SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2,4,6-tri-tert-butylphenol

EC No 211-989-5

CAS No 732-26-3

Evaluating Member State(s): Belgium

Dated: 11 October 2018

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2017

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

2,4,6-tri-tert-butylphenol (2,4,6-TTBP) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB

- Exposure of environment.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

NA

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	X
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Currently, the substance is self-classified as STOT RE 1, Skin Sens. 1B, Acute Tox. 4 and Aquatic Chronic 2. The eMSCA considers that the PBT criteria for 2,4,6-TTBP are fulfilled. Annex XIII section 1.1.3 (c) states that a substance fulfils the toxicity criterion (T) if there is evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to Regulation EC No 1272/2008. Therefore, the eMSCA will prepare a harmonised C&L proposal for this (and other relevant) endpoints.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

The (v)P, (v)B and T criteria according to annex XIII or REACH are considered fulfilled and an Risk Management Option Analysis will be performed. One possible option is to proceed with the SVHC identification of 2,4,6-TTBP according to article 57(d) of REACH.

4.1.3. Restriction

NA

4.1.4. Other EU-wide regulatory risk management measures

NA

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

NA

5.2. Other actions

NA

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Harmonised C&L	October 2018	Belgium
RMOA	November 2020	Belgium
SVHC identification	February 2021	Belgium

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

2,4,6-tri-tert-butylphenol (2,4,6-TTBP) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB

- Exposure of environment.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
PBT/vPvB	Persistency: The P screening criterion is fulfilled. Based on a weight of evidence approach it can be shown that the P and vP criteria are fulfilled for the marine environment. Bioaccumulation: The REACH Annex XIII criterion for B/vB is fulfilled. Toxicity: The substance is self-classified as STOT RE 1. The REACH Annex XIII criterion for T is considered fulfilled. A Harmonised C&L proposal will be submitted to confirm this consideration.
Exposure of environment	Exposure to the environment is expected due to the use of the substance.

7.2. Procedure

Link with SEV evaluation 2015 for reaction mass of 2,6-di-tert-butylphenol and 2,4,6-tri-tert-butylphenol:

- March 2015: eMSCA started evaluation of the reaction mass of 2,6-di-tert-butylyphenol and 2,4,6-tri-tert-butylphenol (EC 907-745-9) resulting in concern for 2,4,6-tri-tert-butylphenol (at the time this evaluation started, there was no registration for 2,4,6-tri-tert-butylphenol).

- 28 April 2015: 2,4,6-tri-tert-butylphenol was registered under REACH.

- 22 January 2016: An update of the registration dossier for 2,4,6-tri-tert-butylphenol was submitted (containing additional endpoint data).

Regarding evaluation of 2,4,6-tri-tert-butylphenol:

- 7 March 2017: First contact between registrant(s) and eMSCA. Full study reports were requested.

- 21 March 2017: 2,4,6-tri-tert-butylphenol was officially added to the CoRAP and the evaluation started.

- 12 May 2017: Most full study reports were received and analysed. Furthermore, the OSPAR background document from 2006[2], the screening assessment by Environment/Health Canada of 2008[3] and the Environment Tier II Assessment from IMAP updated in 2017[4] were analysed.

- 27 July 2017: Meeting between eMSCA and registrant(s).

- September 2017: Way forward on 2,4,6-TTBP was discussed in the PBT expert group.

- In January 2018, the PBT expert group was consulted via written procedure on the biodegradation potential of the substance and comments were received from several members.

- As an outcome of the discussions with the PBT expert group, the eMSCA concluded that sufficient information is available to build a weight of evidence approach to demonstrate that the substance is persistent.

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	2,4,6-tri-tert-butylphenol
EC number:	211-989-5
CAS number:	732-26-3
Index number in Annex VI of the CLP Regulation:	NA
Molecular formula:	$C_{18}H_{30}O$
Molecular weight range:	262.4302
Synonyms:	NA

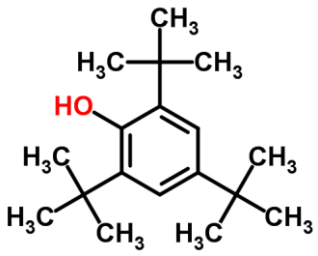
Type of substance X Mono-constituent ☐ Multi-constituent ☐ UVCB

[2] OSPAR Commission, 2006 Update: OSPAR background document on 2,4,6-tri-tert-butylphenol; Publication number 274/2006

[3] Screening Assessment for the Challenge Phenol, 2,4,6-tris(1,1-dimethylethyl)-(2,4,6-tri-tert-butylphenol). Environment Canada/Health Canada November 2008

[4] Environment Tier II Assessment for Phenol, 2,4,6-Tris(1,1-dimethyl). IMAP (accelerated assessment of industrial chemicals in Australia) Last updated 26 April 2017

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Slightly yellow powder with lumps
Vapour pressure	0.035 Pa (0.00026 mm Hg) at 20° C 0.073 Pa (0.00055 mmHg) at 25° C According to OECD 104 (Effusion method: isothermal thermogravimetry) EPI Suite estimation (MPBPVP v1.43): 0.0266 Pa at 25°C and 0.0002 mmHg at 25° C (modified Grain method)
Water solubility	0.063 mg/L at 20°C According to OECD 105 (column elution method) EPI Suite estimation: REG: (WSKOW v1.42): 0.512 mg/L at 25° C
Partition coefficient n-octanol/water (Log Kow)	Log Pow= 7.1 According to OECD 117 (HPLC method) Epi Suite estimation: REG: KOWWIN (v1.68): Log Kow: 6.39
Flammability	Non-flammable According to EU Method A.10
Explosive properties	Non-explosive (based on the substance's structure)
Oxidising properties	Non-oxidising (based on the substance's structure)
Granulometry	Test waived due to technical infeasibility. Substance cannot be analysed by laser diffraction due to the moisture of the substance; Moisture droplets formed in dispersing solvent interfere with the measurement of particle size of crystals. No alternative available (substance sticks to sieves).

Dissociation constant	PALLAS prediction: pKa= 12.62

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 7 (dissemination website consulted on 06-07-2017)

USES	Use(s)
Uses as intermediate	Industrial use as intermediate
Formulation	Industrial formulation of fuel additives and fuel blends.
Uses at industrial sites	Industrial use of fuel additives and additised fuels.
Uses by professional workers	Professional use of fuel additives and additised fuels (ERC: Widespread use of functional fluid indoor and outdoor)
Consumer Uses	NA
Article service life	NA

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

NA

7.6.2. Self-classification

- In the registration(s)*:*

Acute Tox. 4; H302 : Harmful if swallowed

Skin Sens. 1B; H317: May cause an allergic skin reaction

STOT RE 1; H372 (Liver): Causes damage to organs (liver) through prolonged or repeated exposure

Aquatic Chronic 2; H411: Toxic to aquatic life with long lasting effects

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory (consulted on 06-07-2017):

Skin Irrit. 2; H315 : Causes skin irritation

Eye Irrit. 2; H319 : Causes serious eye irritation

STOT SE 3; H335: May cause respiratory irritation

Aquatic Acute 1; H400: Very toxic to aquatic life

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

Aquatic Chronic 4; H413: May cause long lasting harmful effects to aquatic life

Not classified

7.7. Environmental fate properties

7.7.1. Degradation

Hydrolysis:

Due to low water solubility (0.063 mg/L) and lack of hydrolysable functional groups, no hydrolysis study is available.

Estimated data for biodegradation:

1. The ready biodegradability of 2,4,6-TTBP was estimated using the BIOWIN model v4.10.

BIOWIN 2: 0.0068 (Does not biodegrade fast)

BIOWIN 3: 2.0392 (ultimate degradation – months)

BIOWIN 4: 3.0485 (primary degradation – weeks)

BIOWIN 6: 0.0497 (Not readily biodegradable)

The PBT Guidance Table C.4-1 indicates that a substance is potentially P or vP if the substance doesn't biodegrade fast (BIOWIN 2) and the ultimate biodegradation frame prediction is ≥ months (BIOWIN 3). A substance is also potentially P or vP if the substance doesn't biodegrade fast (BIOWIN 6) and the ultimate biodegradation timeframe is ≥ months (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)

This submodel 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 Kow and molecular weight are within the specified ranges and its atom-centered fragments are present in the training set.

- Half-life for primary biodegradation: 171 days.
  As this predicted value largely exceeds the vP-criterion in water (60 days), it is likely that 2,4,6-TTBP meets the vP-criterion.

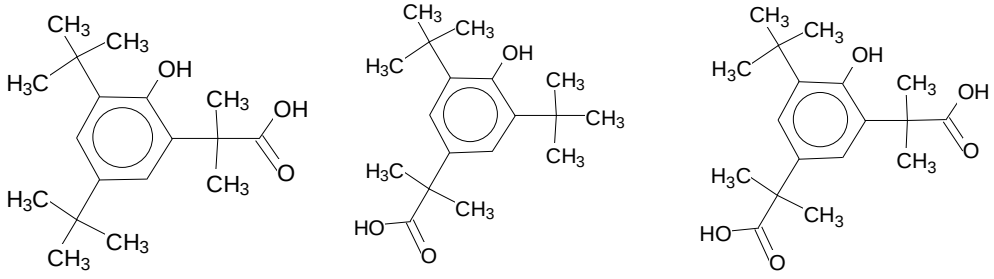
- Half-life for ultimate biodegradation: more than 10 years
  This prediction supports the analysis that not only the parent compound but also the potential interim degradation products show a vP-character.

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

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 eMSCA concluded that the model is 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%:



There are three compounds with observed maps, where the oxidation of the tertiary butyl group was recorded. However, the <u>probability for this to happen is low</u> (ca 10%), which results in low quantities of the predicted transformation product.

Qualitative conclusion: biodegradation of the parent compound proceeds slowly.

Measured data for biodegradation:

In an inherent biodegradability study from 1992 (OECD 302C; Modified MITI Test (II)) 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.

Result measured as oxygen uptake in % ThOD.

Result : maximum of <u>17 % degradation after 5 days</u>, afterwards decline/steady state to <u>13 % after 28 days.</u> The reference substance aniline degrades in a continuous way to 26 % after 5 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.

Because no degradation is observed after 5 days in this inherent test, it could be concluded that the substance is persistent (cf. ECHA Guidance, Chapter R.11, version 3.0, June 2017, p. 51). It is noted that the solubility of 2,4,6-TTBP is quite low (measured value = 0.063

mg/L, estimated value = 0.5 mg/L); it is recognized that this low water solubility may cause a reduced degradation rate, but the absence of any degradation under these optimum conditions in the time period between day 5 and 28, provides nevertheless a reliable indication of the persistent character of 2,4,6-TTBP.

In Chapter R.11 (PBT/vPvB assessment version 3.0; June 2017) of the ECHA guidance on Information Requirements and Chemical Safety Assessment it is indicated 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. Additionally, in specific cases it may be possible to conclude that the vP-criteria are fulfilled with this result if there is additional specific information supporting it (e.g., specific stability of the chemical bonds). The tests provide optimum conditions to stimulate adaptation of the micro-organisms thus increasing the biodegradation potential, compared to natural environments. A lack of degradation therefore provides evidence that degradation in the environment would be slow. Care should be taken in the interpretation of such tests, however, since, for example, a very low water solubility of a test substance may reduce the availability of the substance in the test medium. These issues are discussed in more detail in Sections R.7.9.4 and R.7.9.5 of Chapter R.7b of the Guidance on IR&CSA.'

Based on this, it could be concluded that sufficient information is already available to conclude that 2,4,6-TTBP is persistent/very persistent.

However, in Chapter R.11 (PBT/vPvB assessment version 3.0; June 2017) of the ECHA Guidance on Information Requirements and Chemical Safety Assessment it is also indicated that:

'*A lack of degradation in an inherent biodegradation test (≤ 20%) can provide evidence that degradation in the environment would be slow. It should however be noted that the very low solubility of many PBT/vPvB substances may reduce their availability and hence their degradability in the test. The lack of degradation in an inherent test does not always imply that the substance is intrinsically persistent and in some cases further testing might be needed.'*

Therefore, it was considered that there are strong indications that the substance is persistent, but some further elements would be needed to strengthen this assessment.

In Lofthus *et al.,* 2016 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 immobilized to hydrophobic adsorbents submerged in natural seawater. The experiment was performed at 20 °C in darkness without agitation.

The test is carried out at 20 °C, while it is agreed that the mean seawater temperature for Europe = 9 °C (see pag. 51 of ECHA Guidance, Chapter 11, PBT/vPvB assessment, version 3.0, June 2017).

So, the presented half-life in the article (32.3 days) must be corrected. For the correction, the recommendations in ECHA Guidance R.7b Endpoint specific guidance, version 4.0, June 2017, pag. 222 were applied. With an activation energy (Ea) of 65.4 kJ/mole, the correction factor should be 2.85 (from 20 °C to 9 °C). Therefore, the half-life for 2,4,6-TTBP at relevant EU-temperature = 92 days (i.e. > 60 d). Based on this argumentation 2,4,6-TTBP meets the vP-criterion.

It is noted that there are some insufficiencies/unknown elements in the execution of this experiment. No mass balance is presented and the removal of the parent compound could also be partially caused by dissipation. Potentially, biodegradation is even overestimated and real degradation half-lives could be greater than the values presented. Also, other potentially persistent transformation products have not been investigated.

Based on the above considerations, the biodegradation of 2,4,6-TTBP can even be less if it is considered that removal of the parent compound could also have occured through other means.

Not all details on the test water are given, e.g. concentration of suspended particulate matter (SPM) and organic carbon content is not provided. It is possible that part of the substance was adsorbed to SPM or complexed with organic matter. Still, assuming that the same extraction and analytical methods were applied for test samples and controls, adsorption to SPM is unlikely to prevent biodegradation, because it is stated that the depletion of the total amount of the parent substance in the sterilised control was less than 1% at the end of the experiment. It means that if adsorption to SPM occurred, their extraction method could still retrieve the almost totality of the substance.

Therefore, although some details and information on this study are missing, conditions for biodegradation are considered to be optimal in that study, thus it is unlikely that biodegradation in a common simulation study would be higher. Based on all these considerations, this study demonstrates that the half-life of 2,4,6-TTBP in seawater is > 60 days.

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

7.7.2. Environmental distribution

The adsorption coefficient of 2,4,6-TTBP was determined in an OECD 121 study (Registration data, 2015). Log Koc = 5.3 at 35°C.

The high pKa value (12.6) indicates that at environmentally relevant pH, the substance will be undissociated. The moderate to low vapour pressure (0.073 Pa) indicates that the substance is unlikely to partition to air. When 2,4,6-TTBP is released into water, it is expected to strongly adsorb to suspended solids and sediment based on the adsorption coefficient of 5.3.

The following distribution is predicted by the Level III fugacity Model (Episuite v4.10) based on a water solubility of 0.063 mg/L and Log Kow of 7.1:

Mass Amount		Half-Life	Emissions
	(percent)	(hr)	(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

Persistence Time: 2.16e+003 hr

Based on the above considerations, the eMSCA concludes that 2,4,6-TTBP is expected to partition mainly to soil and sediment.

7.7.3. Bioaccumulation

The predicted bioconcentration factor (BCF) value (regression-based method) is 7129 L/kg wet-wt (Log BCF = 3.853) based on a water solubility of 0.063 mg/L and a LogKow of 7.1 (BCFBAF v3.01; Episuite 4.1).

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. Carp were exposed at concentrations of 0.01 and 0.001 ppm w/v at 25°C with flow-through conditions for 8 weeks.

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 desalted water was added till 1L in total to prepare a dispersion liquid of 1000 ppm. This dispersing water was diluted to two concentrations: 4 ppm (w/v) and 0.4 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 chlorotetracycline 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 ionization 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 range between 4320 after 1 week to 23200 L/kg after 4 weeks at 0.001 ppm w/v and 4830 after 2 weeks to 16000 L/kg after 6 weeks at 0.01 ppm w/v.

Therefore, based on the available information, the eMSCA concludes that 2,4,6-TTBP has a high potential to bioaccumulate.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Experimental data:

Method	Results	Remarks	Reference
Cyprinus carpio freshwater semi-static OECD Guideline 203 (Fish, Acute Toxicity Test) EU Method C.1 (Acute Toxicity for Fish) + Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment nr. 23	96h LC50 >0.048 mg/L (meas. arithm. mean) based on: mortality	1 (reliable without restriction) GLP Water Soluble Fraction was prepared at loading rate of 100 mg/L (highest concentration) Average exposure concentration	Registration dossier (study report, 2015)

Method	Results	Remarks	Reference
		was calculated to be 0.048 mg/L for the highest test concentration.	
Oncorhynchus mykiss freshwater semi-static Equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test)	96h LC50 >0.1 mg/L (nominal) based on: mortality	2 (reliable with restrictions) Non-GLP The reported LC50 value is higher than the water solubility and refers to a nominal value instead of measured concentration.	Registration dossier (study report, 1992)

An acute fish toxicity test (OECD 203) with 2,4,6-TTBP was performed. No effects were seen with *Cyprinus carpio* up to 0.048 mg/L.

In another non-GLP acute toxity test (OECD 203) with *Oncorhynchus mykiss* no effect up to the water solubility was seen.

The eMSCA accepts the use of the LC50 value of 0.048 mg/L for the PNEC derivation.

Long-term toxicity to fish hasn't been investigated since it is indicated in the registration data that the substance is already treated as a PBT substance, therefore the environmental releases are considered strictly controlled and no emission to the environment takes place.

The eMSCA however considers that the use of the substance in fuel can lead to exposure of the environment. Confirmation of the PBT/vPvB properties of 2,4,6-TTBP is most relevant to further limit exposure to the environment (additional risk management measures could come in place). Since the REACH Annex XIII 1.1.3 (c) criterion for T is fulfilled, no further information has been requested under this SEV process.

7.8.1.2. Aquatic invertebrates

Experimental data:

Method	Results	Remarks	Reference
Daphnia magna freshwater	48h EC50 >0.072 mg/L (meas. (initial))	1 (reliable without restriction)	Registration dossier (study report, 2015)

Method	Results	Remarks	Reference
static OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) EU Method C.2 (Acute Toxicity for Daphnia) + Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment nr. 23	based on: mobility	GLP Water Soluble Fraction was prepared at loading rate of 100 mg/L (highest concentration) Actual measured concentration at start was 0.092 mg/L and remained stable during the exposure. Reported EC50 value is higher than the water solubility.	

An acute invertebrates toxicity test (OECD 202) with 2,4,6-TTBP was performed. No effects were seen with *Daphnia magna* up to the water solubility.

Long-term toxicity to aquatic invertebrates has been investigated in accordance with OECD guideline 211. Only the results are available as the original study data are in Japanese and the source data is not available. The study is given an Klimisch score of 4. A 21d NOEC of 0.36 mg/L was determined in this study, which can only be used as supporting data.

Long-term toxicity to aquatic invertebrates hasn't been investigated further since it is indicated in the registration data that the substance is already treated as a PBT substance, therefore the environmental releases are considered strictly controlled and no emission to the environment takes place.

The eMSCA however considers that the use of the substance in fuel can lead to exposure of the environment. Confirmation of the PBT/vPvB properties of 2,4,6-TTBP is most relevant to further limit exposure to the environment (additional risk management measures could come in place). Since the REACH Annex XIII 1.1.3 (c) criterion for T is considered fulfilled, no further information has been requested under this SEV process.

7.8.1.3. Algae and aquatic plants

Experimental data:

Method	Results	Remarks	Reference
Pseudokirchneriella subcapitata freshwater	72h NOEC = 0.04 mg/L (meas. (TWA))	1 (reliable without restriction)	Registration dossier (study report, 2015)

Method	Results	Remarks	Reference
static OECD Guideline 201 (Alga Growth Inhibition Test) EU Method C.3 (Algal inhibition test) + Guidance document on aquatic toxicity testing of difficult substances and mixtures, OECD series on testing and assessment nr. 23	72 h ErC50 and EyC50 > 0.04 mg/L (meas. (TWA)) based on: growth rate and yield inhibition	GLP Water Soluble Fraction was prepared at loading rate of 100 mg/L (highest concentration) Time weighted actual concentration for the highest concentration was 0.04 mg/L	

Due to the low water solubility of 2,4,6-TTBP no toxic concentration levels for algae were reached.

The eMSCA agrees that the 72h NOEC for growth rate inhibition and yield inhibition is 0.04 mg/L. This value can be used for PNEC determination.

7.8.1.4. Sediment organisms

No data

7.8.1.5. Other aquatic organisms

No data

7.8.2. Terrestrial compartment

No data

7.8.3. Microbiological activity in sewage treatment systems

Experimental data:

Method	Results	Remarks	Reference
Activated sludge of a predominantly domestic sewage freshwater static equivalent or similar to OECD Guideline 209 (Activated	3h EC50 > 1000 mg/L (nominal) based on: growth inhibition	2 (reliable with restrictions) GLP	Registration dossier (study report, 1992)

Method	Results	Remarks	Reference
sludge, respiration inhibition test)			

2,4,6-TTBP caused 9% growth inhibition at 1000 mg/L. The EC50 was determined to be >1000 mg/L.

The eMSCA agrees that the EC50 value of >1000 mg/L can be used for PNEC determination for aquatic microorganisms.

7.8.4. PNEC derivation and other hazard conclusions

Not evaluated.

7.8.5. Conclusions for classification and labelling

In the registration data, the substance is classified for the environment as:

Aquatic Chronic 2; H411: Toxic to aquatic life with long lasting effects

The eMSCA considers that 2,4,6-TTBP is a poorly soluble substance for which no acute toxicity is recorded at levels up to the limit of water solubility, that it is not rapidly biodegradable and has a high potential for bioaccumulation. According to the guidance of the application of the CLP criteria (section 4.1.3.3.2), 2,4,6-TTBP could be classified as:

Aquatic Chronic 4; H413: May cause long lasting harmful effects to aquatic life

The eMSCA has no further concern regarding the environmental classification and labelling.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Method	Results	Rel.	Reference
Basic toxicokinetics *in vivo* In male rat (SD) Single dose : 260 mg/kg by gavage or 0.2% by diet Exposure : gavage and in the diet Vehicule : soya oil No guideline followed	Absorption : rapid (peak concentration 15 to 60 min after exposure Blood half-live : 18.2 min for the rapid α-phase and 11.8 hours for the slower β-phase Distribution : in starved rats : max concentration : blood > liver > spleen > kidneys > fat. In testes : trace amounts Excretion : not in urine. A metabolite was detected in the faeces (considered to be 2,4,6-tri-tbutylphenoxy radical)	2	Takahashi O. and Hiraga K., 1983

7.9.2. Acute toxicity and Corrosion/Irritation

Acute toxicity :

Method	Results	Rel.	Reference
Oral route : gavage In rats (SD) (5/sex/dose) Doses : 200 and 2000 mg/kg bw OECD Guideline 401 Vehicle : arachis oil	LD50 : > 200 - < 2000 mg/kg bw 200 mg/kg bw : No observed effects At 2000 mg/kg bw : 2 ♀ were found dead 1D after exposure and 3 ♀ and 1 ♂ were killed 1 or 4D after exposure Clinical signs : 2000 mg/kg bw : ataxia, hunched posture, lethargy, decrease respiratory rate, laboured respiration Gross pathology examination : 2000 mg/kg bw : haemorrhagic lungs, dark or pale liver, haemorrhagic or pale gastric mucosa	1	Registration dossier (study report, 1992)
Dermal route : occlusive In rats (Wistar) (5/sex/dose) Doses : 2000 mg/kg bw Exposure : 24 h OECD Guideline 402 Vehicle : corn oil	LD50 : > 2000 mg/kg bw No mortality Clinical signs : 1 ♀ with erythema No bw change and no abnormalities observed at the gross pathology examination	1	Registration dossier (study report, 2015)

Based on the results of the studies, the substance 2,4,6-TTBP is classified by the registrant as Acute Tox. 4, H302 (Harmful if swallowed).

Based on the available information, the eMSCA supports this conclusion and considers that there is no concern for acute toxicity and thus no need to request further information under this substance evaluation.

Irritation :

Method	Results	Rel.	Reference
Skin irritation study : semi-occlusive In 3 rabbits	Erythema score (mean of the 24, 48 and 72h examinations) : 0.22/4 and fully reversible within 72h Edema score (mean of the 24, 48 and	1	Registration dossier (study report, 1992)

(NZW) Doses : 0.5 g Exposure : 4 H OECD Guideline 404	72h examination) : 0/4 PII : 0.2 Slight irritant		
Eye irritation study In rabbits (NZW) (2 males and 1 females) Doses : 62 mg OECD Guideline 405	Mean score of the 24, 48 and 72h examination : Cornea opacity score: 0/4 Iris score : 0/2 Conjunctivae score (redness) : 0.22/3 Chemosis score : 0/4 Discharge : 0.11/3 Not irritating	1	Registration dossier (study report, 1992)

Based on the results of the studies, the substance 2,4,6-TTBP is not classified by the registrant as skin irritation or eye irritation.

Based on the available information, the eMSCA supports this conclusion and considers that there is no concern for skin and eye irritations and thus no need to request further information under this substance evaluation.

7.9.3. Sensitisation

Method	Results	Rel.	Reference
Local Lymph Node Assay In 5 female mice (CBA) Doses : 0, 10, 25 and 50 % OECD Guideline 429 Vehicle : dimethylformamide	SI : 1.7, 3.3 and 4.6 respectively at 10, 25 and 50% EC3 (estimated) : 22.2% Sensitising	1	Registration dossier (study report, 2015)

Based on the results of the studies, the substance 2,4,6-TTBP is classified by the registrant as Skin Sens. 1B, H317 (May cause an allergic skin reaction).

Based on the available information, the eMSCA supports this conclusion and considers that there is no need to request further information under this substance evaluation.

7.9.4. Repeated dose toxicity

Method	Results	Rel.	Reference
Combined repeated dose toxicity study with the	No mortality and no clinical signs	1	Registration dossier

reproduction/developmental toxicity screening test Oral route : gavage In rats (Wistar) (10/sex/dose) Doses : 0, 3, 10 and 30 mg/kg bw/d Exposure : 29 D for males and 41 to 56 D for females (2w prior mating and until D4 of lactation) OECD Guideline 422, 421 and 407 Vehicle : corn oil	observed BW : slight modifications (at 10 mg/kg bw/d : - 4-9% in 3 ♀ and at 30 mg/kg bw/d : - 5-9% in 3 ♀) Some slight changes in the haematology and clinical biochemistry examination (lower neutrophil count, higher lymphocyte count and RBC count at the highest dose, and at the 2 highest dose lower MCV and MCH) Liver : enlargement in 3 ♂ and 1 ♀ at 30 mg/kg bw/d, increase abs. weight (in ♂ : 8.07, 8.68, 9.24 and 10.38** and in ♀ 7.09, 7.98, 8.95** and 12.08** mg respectively at 0, 3, 10 and 30 mg:kg bw/d), increase relative weight (39 and 63% in ♂ and ♀ at 30 mg/kg bw/d and 21% in ♀ at 10 mg/kg bw/d), hepatocellular hypertrophy in ♂ and ♀ at 10 and 30 mg/kg bw/d, hepatocellular necrosis in 1 ♂ and 1 ♀ at 30 mg/kg bw/d Cecum : mucosal hypertrophy in ♂ at 10 and 30 mg/kg bw/d Spleen : decreased haematopoiesis in ♀ at 10 and 30 mg/kg bw/d (but increase RBC counts) No changes in reproductive parameters Development : increased postnatal loss (in 3 dams at 10 and in 5 dams at 30 mg/kg bw/d), ↓ mean pup bw on D4 (-16 and -20% resp. at 10 and 30 mg/kg bw/d) and lower viability index at 10 and 30 mg/kg bw/d (100, 100, 93.4** and 87.2** respectively at 0, 3, 10 and 30 mg/kg bw/d) NOAEL : 3 mg/kg bw/d NOAEL (reproduction) : > 30 mg/kg bw/d NOAEL (development) : 3 mg/kg bw/d		(study report, 2015)

Chronic toxicity study Oral route : feed In rats (Wistar) (40/sex/dose) Doses : 0, 30, 100, 300 and 1000 ppm Exposure : 24 m OECD Guideline 452	No mortality and no clinical signs BWG : significant decrease in ♀ at 1000 pm from 12 m onward Some changes in haematology examination : Haemoglobin (↓), MCV (↓), platelet count (↑) Liver : sign ↑ in relative weight in ♂ (at 300 and 1000 ppm) and in ♀ (in all dose groups) + swelling, focal necrosis and vacuolization of liver cells at 300 and 1000 ppm Kidney : sign ↑ in weight in ♂ (at 1000ppm) and in ♀ (at 100, 300 and 1000ppm) at 24m Adrenal : sign ↑ in weight at 1000 ppm NOAEL : 30 ppm LOAEL : 100 ppm	2	Matsumoto K. *et al.*, 1991
Subacute toxicity study Oral route : feed In 10 male rats (SD) Doses : 1.98 mmol/kg/d Exposure : 3w No guideline followed	All animals died during the exposure period (between D5 and 11) Gross pathology examination : haemothorax, haematocoelia, intracranial haematoma, intranasal haemorrhage, intramuscular haematoma, intratesticular haematoma and intraepididymis haemorrhage LT50 (lethal time) : 7.4D	2	Takahashi O. and Hiraga K., 1978

Based on the results of the studies, the substance 2,4,6-TTBP is classified by the registrant as STOT RE 1, H372 (Cause damage to organs through prolonged or repeated exposure). The registrant indicated that the affected organ is the liver.

Based on the available information, the eMSCA supports this conclusion and considers that there is no further concern to be clarified for repeated dose exposure toxicity and thus no need to request further information under this substance evaluation.

7.9.5. Mutagenicity

In vitro data:

Method	Result	Rel.	Reference
Bacterial reverse mutation assay *S. Typh.* TA 1535, 1537, 98 and	Genotoxicity : negative (no increase in the number of revertants)	1	Registration dossier (study report, 2015)

100 + *E. Coli* WP2 uvr A With and without S9 mix OECD guideline 471 Vehicle : DMSO	Cytotoxicity : only in tester strains *S. Typh.* TA1535 and 1537 without S9-mix		
In vitro mammalian cell gene mutation test Mouse lymphoma L5178Y cells With and without S9-mix OECD Guideline 476 Vehicle : DMSO	Genotoxicity : negative (no increase in the mutation frequency) Cytotoxicity : yes	1	Registration dossier (study report, 2015)
In vitro mammalian chromosome aberration test Chinese hamster ovary With and without S9-mix Japanese guideline Vehicle : DMSO	Genotoxicity : negative (no increase in structural or numerical chromosome aberrations) Cytotoxicity : yes	1	Registration dossier (study report, 1998)

In vivo data:

No data available.

Conclusion:

Based on the results of the studies, the substance 2,4,6-TTBP is not classified by the registrant as mutagen.

Based on the available information, the eMSCA supports this conclusion and considers that there is no concern for mutagenicity and thus no need to request further information under this substance evaluation.

7.9.6. Carcinogenicity

Method	Result	Rel.	Reference
Chronic study Oral route : feed In rats (Wistar) (40/sex/dose) Doses : 0, 30, 100, 300 and 1000 ppm	No neoplastic effects observed NOAEL : 1000 ppm	2	Matsumoto K. *et al.*, 1991

Exposure : 24m No guideline followed			

Based on the results of the studies, the substance 2,4,6-TTBP is not classified by the registrant as carcinogen.

Based on the available information, the eMSCA supports this conclusion and considers that there is no concern for carcinogenicity and thus no need to request further information under this substance evaluation.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Method	Result	Rel.	Reference
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test Oral route : gavage In rats (Wistar) (10/sex/dose) Doses : 0, 3, 10, 30 mg/kg bw/d Exposure : males : 29D (beginning : 2w prior mating), females : 41 to 56d (2w prior mating and until at least D4 of lactation) OECD Guideline 422 Vehicle : corn oil	P0 : No mortality and no clinical sign observed BW : decrease at 10 and 30 mg/kg bw/d in ♀ during lactation No reproductive parameters changes observed (mating, fertility index, number of corpora lutea, implantation sites, spermatogenic profil and histopathological examination of the reproductive organs) As mentioned in the section 7.9.4 : some changes in the liver were observed NOAEL (parental) : 3 mg/kg bw/d NOAEL (reproduction) : > 30 mg/kg bw/d F1 : Increased postnatal loss (in 3 dams at 10 mg and in 5 dams at 30 mg/kg bw/d) and lower viability index (100, 100, 93.4* and 87.2* respectively at 0, 3, 10 and 30 mg/kg bw/d) Lower mean bw at 10 and 30 mg/kg bw/d at D 4 of lactation (-16 and -20%	1	Registration dossier (study report, 2015)

	compared to control group) NOAEL (developmental) : 3 mg/kg bw/d		

Based on the results of the study, 2,4,6-TTBP is not classified by the registrant as toxic for the reproduction.

Based on the available information, the eMSCA considers that there is no concern for reproductive toxicity and thus no need to request further information under substance evaluation.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not evaluated.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

In the registration data, the substance is classified for human health as:

Acute Tox. 4; H302 : Harmful if swallowed.

Skin Sens. 1B; H317: May cause an allergic skin reaction.

STOT RE 1; H372 (Liver): Causes damage to organs (liver) through prolonged or repeated exposure.

The eMSCA has no further concern regarding the human health hazard classification and labelling. The eMSCA will prepare a harmonised C&L proposal for these endpoints.

7.10. Assessment of endocrine disrupting (ED) properties

In recent decades, focus has been placed on alkylphenols due to their ability to cause feminization and inhibition of testicular growth in aquatic vertebrates such as fish (Jobling *et al.*, 1996; Sumpter, 1995).

Routledge and Sumpter (1997) examined alkylphenols for their estrogenic potential. The study indicates that the size and degree of branching of the alkyl group, as well as its position relative to the hydroxyl group on the phenyl ring, are important features for estrogenic activity of alkylphenols. The estrogenicity potential increased with the number of carbon atoms in the alkyl chain beginning with 4 carbon atoms up to 8 carbon atoms. Activity seems to decrease again when the carbon number exceeds 8. Also the position of the alkyl chain was examined. Estrogenicity increases as the alkyl group is moved from ortho to meta to para, respectively. An alkyl chain in para-position exerted the highest effect.

When assessing the role of the number of substituted butyl groups, Tollefsen Knut-Erik and Nilsen Anja Julie (2008) found large differences between mono-substituted butylphenol, 2,4-di-tert-butylphenol, and 2,4,6-tri-tert-butylphenol. 4-tert-butylphenol exhibited a 2.5 fold higher affinity to the hepatic estrogen receptors (rtER) (RBA = 4.10^{-3}

) than 2,4-di-tert-butylphenol (RBA = $1.6.10^{-3}$), whereas additional alkylation namely 2,4,6-tri-tert-butylphenol caused a 26-fold reduction in ER affinity (RBA = $1.6.10^{-4}$). This again could give an indication that 2,4,6-TTBP has a very low binding affinity.

Moreover, it is indicated in Tollefson *et al*. (2008) that not only substitution with multiple alkyl groups, but also the presence of substituents in the ortho- and meta-position reduced the estrogenic activity.

2,4,6-TTBP has 2 substituents in the ortho position and is therefore likely to exert only very weak endocrine effects.

It should be noted however that for the structural similar substance butylated Hydroxytoluene (BHT, CAS 128-37-0) some indications for an estrogenic mode of action exist (Journal of Dental Research, Volume 83, Issue 3, March 2004, Pages 222-226, In vitro estrogenicity of resin composites, Wada, H. *et al*.) BHT is currently being evaluated by France for potential ED concern (see the Public Activities Coordination Tool (PACT) list and the CoRAP list for 2016 evaluation). This will be further followed-up.

7.10.1. Conclusion on endocrine disrupting properties (combined)

Overall, no additional concern for endocrine disruption was identified for 2,4,6-TTBP based on this preliminary assessment and the currently available information. Moreover, based on the evaluation of all test results, there are currently no indications of ED mediated effects. This doesn't prevent that any further information becoming available on 2,4,6-TTBP or a structurally similar substance could trigger the need for future more detailed evaluation/clarification of the potential ED properties.

7.11. PBT and VPVB assessment

1) Persistence

2,4,6-TTBP is not readily biodegradable according to QSAR estimations (BIOWIN 4.10).

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

Further data indicate that 2,4,6-TTBP is persistent and very persistent, based on a weight-of-evidence approach.

2) Bioaccumulation

2,4,6-TTBP has the potential to bioaccumulate according to QSAR estimations (BCBAF v.3.01).

The B/vB criterion (Annex XIII of REACH) is fulfilled for 2,4,6-TTBP based on a Japanese Guideline Study (BCF values range from 4320 to 23000 L/kg at 0.001 ppm w/v and 4830 to 16000 L/kg at 0.01 ppm w/v).

3) Toxicity

The T criterion (Annex XIII of REACH) is fulfilled for 2,4,6-TTBP based on the results of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD 422, 421 and 407; 2015), supported by data from a chronic toxicity study (OECD 452; 1991). Based on the results of these studies, 2,4,6-TTBP is self-classified as STOT RE 1, H372 to cause damage to the liver through prolonged or repeated exposure. A harmonized C&L proposal will be submitted by the BE CA to confirm this.

4) Overall conclusion

In the registration dossier the registrant(s) indicate that 2,4,6-TTBP is considered to be a PBT and vPvB substance although no definitive study on persistence is available. The eMSCA concludes that according to REACH Annex XIII, 2,4,6-TTBP meets the P, vP, B, vB and T criteria.

7.12. Exposure assessment

7.12.1. Human health

Public information

In a Chinese study[5], 2,4,6-TTBP was found in urban and rural indoor dust samples collected from Shandong province in China. Results showed that 2,4,6-TTBP was positively identified in all the collected urban and rural dust samples with concentrations of 10.3-1160 ng/g and 208-2870 ng/g respectively.

7.12.2. Environment

Public information:

In a report from the Swedish Environmental Research Institute (2008)[6], the highest concentrations of 2,4,6-TTBP in surface sediment at the Swedish Göta Älv estuary were 0.21 ng/g DW in Eriksberg and 0.17 ng/g DW in Rivö. It is mentioned that in a screening study performed in 2003 (coastal sediments from the Stockholm municipality; central Stockholm), the concentration of 2,4,6-TTBP varied between <0.02 and 0.45 ng/g DW.

In a review statement for the OSPAR background document on 2,4,6-TTBP (OSPAR commission, 2009) it is stated that UK has developed a monitoring strategy and as part of this has carried out a one-off survey on 2,4,6-TTBP in sediments in industrial estuaries around the UK coast. A number of samples were below the detection limit, but there were also several positives ranging from 0.01 0.09 µg/g of dry sediment.

Environment Canada (2008)[7] estimated that 2% of the 2,4,6-TTBP that is in commerce in Canada is being released to the environment.

7.12.3. Combined exposure assessment

Not assessed

[5] Occurrence of synthetic phenolic antioxidants and transformation products in urban and rural indoor dust. Runzeng Liu, Yongfeng Lin, Ting Ruan and Guibin Jiang; Environmental Pollution (2016)

[6] One-off survey of 2,4,6-tri-tert-butylphenol and short chained chlorinated parafiins in the Göta Älv estuary, Sweden (2008)

[7] Environment Canada/Health Canada; Screening Assessment for the Challenge Phenol, 2,4,6-tris(1,1-dimethylethyl); November 2008

7.13. Risk characterisation

Not assessed

7.14. References

Jobling, S., Sheahan, D., Osborne, J.A., Matthiessen, P. and Sumpter, J.P. ,1996, Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ. Toxicol. Chem., 15:194-202.

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7.15. Abbreviations

♂ : Male

♀ : Female

Abs. : Absolute

BW : body weight

DMSO : dimethyl sulfoxide

MCV : mean corpuscular volume

NZW : New Zealand White

PII : Primary irritation index

RBC : Red blood cell

Rel. : Reliability

SD : Sprague-Dawley

SI : Simulation index

Sign. : significant