was only 5% (i.e. negligible). This study is merely used as supportive information.

2,4,6-TTBP is not inherently biodegradable based on the results of an OECD TG 302C (Inherent Biodegradability: Modified MITI Test (II); Klimisch score 2) study; a maximum of 17% degradation was observed after 5 days and 13% degradation after 28 days. It is worth noting that REACH guidance R.11 states "Lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled without the need for further simulation testing for the purpose of PBT/vPvB assessment." The OECD TG 302C study provides strong indications that 2,4,6-TTBP can be concluded as persistent.

In accordance with REACH Annex XIII Section 3.2.1. (d), information from a laboratory study using natural seawater (Klimisch score 4) indicates that the half-life of 2,4,6-TTBP in seawater at relevant EU-temperature (9 °C) could be 92 days when applying a temperature correction (although recognizing some uncertainties). Due to the uncertainties, this study is merely used as supportive information.

Overall, based on the available information and considering a weight-of-evidence approach, it is concluded that 2,4,6-tri-*tert*-butylphenol meets the 'persistence' (P) criterion of REACH Annex XIII Section 1.1.1.

<u>Bioaccumulation</u>

The screening criterion for bioaccumulation for aquatic organisms is fulfilled for 2,4,6-TTBP (log K_{ow} > 4.5).

The B/vB criterion of REACH Annex XIII (BCF > 5,000 L/kg) is fulfilled for 2,4,6-TTBP based on a Japanese Guideline Study (Klimisch score 2) with BCF values in common carp (*Cyprinus carpio,* average lipid content of 4.5%) in the range of 4,320 to 23,200 L/kg. QSAR predictions (EPI Suite software, Klimisch score 2) for 2,4,6-TTBP support the findings of the laboratory study with an estimated BCF > 5,000 L/kg.

Supporting evidence is provided in a study investigating the relationship between bioaccumulation and lipid content of fish, which measured the BCFs of a series of 12 highly lipophilic substances (Klimisch score 2). 2,4,6-TTBP was one of these substances, and common carp were exposed to the substance in batches with different lipid contents. The study resulted in a lipid normalised BCF-value of 12,620 for 2,4,6-TTBP (recalculated to 5% lipid content). More than 20 BCFs were determined for 2,4,6-TTBP in the study, which all varied from 5,000 to 20,000.

Moreover, 2,4,6-TTBP screens as bioaccumulative in air-breathing organisms (based on a log K_{ow}> 2 and a log K_{oa} > 5). Biomonitoring data confirm presence of 2,4,6-TTBP in air-breathing organisms with detection in livers of rats and quantification in human serum.

On the basis of the absence of 2,4,6-TTBP in urine and faeces, and rapid clearance from the blood, absorption across the gastrointestinal tract was considered to be 100% in a toxicokinetics study performed with rats (Klimisch score 2). After clearance from the blood 2,4,6-TTBP was found in adipose tissue.

Based on the available information and considering a weight-of-evidence approach, it is concluded that 2,4,6-tri-*tert*-butylphenol meets the 'bioaccumulation' (B) and 'very bioaccumulative' (vB) criteria of REACH Annex XIII Section 1.1.2 and Section 1.2.2.

Toxicity

2,4,6-tri-*tert*-butylphenol is covered by index number 604-097-00-6 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances). It is classified in the hazard class toxic for reproduction category 1B (H360D: May damage the unborn child) and STOT RE category 2 (H373: May cause damage to organs (liver) through prolonged or repeated exposure).

2,4,6-tri-*tert*-butylphenol is concluded to meet the toxicity criteria of REACH Annex XIII Section 1.1.3 (b) and Section 1.1.3 (c).

Conclusion

In conclusion, 2,4,6-tri-*tert*-butylphenol meets the criteria for a PBT substance according to Art. 57(d) of REACH by comparing all relevant and available information with the criteria set out in Annex XIII of REACH, in a weight-of-evidence determination.

Registration dossiers submitted for the substance: The registration dossiers submitted for 2,4,6-tri-*tert*-butylphenol include information indicating that the registrants do not manufacture/import the mono-constituent substance in the EU as such. The purpose of the registrations is to make use of the option[3] allowing the registration of individual constituents for multi-constituent substances. The compositions reported in the registration dossiers correspond to the multi-constituent substances "Reaction mass of 2,6-di-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol" and "Reaction mass of 2,6-di-*tert*-butylphenol, 2-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol".

[3] The option of registering individual constituents of a multi-constituent substance is provided in section 4.2.2 of the guidance for identification and naming of substances under REACH and CLP (ECHA, 2017c).

Justification

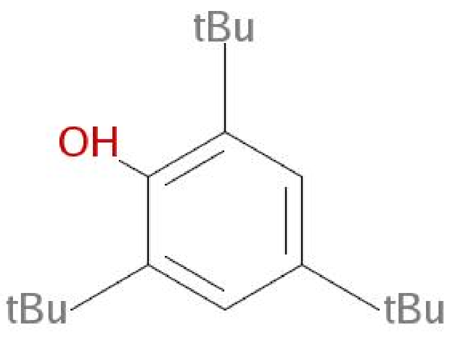
1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	211-989-5
EC name:	2,4,6-tri-*tert*-butylphenol
CAS number (in the EC inventory):	732-26-3
CAS number:	732-26-3
IUPAC name:	2,4,6-tri-*tert*-butylphenol
Index number in Annex VI of the CLP Regulation	604-097-00-6
Molecular formula:	$C_{18}H_{30}O$
Molecular weight range:	262.43 g/mol
Synonyms:	NA

Structural formula:



1.2 Composition of the substance

Name: 2,4,6-tri-*tert*-butylphenol

Description: As the name suggests 2,4,6-tri-*tert*-butylphenol consists of a phenol with a *tert*-butyl group attached on the second, fourth and sixth position starting from the hydroxyl group (three *tert*-butyl groups in total).

Substance type: Mono-constituent

The registration dossiers submitted for 2,4,6-tri-*tert*-butylphenol include information indicating that the registrants do not manufacture/import the mono-constituent substance in the EU as such. The purpose of the registrations is to make use of the option[4] allowing the registration of individual constituents for multi-constituent substances. The compositions reported in the registration dossiers correspond to the multi-constituent substances: "Reaction mass of 2,6-di-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol" and "Reaction mass of 2,6-di-*tert*-butylphenol, 2-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol".

All studies were performed with the mono-constituent substance 2,4,6-tri-*tert*-butylphenol. Almost all of them were performed with a purity of 99.8%.

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

NA

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

NA

[4] The option of registering individual constituents of a multi-constituent substance is provided in section 4.2.2 of the guidance for identification and naming of substances under REACH and CLP (ECHA, 2017c).

1.5 Physicochemical properties

Table 2: Overview of physicochemical properties

Property	Description of key information	Value	Reference (source of information)[5]
Physical state at 20 °C and 101.3 kPa	Observed GLP Purity: ≥99 - ≤100% (w/w)	Slightly yellow powder with lumps	Unpublished study report, 2015[6] (ECHA dissemination website)
Melting/freezing point	OECD TG 102 GLP Purity: ≥99 - ≤100% (w/w)	131 °C (at 1012 ± 1 hPa)	Unpublished study report, 2015 (ECHA dissemination website)
Boiling point	OECD TG 103 GLP Purity: ≥99 - ≤100% (w/w)	278 °C (at 1012 ± 1 hPa)	Unpublished study report, 2015 (ECHA dissemination website)
Vapour pressure	OECD TG 104 GLP Purity: ≥99 - ≤100% (w/w)	3.5 x 10^{-2} Pa (2.6 x 10^{-4} mm Hg) at 20 °C 7.3 x 10^{-2} Pa (5.5 x 10^{-4} mm Hg) at 25 °C	Unpublished study report, 2015 (ECHA dissemination website)
Density	OECD TG 109 GLP Purity: ≥99 - ≤100% (w/w)	0.977 g/cm^3 (at 20 °C)	Unpublished study report, 2015 (ECHA dissemination website)
Water solubility	OECD TG 105 GLP Purity: ≥99 - ≤100% (w/w)	0.063 mg/L (at 20 °C)	Unpublished study report, 2015 (ECHA dissemination website)
Partition coefficient n-octanol/water or kow (log value)	OECD TG 117 GLP Purity: ≥99 - ≤100% (w/w)	Log K_{ow} = 7.1 (at 35 °C column temperature and neutral pH)	Unpublished study report, 2015 (ECHA dissemination website)
Dissociation constant pKa	Estimation using the pKalc function of the PALLAS estimation program (CompuDrug, version 3.6.2.1).	pKa at 20 °C = 12.62	Unpublished study report, 2015 (ECHA dissemination website)

[5] ECHA dissemination website: Registration dossier of 2,4,6-tri-*tert*-butylphenol. (https://echa.europa.eu/en/registration-dossier/-/registered-dossier/5641)
[6] Klimisch score 1.

2. Harmonised classification and labelling

2,4,6-tri-*tert*-butylphenol is covered by Index number 604-097-00-6 in part 3 of Annex VI to the CLP Regulation. It has been included in the 18th Adaptation to Technical Progress of 16 February 2022. The new harmonised classification and labelling shall apply from 23 November 2023 (EC, 2022) as follows:

Table 3: Classification according to Annex VI, Table 3 (list of harmonised classification and labelling of hazardous substances) of Regulation (EC) No 1272/2008

Index No	Chemical name	EC No	CAS No	Classification		Labelling			Spec. Conc. Limits, M-factors and ATEs[7]	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)	Pictogram, Signal Word Code(s)	Hazard statement code(s)	Suppl. Hazard statement code(s)		
604-097-00-6	2,4,6-tri-*tert*-butyl phenol	211-989-5	732-26-3	Repr. 1B Acute Tox. 4 STOT RE2 Skin Sens. 1B	H360D H302 H373 (liver) H317	GHS08 GHS07 Dgr	H360D H302 H373 (liver) H317		Oral: ATE = 500 mg/kg bw	

[7] Acute Toxicity Estimate

3. Environmental fate properties

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

No hydrolysis study is available for 2,4,6-TTBP. However, due to a lack of hydrolysable functional groups, 2,4,6-TTBP is not expected to hydrolyse.

Also, the EPI Suite software (EPIWEB v4.1; HYDROWIN v2.00; US EPA, 2012) cannot estimate a hydrolysis rate constant for 2,4,6-TTBP as this type of chemical structure is not within the applicability domain.

Due to its low water solubility and high log K_{oc}, 2,4,6-TTBP is expected to sorb to particles and to mainly distribute to sediment in the aquatic environment. Hydrolysis is expected to be hindered by adsorption potential of 2,4,6-TTBP onto sediment and particulate matter. Therefore, hydrolysis is not considered to be a relevant degradation mechanism for 2,4,6-TTBP.

3.1.1.2 Summary on abiotic degradation

2,4,6-TTBP is not expected to hydrolyse.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in aqueous media or aqueous environment

3.1.2.1.1 Estimated data

1. The ready biodegradability of 2,4,6-TTBP was estimated using EPI Suite (EPIWEB v4.1; BIOWIN v4.10; US EPA, 2012) (Klimisch score 2; Klimisch *et al*., 1997).

    - BIOWIN 2: 0.0068 (does not biodegrade fast)
    - BIOWIN 3: 2.0392 (ultimate degradation – months)
    - BIOWIN 6: 0.0497 (not readily biodegradable)

The ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11-4, Table R.11-4 (ECHA, 2017b), indicates that a substance is potentially P or vP if the substance does not biodegrade fast (probability <0.5; BIOWIN 2) and the ultimate biodegradation timeframe prediction is ≥ months (value <2.25 (to 2.75); BIOWIN 3). A substance is also potentially P or vP if the substance does not biodegrade fast (probability <0.5; BIOWIN 6) and the ultimate biodegradation timeframe prediction is ≥ months (value <2.25 (to 2.75); BIOWIN 3).

As these criteria are fulfilled, 2,4,6-TTBP is considered to be potentially P or vP according to the PBT guidance.

2. Assessment with Catalogic v5.12.1, based on 301C ready tests (v10.14) (Klimisch score 2).

This sub-model of Catalogic is the most useful as it allows to predict quantitative half-life values for biodegradation. 2,4,6-TTBP is considered to be in the applicability domain as its log K_{ow} and molecular weight are within the specified ranges and its atom-centred fragments are present in the training set.

- Half-life for primary biodegradation: 171 days.
  The substance is therefore predicted as non-biodegradable.

- Half-life for ultimate biodegradation: more than 10 years
  This prediction supports the analysis that not only the parent compound but also the potential interim degradation products show a vP-character.

3. Assessment with Catalogic v5.12.1, based on the soil model (v3.8) (Klimisch score 2).

This model does not provide half-life values, only probable degradation routes.

Applicability domain: 79% of the fragments are within the domain, the software identifies the remaining 21% as unknown. There are no incorrect fragments. Looking at the fragments within the domain, the tertiary butyl group connected to a benzene ring and the phenol group are covered. Although not all fragments are within the domain, the model is considered generally applicable for the substance.

According to the degradation map, 71% of the parent substance remains, and three metabolites are predicted to be formed in following quantities: 17%, 8% and 2.7% (Figure 1):

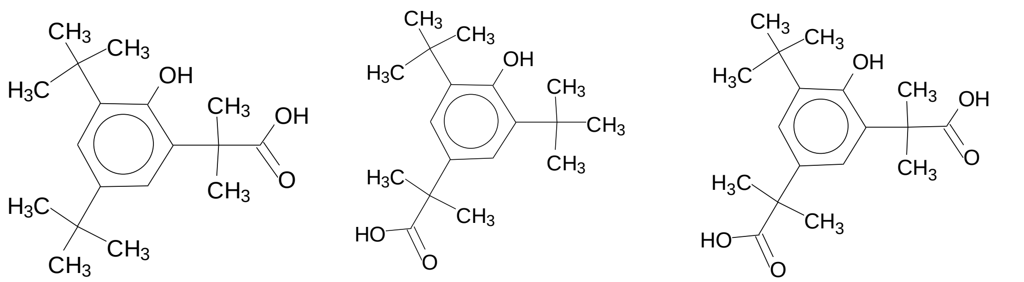


Figure 1: Potential metabolites of 2,4,6-TTBP; predicted by the degradation map of Catalogic v5.12.1

There are three compounds with observed maps, where the oxidation of the tertiary butyl group was recorded. However, the probability for this to happen is low (ca. 10%), which results in low quantities of the predicted transformation product. Therefore, biodegradation of the parent compound will proceed slowly.

3.1.2.1.2 Screening tests

In Lofthus *et al.* (2016), the results of a standard seawater-based BOD test (OECD TG 306) are reported (Klimisch score 4). No information is available regarding the use of shake flask or closed bottle method. The test was performed with seawater collected from a depth of 80 m (below thermocline) in a non-polluted Norwegian fjord (Trondheimsfjord). The salinity of the seawater was 3.4% with a water temperature of 6-8 °C and dissolved oxygen of approximately 8 mg/L. The seawater was acclimated to 20 °C (5-7 days before the start of the experiment), but the water was amended with inorganic nutrients, as recommended in the guideline. Ultrasonification was used to disperse 2,4-6-TTBP (5 mg/L). No ultimate biodegradation (<1%) was measured after 64 days of incubation at 20 °C. Information in order to check the validity of the study is limited (use of a reference compound is not mentioned, no information on blank respiration not exceeding 30%, no information on microbial activity). Due to this limited information this test is merely used as supportive information.

2,4,6-TTBP is not readily biodegradable based on the results of an OECD TG 301C (Ready biodegradability; MITI Test (I); Unpublished study report, 1981; Klimisch score 4) test. The test was performed at 25 °C and 30 mg of test substance was added. The test substance remained undissolved at the end of the test. The percentage degradation of the reference substance aniline was 67% after 7 days. Degradation was calculated based on the BOD. After 28 days, the test item showed 0% degradation as measured by oxygen consumption and 5% degradation as measured by HPLC analysis. In conclusion, 2,4,6-TTBP is not readily biodegradable. This test is used as supportive information.

In an inherent biodegradability study (OECD TG 302C; Modified MITI Test (II)) (Unpublished study report, 1992; Klimisch score 2) 13% degradation of 2,4,6-TTBP was observed after 28 days (O_2 uptake; % of ThOD). 2,4,6-TTBP is therefore considered not inherently biodegradable.

Study details:

The test was carried out in the darkness at 25 ± 1 °C. Oxygen consumption was measured by direct manometer reading.

Agitation: By magnetic stirrers.

Test item = 2,4,6-TTBP at 30 mg/L, aniline as reference substance at 100 mg/L.

Inoculum = mixture of activated sewage sludge at 100 mg dry weight/L. The mixed sludge was prepared by sampling 10 different sites around the UK in accordance with the guideline.

Degradation was measured as oxygen uptake in % ThOD (oxygen consumption was measured by direct manometer reading).

Result: maximum of 17 % degradation after 5 days, afterwards decline/steady state to 13 % after 28 days (all % biodegradation values have been corrected for blank values). The reference substance aniline degrades in a continuous way to 42 % after 7 days, 70% after 14 days and 95 % after 28 days. Total Organic Carbon analysis was not possible for 2,4,6-TTBP as a result of the low water solubility.

It is noted that the solubility of 2,4,6-TTBP is quite low (measured value = 0.063 mg/L; Unpublished study report, 2015; Klimisch score 1). It is recognized that this low water solubility may cause a reduced degradation rate and may require further testing in some cases (ECHA, 2017b: ECHA Guidance on PBT/vPvB assessment, p. 42).

However, because no substantial degradation was observed after 5 days in this inherent test, it could be concluded that the substance is persistent, as the absence of any degradation under these optimum conditions in the time period between day 5 and 28, provides evidence that degradation in the environment would be slow (ECHA, 2017b: ECHA Guidance on PBT/vPvB assessment, Chapter R.11).

Based on this inherent study, it could be considered that sufficient information is already available to confirm that the P-criterion is fulfilled for the purpose of PBT assessment of 2,4,6-TTBP.

This is further supported by modelling data (see section 3.1.2.1.1 Estimated data), other screening tests and information from the scientific literature as reported in the next section.

3.1.2.1.3 Simulation tests

3.1.2.1.3.1 Biodegradation in water

In Lofthus *et al.* (2016) (Klimisch score 4), biotransformation of three poorly water-soluble alkylphenols including 2,4,6-TTBP was investigated by adopting a new methodology, in which the test substances were immobilised as thin films on hydrophobic adsorbents (tetrafluoroethylene and ethylene polymer (ETFE) monofilament fabrics) which were then submerged in natural seawater during a 64-day test period. The objective of the study was to develop a methodology for the determination of biodegradation of low solubility components like 2,4,6-TTBP.

In this study, seawater was collected from a depth of 80 m (below thermocline) in a non-polluted Norwegian fjord (Trondheimsfjord). The salinity of the seawater was 3.4% with a water temperature of 6-8 °C and dissolved oxygen of approximately 8 mg/L. The seawater was acclimated to 20 °C (5-7 days before the start of the experiments). The experiment was performed in the laboratory at 20 °C in darkness without agitation and 2,4,6-TTBP was tested separately. The test was executed for 64 days and samples (triplicates) were collected after 0, 5, 14, 28, 42 and 64 days of incubation.

Immobilisation of 2,4,6-TTBP resulted in initial amounts of around 21.7 µg/ETFE fabric. Related to the volume of seawater in the flasks (275 mL) this amount represented a concentration of around 79 µg/L. All experimental blanks (ETFE fabrics without the alkylphenols) included in the experiments showed results below the detection limit. Alkylphenols on the fabric and in seawater were quantified separately by GC-MS analysis.

Biotransformation of alkylphenols in natural seawater (primary biodegradation) was determined by first-order decay rates after lag periods after correction for depletion in sterilised controls. (This depletion of total amounts of 2,4,6-TTBP in sterilised controls at the end of the experiment (63 days) was <1%). The biotransformation half-life determined for 2,4,6-TTBP was 32.2 days.

It must be noted that the study is carried out at 20 °C, while it is agreed that the mean seawater temperature for Europe is 9 °C. This is stated in Chapter R.11 (ECHA, 2017b: ECHA Guidance on PBT/vPvB assessment, p. 59): '*The reference temperature for providing results on higher tier tests (and carrying out tests, where relevant) is 12 °C for surface water environment and 9 °C for marine environment*'. If the advice of the guidance is followed, the presented half-life of 32.3 days in Lofthus *et al*. (2016) needs to be corrected to an EU reference temperature of 9 °C for marine water. For the temperature correction, reference is made to Chapter R.7b (ECHA, 2017a: ECHA Endpoint specific guidance, p. 221). Based on a suggested default activation energy (E_a) of 65.4 kJ/mole, the correction factor to convert the half-life (at 20 °C to 9 °C) should be 2.85. Therefore, using this conversion, the half-life in marine water for 2,4,6-TTBP at relevant EU temperature (9 °C) would be 92 days.

It is noted that not all details on the test conditions are available for this study. For example, a mass balance is not presented, other potentially persistent transformation products have not been investigated, and the concentration of suspended particulate matter (SPM) and organic carbon content of the water is not provided. The removal of the parent compound could also be partially caused by dissipation such as adsorption to suspended particulate matter (SPM) in the water column. The use of the adsorbent provides uncertainties.

Therefore, since some details and information on this study are missing, the result (calculated half-life at 9°C of 2,4,6-TTBP > 60 days in seawater) can merely be used as supportive information in a weight-of-evidence approach on the persistence of 2,4,6-TTBP.

3.1.2.3 Summary and discussion on biodegradation

- 2,4,6-TTBP is not readily biodegradable according to QSAR estimations (EPIWEB v4.1; BIOWIN v4.10 and CATALOGIC v.5.12.1) and thus it is concluded that the substance is potentially P/vP.

- 2,4,6-TTBP is not readily biodegradable based on the results of an OECD TG 301C study (Unpublished study report, 1981).

- In a study according to OECD TG 306 (Lofthus *et al.*, 2016) no ultimate biodegradation (<1%) was measured after 64 days of incubation.

- 2,4,6-TTBP is not inherently biodegradable based on the results of an OECD TG 302C study (Unpublished study report, 1992).

- Further data (Lofthus *et al.*, 2016) support that the half-life of 2,4,6-TTBP in seawater at relevant EU-temperature (9 °C) could be 92 days (although recognizing some uncertainties.

3.1.3 Field data

No data available.

3.1.4 Summary and discussion of degradation

Hydrolysis is not considered to be a relevant degradation mechanism for 2,4,6-TTBP.

In a standard seawater-based BOD test (Klimisch score 4), according to OECD TG 306 (Lofthus *et al*., 2016), no ultimate biodegradation (<1%) was measured after 64 days of incubation at 20 °C. Modelling data (performed with EPI Suite software; Klimisch score 2) support that 2,4,6-TTBP is not rapidly degradable and that the substance is potentially P or vP.

2,4,6-TTBP is not readily biodegradable based on the results of an OECD TG 301C study (Unpublished study report, 1981; Klimisch score 4).

2,4,6-TTBP is not inherently biodegradable based on the results of an OECD TG 302C study (Unpublished study report, 1992; Klimisch score 2). It is worth noting that REACH guidance R.11 states that "Lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled without the need for further simulation testing for the purpose of PBT/vPvB assessment."

Lofthus *et al*. (2016) (Klimisch score 4) provides further supporting information, indicating that 2,4,6-TTBP has a calculated half-life in seawater of 92 days at 9 °C when applying a temperature correction (although recognizing some uncertainties).

Altogether, based on a weight-of-evidence consideration it can be concluded that 2,4,6-TTBP meets the P criterion of REACH Annex XIII.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

The adsorption coefficient of 2,4,6-TTBP was determined to be Log K_{oc} = 5.3 in an OECD TG 121 study (Unpublished study report, 2015; Klimisch score 1) at neutral pH and a column temperature of 35 °C. Therefore, when 2,4,6-TTBP is released into water, it is expected to strongly adsorb to suspended solids and sediment.

3.2.2 Volatilisation

The moderate to low vapour pressure (OECD TG 104, 0.073 Pa at 25 °C, Unpublished study report, 2015; Klimisch score 1) indicates that the substance is unlikely to partition to air. Indeed, the distribution modelling in section 3.2.3 below shows that only a very small fraction will partition to air.

3.2.3 Distribution modelling

The following distributions are predicted by the Level III fugacity Model (EPIWEB v4.1; Klimisch score 2), based on a water solubility of 0.063 mg/L and Log K_{ow} of 7.1:

2,4,6-TTBP is expected to mainly partition to soil and sediment when equal release to air, water and soil takes place:

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.292	16	1000
Water	8.99	1.44e+003	1000
Soil	64.5	2.88e+003	1000
Sediment	26.2	1.3e+004	0

2,4,6-TTBP is expected to mainly partition to sediment when released to water:

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.0187	16	0
Water	25.5	1.44e+003	1000
Soil	0.0302	2.88e+003	0
Sediment	74.4	1.3e+004	0

2,4,6-TTBP is expected to mainly partition to soil when released to soil:

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.000105	16	0
Water	0.0153	1.44e+003	0
Soil	99.9	2.88e+003	1000

Sediment	0.0446	1.3e+004	0

3.2.4 Field data

Environmental exposure

The OSPAR Commission (2003, 2006 Update) identified 2,4,6-TTBP as a substance for priority action. According to the OSPAR Commission (2003, 2006 Update), 2,4,6-TTBP used as an intermediate is likely to reach the marine environment via a single main route, namely the discharge of wastewater from the limited number of land-based production sites where the substance is produced or formulated/processed into products. Diffuse sources might include releases due to its presence as an impurity in, or degradation product of, final formulations and/or articles, and use of products in the transport industry.

OSPAR also predicted the total continental releases of 2,4,6-TTBP using the model EUSES. The values obtained are the summed values of those estimated using EUSES default modelling for each of the life-cycle stages of 2,4,6-TTBP. Main emissions are expected towards wastewater (0.069 kg/day) and surface water (0.029 kg/day). Emissions towards industrial soil are expected to be lower (0.0014 kg/day). Emissions to air are considered to be negligible (0 kg/day) (OSPAR Commission; 2003, 2006 Update).

In a screening assessment by Environment Canada (2008) potential releases of 2,4,6-TTBP to the environment were estimated with a Mass Flow Tool. The substance was expected to be mostly chemically transformed (93.2%) through the combustion of fuel. It was estimated with the Mass Flow Tool that 2% of the substance may be released to the environmental compartments (soil, sewer (untreated wastewater), and air) during its processing, distribution and use as fuel additive, oil and possibly oil/lubricant additive. A proportion (4.8%) is also estimated to be transferred to waste disposal sites (e.g., a landfill) as a result of this substance's possible use as an oil/lubricant and possible use as a lubricant additive (i.e., packaging wastes, used oil).

Other releases to the environment of 2,4,6-TTBP may include the transport and use of for example gasoline for home uses (lawn mowers, generators). Releases to the water compartment could occur via incidental spills during recreational activities (boating). Also releases at the gas station (fugitive emissions, accidental spills) could be expected. However, these uses were considered minor and were therefore not taken into account by the Mass Flow Tool (Environment Canada, 2008).

Remberger *et al*. (2003) investigated air, sludge, sediment, soil, water and fish samples taken from several study sites in Sweden (table 4). The maximum concentration measured in water was 0.012 µg/L, in sewage sludge 0.15 µg/g dw and in sediment 3.5 ng/g dw. 2,4,6-TTBP was detected in around 65% of the samples.

Table 4: Range of concentrations of 2,4,6-TTBP in Swedish water, sewage sludge, sediment, soil, air and fish samples (Remberger *et al*., 2003)

Concentrations of 2,4,6-TTBP in Swedish samples	
Water	0.000033 – 0.012 µg/L
Sewage sludge	<0.0001 – 0.15 µg/g dw
Sediment	0.01 – 3.5 ng/g dw
Soil	<0.0001 mg/kg dw
Air	<0.01 ng/m^3
Fish	<0.1 – <0.2 ng/g dw

Hansson *et al.* (2008) collected sediment samples at three sites in the Göta Älv estuary close to Göteborg at the Swedish west coast in September 2007. Two samples were taken at the sites Eriksberg and Rivö, and one sample at the reference site Klinten. In the first sample at the first site (Eriksberg, clay underground, close to harbour of Göteborg) 2,4,6-TTBP was detected in a concentration of 0.21 ng/g dw. In the second sample, the concentration was however <0.1 ng/g dw. Also, at the second site, (Rivö, clay sites with low contents of sand) 2,4,6-TTBP was detected at a concentration of 0.17 ng/g dw in the first sample while the concentration was <0.1 ng/g dw in the second sample. At the third site, (Klinten, clay underground mixed with silt, scent of hydrogen sulphide, relatively far from common waterways) the 2,4,6-TTBP concentration was <0.1 ng/g dw.

2,4,6-TTBP was the third most dominant synthetic phenolic antioxidant in a Chinese study on sludge samples (Liu *et al.*, 2015). 56 sludge samples were taken from individual wastewater treatment plants to investigate the occurrence and composition profiles of 12 synthetic phenolic antioxidants and three 2,6-di-*tert*-butyl-4-methylphenol metabolites. The study demonstrated the presence of 2,4,6-TTBP in sludge at a mean concentration of 98.1 ng/g dw.

In a screening programme organised in 2016 by the Norwegian Environment Agency (Konieczny *et al.*, 2017), 80 prioritised compounds were selected for target analysis, among which was 2,4,6-TTBP. Concentrations of 2,4,6-TTBP in sludge from municipal wastewater ranged from 5 to 27 µg/kg dw at the Slemmestad site and ranged from 34 to 47 µg/kg dw at the Hamar site.

Furthermore, 2,4,6-TTBP was detected in livers from rats in the sewage system and city of Oslo, in concentrations of <14 to 40 µg/kg dw. Fish cod liver concentrations were measured ranging from <12 to 50 µg/kg dw. Finally, in invertebrates 2,4,6-TTBP was present in the common shore crab (<0.65 - 2.3 µg/kg dw) and in winkle (0.89 - 2 µg/kg dw), at Indre Oslofjorden (Konieczny *et al.*, 2017).

The same team (Konieczny *et al*., 2018) organised another screening programme in 2017. Here, 27 prioritised substances (suspected PBT candidates) were subjected to screening. In this study, a single occurrence of 2,4,6-TTBP was measured in landfill run-off water at a maximal concentration of 96 ng/POCIS *(*polar organic chemical integrative sampler).

In the framework of the 2020 screening programme on plastic additives and REACH compounds carried out by the Norwegian Institute for Water Research (NIVA) and NILU-Norwegian Institute for Air Research, 2,4,6-TTBP has been measured in a maximum concentration of 6 ng/g dw in samples of sediment from leisure boat marinas (NIVA, 2021).

<u>Human exposure</u>

Human exposure to 2,4,6-TTBP can occur via drinking water, food and indoor air (mainly via dust).

2,4,6-TTBP was detected in sludge of wastewater treatment plants, as mentioned above (Liu *et al*., 2015; Konieczny *et al*., 2017). Humans could be exposed to the substance via drinking water that has passed through municipal wastewater treatment plants.

2,4,6-TTBP has been detected in Norwegian house dust samples in concentrations of <1.8 to 6.2 µg/kg dw at a furniture centre, and 9.9 to 14 µg/kg dw at a hotel, both in Oslo (Konieczny *et al.*, 2017). Furthermore, a Chinese study was conducted in Shandong province investigating individual dust samples from 75 resident houses from the city (n=55) and the surrounding rural area (n=20) (Liu *et al.*, 2017). 2,4,6-TTBP was the second most dominant synthetic phenolic antioxidant (SPA) analogue for all dust samples, accounting for 22% of the total amount of SPA analogues. 2,4,6-TTBP was detected in mean concentrations of 229 ng/g in dust from urban houses and 834 ng/g in dust from rural houses. The mean concentration in all dust samples (n=75) was 323 ng/g (Liu *et al.*, 2017).

The presence of 2,4,6-TTBP has also been demonstrated in food products. Nemoto *et al*. (2001) determined concentrations of five phenolic compounds by GC/MS (SIM) after extraction from food samples, using a steam distillation technique. With this analytical method, 2,4,6-TTBP was found in meat (trace - 0.50 ng/g), liver (trace) and seafood (muscle) (trace - 1.83 ng/g).

In a Swedish monitoring study (Weiss *et al*., 2022), low but quantifiable levels of 2,4,6-TTBP (0.08 ±0.02 ng/mL) were found in pooled serum samples. The target analysis was performed on a pooled human serum sample, constituted from 100 individuals donating blood at Blodcentralen in Odenplan, Stockholm in February 2020.

3.2.5 Summary and discussion of environmental distribution

2,4,6-TTBP is expected to partition mainly to soil and sediment in the environment.

The substance was detected in different environmental and biota matrices, thus indicating the occurrence of the substance in the environment.

3.3 Data indicating potential for long-range transport

Regarding the long-range transport potential of the substance, screening information as set out in the Stockholm Convention on Persistent Organic Pollutants (POPs) (cf. screening criteria for potential for long-range environmental transport in Annex D, Section 1 (d) of the Stockholm Convention[8]) were considered:

There are limited monitoring data/measured levels of the substance available (See paragraph 3.2.4). Moreover, due to the low vapour pressure, transport through air seems unlikely: the log K_{oc} of 5.3 (OECD TG 121, Unpublished study report, 2015; Klimisch score 1) and the distribution modelling (EPIWEB v. 4.1, Section 3.2.3) indicate that 2,4,6-TTBP will mainly partition to soil and sediment.

Based on the current information available on the substance it is not possible to conclude on its long-range transport potential as monitoring data in remote areas is not currently available.

3.4 Bioaccumulation

[8] The criteria for LRTP in Annex D Section 1: (d) Potential for long-range environmental transport:
(i) Measured levels of the chemical in locations distant from the sources of its release that are of potential concern;
(ii) Monitoring data showing that long-range environmental transport of the chemical, with the potential for transfer to a receiving environment, may have occurred via air, water or migratory species; or
(iii) Environmental fate properties and/or model results that demonstrate that the chemical has a potential for long-range environmental transport through air, water or migratory species, with the potential for transfer to a receiving environment in locations distant from the sources of its release. For a chemical that migrates significantly through the air, its half-life in air should be greater than two days.

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

The screening criterion for bioaccumulation for aquatic organisms (log K_{ow} > 4.5) is fulfilled for 2,4,6-TTBP**,** based on the measured log K_{ow} of 7.1 (OECD TG 117, Unpublished study report, 2015; Klimisch score 1).

The predicted bioconcentration factor (BCF) value (regression-based method) is 11,580 L/kg wet-wt (Log BCF = 4.064) (EPIWEB v4.1; BCFBAF v3.01; Klimisch score 2), based on a Log K_{ow} of 7.

CATALOGIC v.5.15.2 BCF Baseline model 5.12 (Klimisch score 2) using a log K_{ow} = 7.1 as input predicts a BCF of 18,621 L/kg (Log BCF = 4.27). The prediction is fully within the applicability domain of the model.

The bioaccumulation potential of 2,4,6-TTBP was investigated in a study conducted according to the Japanese Guideline 'Bioaccumulation study of chemicals in fish and shellfish' (Kanpogyo No. 5, Yakuhatsu No. 615, 49 Kikyoku No. 392) in 1981-1982 (Unpublished study report, 1982; Klimisch score 2). Carp were exposed to concentrations of 0.01 and 0.001 ppm w/v at 25 °C with flow-through conditions for 8 weeks. Following the exposure period, no abnormal observations were made for the fish. The fish were then sacrificed and prepared for analysis.

Glass aquaria with a capacity of 100 L, water flow velocity of 1155 L/d, with a dilution of 2 mL stock/min with 800 mL water/min hydrogenated castor oil (HCO-40) has been used as dispersant. The test substance (1 g) and 40 g of HCO-40 were dissolved in acetone, after which acetone was distilled off, and demineralised water was added until 1 L in total to prepare a dispersion liquid of 1000 ppm. This dispersing water was finally diluted in order to perform the bioaccumulation study with the following test item concentrations: 10 µg/L (0.01 ppm w/v) and 1 µg/L (0.001 ppm w/v).

Test species was common carp (*Cyprinus carpio*), with an average weight of 27.7 g, an average length of 10.3 cm and an average lipid content of 4.5%. Fish were disinfected for 24 h in a solution of 10 ppm aqueous chlorotetracycline hydrochloride under static conditions, as specified in the Japanese Guideline, before the start of the test and were acclimated at 25 °C for 14 days. Test temperature was 25 ± 1 °C.

Analysis of 2,4,6-TTBP was carried out by GC-MS with a 5% OV-17, Chromosorb W HP glass column of 1 m x 2 mm Ø, with helium as carrier gas. Conditions of the mass spectrometer were a separator temperature of 250 °C with an ionisation voltage of 70 eV, an accelerating voltage of 3 kV, ion generator temperature of 230 °C and M/e measurement of 247.

The BCF values ranged from 4,320 after 1 week to 23,200 L/kg after 4 weeks (at 0.001 ppm w/v or 1 µg/L) and ranged from 4,830 after 2 weeks to 16,000 L/kg after 6 weeks (at 0.01 ppm w/v or 10 µg/L).

Table 5: Bioconcentration factors of 2,4,6-TTBP as presented in the report (Unpublished study report, 1982)

Group (ppm w/v)	Week				
	1	2	4	6	8
0.01	5130 5490	4830 5010	13 800 11 400	16 000 7100	5930 12 200

0.001	4320 4630	10 400 8330	23 200 16 400	8220 13 400	8430 20 200 10 200

In their study on the dependence of experimental bioconcentration factors on the lipid content of fish, Tadokoro and Tomita (1987) measured the BCFs of a series of 12 highly lipophilic substances, among which also 2,4,6-TTBP (Klimisch score 2). The study was performed with common carp (*Cyprinus carpio*) according to OECD TG 305C. 24 fish were held in a test tank and exposed to a nominal concentration of 20 µg/L (measured concentration of 13.9 µg/L) of 2,4,6-TTBP for 4 weeks. This test tank was again divided into three batches, containing fish with varying lipid contents. Different feeding regimes and lipid contents in food were used to maintain the different lipid contents in the batches during the exposure period.

More than 20 BCFs were determined for 2,4,6-TTBP using fish whose lipid content varied from 1.5 to 9% and the corresponding BCFs varied from 5,000 to 20,000. Based on these measured BCFs, the authors applied a 'least square linear regression' approach which led to a BCF normalised to 1% lipid content for 2,4,6-TTBP of 2,524. In the bioaccumulation assessment performed in the framework of REACH, BCFs must be normalised to a fish lipid content of 5 % (ECHA, 2017b: ECHA Guidance on PBT/vPvB assessment, p. 69). If the BCF value above (2,524; 1% lipid content) is recalculated to correspond to a lipid content of 5%, a BCF of 12,620 is derived for 2,4,6-TTBP.

It is recognized that in this BCF determination, lipid normalisation is applied twice (once by the authors to 1% lipid content, for which they applied square linear regression; and once by the dossier submitter from 1% to 5%), which could lead to some uncertainty on the value. However, it should be noted that the BCFs which were calculated for the individual fish are also greater than 5,000.

Based on the EPI Suite estimated BCF-values, the final BCF-values derived from the experimental Japanese Guideline study (1982), and the BCF-value recalculated to a lipid content of 5% in the Tadokoro and Tomita (1987) study, which exceed both 2,000 and 5,000 L/kg, it can be concluded that 2,4,6-TTBP fulfils the B- and vB-criteria of REACH Annex XIII (BCF > 5,000 L/kg).

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

The screening criteria for air-breathing organisms, as stated in the ECHA Guidance on PBT/vPvB assessment, Chapter R.11 (ECHA, 2017b), are fulfilled:

Log K_{ow} (experimental; Unpublished study report, 2015; Klimisch score 1) = 7.1 (> 2) and Log K_{oa} (EPIWEB v4.1; KOAWIN v1.10; Klimisch score 2; water solubility of 0.063 mg/L and Log K_{ow} of 7.1) = 10.5 (> 5).

Therefore; 2,4,6-TTBP is potentially bioaccumulative in air-breathing organisms.

In Chapter R.11 (ECHA, 2017b: ECHA Guidance on PBT/vPvB assessment, p. 83) it is stated that:

'In case a substance screens to be potentially bioaccumulative in air-breathing organisms and aquatic bioaccumulation testing indicates no bioaccumulation, further information and potentially further assessment on bioaccumulation in air-breathing organisms may be necessary.'

In this case, further assessment is not necessary, as aquatic bioaccumulation testing already demonstrated that 2,4,6-TTBP has a high potential for bioaccumulation.

It is noted that a concise toxicokinetics study with air-breathers has been performed with 2,4,6-TTBP by Takahashi and Hiraga (1983) (Klimisch score 2). In this study, a single oral dose (up to 260 mg/kg bw 2,4,6-TTBP) was administered to Sprague Dawley rats. It was found that the maximum tissue concentrations were reached after 1.5 to 6 hours in the liver, kidneys and spleen, but the maximum concentration was not reached within 24 hours in adipose tissues. This observation indicates that 2,4,6-TTBP, after disappearing from the blood, moved into adipose tissue. Further, it is noted that 2,4,6-TTBP is not excreted in urine and that it is not detected in the faeces. On the basis of the absence of 2,4,6-TTBP in urine and faeces, and the rapid clearance from the blood, absorption across the gastrointestinal tract is considered to be 100%. After clearance from the blood, 2,4,6-TTBP was found in adipose tissue. From this study, it is not possible to derive an overall elimination half-life in rats, but it confirms that 2,4,6-TTBP is shown to accumulate in adipose tissue and is therefore potentially (very) bioaccumulative in air-breathers.

Biomonitoring data confirm presence of 2,4,6-TTBP in air-breathing organisms as the substance was detected in rats' livers (Konieczny *et al*., 2017) and quantified in human serum samples (Weiss *et al*., 2022). See section '3.2.4 Field data' for further details.

3.4.3 Summary and discussion of bioaccumulation

The screening criterion for bioaccumulation for aquatic organisms is fulfilled for 2,4,6-TTBP (log K_{ow} > 4.5; Unpublished study report, 2015; Klimisch score 1).

2,4,6-TTBP has the potential to bioaccumulate according to QSAR estimations (EPIWEB v4.1; BCFBAF v3.01 and CATALOGIC v.5.15.2 BCF Baseline Model 5.12; Klimisch score 2).

The B/vB criterion of REACH Annex XIII (BCF > 5,000 L/kg) is fulfilled for 2,4,6-TTBP based on a Japanese Guideline Study (Unpublished study report, 1982; Klimisch score 2) which demonstrated that BCF values range from 4,320 to 23,200 L/kg at 0.001 ppm w/v or 1 µg/L and 4,830 to 16,000 L/kg at 0.01 ppm w/v or 10 µg/L. Supporting evidence is provided in a study by Tadokoro and Tomita (1987; Klimisch score 2), which resulted in a BCF-value of 12,620 for 2,4,6-TTBP (recalculated to 5% lipid content).

Moreover, 2,4,6-TTBP screens as bioaccumulative in air-breathing organisms. Furthermore, biomonitoring data confirm presence of 2,4,6-TTBP in air-breathing organisms as the substance was detected in the liver of rats and quantified in human serum samples.

Absorption of 2,4,6-TTBP across the gastrointestinal tract was also considered to be 100% in a toxicokinetics study performed with rats (Takahashi and Hiraga, 1983; Klimisch score 2).

4. Human health hazard assessment

The classification of the Substance in Regulation (EC) No 1272/2008 shows that it meets the criteria for classification in the hazard class:

- Toxic for reproduction category 1B in accordance with Article 57 (c) of REACH.

5. Environmental hazard assessment

The toxicity criteria of REACH Annex XIII Section 1.1.3 (b) and Section 1.1.3 (c) are fulfilled for 2,4,6-TTBP due to its harmonised classification as Repr. 1B and STOT RE 2.

6. Conclusions on the SVHC Properties

6.1 CMR assessment

2,4,6-tri-*tert*-butylphenol is covered by index number 604-097-00-6 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) and it is classified in the hazard class toxic for reproduction category 1B (H360D: May damage the unborn child)[9].

Therefore, this classification of the substance in Regulation (EC) No 1272/2008 shows that it meets the criteria for classification in the hazard class:

- Toxic for reproduction category 1A or 1B in accordance with Article 57 (c) of REACH.

[9] The harmonised classification has been included in the Commission Delegated Regulation (EU) 2022/692 of 16 February 2022 amending, for the purposes of its adaptation to technical and scientific progress, Part 3 of Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures (the 18th ATP to CLP). Pursuant to the second paragraph of Article 2 of this Regulation, this new harmonised classification applies from 23 November 2023. However, pursuant to the third paragraph of that provision substances and mixtures may already be classified, labelled and packaged in accordance with this classification.

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 was used to identify the substance as PBT. All available information (such as the results of standard tests, non-standard tests and (Q)SAR results) was considered together in a weight-of-evidence approach.

6.2.1.1 Persistence

2,4,6-TTBP is not readily biodegradable according to QSAR estimations (EPIWEB v4.1; BIOWIN v4.10 and CATALOGIC v.5.12.1) and thus it is concluded that the substance is potentially P/vP.

2,4,6-TTBP is not readily biodegradable based on the results of an OECD TG 301 C study.

In a study according to OECD TG 306 no ultimate biodegradation (<1%) was measured after 64 days of incubation.

2,4,6-TTBP is not inherently biodegradable based on the results of an OECD TG 302C study.

Further data support that the half-life of 2,4,6-TTBP in seawater at relevant EU-temperature (9 °C) could be 92 days (although recognizing some uncertainties).

6.2.1.2 Bioaccumulation

The screening criterion for bioaccumulation for aquatic organisms is fulfilled for 2,4,6-TTBP (log K_{ow} > 4.5).

2,4,6-TTBP has the potential to bioaccumulate according to QSAR estimations (EPIWEB v4.1; BCFBAF v3.01 and CATALOGIC v.5.15.2 BCF Baseline model 5.12).

The B/vB criterion of REACH Annex XIII is fulfilled for 2,4,6-TTBP based on a Japanese Guideline Study (BCF values range from 4,320 to 23,200 L/kg at 0.001 ppm w/v or 1 µg/L and 4,830 to 16,000 L/kg at 0.01 ppm w/v or 10 µg/L). Supporting evidence is provided in another study, which resulted in a BCF-value of 12,620 for 2,4,6-TTBP (recalculated to 5% lipid content).

Moreover, 2,4,6-TTBP screens as bioaccumulative in air-breathing organisms. Biomonitoring data confirm presence of 2,4,6-TTBP in air-breathing organisms as the substance was detected in the liver of rats and quantified in human serum samples.

Absorption of 2,4,6-TTBP across the gastrointestinal tract was also considered to be 100% in a toxicokinetics study performed with rats.

6.2.1.3 Toxicity

2,4,6-tri-*tert*-butylphenol is covered by index number 604-097-00-6 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances). It is classified in the hazard class toxic for reproduction category

1B (H360D: May damage the unborn child) and STOT RE category 2 (H373: May cause damage to organs (liver) through prolonged or repeated exposure).

6.2.2 Summary and overall conclusions on the PBT and vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as PBT. All available relevant information (such as the results of standard tests, non-standard tests and (Q)SAR results) were considered together in a weight-of-evidence approach.

Persistence

No hydrolysis study is available for 2,4,6-TTBP. However, due to its low water solubility and high log K_{oc}, 2,4,6-TTBP is expected to sorb to particles and to mainly distribute to sediment in the aquatic environment. Hydrolysis is expected to be hindered by adsorption potential of 2,4,6-TTBP onto sediment and particulate matter. Therefore, hydrolysis is not considered to be a relevant degradation mechanism for 2,4,6-TTBP. Moreover, due to a lack of hydrolysable functional groups, 2,4,6-TTBP is not expected to hydrolyse.

2,4,6-TTBP is not readily biodegradable according to QSAR estimations (EPIWEB v4.1; BIOWIN v4.10 and CATALOGIC v.5.12.1; Klimisch score 2) and thus it is concluded that the substance is potentially P/vP.

In a standard seawater-based BOD test (Klimisch score 4), according to OECD TG 306 no ultimate biodegradation (<1%) was measured after 64 days of incubation at 20 °C. This study is merely used as supporting information due to limited information.

2,4,6-TTBP is not readily biodegradable based on the results of an OECD TG 301C (Ready biodegradability; MITI Test (I); Klimisch score 4) test. In this study not only BOD, but also parent compound was followed by HPLC. It appeared that after 28 days, biodegradation as percentage of ThoD was 0% and disappearance of the parent compound was only 5% (i.e. negligible). This study is merely used as supportive information.

2,4,6-TTBP is not inherently biodegradable based on the results of an OECD TG 302C (Inherent Biodegradability: Modified MITI Test (II); Klimisch score 2) study; a maximum of 17% degradation was observed after 5 days and 13% degradation after 28 days. It is worth noting that REACH guidance R.11 states "Lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD TG 302 series may provide sufficient information to confirm that the P-criteria are fulfilled without the need for further simulation testing for the purpose of PBT/vPvB assessment. The OECD TG 302C study provides strong indications that 2,4,6-TTBP can be concluded as persistent.

In accordance with REACH Annex XIII Section 3.2.1. (d), information from a laboratory study using natural seawater (Klimisch score 4) indicates that the half-life of 2,4,6-TTBP in seawater at relevant EU-temperature (9 °C) could be 92 days when applying a temperature correction (although recognizing some uncertainties). Due to the uncertainties, this study is merely used as supportive information.

Overall, based on the available information and considering a weight-of-evidence approach, it is concluded that 2,4,6-tri-*tert*-butylphenol meets the 'persistence' (P) criterion of REACH Annex XIII Section 1.1.1.

Bioaccumulation

The screening criterion for bioaccumulation for aquatic organisms is fulfilled for 2,4,6-TTBP (log K_{ow} > 4.5).

The B/vB criterion of REACH Annex XIII (BCF > 5,000 L/kg) is fulfilled for 2,4,6-TTBP based on a Japanese Guideline Study (Klimisch score 2) with BCF values in common carp (*Cyprinus carpio,* average lipid content of 4.5%) in the range of 4,320 to 23,200 L/kg. QSAR predictions (EPI Suite software, Klimisch score 2) for 2,4,6-TTBP support the findings of the laboratory study with an estimated BCF > 5,000 L/kg.

Supporting evidence is provided in a study investigating the relationship between bioaccumulation and lipid content of fish, which measured the BCFs of a series of 12 highly lipophilic substances (Klimisch score 2). 2,4,6-TTBP was one of these substances, and common carp were exposed to the substance in batches with different lipid contents. The study resulted in a lipid normalised BCF-value of 12,620 for 2,4,6-TTBP (recalculated to 5% lipid content). More than 20 BCFs were determined for 2,4,6-TTBP in the study, which all varied from 5,000 to 20,000.

Moreover, 2,4,6-TTBP screens as bioaccumulative in air-breathing organisms (based on a log K_{ow}> 2 and a log K_{oa} > 5). Biomonitoring data confirm presence of 2,4,6-TTBP in air-breathing organisms with detection in livers of rats and quantification in human serum.

On the basis of the absence of 2,4,6-TTBP in urine and faeces, and rapid clearance from the blood, absorption across the gastrointestinal tract was considered to be 100% in a toxicokinetics study performed with rats (Klimisch score 2). After clearance from the blood 2,4,6-TTBP was found in adipose tissue.

Based on the available information and considering a weight-of-evidence approach, it is concluded that 2,4,6-tri-*tert*-butylphenol meets the 'bioaccumulation' (B) and 'very bioaccumulative' (vB) criteria of REACH Annex XIII Section 1.1.2 and Section 1.2.2.

<u>Toxicity</u>

2,4,6-tri-*tert*-butylphenol is covered by index number 604-097-00-6 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances). It is classified in the hazard class toxic for reproduction category 1B (H360D: May damage the unborn child) and STOT RE category 2 (H373: May cause damage to organs (liver) through prolonged or repeated exposure).

2,4,6-tri-*tert*-butylphenol is concluded to meet the toxicity criteria of REACH Annex XIII Section 1.1.3 (b) and Section 1.1.3 (c).

<u>Conclusion</u>

In conclusion, 2,4,6-tri-*tert*-butylphenol meets the criteria for a PBT substance according to Art. 57(d) of REACH by comparing all relevant and available information with the criteria set out in Annex XIII of REACH, in a weight-of-evidence determination.

6.3 Assessment under Article 57(f)

This section is not relevant for the identification of the substance as SVHC in accordance with Article 57 (a) to (e) of the REACH Regulation.

References

EC (2022). COMMISSION DELEGATED REGULATION (EU) 2022/692. European Commission, Brussels.

ECHA. (2017a). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7b: Endpoint specific guidance. Version 4.0 – June 2017.

ECHA. (2017b). Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.11: PBT/vPvB assessment. Version 3.0 – June 2017.

ECHA. (2017c). Guidance for identification and naming of substances under REACH and CLP. Version 2.1 – May 2017.

EPIWEB v4.1. United States Environmental Protection Agency, Washington, DC, USA. EPI Suite™ - Estimation Program Interface. Accessible at https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

Environment Canada. Health Canada. (2008). Screening assessment for the challenge Phenol, 2,4,6-tris(1,1-dimethylethyl)-(2,4,6-tri-*tert*-butylphenol). Chemical Abstracts Service Registry Number 732-26-3.

Hansson, K., Kaj, L. and Brorström-Lundén, E. (2008). One-off survey of 2,4,6-tri-*tert*-butylphenol and short chained chlorinated paraffins in the Göta Älv estuary, Sweden. For Swedish Environmental Protection Agency. IVL Swedish Environmental Research Institute Ltd. IVL Report U2263.

Klimisch, H.-J., Andreae, M. and Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory toxicology and pharmacology, 25: 1-5.

Konieczny, R.M., Henninge, L.B., Dalen, H., Grabic, R., Ferenčík, M., Bergqvist, P.-A., Lyngstad, E., Berger, J., Haukelidsæter, S. and Randall, S. (2018). Screening programme 2017: Suspected PBT compounds. For The Norwegian Environment Agency. COWI AS. M-1063.

Konieczny, R.M., Horvath, A., Lyngstad, E., Dalen, H., Blytt, L.D., Henninge, L.B., Ferenčík, M., Nilan, M.S., Bergqvist, P.-A., Grabic, R., Haukelidsæter, S. and Randall, S. (2017). Screening programme 2016: Suspected PBT compounds. For The Norwegian Environment Agency. COWI AS. M-806.

Liu, R., Lin, Y., Ruan, T. and Jiang, G. (2017). Occurrence of synthetic phenolic antioxidants and transformation products in urban and rural indoor dust. Environmental Pollution, 221: 227-233.

Liu, R., Song, S., Lin, Y., Ruan, T. and Jiang, G. (2015). Occurrence of synthetic phenolic antioxidants and major metabolites in municipal sewage sludge in China. Environmental Science & Technology, 49: 2073-2080.

Lofthus, S., Almås, I.K., Evans, P., Pelz, O. and Brakstad, O.G. (2016). Biotransformation of potentially persistent alkylphenols in natural seawater. Chemosphere, 156: 191-194.

Nemoto, S., Omura, M., Takatsuki, S., Sasaki, K. and Toyoda, M. (2001). Determination of 2,4,6-tri-*tert*-butylphenol and related compounds in foods. Journal of the Food Hygienic Society of Japan, 42: 359-366.

NIVA (Norwegian Institute for Water Research) (2021). Screening Programme 2020, Part 1 and 2: Plastic additives and REACH compounds. https://www.miljodirektoratet.no/sharepoint/downloaditem/?id=01FM3LD2UCFOEZF4ANLNGKBLJMHB5CDOGO

OSPAR Commission. (2003, 2006 Update). OSPAR background document on 2,4,6-tri-*tert*-butylphenol. Hazardous Substances Series. Publication Number: 274/2006.

Remberger, M., Kaj, L., Palm, A., Sternbeck, J., Kvernes, E. and Brorström-Lundén, E. (2003). Screening tertiary butylphenols, methylphenols, and long-chain alkylphenols in the Swedish environment. For Swedish Environmental Protection Agency. IVL Swedish Environmental Research Institute Ltd. IVL Report B1594.

Tadokoro, H. and Tomita, Y. (1987). The relationship between bioaccumulation and lipid content of fish. In: Kaiser, K.L.E. QSAR in Environmental Toxicology – II. Proceedings of the 2nd International Workshop on QSAR in Environmental Toxicology, held at McMaster University, Hamilton, Ontario, Canada, June 9-13, 1986 (pp. 363-373). Dordrecht, Holland: D. Reidel Publishing Company.

Takahashi, O. and Hiraga, K. (1983). Metabolic studies in the rat with 2,4,6-tri-t-butylphenol: a haemorrhagic antioxidant structurally related to butylated hydroxytoluene. Xenobiotica, 13: 319-326.

Unpublished study report. (1981). Assessment of ready biodegradability, MITI (I).

Unpublished study report. (1982). Bioaccumulation Test Report.

Unpublished study report. (1992). Assessment of inherent biodegradability, Modified MITI (II).

Unpublished study report. (2015). Determination of physico-chemical properties of 2,4,6-tri-*tert*-butylphenol.

US EPA (2012). Estimation Programs Interface (EPI) Suite™, v 4.1. United States Environmental Protection Agency, Washington, DC, USA.

Weiss, J., Engelhardt, J., Fischer, S. and Eriksson, C. (2022). Identification of new and emerging risk chemicals and validation of the exposure index with a focus on humans.