baua:
Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
Federal Institute for Occupational Safety and Health

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Phenol, styrenated

EC number: 262-975-0 | CAS RN: 61788-44-1

&

Reaction mass of 2,6-bis(1-phenylethyl) phenol and 2,4,6-tris(1-phenylethyl) phenol

List No number: 701-171-0

[formerly identified as Reaction mass
of 2,4,6-tris(1-phenylethyl) phenol and Bis(1-phenylethyl) phenol
List No number: 915-333-5]

Evaluating Member State: Germany
(Following the initial assessment by the UK)

Dated: 6 March 2023

Evaluating Member State Competent Authority

Conclusion of the substance evaluation process

BAuA
Federal Institute for Occupational Safety and Health
Division 5 - Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Initial evaluation and follow-up

UK REACH CA
Health and Safety Executive
Redgrave Court
Merton Road
Bootle
Merseyside
L20 7HS
Email: ukreachca@hse.gov.uk

Chemicals Assessment Unit
Environment Agency
Red Kite House, Howbery Park
Wallingford
Oxfordshire, OX10 8BD
Email: UKREACHENV@environment-agency.gov.uk

Year of evaluation in CoRAP: 2014

Before concluding the substance evaluation, a Decision to request further information was issued on: 25 July 2016

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

The following substances (hereafter 'the Substances') were evaluated:

1. Phenol, styrenated (EC No. 262-975-0) and
2. Reaction mass of 2,6- bis(1-phenylethyl) phenol and 2,4,6-tris(1-phenylethyl) phenol ("TSP", List No. 701-171-0, formerly identified as Reaction mass of 2,4,6-tris(1-phenylethyl) phenol and bis(1-phenylethyl) phenol, List No. 915-333-5)

The Substances were originally selected for substance evaluation to clarify concerns about:

- Suspected PBT properties
- Potential environmental endocrine disrupting properties
- Exposure/Cumulative exposure.

During the evaluation another concern was identified by the previous evaluating Member State Competent Authority (MSCA), which was the United Kingdom Competent Authority (UK CA).

The additional concern was:

- Uncontrolled risks from some uses of the Substance.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Phenol, styrenated has been evaluated previously by the former Technical Committee for New and Existing Substances (TCNES) PBT working group due to concerns for potential PBT and vPvB properties (ECHA, 2009). ECHA concluded a targeted compliance check on both EC 262-975-9 and List No. 915-333-5 without requiring further information.[2] There was a substance identity (SID) clarification in 2018 and a new List No. 701-171-0 ("TSP") was created for the substance formerly identified as List No. 915-333-5. For List No. 915-333-5, there are no longer active registrations under REACH.

3. CONCLUSION OF SUBSTANCE EVALUATION

Germany was appointed the eMSCA for the Substances following the withdrawal of the United Kingdom from the European Union.

The evaluation of the available information on the Substance has led the DE CA as the eMSCA to the following conclusions, as summarised in Table 1.

[2] https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e1814b5cab
https://echa.europa.eu/de/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e1814b7307

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
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

Based on the evaluation of the Substances and its conclusion, the eMSCA will prepare a further risk management option analysis (RMOA) in which the appropriate options will be clarified, and the most relevant Risk Management Measures (RMMs) identified (see below).

4.1.1. Harmonised Classification and Labelling

The Substances are self-classified as Aquatic Acute 1, H400 and Aquatic Chronic 2, H411 by the respective registrants. Based on all available information on toxicity to aquatic organisms, the eMSCA considers that a stricter self-classification of Aquatic Chronic 1, H410 (M = 10) should be applied. The harmonisation of the classification at EU level via a CLH proposal is currently not considered a priority by the eMSCA. However, if the new hazard classes for endocrine disruptors are available with the corresponding guidance, classification of the Substances as endocrine disruptors for the environment will be the first step to come to an EU-wide agreement on the ED properties of the Substances. Based on this ED identification subsequent adequate regulatory measures will be analysed in a RMOA.

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

Most data generated during the substance evaluation process seem to suggest that the assessed constituents of the Substances do not fulfil the PBT or vPvB criteria of Annex XIII REACH. However, some data generated for the tristyrenated phenol constituent is not conclusive and does not allow to conclude on persistency of this constituent. Overall, SVHC identification of the Substances based on Article 57 d) or e) is not considered warranted by the eMSCA.

However, available information from *in vitro* and *in vivo* tests on 4-monostyrenated phenol (4-MSP), the main constituent of the Substances, is considered sufficient by the eMSCA to identify 4-MSP as an endocrine disruptor for the environment according to the WHO/IPCS definition.

Based on this information, the eMSCA will conduct a Regulatory Management Option Analysis (RMOA) for the Substances. The RMOA will determine whether, based on the presence of 4-MSP, SVHC identification is warranted according to REACH Article 57 f), if the endocrine disrupting effects to the environment give rise to an equivalent level of concern as SVHC identified according to REACH Article 57 a)-e).

4.1.3. Restriction

Emissions to the environment should be minimised for an endocrine disrupting compound. Depending on the outcome of the RMOA process and a potential SVHC identification, the eMSCA considers that a restriction might be a further step in case further reduction of emissions to the environment are needed.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

Not applicable.

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

Indication of a tentative plan is not a formal commitment by the eMSCA. 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**
RMOA	2023	DE

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substances were originally selected for substance evaluation to clarify concerns about:

- Suspected PBT properties
- Potential environmental endocrine disrupting properties
- Exposure/Cumulative exposure.

During the evaluation another concern was identified by the eMSCA (UK). The additional concern was:

- Uncontrolled risks from some uses of the substance.

The DE CA did not identify further concerns after taking over the role of eMSCA.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Suspected PBT	**No Concern.** Available information suggests that neither the Substances nor its constituents fulfil the PBT/vPvB criteria according to Annex XIII REACH. However, a conclusion regarding persistency based on the available data is not possible for the tristyrenated phenol constituent. As the eMSCA considers the endocrine disrupting properties for the environment of the monostyrenated component as the driving hazard for further risk management measures, no further considerations on the PBT assessment beyond those documented have been made during the follow-up assessment. No further action necessary.
Potential environmental endocrine disruptor	**Concern confirmed.** During the evaluation, additional information from *in vivo* and *in vitro* tests was generated for constituents of the Substances. The information is sufficient to assess 4-MSP, a constituent of the Substances, as an endocrine disruptor (ED) according to the WHO/IPCS definition. **Further regulatory action necessary.**
Exposure/Cumulative exposure	**Concern confirmed.** A threshold for endocrine disrupting effects in environmental organisms (i.e., a Predicted no-effect concentration, PNEC) cannot be derived with sufficient confidence for this substance. Therefore, a safe level in the environment cannot be determined. The eMSCA considers minimisation of emissions to the environment of the substance as necessary due to the endocrine disrupting properties of one of its main constituents. **Further regulatory action necessary.**
Uncontrolled risks from some uses of the substance	
Aquatic toxicity - acute	**Concern confirmed** for DSP, i.e., one of the main constituents of the Substances. From the data available (experimental data on short-term toxicity to fish and daphnia for the Substances, complemented by QSARs estimations), the eMSCA concludes that DSP is very toxic to aquatic life.
Aquatic toxicity - chronic	**Concern confirmed**. From the data available (experimental data on long-term toxicity to fish, daphnia, and algae, complemented by QSARs estimations), on the following main constituents of the Substances, the eMSCA concludes that:

	• MSP is very toxic to aquatic life with long-lasting effects • DSP is very toxic to aquatic life with long-lasting effects • TSP is very toxic to aquatic life with long-lasting effects.

7.2. Procedure

The substance evaluation of the Substances was initiated by the UK CA on 1 March 2014 and focussed on the initial grounds for concern.

A decision with further information requirements was sent to the registrants of the Substances in July 2016 requiring further information to clarify the PBT/vPvP and ED concern for the Substance.[3]

By November 2018, the registrants had updated their registration dossiers to address the requests in the decision.

Following the withdrawal of the United Kingdom from the European Union on 31 January 2020, Germany was appointed the evaluating Member State and took over the substance evaluation for the Substances in the conclusion stage. The evaluation of the available test results relies mainly on the UK's assessment while the regulatory actions have been proposed by the German eMSCA. The German eMSCA reassessed the outcome of the OECD TG 234 study requested to investigate the endocrine disrupting properties. The eMSCA considered the available information as sufficient to conclude the substance evaluation process.

7.3. Identity of the substances

Table 4

SUBSTANCE IDENTITY OF EC 262-975-0	
Public name:	Phenol, styrenated
EC number:	262-975-0
CAS number:	61788-44-1
Index number in Annex VI of the CLP Regulation:	n/a
Molecular formula:	See constituents
Molecular weight range:	198.3-406.6 (based on main constituents)
Synonyms:	Styrenated phenol

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

[3] https://www.echa.europa.eu/documents/10162/75543127-f6c1-5c36-feee-d015e5dd5107

Table 2

SUBSTANCE IDENTITY OF LIST No. 701-171-0 (previously List No. 915-333-5)	
Public name:	Reaction mass of 2,6-bis(1-phenylethyl) phenol and 2,4,6-tris(1-phenylethyl) phenol
List No number:	701-171-0 (the substance was previously identified as List No 915-333-5)
CAS number:	none
Index number in Annex VI of the CLP Regulation:	n/a
Molecular formula:	See constituents
Molecular weight range:	302.4-406.6 (based on main constituents)
Synonyms:	TSP

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

Table 6

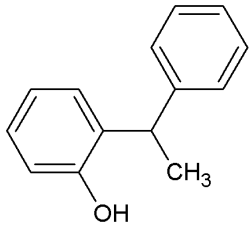
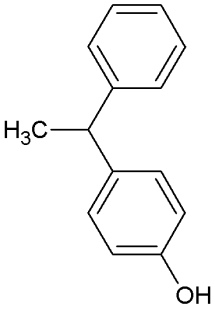
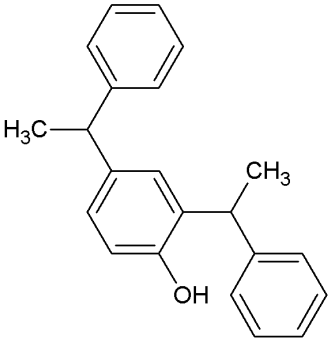
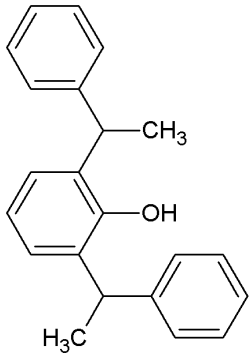
SUBSTANCE IDENTITY OF LIST NO. 915-333-5 (substance previously listed in CoRAP)	
Public name:	Reaction mass of 2,4,6-tris(1-phenylethyl) phenol and Bis(1-phenylethyl) phenol
List No number:	915-333-5 (List No. changed to 701-171-0)
CAS number:	none
Index number in Annex VI of the CLP Regulation:	n/a
Molecular formula:	See constituents
Molecular weight range:	198.3-406.6 (based on main constituents)
Synonyms:	Styrenated phenol

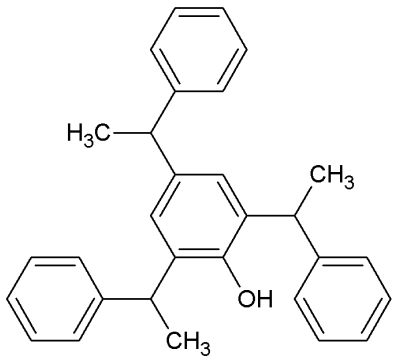
Type of substance ☐ Mono-constituent ☒ Multi-constituent ☐ UVCB

Structural formula:

The registered Substances are essentially multi-constituent Substances containing different proportions of (1-phenylethyl) phenols (these will be called "monostyrenated phenols" from this point forwards), bis(1-phenylethyl) phenols ("distyrenated phenols") and tris(1-phenylethyl) phenols ("tristyrenated phenols"). The main components present are shown below.

Table 7

Main components of the Substances	
Monostyrenated phenols (MSP) [molecular weight 198.3 g/mol, molecular formula $C_{14}H_{14}O$] *	
	*o*-(1-phenylethyl) phenol [2-(1-phenylethyl) phenol] – this is referred to as 2-monostyrenated phenol (abbreviated to 2-MSP) in the remainder of this evaluation. SMILES: c1(O)c(C(C)c2ccccc2)cccc1)
	*p*-(1-phenylethyl) phenol [4-(1-phenylethyl) phenol] – this is referred to as 4-monostyrenated phenol (abbreviated to 4-MSP) in the remainder of this evaluation. SMILES: Oc(ccc(c1)C(c(cccc2)c2)C)c1)
Distyrenated phenols (DSP) [molecular weight 302.4 g/mol, molecular formula $C_{22}H_{22}O$]	
	2,4-bis(1-phenylethyl) phenol – this is referred to as 2,4-distyrenated phenol (abbreviated to 2,4-DSP) in the remainder of this evaluation. SMILES: Oc(c(cc(c1)C(c(cccc2)c2)C)C(c(cccc3)c3)C)c1)
	2,6-bis(1-phenylethyl) phenol – this is referred to as 2,6-distyrenated phenol (abbreviated to 2,6-DSP) in the remainder of this evaluation. SMILES: c1(C(C)c3ccccc3)c(O)c(C(C)c2ccccc2)ccc1)

Tristyrenated phenols (TSP) [molecular weight 406.6 g/mol, molecular formula $C_{30}H_{30}O$]	
	2,4,6-tris(1-phenylethyl) phenol – this is referred to as 2,4,6-tristyrenated phenol (abbreviated to 2,4,6-TSP) in the remainder of this evaluation. SMILES: Oc(c(cc(c1)C(c(cccc2)c2)C)C(c(cccc3)c3)C)c1C(c(cccc4)c4)C)

* According to the registration data on TSP (List no. 701-171-0), monostyrenated phenols are not present in concentrations >0.1% in the substance.

7.4. Physico-chemical properties

Table 8

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF EC 262-975-0	
Property	**Value**
Physical state at 20°C and 101.3 kPa	liquid
Vapour pressure	*Data lacking*
Water solubility	1.95 mg/L at 22°C at pH 6.5 The value was obtained by a UV-spectrometric method. Hence no differentiation between the solubility of the different constituents was done.
Partition coefficient n-octanol/water (Log Kow)	Results for two constituents, both monostyrenated, are available: 2-monostyrylphenol (2-MSP): log K_{OW} 3.13 at 23.6°C 4-monostyrylphenol (4-MSP): log K_{OW} 2.99 at 23.6°C
Flammability	Non-flammable
Explosive properties	Data lacking: waiver used as for Phenol, styrenated there are no chemical groups associated with explosive properties.
Oxidising properties	No.
Granulometry	Not required: Substance is a liquid
Stability in organic solvents and identity of relevant degradation products	Phenol, styrenated was determined to be unstable in organic solvent dichloromethane and degradation product o-(methylbenzyl) phenol was formed after 24 hours as evident from the GC-MS chromatogram obtained at 0 hours and that obtained after 24 hours.
Dissociation constant (pKa)	pKa 3.69 [-7] at 37°C (Test was conducted with "Phenol, styrenated" as a whole. No information available on the constituents.)

Surface tension	Result available for the 4-monostyrenated phenol (4-MSP) constituent: 48.45 mN/m at 20°C of a 90% saturated aqueous solution It is expected that according to the chemical structure of the different constituents the para-mono-styrenated structure have the highest potential of surface activity.

Table 9

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF LIST NO. 701-171-0	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Viscous liquid
Vapour pressure	less than 0.1 Pa at 20°C (Test was conducted with 'Phenol, styrenated' as a whole.)
Water solubility	Tristyrenated constituent: 7.07 µg /L at 20°C and pH 7-8 Distyrenated constituent (not specified which isomer or mixture of isomers respectively): 0.665 mg/L at 20°C and pH 6.96
Partition coefficient n-octanol/water (Log Kow)	Tristyrenated constituent: log K_{OW} 7.77 at 25°C and pH 6 (OECD TG 123, slow stirring method) Distyrenated constituent: log K_{OW} 6.24 at 25°C and pH 6 (OECD TG 123, slow stirring method) 2-monostyrenated phenol (2-MSP): log K_{OW} 3.13 at 23.6°C 4-monostyrenated phenol (4-MSP): log K_{OW} 2.99 at 23.6°C
Flammability	No data or waived.
Explosive properties	Not explosive.
Oxidising properties	Not oxidising.
Granulometry	Not required: Substance is a liquid
Stability in organic solvents and identity of relevant degradation products	Data lacking
Dissociation constant (pKa)	Data lacking
Surface tension	4-monostyrenated phenol (4-MSP): 48.45 mN/m (SD ± 0.05 mN/m) at 20°C of a 90% saturated aqueous solution It is expected that according to the chemical structure of the different constituents the para-mono-styrenated structure have the highest potential of surface activity. Distyrenated constituent: data lacking Tristyrenated constituent: data lacking

7.5. Manufacture and uses

7.5.1. Quantities

Table 10

AGGREGATED TONNAGE (PER YEAR) FOR EC 262-975-0				
☐ 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

Currently, there are 10 active registrations for phenol, styrenated (EC No. 262-975-0).[4]

Table 11

AGGREGATED TONNAGE (PER YEAR) FOR LIST NO. 701-171-0				
☐ 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

TSP (List No. 701-171-0) is solely registered as an intermediate with no disseminated aggregated tonnage.[5]

Following the SID clarification in 2018 for List No. 915-333-5, there no longer are active registrations for this substance under REACH.[6]

[4] ECHA dissemination database on EC 262-975-0: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/11376/1/2 Last accessed 2021-01-12.

[5] ECHA dissemination database on EC 701-171-0: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/21701/1/2 Last accessed 2021-01-12.

[6] ECHA dissemination database on EC 915-333-5: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2055/1/2 Last accessed 2021-01-12.

7.5.2. Overview of uses

Table 12

REGISTERED USES FOR EC No. 262-975-0	
	Use(s)
Uses as intermediate	Use as intermediate under strictly controlled conditions
Formulation	Formulation of coatings, paints, adhesives, and rubber preparations
Uses at industrial sites	Manufacture of substance
Uses by professional workers	Use in coatings/paints - outdoor and indoor use Coatings and paints, thinners, paint removers Cleaning of uncured photosensitive resins
Consumer Uses	Coatings and paints, thinners, paint removers – indoor use
Article service life	Rubber products Wide dispersive indoor/outdoor use of long-life articles and materials (low release) Coatings and paints, thinners, paint removers
Formulation	Formulation of coatings, paints, adhesives, and rubber preparations

Table 13

REGISTERED USES FOR LIST No. 701-171-0	
	Use(s)
Uses as intermediate	Use as intermediate under strictly controlled conditions
Uses at industrial sites	Manufacturing of the substance

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

None of the substances is included in Annex VI of the CLP Regulation. The individual main constituents are also not included in Annex VI of the CLP Regulation.

7.6.2. Self-classification

In the registration, EC 262-975-0 is classified as:

Aquatic Acute 1	H400
Aquatic Chronic 2	H411

In addition, the following hazard classes are notified in the C&L inventory:

Skin Irrit. 2	H315
Eye Irrit. 2	H319
Skin Sens. 1	H317
STOT SE 3	H335

In the registration, TSP is classified as:

Aquatic Acute 1	H400
Aquatic Chronic 2	H411

No further notifications in the C&L inventory exist.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Hydrolysis

Based on the available information, the Substances are not considered to undergo significant hydrolysis in the environment. A similar conclusion was reached in the EU RAR prepared by the UK (EU RAR 2009).

The eMSCA concurs with this conclusion in the follow-up assessment.

7.7.1.2. Phototransformation/photolysis

7.7.1.2.1. Phototransformation in air

The most relevant information on phototransformation in air is contained in the EU RAR prepared by the UK (EU RAR 2009). The half-life for reaction with atmospheric hydroxyl radicals was estimated to be 8.2 hours for monostyrenated phenol, 6.2 hours for distyrenated phenol and 10.1 hours for tristyrenated phenol. The rate constants were re-estimated by the eMSCA (UK) during the substance evaluation using AOP Program (v1.92) within the latest version of the EPI Suite (v4.11). The results were similar to the values reported in the EU RAR and confirm that

the main constituents of the registered Substances will degrade in the atmosphere with a half-life of around 6-12 hours.

The eMSCA concurs with this conclusion in the follow-up assessment.

7.7.1.2.2. Phototransformation in water

No data are available on phototransformation in water in the registration dossiers for the Substances.

7.7.1.2.3. Phototransformation in soil

No data are available on phototransformation in soil in the registration dossiers for the Substances.

7.7.1.3. Biodegradation

7.7.1.3.1. Biodegradation in water

Estimated data

Results available from the BIOWIN and CATALOGIC models available both in the registration dossiers as well as data obtained by the eMSCA (UK) during the initial assessment indicate that components of the Substances are not expected to be readily biodegradable.

Screening tests

The available experimental data suggest that styrenated phenol is not readily biodegradable. Two studies are available (Unpubl. 1997; EC 2000) which showed little or no degradation over 28 days in an OECD TG 301B and OECD TG 301C study.

7.7.1.3.2. Simulation tests (water and sediments)

No valid data are available for simulation tests in water and sediment.

Overall, there are uncertainties over the persistence of styrenated phenol and the constituents therein. The available evidence suggests that the distyrenated and tristyrenated phenol constituents may meet the Annex XIII screening criteria for persistence in water and sediment.

In the Decision sent to the registrants following the initial substance evaluation, an OECD TG 309 study was requested[7]. In a meeting between the eMSCA (UK) and the SPTF consortium of registrants in December 2018, the registrants said that they considered that the OECD TG 309 with TSP was not technically feasible. They explained that this was based on a review of the objective of an OECD TG 309 (water) test. This is to provide biodegradation kinetics (half-life) based on mineralization and primary degradation including the identification and quantification of major transformation products. They indicated that mineralisation of TSP was known to be slow (ready biodegradation test), and the identification and quantification of transformation product requires test concentration of >100 µg/L, but the water solubility of TSP is 7.07 µg/L. Therefore, they concluded that it was not technically feasible to obtain the required information from an OECD TG 309 study. This argumentation is not in the registration dossiers.

[7] Unless the study was not technically feasible, in which case a test according to either OECD TG 307 or 308 should be performed.

7.7.1.3.3. Biodegradation in soil

An aerobic soil simulation study using the tristyrenated phenol constituent has been performed according to OECD 307 and to GLP by some of the registrants in response to the substance evaluation decision. This was submitted to the eMSCA (UK) in November 2018 (Unpubl. 2018c). This used four soils: a sandy loam (18 acres, UK), a silt loam (Gartenacker, Switzerland), another silt loam (Krone, UK) and a loam (Vetroz, Switzerland). Overall, the eMSCA (UK) is satisfied that under the conditions of this study that parent TSP has a worst-case half-life of around 10.7 days at 12 °C, with fast-phase and slow-phase DT_{50} values of, 3.15 d and 38.1 d, respectively. However, as NER formation was high in all four soils (up to the half of applied radioactivity), these half-lives do not only reflect degradation but also dissipation processes. For all soils the dominant transformation products were M_0 (DSP Monoxide) and M_8 (TSP Monoxides), with percentages representative of > 5% applied radioactivity. Half-lives of M_0 and M_8 were calculated by the eMSCA (UK) to be 16.1 d and 17.9 d, respectively. Corresponding transformation DT_{90} for extractable TSP, M_0 and M_8 were 94.3 d, 53.4 d and 59.6 d. However, reservations concerning the kinetic modelling results remain, i.e., the question of seemingly biphasic kinetics and its effect on half live derivation could not be answered while handing over the evaluation process from one eMSCA to the other eMSCA.

7.7.1.3.4. Summary and discussion on degradation

New information provided shows a total half-life well below the persistence criterion for soil. Those transformation products identified and measured at percentages above 5 % also do not reach the persistence criterion. However, the calculated half-lives for the parent compound display degradation as well as dissipation processes, e.g., NER formation of TSP. For the comparison with the P-criteria set out in REACH Annex XIII, degradation half-lives should be used (ECHA R.11). Thus overall, it is concluded that a final conclusion on persistence is not possible based on the available information.

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

No measured data are available for the Koc of styrenated phenol. Predicted values based on the log Kow show that the Koc of the constituents, as would be expected, increases from mono- to di- to tristyrenated phenol.

In line with the approach taken in the EU RAR (2009) the Koc values estimated using the TGD (2003) method have been assumed by the eMSCA for this substance evaluation.

7.7.2.2. Volatilisation

In the registration dossier, the Henry's law constant has been estimated as 0.0364 Pa m³/mol for styrenated phenol from the measured vapour pressure and water solubility of the registered substance and an average molecular weight based on the proportion of constituents (Unpubl. 2016f). These data relate to the technical product as supplied rather than the individual constituents of the substance and so may not be appropriate for all constituents of the registered substance.

Discussion and analysis of available data

A Henry's law constant of 0.0364 Pa m³/mol has been estimated for the technical styrenated phenol product by one registrant. However, this value may not be appropriate for all constituents of the registered substance.

7.7.2.3. Distribution modelling

Not part of the follow-up assessment of the eMSCA.

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

Estimated data and measured data on bioaccumulation in fish is available in the registration and has been previously discussed in the EU RAR on styrenated phenol.

7.7.3.2. Terrestrial bioaccumulation

No information is given in the registration dossiers on terrestrial bioaccumulation.

7.7.3.3. Summary and discussion of bioaccumulation

The available evidence suggests that the mono- and distyrenated phenol constituents of the registered substance have a low potential for bioaccumulation. Predictions for monostyrenated phenol indicate that the BCF in fish is likely to be around 263 L/kg or lower and a dietary accumulation study with distyrenated phenol (which included both 2,4-DSP and 2,6-DSP) indicates that these Substances are rapidly eliminated from fish.

For tristyrenated phenol, the growth corrected and lipid normalised BMF value has been determined to be around 0.355 (range 0.32-0.49). Depuration of the substance from the fish has been found to be relatively slow (half-life of around 18.4 days) and predictions suggest that there is a high probability of the BCF value being above 5,000 l/kg (BCF is 10,395 l/kg using the method recommended in the REACH Guidance; range of predicted values using other available methods is 8,607-36,320 l/kg).

The eMSCA considers tristyrenated phenol as meeting the Annex XIII criteria for being both bioaccumulative (B) and very bioaccumulative (vB).

7.7.4. Secondary poisoning

7.7.4.1. Toxicity to birds

No data are included in the registration dossiers.

7.7.4.2. Toxicity to mammals

Not part of the follow-up assessment of the eMSCA.

7.8. Environmental hazard assessment

As for the other endpoints, as each group of registrants has generally provided different data for this endpoint the available data are considered as a combined data set.

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

The available short-term toxicity data to fish for styrenated phenol are, in the main, difficult to interpret in terms of the toxicity of the substance. There is evidence from one study that di- and/or tristyrenated phenol are not toxic over 96 hours to *Danio rerio* at concentrations up to their solubility limit. However, another study with a mixture of mono- and distyrenated phenol gave an LL_{50} of 14.8 mg/L, suggesting that mono- and possibly distyrenated phenol are acutely toxic to fish at concentrations below their water solubility limits. The interpretation of the results of this study in terms of the actual LC_{50} of the constituents of the substance tested is difficult. A study with 4-MSP indicates that the 21-day LC_{50} would lie between 0.2 and 2 mg/L based on nominal exposure concentrations.

The other available acute toxicity data in the registration dossiers suffer from deficiencies. Therefore, they are not considered further here.

In the absence of suitable data on the short-term toxicity to fish, Brooke et al. (2009) considered the use of QSAR estimates and a similar approach is considered here. Brooke et al. (2009) concluded that the most appropriate method for estimating the toxicity to fish was the ECOSAR program, using calculated values of the log Kow (as the equations within the program were developed using predicted rather than measured log Kow values). The original predictions in Brooke et al. (2009) were carried out using ECOSAR v0.99h. The calculations here have been re-done using ECOSAR v1.11 in EPI Suite v4.11. The calculations were carried out using the QSAR for phenols.

Substance	Log Kow	96h-LC_{50} for fish
2-MSP	3.67	1.85 mg/L
4-MSP	3.67	1.85 mg/L
2,4-DSP	5.83	0.065 mg/L
2,6-DSP	4.98	0.287 mg/L
2,4,6-TSP	7.13	0.009 mg/L (> water solubility)

The predictions are considered to be reliability 2 (reliable with restriction). The QSAR for phenols for acute aquatic toxicity to fish was developed using a set of 188 data points (covering over 60 different substances) and is valid for substances with a molecular weight of up to 1,000 g/mole and substances with a log Kow of up to 7. For substances with a log Kow >7 the LC_{50} is predicted to be greater than the water solubility limit. The method is considered appropriate for the constituents of styrenated phenol.

The predictions suggest that the LC_{50} for tristyrenated phenol is close to, but just above, the water solubility of the substance, and that both monostyrenated phenols and distyrenated phenols are likely to be acutely toxic at concentrations below their respective water solubilities. This is consistent with the available experimental data. In the absence of other suitable data, the predicted LC_{50}s will be considered in this substance evaluation in relation to the PNEC derivation and PBT assessment.

7.8.1.1.2. Long-term toxicity to fish

The Fish Sexual Development Study (FSDT) requested in the decision on the Substances conducted with 4-MSP is discussed in Section 7.10.1. From this test a NOEC resulted based on significant changes in the proportion of undifferentiated fish (NOEC = 2.1 µg 4-MSP/L).

No suitable chronic fish toxicity data is available for the di- or tristyrenated components. In the absence of suitable data on the short-term toxicity to fish, Brooke et al. (2009) considered the use of QSAR estimates and a similar approach is considered here. Brooke et al. (2009) concluded that the most appropriate method for estimating the toxicity to fish was the ECOSAR program, using calculated values of the log Kow (as the equations within the program were developed using predicted rather than measured log Kow values). The original predictions in Brooke et al. (2009) were carried out using ECOSAR v0.99h. The calculations here have been re-done using ECOSAR v1.11 in EPI Suite v4.11. The calculations were carried out using the QSAR for phenols. It should be noted that the program estimates a chronic value (ChV) which the eMSCA understands to be the geometric mean of the NOEC and LOEC. Using the approach in Brooke et al. (2009) the NOEC has been estimated from the ChV by dividing by √2 (this effectively assumes that the NOEC and LOEC are separated by a factor of 2).

Substance	Log Kow	30d-ChV	30d-NOEC
2-MSP	3.67	0.239 mg/L	0.17 mg/L
4-MSP	3.67	0.239 mg/L	0.17 mg/L
2,4-DSP	5.83	0.011 mg/L	0.0078 mg/L
2,6-DSP	4.98	0.045 mg/L	0.032 mg/L
2,4,6-TSP	7.13	0.00189 mg/L	0.0013 mg/L

The predictions are considered to be reliability 2 (reliable with restriction). The QSAR for phenols for chronic aquatic toxicity to fish was developed using a set of 35 data points (covering 13 substances) and is valid for substances with a molecular weight of up to 1,000 g/mole and substances with a log Kow of up to 8. The method is considered appropriate for the constituents of styrenated phenol, although it is relevant to note that the method is based on data for relatively few substances.

The predicted NOECs are all below the water solubilities of the respective constituents. In addition, the experimental chronic NOEC for 4-MSP is below that calculated here. Therefore, the possibility of adverse effects following long-term exposure to each constituent cannot currently be ruled out.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

Data in registration dossiers

An experimental study carried out using the read-across substance 2,6-di-tert-butyl-4-methylphenol (EC No. 204-881-4), is included as a supporting study (Passino and Smith, 1987). No justification is provided for this read across approach. However, the eMSCA does not consider that this substance is appropriate for read across for any of the components of styrenated phenol as this substance is a reaction mass. Additionally, the authors report solubility problems at higher concentrations and vehicle concentrations above the OECD guidelines limit of 0.1 mL/L. Therefore, the data are not considered further.

An OECD TG 202 study carried out using *Daphnia magna* (not to GLP) is included as part of a weight of evidence approach (Unpubl. 2000). The substance tested was styrenated phenol (EC No. 262-975-0, CAS RN. 61788-44-1). The composition was not given but was stated to be the same as the registered substance. The test was carried out using water accommodated fractions (WAFs) prepared by addition of various amounts of the test substance dissolved in dichloromethane to the flask, evaporating the solvent, then adding

dilution water and allowing to equilibrate for 20 hours with stirring. After 20 hours the pH of the water was adjusted to pH 7.0 and the solutions were used directly for the toxicity testing. The initial nominal loadings were 1.0, 10, and 100 mg/L and the WAFs obtained were reported to be clear. Total organic carbon (TOC) measurements of the solutions were carried out at the start of the test and after 7 days to establish the stability of the test substance under the conditions of the test. These showed that the solutions were stable and that the amount of test substance in solution was around 0.5 mg C/L at the 1.0 mg/L nominal loading, about 4.5 mg C/L at the 10 mg/L loading and 40 mg C/L at the 100 mg/L loading. The solution for the 100 mg/L loading was reported to show a "slight turbidity" which suggests that not all the substance was in solution. The test was carried out using a static test system. The loading rate resulting in 0% immobilisation compared with the control (48h-EL_0) was 1 mg/L and the loading rate resulting in 100% immobilisation compared with the control (48h-EL_{100}) was 10 mg/L. The loading rate leading to 50% immobilisation (48h-EL_{50}) could not be estimated owing to the limited number of concentrations tested but was >1 mg/L and <10 mg/L. The registrant gives the study a reliability rating of 2 (valid with restriction) as not all details of the test were available. The eMSCA agrees with this rating. However, the data are difficult to interpret in terms of the toxicity of the constituents of styrenated phenol as the precise composition of the substance tested is not given and the method of preparation of the WAFs does not preclude the presence of undissolved test substance, particularly at the higher loading rates.

A poorly reported OECD TG 202 study using *Daphnia magna* is included as part of the weight of evidence (NITE 1997c; NITE 2018). The substance tested was stated to be mono (or di or tri) -(alpha-methylbenzyl) phenol (CAS Number 61788-44-1). The 48h-EC_{50} for immobilisation was reported to be 4.6 mg/L, based on measured concentrations. Few other details are available. The registrant considered the reliability rating for this study to be 2, as, although few details are available, the study was carried out by a reputable laboratory as part of a national chemicals programme (National Institute of Technology and Evaluation (NITE) of Japan[8]). The eMSCA has reviewed this study previously in Brooke et al. (2009) and has concerns over the study as the composition and identity of the substance tested is not entirely clear, and the toxicity value reported is above the water solubility of di- and tristyrenated phenol. Therefore, the eMSCA considers that the reliability rating of 4 (not assignable) is more appropriate and the data are not considered further in this evaluation.

An OECD TG 202 study carried out to GLP using methylstyrenated phenol (EC No. 270-966-8, CAS RN. 68512-30-1) is included as part of the weight of evidence (Unpubl. 2010j). The species used in the test was *Daphnia magna*. The test was carried out using WAFs prepared by direct addition of the test solution to water, stirring for 24 hours at a speed slow enough to avoid emulsification of the undissolved fraction, settling for 1 hour and then siphoning off the dissolved phase. The test was carried out using a static method at 20.8-21.5 °C and pH 7.8-8.0. The initial nominal loading rates were 2.5, mg/L, 5.0 mg/L, 10 mg/L, 25 mg/L and 50 mg/L and TOC analysis showed that the amount of test substance in solution was around 0.8-1.4 mg C/L at the 2.5 mg/L loading rate, around 1.1-1.2 mg C/L at the 10 mg/L loading rate and around 2.8-2.9 mg/L at the 50 mg/L loading rate. The TOC level in the control solution was 0.4 mg C/L. The 48h-EL_{50} was determined to be 17 mg/L based on the nominal loading rate and the no effect loading rate was 10 mg/L (based on immobilisation). It should be noted that the EL_{50} was interpolated between the no effect loading rate of 10 mg/L and the next highest loading rate tested (25 mg/L, at which 19 out of 20 daphnids were immobile). The registrant gives the study a reliability rating of 1 (reliable without restriction) and the eMSCA agrees with this rating. However, given the compositional and structural differences between the substance tested and the registered substance, and given that the use of a WAF makes it difficult to determine exactly which constituent(s) of the substances tested were responsible for the toxicity seen, it is difficult to draw definitive information from the study with methylstyrenated phenol on the toxicity

[8] http://www.safe.nite.go.jp/jcheck/detail.action?cno=61788-44-1&mno=4-0198&request_locale=en

of styrenated phenol, other than the fact that the two substances show a broadly similar level of toxicity when tested as WAFs. It is suggested that the registrants should include more justification in the registration dossier for the read-across from the substance tested to the registered substance.

An OECD TG 202 study with *Daphnia magna* using WAFs of a different composition of methylstyrenated phenol (EC No. 270-966-8, CAS RN.68512-30-1) is included as part of the weight of evidence approach (Unpubl. 2008e). The initial nominal loading rates for the WAFs were 5.0 mg/L, 10 mg/L, 25 mg/L, 50 mg/L and 100 mg/L and TOC analysis showed that the amount of test substance in solution was around 0.4-0.7 mg C/L at the 5 mg/L loading rate and around 1.2-1.3 mg C/L at the 50 mg/L loading rate. The TOC level in the control solution was 0.1-0.3 mg C/L. The 48h-EL_{50} was determined to be 51 mg/L based on the nominal loading rate and the 48h-EL_{10} was 20 mg/L (based on immobilisation). The registrant gives the study a reliability rating of 1 (reliable without restriction) and the eMSCA agrees with this rating. However, as before, given the compositional and structural differences between the substance tested and the registered substance, and given that the use of a WAF makes it difficult to determine exactly which constituent(s) of the substances tested were responsible for the toxicity seen, it is difficult to draw definitive information from the study with methylstyrenated phenol on the toxicity of styrenated phenol, other than the fact that the two substances show a broadly similar level of toxicity when tested as WAFs. It is suggested that the registrants should include more justification in the registration dossier for the read-across from the substance tested to the registered substance.

An OECD TG 202 guideline study carried out to GLP using *Daphnia magna* (Unpubl. 1997c). The composition of the substance tested is confidential but contained tristyrenated phenol with a smaller amount of distyrenated phenol. The test was carried out using a saturated solution of the substance prepared by adding the substance to the test medium at two loading rates (nominal concentrations of 30 and 100 mg/L), stirring for 48 hours. Following this the mixture was allowed to settle for 2 hours and then was vacuum filtered using a glass microfibre. The dilutions of the resulting solution were used as the test solution (20, 40, 60, 80 and 100% of the saturated solutions). No analytical monitoring/verification was carried out on the solutions. The test was carried out using a static system at 20 °C and pH 7.6-7.7. Around 15% and 5% immobilisation compared to the control was observed for the 100% saturated solutions derived from the loading rates of 100 mg/L and 30 mg/L respectively and so the 48h-EL_{50} was >100% saturation at each loading rate. No immobilisation compared with the controls was evident at using the diluted saturated solutions. The registrant concluded that the saturated solution of the substance was not toxic to *Daphnia magna* and gave the study a reliability rating of 2 (reliable with restrictions) as no analytical verification of the stability of the test solution was carried out. The eMSCA has considered this result previously in Brooke et al. (2009) and agrees with this validity rating.

Discussion and analysis of data on short-term toxicity to aquatic invertebrates

As discussed above, there are shortcomings with most of the data in the registration dossiers for the short-term toxicity to aquatic invertebrates. One study shows that a substance consisting of predominantly tristyrenated phenol and a smaller amount of distyrenated phenol was not toxic to *Daphnia magna* over 48 hours when tested as a saturated solution. However, it is difficult to use the result of this study to derive a PNEC. For the remaining studies in the registration dossiers there are uncertainties over the composition/identity of the substance tested (and how this relates to the registered substance) and/or from the presence of undissolved test substance.

In the absence of suitable data on the short-term toxicity to *Daphnia magna*, Brooke et al. (2009) considered the use of QSAR estimates and a similar approach is considered here. Brooke et al. (2009) concluded that the most appropriate method for estimating the toxicity to *Daphnia magna* was the ECOSAR program, using calculated values of the log Kow (as the equations within the program were developed using predicted rather than measured

log Kow values). The original predictions in Brooke et al. (2009) were carried out using ECOSAR v0.99h. The calculations here have been re-done using ECOSAR v1.11 in EPI Suite v4.11. The calculations were carried out using the QSAR for phenols.

Substance	**Log Kow**	**96h-LC_{50} for *Daphnids***
2-MSP	3.67	1.17 mg/L
4-MSP	3.67	1.17 mg/L
2,4-DSP	5.83	0.107 mg/L
2,6-DSP	4.98	0.325 mg/L
2,4,6-TSP	7.13	0.026 mg/L (> water solubility)

The predictions are considered to be reliability 2 (reliable with restriction). The QSAR for phenols was developed using a set of 63 data points (covering around 46 different substances) and is valid for substances with a molecular weight of up to 1,000 g/mole and substances with a log Kow of up to 7. For substances with a log Kow >7 the LC50 is predicted to be greater than the water solubility limit. The method is considered appropriate for the constituents of styrenated phenol.

The predictions suggest that the E/LC_{50} for tristyrenated phenol is above the water solubility of the substance, but that both monostyrenated phenols and distyrenated phenols are likely to be acutely toxic at concentrations below their respective water solubilities. This is generally consistent with the available experimental data. In the absence of other suitable data, the predicted values will be considered in this substance evaluation in relation to the PNEC derivation and PBT assessment.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

Data in registration dossiers

An OECD TG 211 guideline study (no data on GLP) with *Daphnia magna* is reported (NITE 1997d; NITE 2018). The substance tested was stated to be mono (or di or tri) -(alpha-methylbenzyl) phenol (CAS Number 61788-44-1). The 21d-NOEC was reported to be 0.2 mg/L, and the 21d-LC_{50} was 1.5 mg/L, both said to be based on measured concentrations. Few other details are available. The registrant considered the reliability rating for this study to be 2, as, although few details are available, the study was carried out by a reputable laboratory as part of a national chemicals programme (National Institute of Technology and Evaluation (NITE) of Japan[9]). The eMSCA has reviewed this study previously in Brooke et al. (2009) and has concerns over the study as the composition and identity of the substance tested is not entirely clear, and the toxicity value reported is above the water solubility of di- and tristyrenated phenol. Therefore, the eMSCA considers that the reliability rating of 4 (not assignable) is more appropriate and the data are not considered further in this evaluation.

An OECD TG 211 study carried out to GLP using a semi-static system is included in one registration (Unpubl. 2006b). The study was referenced to the previous assessment of styrenated phenol carried out by the eMSCA (UK) (Brooke et al. 2009). The substance tested was 2,4-bis(1-phenylethyl) phenol (EC Number 220-460-8; CAS RN 2769-94-0) with a purity of 97.21%. This read-across substance is a constituent of the registered substance. The test was carried out using WAFs prepared by direct weight addition of the substance and stirring for 48 hours to produce a saturated solution, followed by dilution of the saturated solution to provide a range of test concentrations. The test system used was a semi-static method (48-hour renewal) and the concentration of the test substance was

[9] http://www.safe.nite.go.jp/jcheck/detail.action?cno=61788-44-1&mno=4-0198&request_locale=en

measured in the new and old test solutions once per week. The nominal concentrations tested were 18.75, 37.5, 75, 150 and 300 µg/L and the corresponding measured concentrations present in these treatment levels were 20.1, 35.7, 56.9, 115 and 249 µg/L respectively. The water used in the test had a hardness of 151-178 mg/L as $CaCO_3$, a temperature of 19-21.7 °C and a pH of 7.59-9. The NOEC was reported to be 115 µg/L based on the geometric mean measured concentration for both reproduction and parent immobilisation and the 21-day EC_{50} was 0.204 mg/L based on parent immobilisation. The results were reported to be based on the arithmetic mean measured concentration. The registrant assigned a reliability of 1 to the study (reliable without restriction). The eMSCA has previously evaluated this study in Brooke et al. (2009) and assigned a reliability of 1 and considers that the result is suitable for use in deriving a PNEC.

A similar GLP compliant OECD TG 211 guideline study with *Daphnia magna* using tristyrenated phenol is available (Unpubl. 2008f). In this case a single exposure concentration was prepared using a generator column method (limit test using a saturated solution). The concentration tested was 35 µg/L (arithmetic mean) and varied between 15.1 and 55 µg/L during the test. The water characteristics were a hardness of 157-178 mg/L as $CaCO_3$, a temperature of 20.2-21.7 °C and a pH of 7.39-8.11. No effects were seen on survival or reproduction in the exposed population compared with the control group and so the 21-day NOEC was ≥35 µg/L. The registrant assigned a reliability rating of 1 to this study (reliable without restriction). The eMSCA has considered this study previously in Brooke et al. (2009) and considers that the study is reliable and interprets the study as tristyrenated phenol showing no effects up to its water solubility limit (7.1 µg/L; the mean measured concentrations in the test were above this value, but no effects were seen).

Discussion and analysis of data on long-term toxicity to aquatic invertebrates

Reliable data are available for the long-term toxicity of two main constituents of styrenated phenol. The 21-day NOEC for distyrenated phenol has been determined as 0.115 mg/L and the 21-day NOEC for tristyrenated phenol is above the water solubility limit of the substance. No reliable data are available for the toxicity of monostyrenated phenol.

To estimate the long-term toxicity of monostyrenated phenol, the eMSCA has made QSAR estimates (given below), along with estimates for the toxicity of di- and tristyrenated phenol for comparison with the available experimental data, using a similar approach to Brooke et al. (2009). Brooke et al. (2009) concluded that the most appropriate method for estimating the toxicity to aquatic invertebrates was the ECOSAR program, using calculated values of the log Kow (as the equations within the program were developed using predicted rather than measured log Kow values). The original predictions in Brooke et al. (2009) were carried out using ECOSAR v0.99h. The calculations presented here have been re-run using ECOSAR v1.11 in EPI Suite v4.11. The calculations were carried out using the QSAR for phenols. The ECOSAR program provides an estimate for the chronic value (ChV), which the eMSCA understands to be the geometric mean of the NOEC and LOEC. Using the approach in Brooke et al. (2009) the NOEC has been estimated from the ChV by dividing by √2 (this effectively assumes that the NOEC and LOEC are separated by a factor of 2).

Substance	**Log Kow**	**21d-ChV**	**21d-NOEC**
2-MSP	3.67	0.223 mg/L	0.158 mg/L
4-MSP	3.67	0.223 mg/L	0.158 mg/L
2,4-DSP	5.83	0.020 mg/L	0.014 mg/L
2,6-DSP	4.98	0.062 mg/L	0.044 mg/L
2,4,6-TSP	7.13	0.005 mg/L	0.0035 mg/L

The predictions are considered to be reliability 2 (reliable with restriction). The QSAR for phenols for chronic aquatic toxicity to invertebrates was developed using a set of 14 data points (covering 14 different substances) and is valid for substances with a molecular weight of up to 1,000 g/mole and substances with a log Kow of up to 8. For substances with a log Kow >8 the ChV is predicted to be greater than the water solubility limit. The

method is considered appropriate for the constituents of styrenated phenol. It is important to note that although the QSAR was developed using only a small data set this did include two alkylated phenols (2,4,6-trimethylphenol and 4-*tert*-octylphenol).

The predictions suggest that the NOEC for all the constituents considered is below their respective water solubility values. When the predicted data for tristyrenated and distyrenated phenol are compared with the available experimental data (NOEC for tristryrenated phenol ≥0.0071 mg/L and NOEC for distyrenated phenol = 0.115 mg/L) the predicted NOECs for these constituents are well below those actually measured[10], suggesting that the QSAR approach may be systematically predicting that the constituents of styrenated phenol are more toxic than actually found. This means that the predictions for monostyrenated phenol should be used with caution as they may overestimate the toxicity.

For the PNEC derivation and PBT assessment, the available measured NOEC values will be considered in preference to the QSAR estimates. However, for monostyrenated phenol, the QSAR estimates will be considered, acknowledging the uncertainty in the values.

7.8.1.3. Algae and aquatic plants

Data in registration dossiers

An OECD TG 201 guideline study carried out (non-GLP) using *Chlorella vulgaris* is used as a key study (Unpubl. 2014g). No further details of the composition of the substance tested are available. As the test substance has a very low solubility in water, solutions were prepared by stirring 100 mg of the test substance in 1 litre of algal media for 48 hrs. The test solutions were made by dilution from this stock. No analytical confirmation of the exposure concentrations was made, so results are based on nominal concentrations (2.5, 3.75, 5.62, 8.43, 12.65 and 18.98 mg/L). After 72 hours of exposure the EL50 for growth rate was determined to be 20.4213 mg/L. A NOEC was not reported. The registrant gave the study a reliability rating of 1 (reliable without restriction). The lack of detail on the identity and composition of the test substance makes it difficult to use the results from this test further in the substance evaluation.

An OECD TG 201 guideline study carried out to GLP using *Desmodesmus subspicatus* is used as a key study (Unpubl. 2010k). The substance tested was styrenated phenol (EC 262-975-0, CAS Number 61788-44-1). The test was carried out using WAFs prepared by addition of various amounts of the test substance on a weighing scoop to water and stirring for 48 hours (the rate of stirring was slow enough to avoid emulsification of the undissolved fraction). After settling for 1 hour the water phase was separated via siphoning. The initial nominal loadings were 0.5, 1.0, 2.5, 5.0, 10, and 25 mg/L and the WAFs obtained were reported to be clear. TOC measurements of the solutions were carried out at points during the test to establish the stability of the test substance under the conditions of the test. These showed that the solutions were stable and that the amount of test substance in solution was around 1.0-1.2 mg C/L at the 1.0 mg C/L nominal loading, about 2.5-2.7 mg C/L at the 5.0 mg/L loading and 5.5-5.6 mg C/L at the 25 mg/L loading. The amount of TOC present in the control was around 0.6-0.9 mg C/L. The test was carried out using a static test system at 20.5- 21.5 °C and a pH of 7.8-8.8. The 72h-NOELR (the loading rate that showed no adverse effect compared with the control) was 1 mg/L based on the initial nominal loading rate for both biomass and growth rate, and the 72h-EL_{50} (the loading rate resulting in 50% effect compared with the control) was 3.14 mg/L for growth rate and 2.3 mg/L for biomass.

The registrant gives the study a reliability rating of 1 (reliable without restriction) and the eMSCA agrees with this rating. However, the data are difficult to interpret in terms of the

[10] In Brooke et al. (2009) it was assumed that 2,4- and 2,6- isomers were present in the ratio 4:5; using this assumption the composite predicted NOEC would be around 0.031 mg/L.

toxicity of the constituents of styrenated phenol as the amount of substance in solution would appear to have been lower than the nominal loading rates (considering the TOC levels found in the controls) and would consist of a mixture of mono- and di-styrenated phenols. The endpoint summary of the registration dossier includes some further analysis of the results of this study, effectively relating the nominal loading rates to the measured TOC levels. This resulted in an estimate of the 72h-NOEC and 72h-EC_{50} of 0.42 mg/L and 1.35 mg/L in terms of the actual substance concentration (these appear to be based on the blank-corrected TOC levels measured at the given loading rates, and assuming that the substance is 85.66% carbon).

A poorly reported OECD TG 201 guideline study using *Desmodesmus subspicatus* is available in one registration (NITE 1997e; NITE 2018). The substance tested was stated to be mono (or di or tri) -(alpha-methylbenzyl) phenol (CAS Number 61788-44-1). The 72h-EC_{50} was reported to be > 10 mg/L for growth rate and the 72h-NOEC for growth rate was 3.2 mg/L, based on measured concentrations. Few other details are available. The registrants considered the reliability rating for this study to be 2 or 4, as, although few details are available, the study was carried out by a reputable laboratory as part of a national chemicals programme (National Institute of Technology and Evaluation (NITE) of Japan[11]). The eMSCA has reviewed this study previously and has concerns over the study as the composition and identity of the substance tested is not entirely clear, and the toxicity value reported is above the water solubility of di- and tristyrenated phenol. Therefore, the eMSCA considers that the reliability rating should be 4 (not assignable) and the data are not considered further in this evaluation.

An OECD TG 201 guideline study carried out to GLP using methylstyrenated phenol (EC 270-966-8, CAS Number 68512-30-1) is included as part of a weight of evidence approach (Unpubl. 2008g) in one registration. The species used in the test was *Desmodesmus subspicatus*. The test was carried out using WAFs prepared by addition of various amounts of the test substance on a weighing scoop to water and stirring for 24 hours (the rate of stirring was slow enough to avoid emulsification of the undissolved fraction). After settling for 1 hour the water phase was separated via siphoning. The initial nominal loadings were 5, 10, 25, 50, 100 and 250 mg/L and the WAFs obtained were reported to be clear. TOC measurements of the solutions were carried out at points during the test to establish the stability of the test substance under the conditions of the test. These showed that the solutions were stable and that the amount of test substance in solution was around 2.2 mg C/L at the 5 mg C/L nominal loading, about 2.7-2.8 mg C/L at the 50 mg/L loading and 3.5-3.6 mg C/L at the 250 mg/L loading. The amount of TOC present in the control was around 1.7-1.8 mg C/L. The 72h-NOELR was determined as 25 mg/L for both growth rate and biomass based on the initial loading rate. The 72h-EL_{50} was determined to be >250 mg/L for growth rate and 178 mg/L for biomass. The registrant gives the study a reliability rating of 1 (valid without restriction) and the eMSCA agrees with this rating. However, given the compositional and structural differences between the substance tested and the registered substance, and given that the use of a WAF makes it difficult to determine exactly which constituent(s) of the substances tested were responsible for the toxicity seen, it is difficult to draw definitive information from the study with methylstyrenated phenol on the toxicity of styrenated phenol, other than the fact that the two substances show a broadly similar level of toxicity when tested as WAFs. It is suggested that the registrants should include more justification in the registration dossier for the read-across from the substance tested to the registered substance.

A GLP compliant OECD TG 201 guideline study with *Desmodesmus subspicatus* is available (Unpubl. 2010l). The substance tested was tristyrenated phenol, but the actual composition is confidential. A saturated solution of the substance was prepared using a column elution method. The concentration of the test substance in solution was determined to be 5.15

[11] http://www.safe.nite.go.jp/jcheck/detail.action?cno=61788-44-1&mno=4-0198&request_locale=en

µg/L (geometric mean; the concentration was found to decrease from around 10.3 µg/L at the start of the test to around 2.8 µg/L at the end of the test; a similar decrease was evident in solutions without algae). The test was carried out as a limit test (saturated solution was tested) using a static system. The water used in the test had a hardness of 0.24 mmol (Ca + Mg)/L, a pH of 7.40-8.74 and a temperature of 21-24 °C. No statistically significant effects on growth rate were found between the exposed population and control population and so the NOEC was ≥5.15 µg/L. However, a statistically significant reduction in biomass (mean inhibition was 16.6% across 6 replicates) was seen in the exposed population compared with the control population and so the NOEC for biomass was <5.15 µg/L. The registrant assigned a reliability of 1 to the study (reliable without restriction). The eMSCA agrees with this rating.

A similar GLP compliant OECD TG 201 guideline study with *Desmodesmus subspicatus* using distyrenated phenol is available (Unpubl. 2010m). The actual composition of the substance tested is confidential. In this case a range of exposure concentrations was prepared using a generator column and diluting the subsequent saturated solution. The concentrations tested were 22.9, 76.4, 140, 309 and 688 µg/L (geometric mean measured concentrations). The water characteristics were a hardness of 0.24 mmol (Ca + Mg)/L, a temperature of 21-24 °C and a pH of 7.59-8.34. The 72h-NOEC was 140 µg/L based on growth rate and biomass, the 72h-EC_{10} was 187 µg/L based on growth rate and 141 µg/L based on biomass and the 72h-EC_{50} was 326 µg/L based on growth rate and 210 µg/L based on biomass. The registrant assigned a reliability rating of 1 to this study (reliable without restriction). The eMSCA agrees with this rating.

Discussion and analysis of data on toxicity to algae and other aquatic plants

The most relevant data for the toxicity to algae of the registered substance and the main constituents therein are the studies with *Desmodesmus subspicatus* using distyrenated phenol and tristyrenated phenol. For distyrenated phenol the 72h-NOEC was determined as 0.14 mg/L based on both growth rate and biomass, and the 72h-EC_{50} was determined as 0.326 mg/L based on growth rate and 0.210 mg/L based on biomass. For tristryrenated phenol the substance was tested as a saturated solution and the effects seen were not sufficient to derive an $EC_{50,}$ but the 72h-NOEC was ≥0.0055 mg/L based on growth rate (i.e., no effects at saturation) and ≤0.0055 mg/L based on biomass.

The REACH Guidance (Chapter 7b) recommends that the preferred observational endpoint for the algal study is growth rate rather than biomass. Therefore, the values based on growth rate are used in in this substance evaluation. No reliable data are available for the monostyrenated phenol constituents of the registered substance.

To estimate the long-term toxicity of monostyrenated phenol, QSAR estimates are given below, along with estimates for the toxicity of di- and tristyrenated phenol for comparison with the available experimental data, using a similar approach to Brooke et al. (2009). Brooke et al. (2009) concluded that the most appropriate method for estimating the toxicity to green algae was the ECOSAR program, using calculated values of the log Kow (as the equations within the program were developed using predicted rather than measured log Kow values). The original predictions in Brooke et al. (2009) were carried out using ECOSAR v0.99h. The calculations here have been re-done using ECOSAR v1.11 in EPI Suite v4.11. The calculations were carried out using the QSAR for phenols. The ECOSAR program provides an estimate for the chronic value (ChV), which the eMSCA understands to be the geometric mean of the NOEC and LOEC. Using the approach in Brooke et al. (2009) the NOEC has been estimated from the ChV by dividing by √2 (this effectively assumes that the NOEC and LOEC are separated by a factor of 2).

Substance	Log Kow	96h-EC_{50}	96h-ChV	96h-NOEC
2-MSP	3.67	4.59 mg/L	2.11 mg/L	1.49 mg/L
4-MSP	3.67	4.59 mg/L	2.11 mg/L	1.49 mg/L
2,4-DSP	5.83	0.34 mg/L	0.15 mg/L	0.11 mg/L
2,6-DSP	4.98	1.12 mg/L*	0.51 mg/L	0.36 mg/L
2,4,6-TSP	7.13	0.073 mg/L*	0.032 mg/L*	0.023 mg/L*

* Value predicted to be above the water solubility of the substance

The predictions are considered to be reliability 2 (reliable with restrictions). The QSAR for the 96h-EC_{50} for algae was developed using a set of 40 data points (covering 27 different substances) and is valid for substances with a molecular weight of up to 1,000 g/mole and substances with a log Kow of up to 6.4. For substances with a log Kow >6.4 the 96h-EC_{50} is predicted to be greater than the water solubility limit. The method is considered appropriate for the constituents of styrenated phenol.

The QSAR for the 96h-ChV for algae was developed using a set of 19 data points (covering 16 substances) and is valid for substances with a molecular weight of up to 1,000 g/mole and substances with a log Kow of up to 8. The method is considered appropriate for the constituents of styrenated phenol. It is important to note that the QSAR was developed using only a small data set and this included only one alkylated phenol (2,4,6-trimethylphenol, although the identities of five other substances are not given for confidentiality reasons).

The predictions suggest that tristyrenated phenol is not toxic to algae at concentrations up to its water solubility. This is consistent with the available experimental data. The predictions for distyrenated phenol suggest that the NOEC is around 0.11-0.36 mg/L[12], which is in good agreement with the experimental data (experimental 72h-NOEC 0.14 mg/L). The EC_{50} for distryrenated phenol is predicted to be around 0.34-1.12 mg/L (the latter value is above the water solubility of distyrenated phenol) which again is in good agreement with the experimental data (experimental 72h-EC_{50} = 0.326 mg/L). This agreement of the predicted data with the experimental data for di- and tristyrenated phenol, along with the fact that a study using a WAF prepared with a mixture of monostyrenated phenol and distyrenated phenol resulted in a 72h-NOEC and 72h-EC_{50} of 0.42 mg/L and 1.35 mg/L respectively, which again is relatively consistent with the available predictions, gives some re-assurance in the predictions for monostyrenated phenol.

For the PNEC derivation, and PBT assessment, the available measured EC_{50} and NOEC values based on growth rate will be considered in preference to the QSAR estimates. However, for monostyrenated phenol, the QSAR estimates will be considered, acknowledging the uncertainty in the values.

7.8.1.4. Sediment organisms

No data on toxicity to sediment organisms was provided in the registration dossiers.

7.8.1.5. Other aquatic organisms

No data on toxicity to other aquatic organisms was provided in the registration dossiers.

[12] Brooke et al. (2009) assumed that the ratio of the 2,4-isomer to the 2,6-isomer was 4:5; using this assumption a composite predicted NOEC of 0.25 mg/L can be estimated.

7.8.2. Terrestrial compartment

No data on toxicity to terrestrial organisms was provided in the registration dossiers.

7.8.3. Microbiological activity in sewage treatment system

The available information suggests that styrenated phenol is of low toxicity to sewage treatment microorganisms.

7.8.4. PNEC derivation and other hazard conclusions

Not part of the follow-up assessment of the eMSCA.

7.8.5. Conclusions for classification and labelling

The registration dossiers generally have not considered the properties of all relevant constituents present in the registered substance and this may have led to an underestimate of the potential hazard to the environment from the substance.

The eMSCA (UK) concludes that the following classification may be appropriate for each constituent.

The conclusion is based on:

- the fact that the main constituents of the Substances are not readily biodegradable
- some are bioaccumulative according to CLP criteria
- available estimates for, and experimental data on, the toxicity of each constituent.

It should be noted that the classifications below depend to a large extent on predictions of toxicity and so are uncertain.

Monostyrenated phenol

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

Distyrenated phenol

Aquatic Acute 1 H400: Very toxic to aquatic life.

M-Factor acute 1

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

M-Factor chronic 1

Tristyrenated phenol

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

M-Factor chronic 10

The eMSCA supports the assessment by the previous assessor. The more protective classification and labelling should be applied for phenol, styrenated and TSP.

7.9. Human Health hazard assessment

Not part of the follow-up assessment by the eMSCA.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Screening data collated at the time of the initial substance evaluation suggested that some constituents of the registered substance, particularly monostyrenated phenols, have the potential to impact the endocrine system. Information came from modelling tools, two experiments on components of the registered substance and read across. As there was insufficient information to allow firm conclusions to be drawn on the endocrine disruption concern, further testing with aquatic organisms was necessary to better define the actual hazard shown.

Based on the weight of evidence, the monostyrenated constituent was concluded of most concern for endocrine effects. In the interests of animal welfare, *in vivo* testing was proposed only on the monostyrenated phenol constituent at this stage, and the Registrant(s) was required to perform a FSDT (OECD TG 234) for the monostyrenated phenol constituent of the registered substance using five test concentrations and appropriate controls.

In their updated dossiers some Registrant(s) have provided the results of *in vitro* assays on a human cell line expressing the oestrogen receptors hERα and hERβ with 2-MSP and 4-MSP, together with further details of the assays conducted prior to the original substance evaluation. In addition, results from *in vitro* assays on a human cell line expressing the Zebrafish oestrogen receptors zfERα, zfERβ1 and zfERβ2 are also provided. All available QSAR and *in vitro* results are summarized below:

Published data identified by the eMSCA

Brooke et al. (2009) Oestrogen receptor binding profiler of styrenated phenol constituents

Modelling carried out in Brooke et al. (2009) using the oestrogen receptor binding profiler tool within the OECD QSAR Toolbox indicated that monostyrenated phenol isomers are predicted to be moderate binders with the 2,4-DSP isomer predicted to be a strong binder. The 2,6-DSP isomer and tristyrenated phenol were identified as having hindered phenol groups, suggesting a lower binding activity. Further details of these predictions are available in Brooke et al. (2009).

Ogawa et al. (2006) yeast two-hybrid assay on different compositions of styrenated phenol

Screening tests carried out by Ogawa et al. (2006) using a yeast two-hybrid assay with three different compositions of styrenated phenol showed signs of estrogenic activity. The strongest activity was found with a mixture consisting of 74% mono- and 26% distyrenated phenol, which showed a similar level of activity to that seen for nonylphenol and bisphenol-A. A mixture consisting of mono-, di- and tristyrenated phenol (of unknown composition) showed a lower activity and a mixture consisting of 93% distyrenated phenol and 7% tristyrenated phenol showed no activity unless treated with rat liver S-9 to generate metabolites. The authors of the study concluded that monostyrenated phenol was predominantly responsible for the activity seen.

Terasaki et al. (2007) oestrogen receptor competitive enzyme-linked immunosorbent assay (ELISA) and yeast two-hybrid assay on 2,4-DSP

The test substance 2,4-DSP was synthesised by the authors of this study and stated to have a purity of ≥98%.

A commercial ELISA system was used to determine the binding affinity of nine test compounds including 2,4-DSP to human ERα. 2,4-DSP was tested alone and in the presence of rat liver S9 mix to generate metabolites. The experiment was run twice for each compound, with each run having two replicates. 2,4-DSP was found to exhibit binding affinity to hERα. In the absence of S9, the IC50 was 15000 nM (4.54 mg/L) and the relative binding efficiency compared to that of the positive control diethylstilbestrol was 0.14%. In the presence of S9, the IC50 was 7900 nM (2.39 mg/L), and the relative binding efficiency was 0.25%. The eMSCA notes that the IC50 are above the limit of water solubility of 2,4-DSP (0.665 mg/L).

The same nine test compounds were screened using a yeast two-hybrid assay with either hERα or the medaka oestrogen receptor (medERα) in the absence and presence of the rat liver S9 mix. All experiments were run three times for each compound, with each run having two replicates. 2,4-DSP was not found to exhibit any estrogenic activity in the hERα test. Estrogenic activity in the medERα could not be quantified, but instead is reported as suspected, as the maximum observed response level even at high test concentration was below 10-fold that of the blank control. No estrogenic activity was observed in the medERα test in the presence of S9. Although the maximum test concentration is not stated, the eMSCA assumes that similar test concentrations were used in the yeast two-hybrid assay and the ELISA. It is therefore assumed that the suspected activity observed in the medERα assay was seen at concentrations above the limit of water solubility of 2,4-DSP (0.665 mg/L).

Data in registration dossiers

Unpubl. (2016g) human cell line oestrogen receptor assay on MSP (mix of isomers), 2-MSP, 4-MSP, 2,4-DSP, 2,6-DSP and 2,4,6-TSP expressing the oestrogen receptors hERα and hERβ

A study using an *in vitro* assay with a human cell line, for which ECHA had only received a summary at the time of the draft evaluation in 2014, concluded that estrogenic effects were seen for monostyrenated phenol (mix of isomers). Effects for 2,6-DSP were seen in the oestrogen receptor assay but indicated to be at concentrations known to induce non-specific effects. No significant activity was seen for 2,4-DSP or 2,4,6-TSP.

Additional details of the assay and additional work on 2-MSP and 4-MSP have now been provided by the Registrant(s). Several constituents of styrenated phenol were tested in a non-guideline *in vitro* transcription activation assay. Estrogenic agonist and antagonist activities were tested using immortalised luciferase-reporter human cell lines expressing the oestrogen receptors hERα and hERβ. Each test concentration had four replicates and was repeated twice using DMSO as a solvent. Estrogenic activity was quantified in percentage relative to the effect induced by 10 nM 17β-oestradiol after 16 hours exposure. Oestrogen antagonistic activity was tested for in the presence of 0.1 nM 17β-oestradiol. The constituents were tested at different concentrations to obtain a dose response curve for substances showing activity, but the number of concentrations tested for each substance are not specified.

The maximum test concentration was set by determining a concentration that would not result in general cytotoxicity after testing cell lines that were not transfected with the oestrogen receptors and quantifying effects relative to the solvent control (DMSO). Cell lines that had not been transfected with the oestrogen receptors were also used to determine the concentration at which any non-specific effects on luciferase expression were observed (i.e., increases in luciferase expression that could not have been due to oestrogen receptor binding) and this was quantified relative to any observed vehicle activity (DMSO).

MSP and its two isomers did not show any non-specific activity up to 10 µM (1.98 mg/L), so the maximum test concentration was set to 33 µM (6.54 mg/L). MSP (as a mixture of isomers) showed significant oestrogen agonist activity. The EC50 for MSP on hERα and hERβ were 5.7 and 5.1 µM (1.13 and 1.01 mg/L) respectively. 4-MSP was found to be the more potent isomer for estrogenic activity. The EC50 for 2-MSP and 4-MSP on hERα were

38.2 (note this is above the maximum exposure concentration) and 3.2 µM (7.57 and 0.63 mg/L) respectively. The EC50 for 2-MSP and 4-MSP on hERβ were 16.0 and 1.4 µM (3.17 and 0.28 mg/L) respectively. As the results are expressed relative to the luciferase expression induced by 10 nM 17β-oestradiol (0.0027 mg/L) this indicates that 4-MSP induced half the effect at a concentration that was two orders of magnitude higher.

2,4-DSP did not show any non-specific activity up to 10 µM (3.02 mg/L), but general cytotoxicity was observed at concentrations greater than 33 µM (9.98 mg/L). The maximum test concentration for the assay was therefore set at 33 µM. No significant oestrogen agonist activity was observed for 2,4-DSP. The eMSCA notes that no estrogenic effects were observed up to the limit of water solubility of 2,4-DSP (0.665 mg/L).

2,6-DSP showed low to high non-specific activity at 10 µM (3.02 mg/L), and general cytotoxicity was observed at concentrations greater than 33 µM (9.98 mg/L). The maximum test concentration for the assay was therefore set at 33 µM, but any effects occurring at 10 µM and above were non-specific. 2,6-DSP showed an apparent oestrogen agonistic activity on hERα, but this was observed at a concentration of 10 µM which was known to induce non-specific effects. The author (Unpubl. 2016g) therefore concludes that no significant hERα agonist activity was observed. No significant hERβ agonist activity was observed for 2,6-DSP. The eMSCA notes that no estrogenic effects or non-specific activity was observed up to the limit of water solubility of 2,6-DSP (0.665 mg/L).

2,4,6-TSP showed low to high non-specific activity at 10 µM (4.07 mg/L), and general cytotoxicity was observed at concentrations greater than 33 µM (13.42 mg/L). The maximum test concentration for the assay was therefore set at 33 µM, but any effects occurring at 10 µM and above were non-specific. No significant oestrogen agonist activity was observed for 2,4,6-TSP. The eMSCA notes that no estrogenic effects or non-specific activity was observed up to the limit of water solubility of 2,4,6-TSP (0.0071 mg/L).

Unpubl. (2018e) human cell line oestrogen receptor assay on 2-MSP, 4-MSP, 2,4-DSP, and 2,6-DSP expressing the oestrogen nuclear receptors zfERα, zfERβ1 and zfERβ2

Unpubl. (2018e) tested oestrogen agonist and antagonist activity in an *in vitro* transcription activation assay. Estrogenic agonist and antagonist activities were tested using immortalised luciferase-reporter HELN cell lines expressing the Zebrafish oestrogen nuclear receptors zfERα, zfERβ1 and zfERβ2. Each test concentration had four replicates and was repeated twice, using DMSO as a solvent. Estrogenic activity was quantified in percentage relative to the effect induced by 10 nM 17β-oestradiol after 16 hours exposure. Oestrogen antagonistic activity was tested for in the presence of 0.1 nM 17β-oestradiol. The constituents were tested at different concentrations to obtain a dose-response curve for substances showing activity, but the number of concentrations tested for each substance are not specified. The maximum test concentration was set by determining a concentration that would not result in general cytotoxicity after testing cell lines that were not transfected with the oestrogen receptors and quantifying effects relative to the solvent control (DMSO). Non-specific activity (an increase in luciferase expression seen in cells not transfected with the oestrogen receptors) was also determined relative to the solvent control (DMSO).

2-MSP and 4-MSP did not show any non-specific activity up to 10 µM (1.98 mg/L), but the maximum concentration tested is not stated. 2-MSP was found to be a zfERα agonist, with EC50 118.6 µM (23.51 mg/L). 2-MSP was not found to be a significant agonist or antagonist for the other receptors. 4-MSP was found to be a zfERα, zfERβ1 and zfERβ2 agonist, with EC50 20.56 µM (4.08 mg/L), 25.51 µM (5.06 mg/L) and 85.27 µM (16.91 mg/L) respectively. As the results are expressed relative to the luciferase expression induced by 10 nM 17β-oestradiol (0.0027 mg/L) this indicates that 4-MSP induced half the effect at a concentration that was at least three orders of magnitude higher.

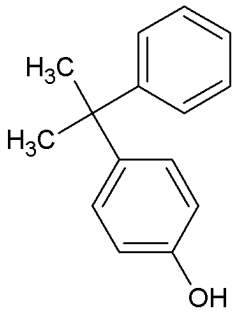
2,4-DSP and 2,6-DSP showed non-specific activity at 10 µM (3.02 mg/L), and general cytotoxicity was observed at concentrations greater than 33 µM (9.98 mg/L). Although both substances showed an increase in luminescence at 10 and 33 µM for zfERα, zfERβ1 and zfERβ2 this was observed at concentrations which were known to induce non-specific effects. The author (Unpubl. 2018e) therefore concludes that no significant agonist or

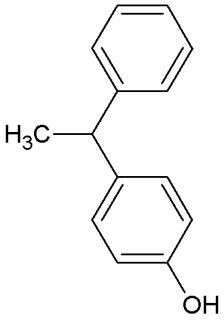
antagonist effects were observed for 2,4-DSP and 2,6-DSP. The eMSCA notes that no estrogenic effects were observed up to the limit of water solubility of 2,4-DSP and 2,6-DSP (0.665 mg/L).

The Registrant(s) therefore decided that the FSDT study would be conducted using 4-MSP as the test substance, as this was found to be the more potent monostyrenated phenol isomer.

Additional data for analogue substances

(4-(α, α-dimethylbenzyl) phenol) (p-cumyl phenol; EC Number 209-968-0; CAS No 599-64-4), the structure of which is shown below, is structurally very similar to 4-MSP and can be regarded as a read-across substance.

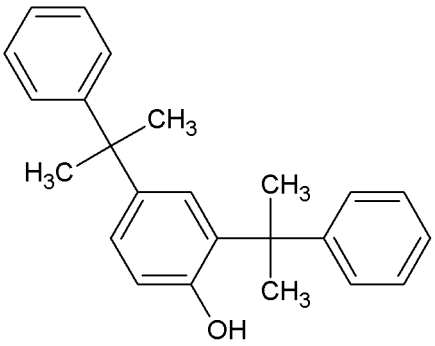


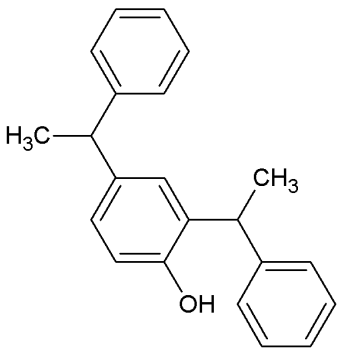


4-(α,α-dimethylbenzyl) phenol (p-cumyl phenol) 4-MSP

As can be seen this substance is structurally similar to the 4-MSP constituent in styrenated phenol (the main difference being that 4-MSP has a tertiary carbon between the two aromatic rings, whereas it is a quaternary carbon in 4-(α,α-dimethylbenzyl) phenol). ECHA (2013) states that 4-(α,α-dimethylbenzyl) phenol has been shown to exhibit endocrine modulating activity *in vitro*, and modelling predicted that the substance would bind to the oestrogen receptor. However, ECHA (2013) concluded that insufficient information was available to carry out a meaningful evaluation of the endocrine disrupting properties.

ECHA (2013) also indicates that the substance 2,4-bis(1-methyl-1-phenylethyl) phenol (EC 220-466-0; CAS No 2772-45-4) has shown activity on juvenile hormone in an assay investigating effects on the settlement and metamorphosis of larvae of the polychaete *Capitella*. However, again it was concluded that it was not possible to draw meaningful conclusions from these data. The structure of 2,4-bis(1-methyl-1-phenylethyl) phenol is shown below. The structure of this substance is similar to the 2,4-DSP constituent of styrenated phenol (the main difference being that there are tertiary carbon atoms between the aromatic rings in 2,4-DSP, whereas they are quaternary carbon atoms in 2,4-bis(1-methyl-1-phenylethyl) phenol).





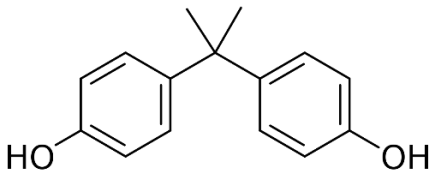
2,4-bis(1-methyl-1-phenylethyl) phenol 2,4-DSP

The information in ECHA (2013) with the read-across substances 4-(α,α-dimethylbenzyl) phenol and 2,4-bis(1-methyl-1-phenylethyl) phenol is therefore supportive that the monostyrenated and 2,4-DSP constituents of the registered substance may have endocrine disrupting properties.

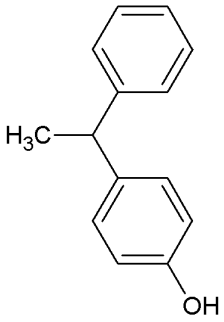
A combined OECD TG 305/229 study was conducted with oligomerisation and alkylation reaction products of 2-phenylpropene and phenol (OAPP) (EC 700-960-7) (previously phenol, methylstyrenated) (ECHA, 2013). The test substance was a UVCB composed of five different constituent groups (monoalkylated phenol, dialkylated phenol, trialkylated phenol, dimers of C9 monomers and trimers of C9 monomers). Similarly, to styrenated phenol, the components considered to have potential endocrine disrupting effects were the mono- and dialkylated forms. Fathead minnow were used as the test species and exposure was via the dietary route. The test was run at a single exposure concentration of 500 µg/g food. A blank control and a positive control of 100 µg hexachlorobenzene/g food plus 100 µg 17β oestradiol /g food were included. The positive control was designed to be a reference for both possible bioaccumulation and endocrine effects. Vitellogenin (VTG) was measured at day 0, 7 and 14 of the uptake phase. The male fish had a statistically significant increase in VTG after 14 days when compared to the control. The female fish did not have significantly increased VTG. The positive control fish showed statistically significantly increased VTG in both the males and females. This study indicates that OAPP may have an estrogenic mode of action, but a higher tier test including apical endpoints would be needed to confirm this. As the test substance was a UVCB it is also not possible to conclude on which of the constituents caused the observed effects.

eMSCA comments

There is a clear structural similarity between 4-MSP and bisphenol A (BPA) (EC 201-245-8, CAS 80-05-7), with 4-MSP having one fewer –OH group and one fewer –CH3 group than BPA. BPA has been identified as a Substance of Very High Concern (SVHC) due to its endocrine disrupting properties for the environment, and has been found to have estrogenic, anti-androgenic and thyroid activity. The results from several *in vivo* fish studies show a female biased sex ratio which could be a result of estrogenic or anti-androgenic activity, but as an increase in male VTG was observed in all studies where VTG was measured it was concluded that the primary mode of action of BPA was estrogenic, with some indications that it is also anti-androgenic and thyroid disrupting.



Bisphenol A (BPA)



4-MSP

The SVHC dossier for BPA references a study by Kitamura et al. (2005) that identified the structural alerts for the different modes of action of BPA and similar compounds.

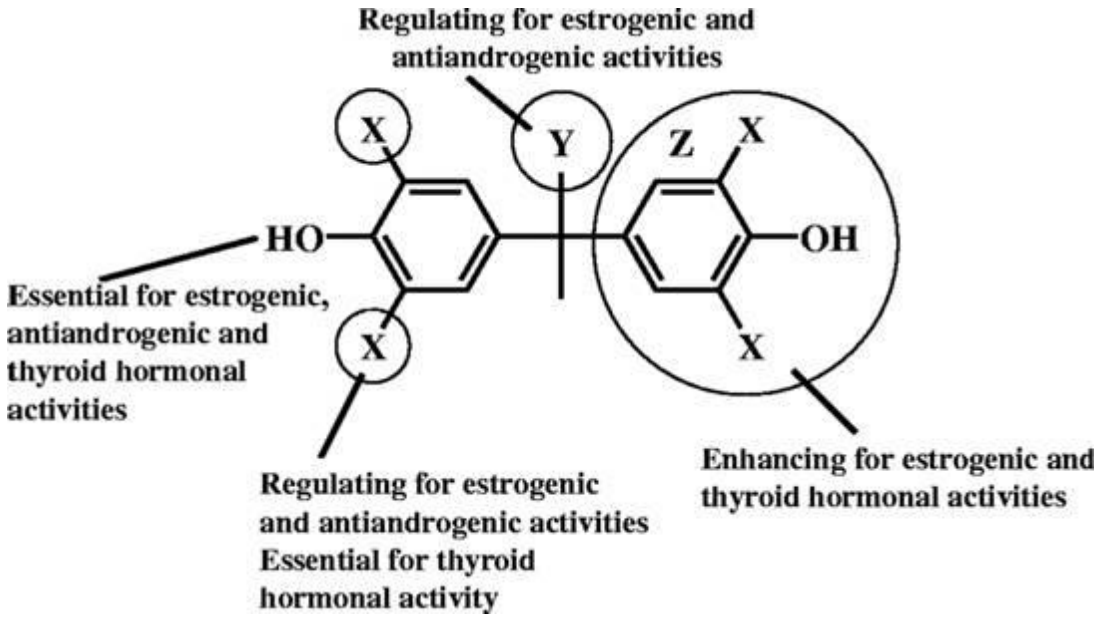
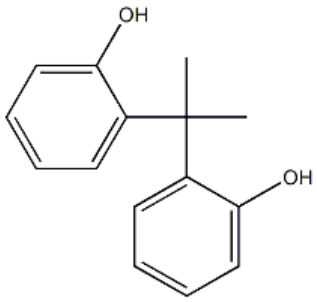


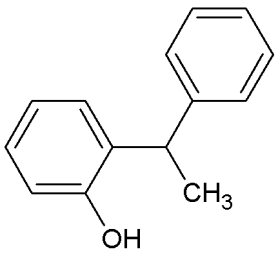
Figure 1. Taken from Kitamura et al. (2005) showing structural alerts for endocrine activity in bisphenol compounds.

4-MSP has the structural alerts for estrogenic and anti-androgenic activity. This is supported by the available *in vitro* data summarised above for oestrogenicity, but no experimental data is available for 4-MSP on its androgenic activity. Although Kitamura et al. (2005) did not test any bisphenols with the hydroxyl group in the 2- position they note that other researchers have found them to have some estrogenic and anti-androgenic activity, but with lower potency than the 4-hydroxyl. This would support the Registrant(s) conclusion that 4-MSP is the most potent isomer.

2-MSP has one fewer –OH group and one fewer –CH3 group than 2,2'-BPA (CAS 7559-72-0). 2,2'-BPA has not been registered under REACH, so the eMSCA searched for any published data on the relative estrogenic activity of BPA and 2,2'-BPA and two studies were located.



2,2'-BPA



2-MSP

A study by Szafran et al. (2017) compared the estrogenic properties of BPA and 2,2'-BPA, amongst other BPA analogues, using non-guideline *in vitro* assays. Estrogenic agonist activity was tested using human cell lines expressing the oestrogen receptors ERα and ERβ fused with green fluorescent protein. Treatment with 5 µM BPA for 1 hour induced significant activity of ERα and ERβ, equivalent to 69% and 76% of the activity of the positive control 17-β-oestradiol. Treatment with 5 µM 2,2'-BPA did not induce any significant ERα activity (<1% 17-β-oestradiol activity) and only a weak ERβ activity (<9% 17β-oestradiol activity). Estrogenic activity was also tested in an oestrogen responsive breast cancer cell line (MCF-7). Treatment with 5 µM BPA for 24 hours induced significant activity, equivalent to 57% of the activity of the positive control 17β-oestradiol. Treatment

with 5 µM 2,2'-BPA induced 19% of the 17-β-oestradiol activity, indicating weak estrogenic activity. The authors also note that 2,2'-BPA has previously been found to be non-estrogenic using a yeast-based screening method, the full results of which have not been published. BPA was found to show clear estrogenic effects, whilst 2,2'-BPA was found to induce non-significant or weak oestrogen activity. Therefore, the results from Szafran et al. (2017) further support the Registrant(s) conclusion that 4-MSP is the more potent isomer.

Van Leeuwen et al. (2019) compared the estrogenic and androgenic properties of BPA and 2,2'-BPA, amongst other BPA analogues, using non-guideline *in vitro* assays. Activity was tested using yeast (*Saccharomyces cerevisiae*) cell lines expressing the human oestrogen receptor ERα or the human androgen receptor fused with green fluorescent protein. For estrogenic activity BPA had a 24h EC50 of 20 µM, whilst 2,2'-BPA had a 24h EC50 of >200 µM. Neither compound was found to have androgenic effects in this study. The anti-estrogenic and anti-androgenic properties of the test substances were also investigated by repeating the study, but with the addition of a non-saturating level of a known agonist (17β-oestradiol in the yeast oestrogen bioassay and 17β-testosterone in the yeast androgen bioassay) and results were reported as the concentration required to inhibit the response to the agonist by 50%. BPA was not found to be anti-estrogenic, and 2,2'-BPA had a 24h IC50 of >500 µM. BPA had an anti-androgenic 24h IC50 of 30 µM and 2,2'-BPA had a 24h IC50 of 20 µM. Van Leeuwen et al. conclude the BPA shows both oestrogen agonist and androgen antagonist effects and that 2,2-BPA shows oestrogen agonist, oestrogen antagonist and androgen antagonist effects. However, for 2,2'-BPA two of these results are unbounded. Therefore, the eMSCA interprets this study as demonstrating that BPA is a more potent oestrogen agonist than 2,2'-BPA and that the anti-androgenic activity of both substances is similar. Therefore, the results from van Leeuwen et al. (2019) further support the Registrant(s) conclusion that 4-MSP is the more potent isomer for estrogenic activity.

The eMSCA agrees with the Registrant(s) assessment of the *in vitro* data and concurs that 4-MSP is the appropriate isomer for testing in the FSDT. The eMSCA also notes that the results from Unpubl. (2016g) and Unpubl. (2018e) confirm the previous finding of Ogawa et al. (2006), that MSP (separate isomers or as a mixture) is an oestrogen agonist in *in vitro* studies. A FSDT is suitable for detecting and differentiating between estrogenic and anti-androgenic activity *in vivo*, so both possible modes of action can be investigated in the same study.

FSDT study on 4-MSP submitted to comply with the substance evaluation request (Unpubl. 2018d).

Factual resumé

The test followed OECD TG 234 and used Zebrafish (*Danio rerio*) which had been bred in the test facility. The main part of the study was performed according to GLP. The test item, 4-MSP, had a purity of 96.9% and no solvent was used to prepare the stock solutions. An initial range finder was carried out to determine the appropriate exposure concentrations for the main study. The range finder was based on OECD TG 210 and exposed 30 fertilised eggs per replicate for 21 days, but with three test concentrations and a control, and using two replicates. The nominal range finder exposure concentrations were 0.02, 0.2 and 2 mg/L. The highest exposure concentration resulted in 100% mortality by the end of the test. At the two lower exposure concentrations no effects were observed on mortality, length, weight or hatching success. It was therefore decided to set the maximum exposure concentration in the main study to 10% of 2 mg/L.

The main study was conducted with nominal concentrations of 2.0, 6.3, 20.0, 63.2 and 200 µg/L together with an untreated control. At each treatment level, four replicates, each with 30 fertilised eggs, were maintained under flow through conditions with a renewal rate of 8 volumes per vessel per day.

Endpoints measured included hatching rates, hatching success and mortalities during the early life stage and juvenile growth. At day 35 post fertilization (pf) and when groups were terminated (day 63 pf) fish lengths and wet weights were determined.

All surviving fish were analysed for VTG. The laboratory took blood samples by cardiac puncture and then normalised VTG by total protein content. Following a query from the eMSCA, the CRO indicated that protein normalisation was applied by them to address the variation in blood volumes collected from the small fish used in Zebrafish endocrine tests.

Sex ratios were determined macroscopically by inspection of the gonads as part of the main study. The sex ratio analysis was based on the histological results, rather than the macroscopic examination and was conducted by a sub-contracted non-GLP laboratory. The histopathological verification was performed using OECD (2010). This means that the histology section of the report is not GLP compliant.

The exposure concentrations were confirmed by GC-MS analysis throughout the study. Samples were taken from all test vessels on Day 0, 7, 14 and at test end. From Day 21 to 56, two of the four replicate vessels at each exposure concentration were alternately sampled weekly. The limit of quantification was 0.75 µg/L. During the study, the mean 4-MSP concentrations per treatment were between 92.0% and 105.7% of the nominal test item concentration. However, as some samples differed from the desired 80–120% of the nominal values, the results were based on mean measured concentrations (2.1, 6.4, 19.7, 61.8, 187.9 µg/L).

The study report states that the validity criteria for OECD TG 234 were met, with two exceptions relating to water temperature and measured concentrations that were not considered to have influenced the overall validity, as:

- the dissolved oxygen concentration was above 60% air saturation value throughout the test.
- the water temperature did not differ by more than ±1.5 °C between test chambers at any time.
    - the water temperature remained within 27±2 °C, except for temperatures as low as 24.5 °C on day 6 in several of the test vessels which the authors did not consider having affected the results
- a validated method for analysis of the exposure concentration with a limit of quantification below the lowest nominal concentration was used.
    - the measured concentrations varied by more than 20% from the mean measured concentration at some time points. In these cases, a second sample was analysed to confirm the exposure concentration in the tank, and a mean value of the original and retained sample used in the calculation of the vessel mean exposure concentration.
- hatching success of eggs in the controls was >80%.
- post hatch survival in the controls was >70%.
- the mean length and mean weight of control fish was >14 mm and >75 mg (wet weight, blotted dry) respectively.
- The sex ratio (% females) in the control fish was between 30 – 70%.

The study report concludes that there were no apical effects observed at the highest test concentration, as there were no statistically significant effects on hatching rate, post-hatch survival, length, or weight. The NOEC for apical effects was therefore reported as ≥187.9 µg/L. Abnormal behaviour was recorded daily, and during the test one fish in the control and one fish in the highest test concentration were noted to have uncoordinated swimming behaviour.

For endocrine related endpoints and biomarkers statistically significant effects were observed. These are summarised in Table 14.

Table 14

ENDOCRINE RELATED NOEC FROM THE STUDY WITH 4-MSP	
Endpoint	**NOEC (µg/L mean measured)**
Sex ratio (% females)	≥187.9
Sex ratio (% males)	61.8
Sex ratio (% undifferentiated)	2.1
VTG (females)	61.8
VTG (males)	≥61.8
VTG (undifferentiated)	61.8

Sex ratio observations

The original study report used different categories to assess sex ratio to those required by the test guideline. Following queries from the eMSCA a revised study report has been provided with the following categories:

- Male
- Female
- Undifferentiated[13]
- Intersex

These categories are the ones specified in the OECD TG 234 test guideline.

A further category of "Unidentified– no gonads" is also included in the report. The numbers of these specimens were 14/15[14], 6, 0, 1, 2 and 2 in the control, 2.1, 6.4, 19.7, 61.8 and 187.9 µg/L exposures respectively. The CRO has clarified that these unidentified fish were due to a technical error by the CRO as the prepared slides were found not to contain any gonads. The sex of these fish could not be determined and, consequently, these individuals may have been males, females, or any of the other categories. Due to this, and as the CRO considered that there was no reason to assume that the error would not have been random across these categories, the CRO has determined the percentage of fish in each category excluding the "no gonads" group (with results reported this way below).

In the controls, 29% male, 60% female and 11% undifferentiated fish were observed. No intersex fish were observed.

Following a request from the eMSCA, the CRO has shared control data from previous long term ED studies to provide information about the historic performance of fish from this colony. The mean percentage males in five previous FSDT tests ranges from 13.6 – 50.4%. The mean percentage of females ranges from 28.5 – 47.8%. The mean percentage undifferentiated ranges from 5.5 – 56.9%. Data were also provided for some fish full life cycle tests, which has a longer duration than the FSDT. In these studies, the mean percentage males ranges from 27.6 – 55.5%, the mean percentage of females ranges from

[13] This is a combination of what the CRO previously reported as fish identified as Female/stage 0 and transition phase individuals ("lacking fully developed germ cells as it was therefore not possible to confirm the sex of these individuals").

[14] Different results tables in the report indicate that there were either 14 or 15 unidentified fish in the control.

44.5 – 72.4%. There were no undifferentiated fish in these longer tests. The results from this study with 4-MSP fall within these historic ranges for male and undifferentiated fish.

No male Zebrafish were found in the highest exposure concentration (187.9 µg/L) at the end of the experiment. The percentage males in the other test concentrations did not differ from the control, so the NOEC sex ratio (%males) is reported as 61.8 µg/L. The percentage undifferentiated increased with test concentration in a dose-dependent manner. A statistically significant difference from the control was observed at concentrations of 6.4 µg/L and above, so the NOEC sex ratio (undifferentiated) is reported as 2.1 µg/L. At 187.9 µg/L it seems that there has been a male-specific shift to sexually undifferentiated fish. This appears to contradict a general developmental toxicity of 4-MSP. The proportion of female fish was not significantly different to the controls at any exposure concentration. No intersex fish were observed at any of the test concentrations.

Table 15

MEAN PROPORTION OF EACH SEX CATEGORY FOR THE STUDY WITH 4-MSP				
Test conc µg/L	**Male**	**Female**	**Undifferentiated**	**Intersex**
0	29	60	11	0
2.1	28	58	14	0
6.4	30	50	21*	0
19.7	22	41	36*	0
61.8	19	51	30*	0
187.9	0*	36	64*	0

*Statistically significantly different from the control.

Vitellogenin

No significant differences in VTG level were found between the male fish in the control and those exposed. VTG in the highest test concentration could not be determined as no males could be identified. Female VTG and undifferentiated VTG was found to be statistically significantly increased in the highest test concentration, but not the other concentrations.

The VTG concentration in undifferentiated fish at the highest test concentration is at the same order of magnitude as the control female fish.

The NOEC VTG (female) and NOEC VTG (undifferentiated) are reported as 61.8 µg/L. However, at the following higher test concentration, which represents the highest dose tested, there is a significant increase in the VTG level in females and the undifferentiated fish. This increase might turn out much more pronounced at slightly higher test concentrations, i.e., at 500 µg/L. The males cannot be observed since at the highest tested dose there are no males present. Thus, it cannot be excluded that in the range from 61.8 µg/L to 187.9 µg/L, there are also significant changes in the VTG levels in males that even lead to a complete loss of male fish at 187.9 µg/L. Owing to the spacing of the dilution series in the present FSDT study, such effects could not have been observed.

The report concludes that 4-MSP has a strong estrogenic mode of action when following the OECD TG 234 interpretation guidance:

- No males were observed at the highest test concentration 187.9 µg/L.
- There was a statistically significant increase in undifferentiated fish from 6.4 µg/L.
- There was a statistically significant increase in female and undifferentiated fish VTG at the highest test concentration.

eMSCA comments

The eMSCA agrees with the conclusions drawn in the study report. Especially the complete loss of male fish at the highest test concentration, as well as the observed increase in undifferentiated fish with increasing test concentration in a dose-dependent manner, provide a biologically plausible and strong evidence for an estrogenic mode of action for the observed effects on sex ratio.

(a) Sex ratio in controls

The percentage of males in control fish was less than 30% and there was a high percentage of undifferentiated and unidentified fish (no gonads) in the control. As discussed in the description of the study above, the amended report addresses the unidentified fish by excluding these from the amended sex ratio calculations. The resulting percentage males in the control is still less than 30%, although the recalculation (to exclude unidentified fish) has raised the percentage to 29% from 26%. The eMSCA notes that the validity criteria of the study are formally met, as the number of female fish in the control replicates always fits to the range required from the OECD 234 test guideline. Additionally, the corrected value of the male fish in the control samples is with 29% quite close to the required 30%.

As female development in Zebrafish in 9-week-old juveniles is not complete and female development is finished first (e.g., see Maack&Segner, 2003), the observed concentration dependent shift in the ratio of male fish to undifferentiated fish (with no male fish at the highest tested dose), is exactly what one would expect for an estrogenic endocrine disrupting substance.

The fluctuation of the number of undifferentiated fish in the control samples, in view of the eMSCA, underlines the observed effects since it requires a pronounced effect to see statistically significant changes to the control here.

(b) VTG results

VTG concentrations in males at four[15] test concentrations (2.1, 6.4, 19.7, 61.8 µg/L) do not differ from the controls and are all within the historic control range. This is despite the indicated 100% loss of males at the highest concentration.

VTG concentrations in females at all test concentrations, except for the highest test concentration, do not differ from the controls and are within the historic control range. Female VTG concentration in the highest test concentration is statistically significantly higher than that in the controls, but the absolute change in concentration is small[16]. VTG concentration increases by less than a factor of two. VTG has a high dynamic range, and a strong oestrogen would be expected to increase this response by several orders of magnitude at these test concentrations, for example ethinyl-oestradiol exposure increased VTG concentrations in Zebrafish by over a factor of 10000 at 25 ng/L (Orn et al. 2003). Furthermore, in the OECD validation reports for the FSDT (OECD 2011a and OECD 2011b) the female zebrafish had VTG levels consistently high because of oestrogen exposure. The CRO notes that the vitellogenin concentration in female fish at this stage of development is relatively low and increases significantly with further development.

The eMSCA notes that at the highest dose tested, there is a significant increase in the VTG level in females and the undifferentiated fish. This increase might turn out much more pronounced at slightly higher test concentrations, i.e., at 500 µg/L. The males cannot be observed at a comparable dosing, since at the highest tested dose there are no males

[15] VTG at the highest test concentration could not be tested as no males were observed

[16] One possibility is whether the absence of a high increase of VTG in female is due to normalization of the protein content. However, it is unclear to the eMSCA why the normalization would obscure results for total protein.

present anymore. Thus, in view of the eMSCA it cannot be excluded that in the range from 61.8 µg/L to 187.9 µg/L, there are also significant changes in the VTG levels in males that even lead to a complete loss of male fish at 187.9 µg/L. Owing to the spacing of the dilution series in the present FSDT study, such effects could not have been observed.

(c) GLP compliance

The histology section of the report is not GLP compliant. GLP covers how studies are organised, planned, performed, reported, reviewed, and archived, thereby ensuring the quality and validity of test data used for determining the safety of chemicals and chemicals products. This was queried with the Registrant(s), and they provided a statement which notes that the histology was used to confirm the macroscopic (and GLP compliant) sex identification. However, there are numerous disagreements between the macroscopic and histological sex identifications and macroscopic identification is unable to identify all the categories defined in the test guideline.

The eMSCA considers that the endpoints should ideally be in line with GLP requirements. However, in this study the eMSCA cannot identify significant failures or inconsistencies that would render the presented results unreliable.

(d) Overall weight of evidence for endocrine effects from the study

There are two core endocrine endpoints of the FSDT: VTG and sex ratio. Below is the table from the test guideline indicating how the two endpoints combine to demonstrate an ED MoA.

Table 16

REACTION OF THE ENDOCRINE ENDPOINTS TO DIFFERENT MODES OF ACTION OF CHEMICALS:				
↑= increasing, ↓=decreasing, -=not investigated MOA	**VTG ♂**	**VTG ♀**	**Sex ratio**	**References**
Weak oestrogen agonist	↑	↑	↑♀ or ↑Undiff	(27) (40)
Strong oestrogen agonist	↑	↑	↑♀ or ↑Undiff, No ♂	(28) (40)
Oestrogen antagonist	-	-	↓♀, ↑Undiff.	(29)
Androgen agonist	↓ or -	↓ or -	↑ ♂, No ♀	(28) (30)
Androgen antagonist	-	-	↑♀ ↑Intersex	(31)
Aromatase inhibitor	↓	↓	↓♀	(33)

Comparing the results for the FSDT conducted using 4-MSP with this table:

(1) For VTG changes

a. In male and female fish, there was no change in VTG at four concentrations. Female VTG concentration in the highest test concentration is statistically significantly higher than that in the controls, but the absolute change in concentration is small.
b. In undifferentiated fish, there was no change in VTG at four concentrations but a statistically significant change in VTG at the highest test concentration

(2) For impacts on sex ratio:
   a. No intersex fish were observed
   b. No increase in female sex ratio was observed
   c. A statistically significant dose-response was observed in an increasing proportion of undifferentiated fish across the four highest concentrations
   d. No males could be observed at the highest concentration

On this basis the ED MoA "strong oestrogen agonist" in the FSDT TG is clearly fulfilled by 4-MSP in the current study.

A high proportion of undifferentiated gonads in a colony that performs optimally may be a sign of an oestrogen agonist or antagonist (i.e., not diagnostic of mode of action); it could also be a result of overt toxicity or reproductive toxicity. As in the current study a male specific shift to sexually undifferentiated fish was observed, this seems to contradict a general developmental toxicity of 4-MSP. Hence this result can be treated as diagnostic of an endocrine action. The validation report (phase 2) for the Fish Sexual Development Test states that, "a skewing toward more undifferentiated fish is not necessarily population relevant but can be so if for example the delay in sexual development of one of the sexes causes an asynchronous mating behaviour that affect breeding" (OECD, 2011).

Considering all these aspects provided by the substance evaluation data discussed above and the additionally available in vitro and in vivo data also from read-across substances, the eMSCA concludes that there is sufficient evidence that 4-MSP acts as an endocrine disruptor in fish via an estrogenic mode of action. In view of the eMSCA, this is also in line with the interpretation given in the OECD 150 GD since for 4-MSP, at least at the highest tested dose, there is a significant increase in the VTG level in female fish (males cannot be concluded at the highest dose since there are no males present), there is an increased number of undifferentiated fish and there are no males present anymore.

7.10.2. Endocrine disruption - Human health

In the Decision registrants were requested to provide "Information on the endocrine disruption potential of their respective substance with respect to human health". The registrants of List No 701-171-0 and the opt-out registrant of EC No. 262-975-0 produced a document summarising the available data.

They concluded as follows; Based on in vitro testing data, 4-MSP was the only styrenated phenol, which was confirmed to result in slight ER activation, with 4-MSP being much more potent than 2-MSP on both hERaα and hERβ. However, when MSP was compared to molecules such as 17-β oestradiol (E2), ethinyl-oestradiol (EE2) and Nonylphenols for ER activity, potency was much lower (activities at the micromolar range versus nanomolar range).

This document also referred to the results of the recent testing on OAPP however, read-across was not sufficiently justified.

7.10.3. Conclusion on endocrine disrupting properties (combined/separate)

The results from the available *in vitro* and *in vivo* studies for all components are summarised in

Table below. The QSAR and *in vitro* assays have only investigated potential estrogenic activity.

Table 17

IN VITRO AND IN VIVO DATA FOR COMPONENTS OF STYRENATED PHENOL						
Study	**MSP (mixed isomers)**	**2-MSP**	**4-MSP**	**2,4-DSP**	**2,6-DSP**	**2,4,6-TSP**
Brooke et al. (2009) QSAR	Not tested	**Moderate oestrogen receptor binder**	**Moderate oestrogen receptor binder**	**Strong oestrogen receptor binder**	Non-binding	Non-binding
Ogawa et al. (2006) In vitro	**Estrogenic**	**Estrogenic**	**Estrogenic**	Not E active	Not E active	Not E active
Terasaki et al. (2007) In vitro	Not tested	Not tested	Not tested	**Oestrogen receptor binder** **Suspected estrogenic**	Not tested	Not tested
Unpubl. (2016g) In vitro	**Estrogenic**	**Estrogenic**	**Estrogenic**	Not E active	Not E active	Not E active
Unpubl. (2018e) In vitro	Not tested	**Estrogenic**	**Estrogenic**	Not E active	Not E active	Not tested
Unpubl. (2018d) FSDT	Not tested	Not tested	**Estrogenic**	Not tested	Not tested	Not tested

2-MSP has been found to show estrogenic activity in three *in vitro* assays. In all cases when 4-MSP was also tested using the same experimental design 2-MSP was less potent than 4-MSP.

2,4-DSP and 2,6-DSP have not been found to show estrogenic activity in *in vitro* assays using yeast and human cell lines (expressing human and Zebrafish oestrogen receptors). In the study using a yeast cell line with medaka oestrogen receptor some activity was observed, but this could not be quantified by the authors as less than a 10-fold effect was seen compared to the blank control even at a high-test concentration (assumed to be more than the water solubility). Some non-specific luciferase activation was observed together with cytotoxicity at concentrations above the water solubility of these two isomers. The eMSCA therefore considers that there is no indication that these two substances are estrogenic, and therefore that no further information is required for these constituents at present.

There are only very limited data on the potential endocrine disrupting properties of 2,4,6-TSP, and no new data are available since the Decision was issued. The available QSAR and *in vitro* data indicate that 2,4,6-TSP is not estrogenic.

Based on the outcome of the OECD 234 together with the additionally available in vitro and in vivo data also from read-across substances, the eMSCA concludes that there is sufficient evidence that 4-MSP acts as an endocrine disruptor in fish via an estrogenic mode of action.

7.11. PBT and vPvB assessment

Although each group of registrants have generally provided different data for this endpoint, the available data are considered as a combined data set.

One registrant concluded that the substance was not P, not B and not T. However, as discussed earlier, much of the information in this registration is of questionable relevance to the main constituents of the registered substance and so the eMSCA disagrees with this assessment and these conclusions are not considered further by the eMSCA.

Other registrations contain PBT assessments based on three of the main constituents of the registered substance. Monostyrenated phenols were not P, not B and not T. Distyrenated phenols were not B and not T, but no definitive conclusion could be drawn on P. 2,4,6-tristyrenated phenol was not P, to meet the criteria for B and vB, but no definitive conclusion could be drawn on T. As discussed in the following Sections, the overall conclusions are in line with the available data for these constituents.

The tristyrenated constituent is considered to meet the criteria for B and vB. However, a definitive conclusion on P, vP and T could not be drawn.

7.11.1. Persistence assessment

The registered substance is predicted to have a relatively short half-life in air (between 6.2 and 21.1 hours; dependent on the constituent). Therefore, the substance is not likely to persist in air.

Predictions for the persistence of the main constituents of styrenated phenol have been carried out by the eMSCA (UK) using the BIOWIN v4.10 Program within EPI Suite v4.11 (see Section 7.7.1.3.1). According to the REACH Guidance Document, the results of these predictions can be used to evaluate whether a substance is likely to be readily biodegradable regarding the screening criteria for a PBT assessment. The following criteria are applied[17] for the identification of a potentially persistent substance based on the screening criteria.

Biowin2 (non-linear model prediction) – Does Not Biodegrade Fast (probability <0.5) **and** Biowin3 (ultimate biodegradation time) - ≥ Months (value < 2.2)
or
Biowin6 (MITI non-linear model prediction) - Does Not Biodegrade Fast (probability < 0.5) **and** Biowin3 (ultimate biodegradation time) - ≥ Months (value < 2.2).

The relevant predictions obtained for the main constituents of styrenated phenol are summarised below (see Section 7.7.1.3.1).

- 2-MSP
  - Biowin2 (Non-Linear Model Prediction): Biodegrades Fast (probability 0.97)

[17] Based on Table R. 11-2 in the Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment, Version 1.1, November 2012.

- Biowin3 (Ultimate Biodegradation Timeframe): Weeks (value 2.76)
- Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (probability 0.21)

- 4-MSP
  - Biowin2 (Non-Linear Model Prediction): Biodegrades Fast (probability 0.97)
  - Biowin3 (Ultimate Biodegradation Timeframe): Weeks (value 2.76)
  - Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (probability 0.21)
- 2,4-DSP
  - Biowin2 (Non-Linear Model Prediction): Biodegrades Fast (probability 0.99)
  - Biowin3 (Ultimate Biodegradation Timeframe): Weeks-Months (value 2.48)
  - Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (probability 0.0268)
- 2,6-DSP
  - Biowin2 (Non-Linear Model Prediction): Biodegrades Fast (probability 0.98)
  - Biowin3 (Ultimate Biodegradation Timeframe): Weeks-Months (value 2.48)
  - Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (probability 0.027)
- 2,4,6-TSP
  - Biowin2 (Non-Linear Model Prediction): Biodegrades Fast (probability 0.99)
  - Biowin3 (Ultimate Biodegradation Timeframe): Months (value 2.20)
  - Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (probability 0.0029)

As can be seen, none of the constituents considered meet the screening criteria for P, although the prediction for tristyrenated phenol is borderline based on the Biowin6 and Biowin3 combination. The REACH Guidance also suggests that care needs to be taken when the Biowin3 prediction is in the range 2.2 to 2.7. Taking this into account, the Biowin6 and Biowin3 combination for 2,4- and 2,6-DSP are also unclear as to whether the substance meets the screening criteria for P.

Information from screening tests is available for commercial styrenated phenol products. This shows that the substance is not readily biodegradable in an OECD TG 301B study, although it is possible that the bioavailability of the substance in the test may not have been optimal. Information from read-across substances also suggests that styrenated phenol is not readily biodegradable, but there are some uncertainties relating these data to the main constituents of the registered substance.

Predictions carried out by the eMSCA (see above) suggest that both MSPs do not meet the Annex XIII screening criteria for persistence, but that the situation with distyrenated and tristyrenated phenols is borderline. The available study with styrenated phenol was carried out using a substance containing both di- and tristyrenated phenol (predominantly

tristyrenated phenol) and the lack of degradation seen in this test is consistent with the prediction for tristyrenated phenol.

A new OECD TG 307 soil simulation study has been performed with TSP as requested in the substance evaluation decision. The study is described in more detail in section 7.6.1.3.3. The results from the study show significant evidence of molecular transformation from the earliest sampling intervals; increasing evolution of $^{14}CO_2$; and increasing radioactive content associated with the non-extractable residues (NER). The calculated DT_{50} and DT_{90} values for TSP are 10.7 d and 94.3 d respectively, and the calculated total transformation DT_{50} and DT_{90} values for the dominant metabolites are 16.1 d and 17.9 d, and 53.4 d and 59.6 d, respectively. Based on the results of the study, the eMSCA concludes that under the conditions of this test, TSP and its associated transformation products do not meet the persistence criteria of REACH Annex XIII.

Modelled results suggest that both distyrenated phenols and tristyrenated phenol have a high potential for long-range transport, but that monostyrenated phenols have a moderate potential for long-range transport. It is important to note that all the predictions for environmental distribution and long-range transport rely on the assumptions made over the biodegradation half-life of the substance in water, sediment, and soil, and could potentially be refined if further information becomes available. Therefore, no firm conclusions on the long-range transport potential can be drawn at present.

The calculated dissipation half-lives are well below the persistence criterion. However, the Annex XIII criteria refer to degradation half-lives. As no degradation half-lives were derived, a final conclusion on persistence is not possible.

7.11.2. Bioaccumulation assessment

The available weight of evidence from experimental studies, QSAR predictions and read across from analogous substances suggests that the mono- and distyrenated phenol constituents of the registered substance have a low potential for bioaccumulation. Predictions for monostyrenated phenol indicate that the bioconcentration factor (BCF) in fish is likely to be around 263 l/kg or lower, and a new log Kow study performed by the registrants provided measured log Kow values of 2.99 for 4-MSP and 3.13 for 2-MSP. This shows that neither MSP constituent screens as B/vB. A dietary accumulation study with distyrenated phenol (which included both 2,4-DSP and 2,6-DSP) indicates that the substance is rapidly eliminated from fish.

For 2,4,6-TSP, the growth corrected, and lipid normalised biomagnification factor (BMF) value has been determined to be around 0.355 (range 0.32-0.49). Depuration of the substance from the fish has been found to be relatively slow (with a growth corrected half-life of around 18.4 days; k_2 = 0.059 day^{-1} and k_{2g} = 0.038 day^{-1}) and predictions suggest that there is a high probability of the BCF value being above 5,000 l/kg (the BCF is 10,395 l/kg using the method recommended in the REACH Guidance; the range of predicted values using other available methods is 8,607-36,320 l/kg).

Although the BCF value for 2,4,6-TSP is uncertain, as it is a predicted value obtained from a dietary study, there are also other indicators of a high potential for bioaccumulation for this constituent:

- Brooke and Crookes (2012) carried out an analysis of depuration rate constants and found that a depuration rate constant of $k_2 \leq 0.065$ day^{-1} or a lipid normalised $k_2 \leq 0.085$ day^{-1} was consistent with a BCF (normalised to a 5% lipid content) of ≥5,000 l/kg. The growth-corrected k_2 value for 2,4,6-TSP was 0.038 day^{-1} (normalising this to a 5% lipid content results in a lipid normalised value of 0.044 day^{-1}). Thus, the low rate of depuration seen in the feeding study is consistent with the BCF for 2,4,6-TSP being >5,000 l/kg.

- Inoui *et al.* (2012) investigated the correlation of the dietary BMF values with BCF values. This indicated that a BMF (growth corrected and lipid normalised) >0.31 corresponds to a BCF (lipid normalised) >5,000 l/kg. Again, the BMF obtained for styrenated phenol of 0.355 (range 0.32-0.49) is strongly suggestive that the BCF for 2,4,6-TSP is >5,000 l/kg.

- A BCF study using aqueous exposure, although not fully valid owing to problems with the control group, indicated uptake in the early stages of the experiment consistent with a BCF >5,000 l/kg.

Overall, it is concluded that neither the mono- nor distyrenated phenol constituents of the registered substance meet the Annex XIII criteria for B or vB, but that the 2,4,6-TSP constituent of the registered substance meets the Annex XIII criteria for both B and vB. This conclusion is consistent with the Brooke et al. (2009) report and the conclusions of the former TCNES PBT Working Group (ECHA, 2009).

7.11.3. Toxicity assessment

There is considerable uncertainty over the environmental hazards presented by some of the main constituents of the registered substance. For the purposes of this substance evaluation, the eMSCA (UK) has filled important data gaps (where appropriate) using QSARs, and the uncertainties in this should be taken into account when considering any conclusions drawn from the data. The available evidence suggests that the 2,4,6-TSP constituent may have a long-term NOEC <0.01 mg/L for fish, meaning that the substance potentially meets the Annex XIII T-criterion. However, this is based on QSAR data only and further testing would be required to confirm this. The aquatic plant and invertebrate data available for both mono- and distyrenated phenols suggest that the long-term NOECs will be >0.01 mg/L. Due to concerns with the reliability and validity of the new FSDT study performed with 4-MSP, these data are not suitable for the T assessment. The remaining 4-MSP data does not indicate that the substance meets the Annex XIII T criteria.

It is concluded that the DSP and TSP constituents do not meet the Annex XIII T-criterion based on available NOEC or EC10 data, although measured and reliable fish data are not available. However, if the Substances are identified as endocrine disruptors in the environment based on all available data this would also be sufficient to fulfil the T-criterion according to Annex XIII.

The substance does not meet the Annex XIII T-criterion based on current self-classifications for human health.

7.11.4. Summary and overall conclusions on PBT and vPvB Properties

For the purposes of this substance evaluation, the eMSCA has considered the main constituents of the registered substance individually.

Based on the available weight of evidence, the MSP and DSP constituents are concluded not to meet the Annex XIII criteria for PBT or vPvB. The MSP constituents do not meet the (screening) criteria for persistence, bioaccumulation, or toxicity. The toxicity of MSP should be re-evaluated when definitive ED data are available. Nevertheless, as it does not screen as P or B, this will not affect the PBT conclusion for this constituent. The DSP constituents do not meet the (screening) criteria for bioaccumulation, but no conclusion can currently be reached in relation to the persistence criteria. Given that these constituents are not B (based on the currently available information) the need to investigate the persistence and fish toxicity further is a low priority.

2,4,6-TSP is confirmed as meeting the Annex XIII criteria for being both bioaccumulative (B) and very bioaccumulative (vB). It is not persistent based on the new soil degradation test. QSAR estimates suggest that the constituent might meet the T-criterion based on the

predicted long-term toxicity to fish. Overall, 2,4,6-TSP is concluded not to be PBT or vPvB as it is not persistent.

In summary:

MSP (both isomers)
- not P, not B, not T based on available data

DSP (both isomers)
- screening P, not B, not T based on available data

- 2,4,6-TSP parent: not P, confirmed vB, no conclusion on T

The eMSCA notes that in addition to the mono-, di- and tristyrenated phenol components there are additional constituents and impurities present at concentrations ≥0.1% that have not been assessed in the PBT assessments included in the registration dossiers. As not all constituents or impurities have been identified it is not possible for the eMSCA to evaluate them further at present. It is recommended that the registrants update their registration dossiers to ensure all constituents and impurities ≥0.1% are evaluated in the PBT assessment.

As the eMSCA considers the ED properties for the environment the driving hazard for further risk management measures, no further considerations on the PBT assessment beyond those documented above have been made during the follow-up assessment.

7.12. Exposure assessment

The Decision required registrant(s) who needed to submit an exposure and risk assessment as part of their registration dossier to update their environmental exposure scenarios to model the mono-, di- and tristyrenated constituents separately, before summing the Risk Characterisation Ratios (RCR) to give the overall risk for each exposure scenario.

Several Registrants have included exposure modelling, but have not modelled the mono-, di- and tristyrenated constituents separately. These Registrants should update their exposure assessment to model each constituent separately and use the PNEC calculated by the eMSCA before summing the RCR.

One Registrant has updated the exposure modelling as requested. However, the aquatic PNEC used are not constituent specific, as they are based on aquatic ecotoxicity data from tests using a mix of constituents. The sediment and soil PNECs are calculated using the Equilibrium Partitioning Method with constituent specific physico-chemical input values which results in differing PNEC for each constituent, but they are all based on the same underlying aquatic ecotoxicity data.

As the PNEC calculated by the eMSCA for water, sediment and soil are lower than those calculated by this Registrant, the RCR for all compartments increase. The eMSCA has made an initial assessment of the effect of using these lower PNECs on the modelled risk. RCRs above 1 are modelled in water, soil, and sediment for all exposure scenarios when the RCR for each component are combined. The highest RCR for the sediment compartment in the manufacturing scenario is 28. The components driving this total risk are:

- For all exposure scenarios modelled by this Registrant, RCR greater than one is observed for the monostyrenated phenol constituent in fresh and marine water.
- For all exposure scenarios modelled by this Registrant, RCR greater than one is observed for the monostyrenated phenol constituent in fresh and marine sediment. In addition, some exposure scenarios also have RCR greater than one in sediment for the distyrenated component.
- For all exposure scenarios modelled by this Registrant, RCR greater than one is observed for the mono- and distryrenated phenol constituent in soil. The eMSCA notes that the Registrant assumed that these two components did not biodegrade in soil.

Based on the results of the tristyrenated phenol soil simulation study that demonstrated a half-life of 10.7 days for this substance the eMSCA considers that this half-life could also be used for the mono- and distyrenated components. This amendment would reduce the modelled RCR in soil.

This Registrant is therefore required to update their exposure assessment using the PNEC calculated by the eMSCA and to refine the modelling to demonstrate RCR below 1.

As noted above, several Registrants have submitted separate exposure scenarios for their individual tonnages. As various Registrants have not modelled the individual components separately, and as RCRs above 1 are modelled for the single Registrant who has modelled the components separately, the eMSCA (UK) has not considered the combined exposure due to aggregated tonnages and combined uses from all different Registrants at this stage. This assessment will be made once all the individual exposure assessments have been updated by the Registrants.

The previous Decision required the Registrant(s) to provide information on whether there are any discharges to marine water. None of the Registrants have provided any further information on this, but all exposure scenarios submitted model both freshwater and marine water emissions. This information request is outstanding, and so currently direct marine emissions are assumed.

The Decision required the Registrant(s) to provide information on emissions to waste. Some Registrants have provided further detail on the production of waste from each exposure scenario and provided justification for why this is considered insignificant for the purposes of the risk assessment. The eMSCA accepts the justification provided. However, some Registrants have not provided this information. This information request is therefore outstanding for some Registrants.

The Decision also required the Registrant(s) to provide information on emissions due to recycling of articles containing the substance. Most Registrants have not addressed this information requirement. One registrant notes that the recycling of articles containing the substance is not a notified use and is therefore not covered by an exposure scenario and that the handling of articles after disposal to waste is outside the scope of a chemical safety assessment. The eMSCA (UK) does not agree with this argument and notes that ECHA R18 provides guidance on how to model the possible emissions from the recycling of articles. This guidance should be followed by the Registrant(s).

The eMSCA has not identified additional observations beyond those contained in the intermediate assessment by the previous eMSCA.

7.12.1. Human health

Not evaluated.

7.12.2. Environment

Not performed.

7.12.3. Combined exposure assessment

Not performed.

7.13. Risk characterisation

Not performed.

7.14. References

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7.15. Abbreviations

%	Percentage
B	Bioaccumulative
BCF	Bioconcentration factor
CLP	Classification, labelling and packaging (of substances and mixtures)
cm	Centimetre
CoRAP	Community Rolling Action Plan
CSR	Chemical Safety Report
d	Day
DMEL	Derived Minimal Effect Level
DNEL	Derived No Effect Level
DSD	Dangerous Substances Directive
ECETOC TRA	European Centre for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment
ECHA	European Chemicals Agency
eMSCA	evaluating Member State Competent Authority
EPA	Environmental Protection Agency
ES	Exposure Scenario
ERC	Environmental release category
EU	European Union
FSDT	Fish Sexual Development Test
g	Gramme
GC	Gas chromatography
GC/FID	Gas chromatography – Flame Ionisation Detection
GC/MS	Gas chromatography – mass spectrometry
GLP	Good laboratory practice
hPa	Hectopascal
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union of Pure and Applied Chemistry
kg	Kilogram
kJ	Kilojoule
km	Kilometre
kPa	Kilopascal
K_{oa}	Octanol-air partition coefficient
K_{oc}	Organic carbon-water partition coefficient
K_{ow}	Octanol-water partition coefficient
L	Litre
LEV	Local Exhaust Ventilation
Log	Logarithmic value
LOD	Limit of detection

LOQ	Limit of quantitation
M	Molar
m	Metre(s)
µg	Microgram
mg	Milligram
min	Minute
mL	Millilitre
mol	Mole
MS	Mass spectrometry
MSCA	Member State Competent Authority
m/z	Mass to charge ratio
nm	Nanometre
NOAEL	No observed adverse effect level
NOEC	No-observed effect concentration
NOEL	No observed effect level
OC	Operational condition
OECD	Organisation for Economic Co-operation and Development
p	Statistical probability
P	Persistent
Pa	Pascal
PBT	Persistent, Bioaccumulative and Toxic
PC	Product category
pg	Picogramme
pKa	Acid dissociation constant
PNEC	Predicted no effect concentration
ppb	Parts per billion
PPE	Personal Protective Equipment
ppm	Parts per million
PROC	Process Category
QSAR	Quantitative structure-activity relationship
r^2	Correlation coefficient
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation No. 1907/2006)
RCR	Risk characterisation ratio
RMM	Risk Management Measures
RPE	Respiratory protective equipment
t	Tonne
T	Toxic (hazard classification)
TCNES	Technical Committee for New and Existing Substances
TG	Test Guideline
UK	United Kingdom
UV	Ultraviolet
vB	Very bioaccumulative
vP	Very persistent
vPvB	Very persistent and very bioaccumulative
wt.	Weight