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, dodecyl-, branched

EC No 310-154-3

CAS No 121158-58-5

Evaluating Member State: Germany

Dated: 18.10.2019

Evaluating Member State Competent Authority

BAuA
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Year of evaluation in CoRAP: 2018

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

Further information on registered substances here:

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

DISCLAIMER

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

Foreword

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

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

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

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

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

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Phenol, dodecyl-, branched (PDB, EC 310-154-3, CAS 121158-58-5) was originally selected for substance evaluation in order to clarify concerns about its endocrine disrupting properties for human health and environment. No additional concerns were identified during the evaluation.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A large portion of PDB is used to manufacture e.g. calcium salts of alkyl phenol sulfides (phenates). The three following registered substances have been subjected to a Risk Management Option Analysis (RMOA) by the Swedish competent authority in 2018:
[1] Phenol, dodecyl-, sulfurized, calcium salts, CAS 68855-45-8.
[2] Phenol, dodecyl-, sulfurized, carbonates, calcium salts, CAS 68784-25-8.
[3] Phenol, para-alkylation products with C10-15 branched olefins (C12 rich) derived from propene oligomerization, carbonate, calcium salts, overbased, sulfurized, including distillates (petroleum), hydrotreated, solvent-refined, solvent dewaxed, or catalytic dewaxed, light or heavy paraffinic C10-C50, EC 701-251-5 (no CAS available).

A smaller amount of PDB is used in the production of aryl-based ZDDP substances as lubricant additives. The two following registered substances have been subjected to an interim Risk Management Option Analysis (RMOA) by the Swedish competent authority in 2019[2]:

[4] Zinc bis[bis(dodecylphenyl)] bis(dithiophosphate), CAS 54261-67-5
[5] Zinc bis[bis(tetrapropylenephenyl)] bis(hydrogen dithiophosphate), CAS 11059-65-7

PDB is a common constituent (impurity) in both aryl-based substances.

Both substances' groups still contain varying amounts of free PDB.

The RMOAs focused on the concern for human health (classification as Repr. 1B). The RMOA conclusion document[3] for the phenates states: "New data on exposure obtained during the SEv process for **[3]** do not indicate an unacceptable risk for the EU population at large. The CSR was updated during the SEv process taking into account the new exposure data. Due to the similar uses for all phenates, it is concluded that there is no indication of an EU-wide risk for workers or consumers for any of the phenates, provided that the registrations for **[1]** and **[2]** are updated in line with **[3]** and all risk management measures are implemented."

It was concluded at the time of finalising the RMOA that no action was currently needed but that the need for SVHC identification of this phenate group should be reassessed after conclusion of the substance evaluation on PDB.

For the RMOA of the aryl-based ZDDP substances no conclusion was drawn until the time of writing this report (17-09-2019).

[2] Public Activities Coordination Tool entry on Swedish RMOA for ZDDP, https://echa.europa.eu/de/rmoa/-/dislist/details/0b0236e18131db29

[3] Risk Management Option Analysis Conclusion Document of the Swedish Competent Authority, November 2018: https://echa.europa.eu/documents/10162/1b972670-390d-7acb-e98a-4d0b732ea08a

3. CONCLUSION OF SUBSTANCE EVALUATION

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

Table 1: Conclusion of substance evaluation.

Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	
Identification as SVHC	X
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Identification as a substance of very high concern, SVHC

The available data for PDB provide sufficient evidence that the substance fulfils the WHO/IPCS definition of an endocrine disruptor (WHO/IPCS, 2002). Furthermore, the nature and type of effects described led the eMSCA to the conclusion that phenol, dodecyl-, branched is of equivalent level of concern compared to CMR, PBT and vPvB substances as described in Art. 57 (f). Thus, to come to an EU-wide agreement on the endocrine disrupting properties of PDB for human health and the environment, SVHC identification is the most appropriate step to initiate further risk management measures. To clarify this, the appropriate measures will be analysed in a RMOA following the conclusion of the substance evaluation process.

The following paragraphs summarise the available evidence that led the eMSCA to the conclusion that PDB has endocrine disrupting properties for humans and the environment.

Human health

There is strong evidence that the adverse effects on fertility and sexual function (which led to classification of PDB as Repr. 1B), particularly in females, are due to the oestrogenic activity of PDB. The eMSCA comes to the conclusion that PDB fulfills the WHO/IPCS definition of an endocrine disruptor (WHO/IPCS (2002) cited in OECD (2018)) with regard to human health.

Environment

Due to evolutionary conservation of the endocrine system, similar effects by oestrogenic active substances can be exerted on humans, rats and aquatic vertebrates like fish. It is concluded that the described mammalian effects are adverse and population-relevant for environmental species.

Therefore, the eMSCA considers it possible to conclude on the endocrine disrupting properties in the environment from the data on human health. There is strong evidence that PDB is also an endocrine disruptor with oestrogenic activity for the environment.

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

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

Table 2: Follow-up.

Follow-up action	Date for intention	Actor
RMOA is to be finalised	in 2020	DE
Annex XV dossier for SVHC identification	not yet decided	DE

Part B. Substance evaluation

6. EVALUATION REPORT

6.1. Overview of the substance evaluation performed

PDB was originally selected for substance evaluation in order to clarify concerns about:

- Endocrine disrupting properties for human health.

- Endocrine disrupting properties for the environment.

Table 3: Evaluated endpoints.

Endpoint evaluated	Outcome/conclusion
Endocrine disrupting properties for human health	According to the assessment of the eMSCA, there is sufficient evidence to conclude that PDB acts as an endocrine disruptor for human health with an oestrogenic mode of action.
Endocrine disrupting properties for the environment	According to the assessment of the eMSCA, there is sufficient evidence to conclude that PDB acts as an endocrine disruptor for the environment with an oestrogenic mode of action.

6.2. Procedure

- On 31 August 2018, a meeting was held with industry including an introduction to the substance evaluation process and an informal discussion. Afterwards, industry submitted further information to a questionnaire of the eMSCA.

- The eMSCA evaluated data from *in vivo* studies in rats and *in vitro* studies (based on mammalian and fish cells).

- After evaluation of the studies regarding human health, the eMSCA concluded that PDB is an endocrine disruptor for human health.

- A comprehensive approach was used to conclude on the endocrine disrupting properties of PDB for the environment. Information on the endocrine disrupting properties of PDB itself for human health was looked at together with information on the endocrine disrupting properties for the environment from supporting *in vitro* studies conducted with an isomeric mixture of p-dodecylphenol (Tollefsen and Nilsen, 2008) as well as with the linear p-dodecylphenol as read-across substance (Knudsen and Pottinger, 1999).

- It was concluded that PDB is an endocrine disruptor not only for human health, but also for the environment.

6.3. Identity of the substance

Phenol, dodecyl, branched (CAS 121158-58-5) is an UVCB (substance of unknown or variable composition, complex reaction products or biological materials) substance for which a complex and variable composition of partly unknown constituents is possible.

Table 4: Substance identity.

SUBSTANCE IDENTITY	
Public name:	Phenol, dodecyl-, branched
EC number:	310-154-3
CAS number:	121158-58-5
Index number in Annex VI of the CLP Regulation:	604-092-00-9
Molecular formula:	n.a. (UVCB)
Molecular weight range:	n.a. (UVCB)
Synonyms:	Phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation; TPP For other synonyms please refer to section 6.4

Type of substance ☒ UVCB

Structural formula:

This substance is a UVCB and cannot be displayed by a single structure. The following structural formula represents the main constituents of the substance.

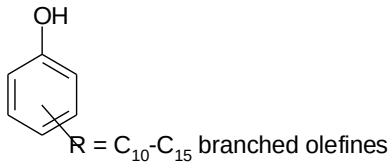


Table 5: Multiconstituent/UVCB substance/others.

Constituents	Typical concentration	Concentration range	Remarks
phenol, alkylation products with alkenes C1-7, branched			CBI
phenol, octyl-, branched			CBI
phenol, nonyl-, branched			CBI
phenol, decyl-, branched			CBI
phenol, undecyl-, branched			CBI

phenol, dodecyl-, branched			CBI
phenol, tridecyl-, branched			CBI
phenol, tetradecyl-, branched			CBI
phenol, pentadecyl-, branched			CBI
phenol, dialkylation products with C10-C14, C12-rich alkenes, branched and linear			CBI
phenol, oxygen-alkylation products (ethers) with C10-C14, C12-rich alkenes, branched and linear			CBI
phenol			CBI
C10-14, C12-rich alkenes, branched and linear			CBI

6.4. Alternative names and synonyms

After checking the description of the manufacturing process in the registration dossiers, it became obvious that the branched dodecyl moiety is mainly a propylene tetramer (3,4,5,6-tetramethyloctan-2-yl)phenol, which is synthesized by an oligomerisation of propene. This fact is supported by the used IUPAC name given on ECHA brief profile: 4-(3,4,5,6-tetramethyloctan-2-yl)phenol as well as the used SMILES notation.
The substance described above is termed using several different chemical substance names.

The following alternative names or synonyms are known for phenol, dodecyl-, branched (EC 310-154-3, CAS 121158-58-5):

- Dodecylphenol T (Based on Tetrapropylen or Tri-n-buten)
- Dodecylphenol, mixed isomers (CAS No. 27193-86-8)
- Maslo Gazpromneft Diesel Extra 10W-40
- Phenol, (tetrapropenyl), derivatives (CAS No. 74499-35-7)
- Phenol, 4-dodecyl, branched (CAS No. 210555-94-5)
- Phenol, tetrapropylene (CAS No. 57427-55-1)
- Phenol, 4-isododecyl; 4-isododecylphenol (CAS No. 27459-10-5)
- Tetrapropenyl phenol
- 2,3,5,6-tetrakis(ethenyl)phenol
- 4-(3,4,5,6-tetramethyloctan-2-yl)phenol
- 4-(3,4,5-trimethylheptyl)phenol
- Dodecylphenol, mixed isomers
- Phenol, alkyl branched (species comprising decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, substituents)

- Phenol, dodecyl-, branched
- Phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation

These names are used as synonyms for the evaluated substance phenol, dodecyl-, branched (CAS 121158-58-5). Formally, these substances are not identical with the registered substance (different CAS numbers and names) and should therefore not be registered in a joint dossier. However, due to the information in the registration dossiers the substances seem to be the same as the registered substance, the registrants just used other names. The specific identity of the substance is of less importance to industry than the critical physico-chemical properties exhibited by the substance. Thus, since industry does not differentiate between the given substances above, it seems to be appropriate to also follow this approach within the substance evaluation to make sure, that the regulatory instrument reflects the "real" situation on the market.

Thus, this substance evaluation was done on phenol, dodecyl-, branched (CAS 121158-58-5). However, all studies submitted for registration using one of the given substances mentioned above were taken into account in this substance evaluation. The isomeric mixture of 4-dodecylphenol was considered to be PDB and was included in the evaluation. Next to this, studies conducted using the substance 4-dodecylphenol (CAS 104-43-8) with supposed linear alkyl chain as test material were evaluated as well, which is justified by applying a read across approach as explained below.

6.5. Justification for read-across from p-Dodecylphenol to Phenol, dodecyl-, branched (PDB)

This read-across is based on p-dodecylphenol (CAS 104-43-8) with a linear alkyl chain as the source substance. Substances with branched p-dodecylphenol isomers are considered as the target substances covered by the substance evaluation of the registered substance by the eMSCA.

The read-across consideration is substantiated due to the chemical structure, physico-chemical properties and effects.

- Structure: p-dodecylphenol (CAS 104-43-8) can be used as read-across substance for PDB as the structure is similar. Both substances have an aromatic ring and an alkyl group in para position[4]. The difference is that p-dodecylphenol (CAS 104-43-8) is supposed to have a non-branched alkyl chain.

- Properties: The physico-chemical properties water solubility and log K_{OW} are comparable, too (based on the assumption that for solubility of phenol, dodecyl-, branched, the value of the distilled substance without the presence of impurities is used).

- Effects: The oestrogenic activity of p-dodecylphenol (CAS 104-43-8) and PDB is comparable based in receptor binding studies and uterotrophic assays.

Competitive binding assays:
The relative binding affinity (RBA) determined in binding assays using mammalian oestrogen receptors (ER) are in the same order of magnitude for p-dodecylphenol, which

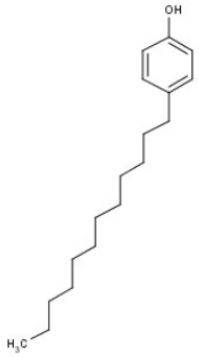
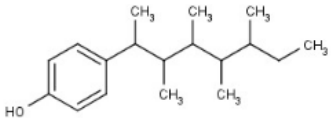
[4] According to the information given by the registrants in the registration dossiers.

is assumed to be linear (RBA = 0.24 % for human ER (hER)) and PDB (0.11 % for ratER). For the isomeric mixture of p-dodecylphenol (also supposed to be branched like PDB)[5], the RBA is 0.019 % for rat ER and 0.016 % for rainbow trout ER (rtER).
For the linear p-dodecylphenol a study using rtER is available, without specified effect value or RBA. The estimated RBA is 0.01 %.

Uterotrophic assay:
Significant increases in uterus weight occur at a similar dose range for linear p-dodecylphenol (40 mg/kg/day) and for PDB (75 mg/kg/day); for the latter it was the lowest tested dose.

[5] For p-dodecylphenol which has a linear alkyl chain in para-position of the aromatic ring, there exists only one possible isomer. Thus, it can be assumed that the substance p-dodecylphenol (isomeric mixture) covers substances with a branched dodecyl chain and is therefore identical to PDB.

Table 6: Read-across considerations on PDB and p-dodecylphenol.

	p-Dodecylphenol	Phenol, dodecyl-, branched
CAS	104-43-8	121158-58-5
EC	203-202-9	310-154-3
Molecular formula	$C_{18}H_{30}O$	$C_{18}H_{30}O$
Structure		UVCB substance, structure e.g.: 
Solubility	14 µg/L[6]	1.54 mg/l at 20°C, flask method (lower alkyl phenols C3 to C9 present in sample) 31 µg/L flask method (distilled substance, without impurities)[7]
log P_{OW}[9]	7.91	7.14
Uterotrophic assay, OECD 440, endpoint uterine weights	40 mg/kg/day onwards	Positive at 75 mg/kg/day (lowest dose tested)
Competitive binding assay using human ER or rat ER	RBA: 0.24 % (hERα)	IC_{50}: 1.1 µM (ratER) RBA: 0.11 % (ratER) Isomeric mixture of p-dodecylphenol: IC_{50}: 4.85 µM (ratER) RBA: 0.019 % (ratER)
Competitive binding assay using rainbow trout ER	10^4-fold more p-dodecylphenol was necessary to obtain the same effect as with 17β-oestradiol (E2; estimated by author). RBA: 0.01 % (estimated)	Isomeric mixture of p-dodecylphenol: RBA: 0.016 %

[6] Information for solubility and log P_{OW} for p-dodecylphenol from https://chem.nlm.nih.gov/chemidplus/rn/104-43-8

[7] Both values on solubility from ECHA web site (the latter from supporting experimental result)

6.6. Physico-chemical properties

Table 7: Overview of physicochemical properties.

Property	Value
Physical state at 20 °C and 101.3 kPa	clear bright to amber/brown viscous liquid
Vapour pressure	0.011 Pa at 25 °C (effusion method, vapour pressure balance)
Water solubility	1.54 mg/L at 20 °C, flask method (lower alkyl phenols C3 to C9 present in sample) 31 µg/L flask method (distilled substance, without impurities)[8]
Partition coefficient n-octanol/water (log K_{OW})	7.14 at 25 °C (for the main component phenol with C12 alkylated branched olefin), slow stirring method (log K_{OW} 6.45 for C9 constituent)
Flammability	n.a., study technically not feasible
Explosive properties	n.a., study scientifically not necessary
Oxidising properties	n.a., study scientifically not necessary
Granulometry	n.a., substance is a liquid
Stability in organic solvents and identity of relevant degradation products	n.a.
Dissociation constant	n.a., no ionisable groups

6.7. Manufacture and uses

6.7.1. Quantities

Table 8: Aggregated Tonnage (per year).

AGGREGATED TONNAGE				
☐ 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

6.7.2. Overview of uses

The UVCB substance PDB, is manufactured and/or imported in the European Economic Area in 10 000 - 100 000 tonnes per year. The substance is used at industrial sites and in manufacturing.

The manufacture of PDB takes place in closed and controlled continuous processes. The substance is predominantly used in the supply chain as a chemical intermediate and the raw material is transformed into other chemical products (e.g. during the synthesis of

[8] Both values from ECHA web site (the latter from supporting experimental result)

polymers). The main end uses of PDB are the preparation of a variety of lubricant additive materials and of fuel system cleaners (PDB is consumed during the use as fuel system cleaner by 95 %; however, it is unclear whether the residual amount may be released to the environment). Depending on the starting materials and production conditions, the additives may contain significant amounts of unreacted PDB. They are used in petrol and diesel powered road vehicles and marine diesel engines and thus a wide dispersive use can be assumed. Furthermore, an application in oil fields as a deemulsifier is indicated, as well as uses for formulation of paints, printing inks, varnishes and coatings. Additionally, there are uses indicated for PDB as a monomer for forming phenol /formaldehyde resins.

According to the information provided at the ECHA dissemination site, the substance is used as an intermediate for the manufacture of chemicals, rubber products and plastic products.

Table 9: Registered uses.

Use(s)	
Uses as intermediate	X
Formulation	
Uses at industrial sites	X
Uses by professional workers	
Consumer Uses	
Article service life	

6.8. Classification and Labelling

6.8.1. Harmonised Classification (Annex VI of CLP)

Table 10: Harmonised Classification according to Annex VI of CLP Regulation (Regulation (EC) 1272/2008).

Index No	International Chemical Identification	EC No.	CAS No.	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)		
604-092-00-9	phenol, dodecyl-, branched	310-154-3	121158-58-5	Skin Corr. 1C	H314	M=10 M(Chronic)=10	
				Eye Dam. 1	H318		
				Aquatic Acute 1	H400		
				Aquatic Chronic 1	H410		
				Repr. 1B	H360F		

6.8.2. Self-classification

The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Skin Corr. 1A, Skin Irrit. 2, Eye Irrit. 2, Acute Tox. 4, Skin Corr. 1B.

6.9. Environmental fate properties

Based on the available information from two key studies in the registration dossier it is concluded that PDB is neither readily nor inherently biodegradable and meets the screening criteria for persistence, P/vP. Therefore, the eMSCA views PDB as potentially fulfilling the persistence criteria according to Annex XIII, but does not consider a request of additional data on persistence as proportionate at this stage of assessment. The available physico-chemical data suggest that PDB is of low volatility and low water solubility and tends to adsorb strongly to organic matter in soils, sediments and sludges. Due to the low vapour pressure of the substance and its Henry law constant, a partitioning into atmosphere will not be a significant pathway.

6.10. Environmental hazard assessment

6.10.1. Aquatic compartment (including sediment)

PDB is acutely very toxic to the aquatic compartment with an EC50 of 0.037 mg/L for *D. magna* as the most sensitive tested species.
The chronic *D. magna* study showed a NOEC of 0.0037 mg/L (LOEC: 0.012 mg/L, nominal) for immobilisation of parents and reproduction after a 21 day exposure. The EC_{50} values (21d) for reproduction and immobilisation were 0.0086 mg/L and 0.0079 mg/L, respectively. EC_{10} values were not provided.

The substance has a harmonised classification as Aquatic acute 1 and Aquatic chronic 1. The information is updated up to January 2019.

6.10.2. PNEC derivation and other hazard conclusions

Not assessed.

6.11. Assessment of endocrine disrupting (ED) properties

6.11.1. This evaluation was performed to address the endocrine disrupting properties of PDB regarding human health and the environment. Endocrine disruption - Human health

For this evaluation the eMSCA took into account all toxicity data of PDB (EC 310-154-3) regarding human health available from the registration dossiers, the CLH dossiers submitted by both Chevron Oronite SAS (Chevron, 2013) and the SI Group-UK, Ltd (SI Group, 2012), respectively, and the RAC-Opinions (ECHA, 2013c; ECHA, 2013d) with the corresponding background documents (ECHA, 2013a; ECHA, 2013b). Furthermore, public scientific literature was considered. The status of information is June 2018.

In silico data (OECD level 1) - QSAR

The QSAR Toolbox (Version 3.4.0.17) gives under the CAS 121158-58-5 the following result regarding ER binding: The substance is marked as strong ER binder due to "cyclic

molecular structure with a single non-impaired hydroxyl group". The effects resulting from ER binding are typically considered reproductive and developmental hazards.

Mechanistic *in vitro* studies (OECD level 2)

For an overview of available *in vitro* studies, see Table 11.

An androgen receptor (AR) binding assay (Thomas, 2012a) according to OPPTS 890:1150 and an ER binding assay (Thomas, 2012b) according to OPPTS 890:1250 were performed using rat prostate cytosol and rat uterine cytosol, respectively. The AR binding assay demonstrated weak competitive binding of phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation to AR with an IC_{50} of 92 µM and a RBA of 0.0016 % compared to the positive control R1881 (a synthetic AR agonist; IC_{50} = 1.44 nM). The RBA of phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation is comparable to that of the weak positive control dexamethasone (IC_{50} = 74 µM; RBA = 0.002 %). The ER binding assay demonstrated weak to moderate competitive binding of PDB to ER with an IC_{50} of 1.1 µM and a RBA of 0.11 % compared to the positive control E2 (IC_{50} = 1.2 nM). The RBA of PDB is about three-times higher than that of the weak positive control 19-norethindrone (IC_{50} = 3.46 µM; RBA = 0.034 %).

For a competitive ER binding assay (cytosolic rat uterine ER preparation) by Blair et al. (2000), the substance 4-dodecylphenol (mixture of isomers, no CAS indicated) was used. Thus, the test material is most likely identical to PDB. The IC_{50} was 4.85 µM corresponding to a RBA of 0.019 % when compared to E2. In comparison, 4-nonylphenol (five different substances from different lots and producers, purity 85 to 95.6 % or technical) had an RBA of 0.019 to 0.037 %. 4-tert-Octylphenol (purity 97 %) had an RBA of 0.015 %. The IC_{50} of 4-dodecylphenol (mixture of isomers) was between the values of 4-nonylphenol and 4-tert-octylphenol and is therefore in the same range.

A further ER binding study is available for p-dodecylphenol (read-across, CAS 104-43-8). Akahori et al. (2008) developed a binding assay using recombinant human ERα (hERα) where the ligand binding domain (LBD) of human ERα (hERα) was fused with glutathione-S-transferase and expressed in E.coli. This binding assay demonstrated competitive binding of p-dodecylphenol (read-across, CAS 104-43-8) to the LBD of hERα with a RBA of 0.24 % when compared to the positive control E2 (no IC_{50} values were reported).

Regarding the thyroid system, dodecylphenol (CAS 27193-86-6) and p-dodecylphenol (read-across, CAS 104-43-8) were tested *in vitro* for inhibition of deiodinases 1, 2, 3 (DIO 1, 2, 3) using high throughput assays (Olker et al., 2018). Recombinant human DIO 1, 2, or 3 was expressed in HEK293 cells and the cell lysates served as the source of the respective enzymes. The assay measured DIO-liberated iodide with the Sandell-Kolthoff reaction. Both substances were positively identified as inhibitors of all three DIOs. The strongest inhibitory effect was exerted on DIO 3. IC_{50} values for DIO 1 were 37.2 µM and 61.4 µM for CAS 104-43-8 and CAS 27193-86-6, respectively. IC_{50} values for DIO2 were 74.2 µM and 84.2 µM for CAS 104-43-8 and CAS 27193-86-6, respectively. IC_{50} values for DIO3 were 11.2 µM and 15.0 µM for CAS 104-43-8 and CAS 27193-86-6, respectively.

Table 11: Mechanistic *in vitro* studies (OECD level 2).

Method	Results	Remarks	Reference
Androgen receptor (AR) binding assay (rat prostate cytosol) according to **OPPTS**	**Phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation:**	1 (reliable without restriction)	(Thomas, 2012a)

(Office of Prevention, Pesticides and Toxic Substances) **890:1150** **GLP compliance** Competition of phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation with 3H-R1881 for AR binding sites in rat prostate cytosol Tissue source: pooled prostate tissue from castrated male Crl:CD(SD) rats Positive control: R1881 Weak positive control: dexamethasone	IC50 = 92 µM RBA = 0.0016 % **Positive control (R1881):** IC50 = 1.44 nM RBA set to 100 % **Weak positive control (dexamethasone):** IC50 = 74 µM RBA = 0.002 %	Test material: Phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation (CAS 121158-58-5) purity: 100 %	
ER binding assay (rat uterine cytosol) according to **OPPTS 890:1250** **GLP compliance** Competition of TPP with 3H-17β-oestradiol (E2) for ER binding sites in rat uterine cytosol Tissue source: pooled uterine tissue from ovariectomised female Crl:CD(SD) rats Positive control: 17β-oestradiol (E2) Weak positive control: 19-norethindrone	**Tetrapropenyl phenol:** IC50 = 1.1 µM RBA = 0.11 % **Positive control (17β-oestradiol (E2)):** IC50 = 1.2 nM RBA set to 100 % **Weak positive control (19-norethindrone):** IC50 = 3.46 µM RBA = 0.034 %	1 (reliable without restriction) Test material: Tetrapropenyl phenol (wrong CAS No given in study) CAS of test material is not correctly provided in the registration. The CAS given for the test material erroneously corresponds to E2 (positive control).	(Thomas, 2012b)

Negative control: octyltriethoxysilane	**Negative control (octyltriethoxysilane):** No binding		
ERα receptor binding assay (hERα ligand binding domain fused with GST and expressed in E.coli) Competition of p-dodecylphenol with 3H-E2 **Concentrations of test chemicals: 10 pM to 100 µM**	**p-Dodecylphenol:** IC50 values not given RBA: 0.24 % **Positive control E2:** IC50 values not given RBA set to 100 %	2 (reliable with restriction) Not in the registration Test material: p-Dodecylphenol (CAS 104-43-8), read-across substance	(Akahori et al., 2008)
Oestrogen receptor (ER) binding assay (rat uterine cytosol) Competition of p-dodecylphenol (isomeric mixture) with 3H-E2 **Concentrations: 1 nM to 100 µM**, Positive control: E2 (33 pM – 100 nM)	**p-Dodecylphenol (isomeric mixture):** IC50: 4.85 µM RBA: 0.019 % **Positive control E2** IC50 = 0.899 nM RBA set to 100 %	2 (reliable with restriction) Not in the registration Test material: p-Dodecylphenol (isomeric mixture, purity 99.7 %) CAS not provided	(Blair et al., 2000)
Deiodinase inhibition assays Source of human deiodinases 1, 2, 3 (DIO 1, 2, 3): Cell lysate of adenovirally expressed enzyme in HEK293 cells Sandell-Kolthoff reaction to measure deiodinase-liberated iodide	**Dodecylphenol and 4-Dodecylphenol:** Reported as inhibitors of DIO 1, 2, and 3 **Dio1** IC50:37.2 µM (CAS 104-43-8); 61.4 µM (CAS 27193-86-6) **Dio2** IC50:74.2 µM (CAS 104-43-8); 84.2 µM (CAS 27193-86-6) **Dio3** IC50:11.2 µM (CAS 104-43-8); 15.0 µM (CAS 27193-86-6) **Positive controls**	2 (reliable with restriction) Not in the registration Test materials: 4-Dodecylphenol (CAS 104-43-8), read-across substance Dodecylphenol (CAS 27193-86-6)	(Olker et al., 2018)

	6-Propyl-2-thiouracil (IC50 of 5.4 µM for DIO1) Xanthohumol (IC50 of 0.8 µM and 0.3 µM for DIO 2 and DIO 3, respectively)		

Mechanistic *in vivo* studies (OECD level 3) - Uterotrophic and Hershberger assays

For an overview of mechanistic *in vivo* studies (OECD level 3) see Table 12.

Data of two uterotrophic assays (according to OECD TG 440) each using six ovariectomised female Crl:CD(SD) rats per treatment group are available (Edwards, 2010a; Edwards, 2010b). These assays tested phenol (tetrapropenyl) derivatives (CAS 74499-35-7) (Edwards, 2010a) or purified phenol (tetrapropenyl) derivatives (impurities more polar than TPP were removed chromatographically) (Edwards, 2010b), respectively. Rats were exposed in both studies to doses of 0, 75, 125, 250, 500 mg/kg/day phenol (tetrapropenyl) derivatives (actually ingested) and positive control groups received 0.2 mg/kg/day 17α-ethinyloestradiol (EE2). Wet and blotted mean uterine weights were dose-dependently increased by phenol (tetrapropenyl) derivatives and both assays demonstrated oestrogenic activity of phenol (tetrapropenyl) derivatives already at the lowest dose tested (75 mg/kg/day). The positive control EE2 similarly showed the expected response, however, the magnitude of increase in uterus weight elicited by EE2 was only reported in one of the two uterotrophic assays (Edwards, 2010a). In that case, based on extrapolation from the dose-response curve, the magnitude of increase in wet and blotted mean uterine weights in the positive control group (0.2 mg/kg/day of EE2) would correspond to a dose of approximately 400 mg/kg/day phenol (tetrapropenyl) derivatives. Thus, based on this assay, the oestrogenic activity of phenol (tetrapropenyl) derivatives is about 2000 times (400/0.2) lower than that of EE2.

General toxicity consisted of mortality (one animal at 500 mg/kg/day phenol (tetrapropenyl) derivatives (Edwards, 2010b)) and lower body weights compared to the control were observed in all dose groups. At 500 mg/kg/day phenol (tetrapropenyl) derivatives, body weight was 11.1 % (Edwards, 2010a) and 12.9 % (Edwards, 2010b) lower than the respective controls. However, also the treatment with EE2 resulted in lower mean body weights (10.7 % (Edwards, 2010a), 9.9 % (Edwards, 2010b)). Lower body weights have been frequently observed in other studies with model oestrogens (NTP, 2008a; NTP, 2008b; NTP, 2010a; NTP, 2010b). Therefore, lower body weights due to phenol (tetrapropenyl) derivatives exposure can be explained, at least in part, by its oestrogenicity.

Furthermore, two immature rat uterotrophic assays (Akahori et al., 2008; Yamasaki et al., 2003) according to OECD TG 440 are available for p-dodecylphenol (read-across, CAS 104-43-8). In the immature rat uterotrophic assay by Akahori et al. (2008), 65 chemicals were tested. 20-day old female rats were assigned to six rats per group. Three doses (single doses not specified) of p-dodecylphenol (read-across, CAS 104-43-8) up to 1000 mg/kg/day (examining the oestrogenic effect) alone or in combination with 0.6 µg/kg/day EE2 (examining the antioestrogenic effect) were administered at three consecutive days. The vehicle control group received olive oil and the positive control group received 0.6 µg/kg/day of EE2. The LED (lowest effective dose) of p-dodecylphenol (read-across, CAS 104-43-8) inducing a significant increase in uterine weight was 40 mg/kg/day. No antioestrogenic effect was observed when rats where co-exposed to EE2. No information about general toxicity was provided.

The study by Yamasaki et al. (2003) investigated chemicals in an uterotrophic and a Hershberger assay. In the uterotrophic assay the dosage was 8, 40 and 200 mg/kg/day p-dodecylphenol (read-across, CAS 104-43-8) (examining the oestrogenic effect) or 8,

40, 200 mg/kg/day p-dodecylphenol (read-across, CAS 104-43-8) and 0.6 µg/kg/day EE2 (examining the antioestrogenic effect). Compared to the control, the absolute and relative uterine weight was significantly increased at ≥ 40 mg/kg/day p-dodecylphenol (read-across, CAS 104-43-8). No antioestrogenic activity was observed when rats where coexposed to EE2. No clinical signs of general toxicity were reported. The body weight was slightly but not significantly decreased by p-dodecylphenol (read-across, CAS 104-43-8) at the highest dose.

In the Hershberger assay from Yamasaki et al. (2003), the dosage was 10, 30, 100 mg/kg/day p-dodecylphenol (read-across, CAS 104-43-8) (examining the androgenic effect) or 10, 30, 100 mg/kg/day p-dodecylphenol (read-across, CAS 104-43-8) and 0.2 mg/kg/day testosterone propionate (TP, examining the antiandrogenic effect). In the androgenic part of the assay, a decrease in the weight of the bulbocavernosus/levator ani muscle (BC/LA) at a dose of 100 mg/kg/day was observed. However, since there was no dose response, no effects on other androgen-sensitive organ weights and no effects in the antiandrogenic part of the test, the Hershberger assay is considered negative. Regarding signs of general toxicity, no clinical abnormalities were detected. The body weight was slightly but not significantly decreased by p-dodecylphenol (read-across, CAS 104-43-8) at the highest dose.

Table 12: Mechanistic *in vivo* studies (OECD level 3) - Uterotrophic and Hershberger assays.

Method	Results	Remarks	Reference
Uterotrophic assay (OECD TG 440) **GLP compliance** Six ovariectomised female Crl:CD(SD) rats per group **0, 75, 125, 250, 500 mg/kg/day** Positive control: 0.2 mg/kg/day EE2 Exposure: oral gavage; once daily during study days 0-2 (three doses)	**Endocrine mediated toxicity** **TPP:** Dose-dependent increases in wet (↑177-508 %) and blotted (↑184-251 %) mean uterine weights at all dose levels **Positive control (EE2):** Increase in wet and blotted mean uterine weight (magnitude not reported) No macroscopic internal findings in the uterus at any dose **General toxicity** One animal in the 500 mg/kg/day group died Lower mean body weight in the 500 mg/kg/day group (↓12.9 %; sign.) compared to the controls **EE2:** ↓9.9 % lower mean body weight compared to the controls	1 (reliable without restriction) not in the registration Test material: TPP (CAS most likely the same as in Edwards et al., 2010a) not as manufactured: impurities more polar than TPP were removed chromatographically.	(Edwards, 2010b)

Uterotrophic assay (according to OECD draft guideline TG 440) **GLP compliance** Six immature female Crl:CD (SD) IGS rats (19 days old) per group Three doses (not specified) up to 1000 mg/kg/day or three doses + EE2 (0.6 µg/kg/day) Exposure: subcutaneous injections of the test chemical into the back for three consecutive days (4 mL/kg)	**Endocrine mediated toxicity** **p-Dodecylphenol:** sign. uterine weight increase No antioestrogenic activity of p-dodecylphenol was detected when co-exposed with EE2 Lowest effective dose (LED): 151 µmol/kg/day (40 mg/kg/day); logLED: 2.18 µmol/kg/day **General toxicity** Not reported	2 (reliable with restriction) not in the registration test material: p-Dodecylphenol (CAS 104-43-8), read-across substance Purity > 95 %	(Akahori et al., 2008)
Uterotrophic assay (according to OECD TG 440) **GLP compliance** Six immature female Crj:CD (SD) rats (PND 19) per group **0, 8, 40, 200 mg/kg/day** or **0, 8, 40, 200 mg/kg/day + EE2 (0.6 µg/kg/day)** Exposure: subcutaneous injections of the test chemical into the back for three consecutive days (2 mL/kg)	**Endocrine mediated toxicity** **p-Dodecylphenol:** Dose-dependent increases in blotted uterine weight at 40 and 200 mg/kg/day (absolute and relative) No antioestrogenic activity of p-dodecylphenol was detected when co-exposed with EE2 **Positive control (EE2)** 0.6 mg/kg/day: increase in blotted uterine weight (absolute and relative) **Tamoxifen** (1 mg/kg/day): mitigation of EE2-induced increase in uterine weight **General toxicity** No clinical abnormalities observed Lower mean body weight in the 200 mg/kg/day group (↓3.9 %;	1 (reliable without restriction) not in the registration test material: p-Dodecylphenol (CAS 104-43-8), read-across substance purity unknown	(Yamasaki et al., 2003)

	not sign.) compared to the controls **EE2**: lower mean body weight (↓4.6 %) compared to the controls		
Hershberger assay (OECD TG 441) **GLP compliance** Six castrated male Brl Han: WIST Jcl (GALAS) rats per group **0, 10, 30, 100 mg/kg/day** or **0, 10, 30, 100 mg/kg/day + testosterone propionate (TP; 0.2 mg/kg/day)** Test substance orally administered by gavage for 10 consecutive days beginning at PND 56 TP was administered by subcutaneous injections into the back	**p-Dodecylphenol:** No androgenic or antiandrogenic effects Decreased weight of the bulbocavernosus/levator ani muscle (BC/LA) at 100 mg/kg/day of p-dodecylphenol (alone); toxicological relevance unclear since no other androgen-dependent organ weights were affected. Test considered negative **Positive control (TP)** 0.2 mg/kg per day: increased weight of ventral prostate, seminal vesicle, BC/LA, glans penis, cowper's gland **Flutamide** (10 mg/kg/day): mitigation of TP-induced weight increases of androgen-dependent organs **General toxicity** No clinical abnormalities observed Lower mean body weight in the 100 mg/kg/day group (↓8.8 %; not sign.) compared to the controls	1 (reliable without restriction) not in the registration p-Dodecylphenol (CAS 104-43-8), read-across substance purity unknown	(Yamasaki et al., 2003)

Mechanistic *in vivo* studies (OECD level 4) - female pubertal assays

Four female pubertal assays (similar to OPPTS 890.1450; see Table 13) were performed using 15 immature female rats per dose (3-4 doses per experiment). Test substances were either phenol (tetrapropenyl) derivatives (CAS 74499-35-7) (Knapp, 2009b), distilled phenol (tetrapropenyl) derivatives (enriched C12 homologues > 85 %) (Knapp, 2009a) or calcium salt of TPP (Knapp, 2007a; Knapp, 2007b). Doses tested in these studies ranged from 10 to 1000 mg/kg/day. Combined results of the four studies indicate oestrogenic activity of phenol (tetrapropenyl) derivatives starting at doses of 50 mg/kg/day. The most diagnostic parameters for an oestrogenic mode of action were an earlier attainment of vaginal patency (≥ 50 mg/kg/day), earlier first oestrus (≥ 60 mg/kg/day), oestrus cycle disturbances (≥ 50 mg/kg/day), reduced ovary weight (≥ 50 mg/kg/day) and absence or reduction of the number of corpora lutea (≥ 200 mg/kg/day). Two studies reported granulosa cell necrosis and oocyte degeneration (severity dose dependent; ≥ 200 mg/kg/day) (Knapp, 2009a; Knapp, 2009b). LH and E2

were measured in two of the pubertal assays (Knapp, 2009a; Knapp, 2009b). For LH, no differences were detected in both studies, whereas E2 was significantly increased at 800 mg/kg/day in one study (Knapp, 2009a). Reduced uterus weight accompanied by hypoplasia/atrophy was observed consistently in all pubertal assays. This finding seems contrary to an oestrogenic response. However, uterus weights and histology have to be considered in the context of the cycling status. Since at termination, rats were not matched by oestrus stage, a different distribution of oestrous stages between control and treatment groups might explain the observed lower uterus weights. Similarly, oestrous stage at the time of blood sampling has significant influence on the levels of reproductive hormones. In order to detect substance-induced changes of E2 and luteinizing hormone (LH), animals at the same oestrous stage should be compared, preferable in di-oestrus (Biegel et al., 1998a; Goldman et al., 2000). Since no individual data are available and hormone levels were not grouped according to oestrous stage, the data regarding E2 and LH are not informative.

Apart from an oestrogenic response, some interaction of phenol (tetrapropenyl) derivatives with the thyroid hormone system was evident. Knapp (2007b) reported a dose-dependent increase in the incidence of thyroid hypertrophy at ≥20 mg/kg/day. Thyroid hypertrophy and colloid depletion was also noted at 800 mg/kg/day (3/15 animals) in Knapp (2009b) and at ≥ 200 mg/kg/day (lower doses were not investigated) in Knapp (2009a). Thyroid-stimulating hormone (TSH) and thyroxine (T4) levels were measured in three of the four pubertal assays (Knapp, 2007b; Knapp, 2009a; Knapp, 2009b). For T4, no differences were detected in any study, whereas TSH was significantly increased at 800 mg/kg/day in one out of three pubertal assays which measured this parameter (Knapp, 2009a).

General toxicity was observed starting at 200 mg/kg/day as indicated by lower body weights, and mortality occurred at ≥ 800 mg/kg/day (Knapp, 2007a; Knapp, 2009a; Knapp, 2009b). Increased weights (absolute and relative to body weight) of liver (≥ 250 mg/kg/day) and decreased weights (absolute and relative to body weight) of thymus (≥ 200 mg/kg/day) and spleen (800 mg/kg/day) were observed in two studies (Knapp, 2009a; Knapp, 2009b). Furthermore, in one study, an increased weight (absolute and relative to body weight) of the adrenals was reported at ≥ 60 mg/kg/day (Knapp, 2007a).

Table 13: Mechanistic *in vivo* studies (OECD level 4) - female pubertal assays.

Method	Results	Remarks	Reference
Female puberty assay similar to **OPPTS 890.1450** 15 immature female Crl:CD (SD) rats per group **0, 5, 20, and 60 mg/kg/day** Exposure: oral gavage once daily for 20 consecutive days, postnatal days 22-41	**Endocrine mediated toxicity** Earlier vaginal opening and decreased body weight at time of vaginal opening (60 mg/kg/day; sign.) Decreased number of corpora lutea (≥ 20 mg/kg/day) Uterine hypoplasia (60 mg/kg/day) Thyroid hypertrophy (≥ 20 mg/kg/day)	1 (reliable without restriction) Not in the registration Test material: Calcium salt of TPP (no CAS given)	(Knapp, 2007b)

	No changes in thyroxine (T4) and thyroid-stimulating hormone (TSH) levels **General toxicity** No significant effects on mean body weights or body weight gains no changes in organ weights (liver, kidneys, adrenal glands, uterus, ovaries, pituitary, thyroid)		
Female puberty assay according to **OPPTS 890.1450** **GLP compliance** 15 immature female Crl:CD (SD) rats per group **0, 10, 50, 200, and 800 mg/kg/day** Exposure: oral gavage once daily for 20 consecutive days, postnatal days 22-41	**Endocrine mediated toxicity** Earlier vaginal opening and decreased body weight at time of vaginal opening (≥ 50 mg/kg/day; sign.) Younger age at first oestrus (dose-dependent; sign. at 800 mg/kg/day) Increased number of females in permanent di-oestrus or oestrus (≥ 50 mg/kg/day) Oestrus cycle length not changed (determined only for a limited number of animals) Decreased uterine weight (relative and/or absolute; wet and/or blotted) (sign. ≥ 10 mg/kg/day) Uterus atrophy (severity dose dependent; ≥ 200 mg/kg/day) Cervix atrophy (800 mg/kg/day) Decreased absolute and/or relative ovarian/oviduct weight (sig. ≥ 10 mg/kg/day) Decreased number of corpora lutea (dose-dependent; ≥ 200 mg/kg/day); complete absence in all animals at 800 mg/kg/day Granulosa cell necrosis and oocyte degeneration (severity dose dependent; ≥ 200 mg/kg/day)	1 (reliable without restriction) Test material: phenol (tetrapropenyl) derivatives (CAS 74499-35-7) Purity: 100 % distilled, laboratory-enriched C12 homologs > 85 %; not as commercially manufactured	(Knapp, 2009a) Due to their young age, the number of females with incomplete cycles was high. Therefore, oestrus cycle length was determined only for a limited number of animals. Histology was done only for the two highest dose groups.

	Decreased absolute pituitary weight (dose dependent; sign. at 800 mg/kg/day) E2 and TSH levels sign. increased at 800 mg/kg/day Luteinizing hormone(LH) and T4 levels unchanged Colloid depletion and hypertrophy of the thyroid at ≥ 200 mg/kg/day (lower dose groups not investigated); ectopic thymus-tissue (1/15 animals at 200 mg/kg/day and 8/15 animals at 800 mg/kg/day) **General toxicity** Clinical signs: Brown material on various body surfaces (800 mg/kg/day). Clear material around the mouth; and salivation (≥ 200 mg/kg/day) 11/15 females in the 800 mg/kg/day group died after two to six days of exposure Lower body weight gain (PND 22-42; not sign.) and body weight (↓5.0-9.8 %; PND 34-41 sign.) in the 200 mg/kg/day group Lower body weight gain (PND 28-42; not sign.) and body weight (↓9.7-24.5 %; PND 24-41; sign.) in the 800 mg/kg/day group Increased absolute and relative liver weights (800 mg/kg/day) Decreased absolute and relative weights of spleen (dose-dependent; sign. at 800 mg/kg/day) and thymus (dose-dependent; sign. at ≥ 200 mg/kg/day)		
Female puberty assay according to **OPPTS 890.1450** **GLP compliance**	**Endocrine mediated toxicity** Earlier vaginal opening and decreased body weight at time of vaginal opening (dose-	1 (reliable without restriction) Test material: phenol (tetrapropenyl)	(Knapp, 2009b) Due to their young age, the

15 immature female Crl:CD (SD) rats per group **0, 10, 50, 200, and 800 mg/kg/day** Exposure: oral gavage once daily for 20 consecutive days, postnatal days 22-41	dependent; ≥ 50 mg/kg/day; sign.) Younger age at first oestrus (dose-dependent; sign. ≥ 200 mg/kg/day) Increased number of females in permanent oestrus (≥ 200 mg/kg/day) Oestrus cycle length not changed (determined only for a limited number of animals) Decreased uterine weight (relative and absolute; wet and blotted; dose-dependent; ≥ 200 mg/kg/day; sign.) Uterus atrophy (800 mg/kg/day; only in animals found dead) Decreased absolute and relative ovarian/oviduct weight (dose-dependent; sig. ≥ 50 mg/kg/day) Absence of corpora lutea (dose-dependent; ≥ 200 mg/kg/day) Granulosa cell necrosis and oocyte degeneration (severity dose dependent; ≥ 200 mg/kg/day) E2, LH, T4, and TSH levels unchanged Thyroid hypertrophy at 800 mg/kg/day (3/15 animals); ectopic thymus-tissue with focal necrosis in thyroid (1 animal found dead/15); minimal focal lymphocytic infiltration in the thyroid (1/15 animals) **General toxicity** Clinical signs: Brown material on various body surfaces (800 mg/kg/day). Clear material around the mouth; and salivation (≥ 200 mg/kg/day) 8/15 females in the 800 mg/kg/day group died after one to four days of exposure.	derivatives (CAS 74499-35-7)	number of females with incomplete cycles was high. Therefore, oestrus cycle length was determined only for a limited number of animals. Due to high mortality in the 800 mg/kg/day group, no statistical analysis Histology was done only for the two highest dose groups

	Reduced body weight gain (PND 22-42; not sign.) and body weight (PND 35-42; ↓5.5-8.8 %; not sign.) in the 200 mg/kg/day group Lower body weight gain (PND 27-28; sign.) and body weight (↓9.7-16.1 %; PND 24-40; 800 mg/kg/day; sign.) Increased absolute and relative liver weights (800 mg/kg/day) Decreased absolute and relative weights of spleen (dose-dependent; sign. at 800 mg/kg/day) and thymus (dose-dependent; sign. ≥ 200 mg/kg/day)		

Repeated-dose toxicity studies (OECD level 4 with restrictions due to old protocols)

Several guideline and non-guideline studies investigating repeated-dose toxicity in rats (Haas, 2012; Harriman, 2004; Reyna, 1988; Vogin, 1970) and one study in dogs (Vogin, 1970) are available for tetrapropenyl phenol, phenol (tetrapropenyl) derivatives or phenol, dodecyl (see Table 14). The guideline studies (OECD TG 407 and 408) were conducted before they were updated with additional sensitive parameters to detect endocrine disruption. Nevertheless, some of the parameters (e.g. weight of reproductive organs and histology) provide relevant information on potential endocrine modes of action of the test substance.

Combined results of the repeated-dose toxicity studies in rats demonstrate changes of female reproductive organs which are indicative of an endocrine mode of action of tetrapropenyl phenol. In two out of three studies which investigated this parameter, decreased ovarian weight starting at doses of 100 - 200 mg/kg/day was observed (Haas, 2012; Harriman, 2004). Reduced numbers of corpora lutea were reported at doses ≥ 150 mg/kg/day in Haas (2012) and at 180 mg/kg/day in Harriman (2004). Decreased uterus weight without histological changes was reported by Haas (2012) at doses ≥ 150 mg/kg/day.

In male rats, decreased weight of testes and sexual accessory organs were evident in several studies at doses ≥ 180 mg/kg/day (Haas, 2012; Harriman, 2004; Reyna, 1988; Vogin, 1970), although organ weight reductions could sometimes be explained by reduced body weights (Haas, 2012). Testes histology revealed germ cell depletion (≥ 180 mg/kg/day (Harriman, 2004; Vogin, 1970)) and/or interstitial atrophy (≥ 180 mg/kg/day (Harriman, 2004)), and tubular hypoplasia (300 mg/kg/day (Reyna, 1988; Vogin, 1970)). Histology of male sexual accessory glands revealed decreased secretion of seminal vesicles and prostate (≥ 150 mg/kg/day (Haas, 2012; Harriman, 2004; Reyna, 1988)), hypoplasia/atrophy of the coagulating glands and prostate (≥ 200 mg/kg bw/d (Haas, 2012; Reyna, 1988)), and hypoplasia and hypospermia in the epididymis (300 mg/kg/day (Harriman, 2004; Reyna, 1988)). Furthermore, a dose-dependent increase in the incidence of thyroid hypertrophy was evident in males in one study (≥ 5 mg/kg bw/d (Harriman, 2004)).

General toxicity was observed in all rat studies. Body weight gain and mean body weights were lower in both sexes mostly at doses ≥ 180 mg/kg/day. Increased liver weights were observed at ≥ 200 mg/kg bw/d (Harriman, 2004; Reyna, 1988; Vogin, 1970), accompanied by vacuolization and centrilobular hepatocellular hypertrophy (≥ 200 mg/kg bw/d (Haas, 2012; Harriman, 2004)). Indications for liver cell degeneration or necrosis were neither observed microscopically nor as elevated liver enzyme activities (ALAT, ASAT). Reduced haematocrit, haemoglobin, white blood cells and lymphocytes were observed at ≥ 200 mg/kg bw/d (Haas, 2012; Harriman, 2004). Effects only observed in females included decreased serum cholesterol (≥ 100 mg/kg bw/d (Haas, 2012; Harriman, 2004)). Serum triglycerides were decreased in males and females (≥ 180 mg/kg bw/d (Harriman, 2004)). In males only, a significant increase in absolute and relative adrenal weight was observed at doses ≥ 100 mg/kg bw/d in one study (Haas, 2012) and at ≥ 180 mg/kg bw/d in two studies (Harriman, 2004; Reyna, 1988). Adrenal cortical hypertrophy was reported in males in one study (Haas, 2012).

Interestingly, in a 90-day repeated-dose toxicity study in dogs (3 animals/dose/sex), no general or reproductive toxicity at a dose up to 143 mg/kg/day (Vogin, 1970) was observed. Thus, this study might indicate a species-specific sensitivity of rat towards phenol, dodecyl. However, the focus of this study was on the male reproductive tract and only three dogs per sex were used in each treatment group.

Table 14: Repeated-dose toxicity studies (OECD level 4 with restrictions due to old protocols).

Method	Results	Remarks	Reference
28-day repeated dose toxicity study according to and in part exceeding **OECD TG 407 (1995)** **GLP compliance** Dose-range finding study for a one-generation study (Knapp, 2006) SD Crl:CD IGS BR rats 10 animals/sex in 0 and 300 mg/kg groups; 5/sex/group in other dose groups From the 10 animals/sex in the control and 300 mg/kg group, 5 animals/sex/group were terminated at 28 days whereas 5 animals/sex/group	**Endocrine mediated toxicity** **Females** Reduced ovarian weight (≥ 180 mg/kg/day; dose-dependent) Reduced corpora lutea (≥ 180 mg/kg/day) **Males** Decreased absolute testes weight and ratios relative to brain or body weights (300 mg/kg/day) Germ cell depletion (300 mg/kg/day) and/or interstitial cell atrophy (≥ 180 mg/kg/day) Decreased absolute weight and ratios relative to brain or body weights of coagulating gland, epididymidis, prostate, seminal vesicles (dose-dependent; ≥180 mg/kg/day; sign.);	1 (reliable without restriction) Test material: tetrapropenyl phenol (CAS 74499-35-7) 100 % purity	(Harriman, 2004) Old OECD TG407 protocol; contains none of the endocrine parameters which have been added in the revised versions from 1998 and 2008

were assigned to a 14-day recovery period; **0, 5, 20, 60, 180 and 300 mg/kg/day** Exposure: oral (gavage) 7 days a week for 28 days	substantially more affected than terminal body weight Hypospermia and cellular luminal debris in the epididymides (300 mg/kg/day) Decreased secretion of prostate and seminal vesicles (≥ 180 mg/kg/day; sign.) thyroid hypertrophy (≥ 5 mg/kg/day; incidence dose-dependent) **General toxicity** **Females** Reduced body weight gain and lower body weight (not sign.) Increased liver weight (300 mg/kg/day; sign.) centrilobular hepatocellular hypertrophy and periportal hepatocellular vacuolisation (≥ 180 mg/kg/day) ALP and ASAT unchanged, GGT slightly elevated at ≥ 180 mg/kg decreased haematocrit and haemoglobin (≥ 180 mg/kg/day, dose-dependent, females only) decreased serum cholesterol, and increased serum triglycerides (≥ 180 mg/kg/day; dose-dependent) **Males** Reduced body weight gain and lower body weight (↓10 % and ↓13 % at 180 and 300 mg/kg/day, sign. respectively) Increased liver weight (300 mg/kg/day; sign.) Centrilobular hepatocellular hypertrophy and periportal hepatocellular vacuolization (≥ 180 mg/kg/day)		

	ALP and GGT unchanged; ASAT decreased at 300 mg/kg/day Increased absolute and relative adrenal weight (≥ 180 mg/kg/day) Decreased absolute weight of the heart and ratio relative to brain (≥ 180 mg/kg/day)		
28-day repeated dose toxicity study comparable to **OECD TG 407 (1981)** **GLP compliance** 10 Sprague-Dawley rats per sex and dose 0, 500, 2500 and 5000 ppm in the diet (nominal in diet) corresponding to approximately **0, 40, 180 and 300 mg/kg bw/day** Exposure: 28 day (7 days/week)	**Endocrine mediated toxicity** **Females** No ovarian weight determined Unclear whether histology of reproductive organs was performed **Males** Small or atrophic prostate, seminal vesicles, and testes; abnormally soft testes (300 mg/kg bw/d; 8/10 males and confirmed histologically in 7 of these 8 animals) Histological findings in testes (tubular hypoplasia); seminal vesicles (no secretion); prostate (decreased secretion, hypoplasia), epididymis (hypoplasia, decreased/absent sperm); all at 300 mg/kg bw/d **General toxicity** **Females** Reduced food intake (≥ 180 mg/kg bw/d; ↓26.9 % at 300 mg/kg bw/d) Reduced body weight gain and lower body weight (≥ 180 mg/kg bw/d; ↓9.6 % at 300 mg/kg bw/d) Increased liver weight; absolute and rel. to body weight (300 mg/kg bw/d) Unusual urine colour (blood-like appearance; (≥ 180 mg/kg bw/d)	2 (reliable with restrictions) Test material: Phenol, (tetrapropenyl) derivatives (CAS 27193-86-8) Purity unknown	(Reyna, 1988) Organ weights: adrenals, brain, kidneys, liver, spleen, testes with epididymides; no ovarian weight was determined Laboratory only reported those findings where organs were statistically different from control as both absolute weights and organ weight/body weight ratios A functional observational battery for neurotoxicity was not performed since this test was not part of the OECD 407 guideline at the time the study was performed

	Increased blood urea nitrogen, increased GGT, and decreased ALAT (all at 300 mg/kg bw/d) Splenic congestion and/or bone marrow hypoplasia of minimal severity (300 mg/kg bw/d) **Males** Reduced food intake (≥ 180 mg/kg bw/d; ↓49.3 % at 300 mg/kg bw/d) Reduced body weight gain and lower body weight (≥ 180 mg/kg bw/d; ↓32.4 % at 300 mg/kg bw/d) Unusual urine colour (blood-like appearance; (300 mg/kg bw/d) Increased absolute and rel. weight of adrenals (300 mg/kg bw/d) Increased blood urea nitrogen (300 mg/kg bw/d), increased chloride (≥ 180 mg/kg bw/d), decreased ASAT (300 mg/kg bw/d), decreased ALAT (300 mg/kg bw/d) Reduced reticulocytes (≥ 180 mg/kg bw/d) Splenic congestion and/or bone marrow hypoplasia of minimal severity (300 mg/kg bw/d)		Old OECD TG407 protocol; contains none of the endocrine parameters which have been added in the revised versions from 1998 and 2008
90-day repeated dose toxicity study (non-guideline) 20 Albino FDRL rats per sex and group **0, 25, 100 and 200 mg/kg/day** Exposure: oral (feed) 7 days/week for 90 days	**Endocrine mediated toxicity** **Females** No effect on ovary weight; no microscopic findings **Males** Reduced absolute and relative testes weights at 200 mg/kg/day (↓23 %) Testicular hypospermia (6/20 animals) at 200 mg/kg/day **General toxicity** **Females**	2 (reliable with restrictions) Test material: Phenol, dodecyl (CAS 27193-86-8) Purity unknown	(Vogin, 1970) No analytical confirmation of dietary concentrations Chronic bronchitis and chronic inflammation of kidneys in all dose groups

	Reduced body weight gain and lower body weight at 200 mg/kg/day (↓11 %; not sign.) Increased liver weight relative to body weight at 200 mg/kg/day; no histological findings **Males** Reduced body weight gain and lower body weight at 200 mg/kg/day (↓18.4 %; not sign.) Increased liver weight relative to body weight at 200 mg/kg/day; no histological findings		
90-day repeated dose toxicity study (non-guideline) Three Beagle dogs per sex and group **0, 18, 71 and 143 mg/kg/day** Exposure: oral, treated feed was available 1 h/day, 6 days/week for 13 weeks	No general toxicity and no effects on reproductive organs observed	2 (reliable with restrictions) Test material: Phenol, dodecyl (CAS 27193-86-8) Purity unknown	(Vogin, 1970)

Reproductive and developmental toxicity studies (OECD level 4 and 5; with restrictions due to old protocols)

One prenatal developmental toxicity study according to OECD TG 414 (Schroeder, 1987), as well as a one- and a two-generation reproductive toxicity study (Edwards, 2012; Knapp, 2006) according to OECD TG 415 and OECD TG 416, respectively, are available for phenol (tetrapropenyl) derivatives (see Table 15). The prenatal developmental toxicity study by Schroeder (1987) was performed according to the outdated protocol of OECD TG 414 from 1981, and no parameters diagnostic for endocrine disruption (regarding oestrogenic/androgenic/thyroid/steroidogenic (EATS) modalities) were examined. Increased resorptions and reduced litter size as reported in this study at 300 mg/kg /day phenol (tetrapropenyl) derivatives could be due to an endocrine mode of action affecting the reproductive hormone axis. Lower body weight gain of dams during pregnancy was evident at this dose level but body weight at day 20 was only 8 % lower than control. This does not explain the effects of phenol (tetrapropenyl) derivatives on resorptions and reduced litter size.

One-generation reproductive toxicity study (Knapp, 2006)

In the one-generation reproductive toxicity study (OECD TG 415) by Knapp (2006), phenol (tetrapropenyl) derivatives (CAS 74499-35-7) was administered to SD Crl:CD rats of the parental generation (30 males and 30 females) in doses of 0 (corn oil vehicle), 5, 25 or 125 mg/kg/day by oral gavage. Dosing was initiated 73 days prior to mating and continued throughout mating, gestation and lactation. Parental (F0) animals were terminated on weaning of the F1 litters at PND 21. F1 pups were sacrificed at weaning or were selected (four per sex and dose if possible) for the assessment of developmental landmarks (time of vaginal opening in females at PND 25, and balano-preputial separation in males at PND 35).

Significant and marked reductions in copulation index and fertility index were observed at 125 mg/kg/day. Only 4/30 females with evidence of copulation became pregnant (control 28/30). Mean litter size at 125 mg/kg/day was 1.7 pups per litter compared to 13 pups per litter in controls. Furthermore, several effects of phenol (tetrapropenyl) derivatives on parameters sensitive for an endocrine mode of action were affected. These included changes in the weight of reproductive organs accompanied by histopathological findings, and oestrus cycle disturbances. In females ovarian weight (absolute and rel. to brain and/or body weight; ≥ 25 mg/kg bw/d) was decreased. Microscopic evaluation revealed a decreased number of corpora lutea and an increase of ovarian cysts at 125 mg/kg/day. Absolute uterus weight was not significantly changed at any dose level.
At 125 mg/kg/day, uterus weight relative to brain weight was significantly increased and an increase of endometrial cysts was detected microscopically. Oestrus cycle length tended to be longer with increasing dose and a higher number of females were in permanent di-oestrus or oestrus at termination (125 mg/kg/day). In males, absolute and relative (to body weight and/or brain) weights of the cauda epididymidis, epididymidis, prostate, and seminal vesicles were significantly lower at ≥ 25 mg/kg bw/d (at 125 mg/kg/day significant for testes). Further findings in males included reduced epididymal sperm concentrations (≥ 25 mg/kg/day), and decreased secretion of prostate (≥ 5 mg/kg/day), coagulating glands (≥ 25 mg/kg/day), and seminal vesicles (125 mg/kg/day). In female offspring, time to vaginal opening was not affected by exposure of dams during gestation and lactation (note that offspring was not exposed post-weaning). In male offspring, balano-preputial separation was delayed (≥ 25 mg/kg/day). However, balano-preputial separation was not associated with higher mean body weight and the timing was within the historical control range of the lab.

Signs of general toxicity were evident in both sexes by lower pre-mating weight gain and lower pre-mating body weight as well as lower terminal body weight. Kidney weight relative to body weight was increased in males (≥ 25 mg/kg/day) and females (≥ 125 mg/kg/day) accompanied by kidney mineralisation (≥ 25 mg/kg/day in males and at 125 mg/kg/day in females). In males only, increased liver weight (relative to body weight) without histological findings (125 mg/kg/day), and increased weight of the adrenals (relative to body weight and brain weight; ≥ 25 mg/kg/day) with adrenocortical hypertrophy (125 mg/kg/day) was reported. F1 offspring at 25 mg/kg/day showed statistically significantly reduced pre-weaning body weight gain compared to controls between PND 4-21. Postnatal survival from birth to PND 0 and birth to PND 4 (each 55.6 % per litter) was reduced when this group was compared to the control group (96.6 % and 95.7 %, respectively). These parameters were not statistically evaluated in the 125 mg/kg/day group due to the small sample size.

Two-generation reproductive toxicity study (Edwards, 2012)

In the two-generation reproductive toxicity study (OECD TG 416; 2001) by Edwards (2012), phenol (tetrapropenyl) derivatives (CAS 27193-86-8) was administered to SD Crl:CD rats of the parental generation (30 males and 30 females) in doses of 0, 1.5, 15, and 75 mg/kg/day by dietary exposure. Premating exposure lasted for a minimum of 70 consecutive days. F0 and F1 males were dosed throughout mating and through the day of euthanasia. F0 and F1 females were dosed throughout mating, gestation, and lactation through the day of euthanasia. Fertility of F1 adults was low in all treatment groups (including the controls). Therefore, the F1 adults were re-bred to produce second litters.

The first litters from the F1 adults were referred to as the "F2 litters" while the second litters from these adults were referred to as the "F2a litters".

The number of pups born and live litter size was lower in the F1 and F2 generations at 75 mg/kg/day compared to the controls but this did not reach statistical significance. On the other hand, in the F2a generation, the number of pups born (13.4 vs 10.1 in controls) and live litter size (13.4 vs 9.5 in controls) was significantly reduced at 75 mg/kg/day. The number of implantation sites was significantly reduced in the F0 adults at 75 mg/kg/day (no investigation of implantation sites in F1 dams due to multiple gestations). Furthermore, effects on endocrine sensitive parameters in adult females of the F0 and F1 generation at 75 mg/kg/day comprised reduced ovary weight (absolute and relative to body weight/brain weight) and reduced numbers of corpora lutea (without data on staging), increased length of the oestrous cycle, and oestrous cycle irregularities (higher number of females in persistent oestrus or dioestrus). Time to vaginal opening was decreased and occurred at a lower body weight in the F1 offspring at 75 mg/kg/day (27.4 days versus 32.4 days in controls).

Epididymal sperm count was significantly reduced at 75 mg/kg/day in the F0 but not in the F1 adult males. In F0 and F1 males, changes in the absolute weight and in organ-to-brain weight ratios (mostly decreases, sometimes increases in F1 males) of several reproductive/accessory reproductive organs were observed at 75 mg/kg/day. These included prostate, seminal vesicles as well as left and/or right testes, epididymides, and cauda epididymides. However, these effects occurred together with lower body weights. When organ to body weight ratios were compared, there were only a few statistically significant decreases (seminal vesicles and prostate in F0 males). No histological findings in male reproductive/accessory reproductive organs were reported. Balano-preputial separation was significantly delayed in F1 males (75 mg/kg/day) but was associated with lower body weight. There was no effect on anogenital distance (AGD) and anogenital distance index (AGDi) in F2 offspring on PND1 (not investigated in F2a).

General toxicity was evident in both sexes of the F0 and F1 generations. Pre-mating weight gain, pre-mating body weight as well as terminal body weight was lower at 75 mg/kg/day. Pup body weight gain and pup body weight was lower in the F1 and F2/F2a generation (mostly at 75 mg/kg/day). Adrenal weight relative to body weight was slightly but significantly increased in both sexes of the F0 and F1 generation (75 mg/kg/day) but no histology was performed. F0 and F1 males showed increased incidences of kidney mineralisation (at 75 mg/kg/day in F0 and at ≥ 15 mg/kg /day in F1).

Relevance of general toxicity

In conclusion, the effects on the female reproductive organs and functional parameters consistent with the oestrogenic mode of action of the test substance could not be attributed to the observed signs of general or non-specific toxicity. Furthermore, a limited degree of lower body weight gain was also seen for EE2 in the uterotrophic assays (Edwards, 2010a; Edwards, 2010b; Yamasaki et al., 2003), or were in some studies identified in parallel with lower food consumption (Reyna, 1988; Schroeder, 1987). Unequivocally, very high doses of phenol (tetrapropenyl) derivatives or the corresponding calcium salt of phenol (tetrapropenyl) derivatives cause general toxicity (e.g. mortalities at 800/1000 mg/kg/ bw/d (Knapp, 2007a; Knapp, 2009a; Knapp, 2009b)). Other effects such as reduced weights of spleen and thymus starting to occur at doses of 200 mg/kg or higher (Knapp, 2009a; Knapp, 2009b) may indicate an immunosuppressive effect (which is not specifically known to cause ED effects as observed) that occurred at dose levels higher than those with effects on the reproductive organs/puberty. In addition, as discussed below, several effects of phenol (tetrapropenyl) derivatives that are usually interpreted as general toxicity are also observed in studies with model oestrogens.

Table 15: Reproductive and developmental toxicity studies (OECD level 4 and 5; with restrictions due to old protocols).

Method	Results	Remarks	Reference
Two-generation reproductive toxicity study according to OECD **TG 416** (2001) **GLP compliance** SD Crl:CD rats (30/sex/group for both generations) **0, 1.5, 15 or 75 mg/kg/day** Exposure: oral (diet) F0 animals were exposed for 129-134 consecutive days and F1 animals were exposed for 210-227 consecutive days	**Endocrine mediated toxicity (P)** **Females (P)** Trend for lower number of pups born and live litter size (75 mg/kg/day) but not sign. and close to historical control range Reduced number of implantation sites (↓12 %; 75 mg/kg/day; sig.) Reduced ovary weight at 75 mg/kg/day (↓29.6 %; absolute; sign.). Decreased ovary weight rel. to body weight (↓21.6 %) and rel. to brain weight (↓26 %; 75 mg/kg/day; sig.) Decreased presence of corpora lutea at 75 mg/kg/day (6/28 vs 1/30 in controls; sign.) Increased length of oestrus cycle (75 mg/kg/day; sign.) Increased number of females in permanent diestrus (75 mg/kg/day) Increased ovarian cysts (≥ 15 mg/kg/day; not sign.) **Males (P)** Decreased epididymal sperm concentration (↓26 %; 75 mg/kg/day; sig.) Decreased absolute seminal vesicle weight (↓30 %; 75 mg/kg/day; sig.). Decreased seminal vesicle weight rel. to body weight (↓14.4 %) and rel. to brain weight (↓29 %; 75 mg/kg/day; sign.)	1 (reliable without restriction) Test material: Phenol (tetrapropenyl) derivatives (CAS 74499-35-7) purity 100 %	(Edwards, 2012) Reduced fertility in the F1 generation including the control group. Therefore, F1 adults were re-bred to produce second litters. First litters of F1 animals were called "F2 litters" and second litters were called "F2a litters"

	Decreased absolute prostate weight (↓22 %; 75 mg/kg/day; sig.). Decreased prostate weight rel. to body weight (↓4.3 %; 75 mg/kg/day; not sign.) and rel. to brain weight (↓21 %; 75 mg/kg/day; sign.) Changes (mostly decreases) of absolute weight and/or relative to brain weight were observed for testes, epididymis, and cauda epididymis. However, when related to body weight, organ weights were either unchanged or even increased compared to the control No histopathological findings in reproductive/reproductive accessory organs Increased pituitary weight (absolute and rel. to body weight, and brain weight at 75 mg/kg/day; sign.) **General toxicity (P)** **Females P** Decreased body weight at initiation of mating (↓12.6 %) and at termination (↓12 % at 75 mg/kg/day; sign.) **Males P** Decreased body weight at initiation of mating (↓17.3 %) and at termination (↓18.5 %; 75 mg/kg/day; sign.) Renal mineralisation (75 mg/kg/day) **Endocrine mediated toxicity (F1)** **Females F1** Reduced number of pups born and decreased live litter size at		

	75 mg/kg/day; sign. in F2a, not sign. in F2 Reduced ovary weight at 75 mg/kg/day (↓38 %; absolute; sign.). Decreased ovary weight rel. to body weight (↓19.2 %) and rel. to brain weight (↓35.7 %; 75 mg/kg/day; sig.) Decreased presence of corpora lutea at 75 mg/kg/day (16/26 vs 6/28 in controls; sign.) Increased length of oestrus cycle (75 mg/kg/day; sign.) Increased number of females in permanent oestrus or di-oestrus (75 mg/kg/day) Earlier vaginal patency (75 mg/kg/day; sign.) **Males F1** Changes (mostly decreases) of absolute weight and/or relative to brain weight were observed for several reproductive/accessory reproductive organs. However, when related to body weight, organ weights were either unchanged or even increased compared to the control No histopathological findings in reproductive/reproductive accessory organs Increased pituitary weight (absolute and rel. to body weight and brain weight at 75 mg/kg/day; sign.) Delayed balano-preputial separation (75 mg/kg/day) not associated with higher mean body weight **General toxicity (F1)** **Females F1** Decreased body weight at initiation of mating (1st mating: ↓12.5 %; 2nd mating: ↓18.8 %) and at termination (↓24 %)		

	Higher absolute adrenal weights (75 mg/kg/day) **Males F1** Decreased body weight at initiation of mating (1st mating: ↓22.5 %; 2nd mating: ↓25.9 %) and at termination (↓28.4 %) at 75 mg/kg/day (sign.) Renal mineralisation (≥ 15 mg/kg/day) **Toxicity (F2/F2a pups)** Reduced survival from birth to PND4 (75 mg/kg/day). Lower birth weights and lower body weight gains (75 mg/kg/day) No effects on anogenital distance (AGD) and anogenital distance index (AGD_i) in F2 (in F2a not investigated)		
Prenatal development toxicity study according to **OECD TG 414 (1981)** **GLP compliance** 24 female Sprague-Dawley rats per dose **0, 20, 100 and 300 mg/kg/day** Exposure: oral: (gavage) once/day, from GD 6 – 15 (females only); termination at GD 20 Foetuses were evaluated for external, visceral, and skeletal alterations	**Endocrine sensitive toxicity** No change in number of corpora lutea and implantations Dose-dependent increase in pre-implantation losses (not sign. at any dose) Increased resorptions and reduced litter size (300 mg/kg bw/d; sign.) **General maternal toxicity** Reduced food consumption and weight gain (300 mg/kg bw/d) during treatment, and reduced weight gain on days 15-20 post treatment. Lower body weight at day 20 (300 mg/kg bw/d; ↓8 %; sign.)	1 (reliable without restriction) Test material: phenol (tetrapropenyl derivatives) (CAS 27193-86-8) 100 % purity	(Schroeder, 1987) Old OECD TG 414 protocol, no endocrine-related measurements in the dams and in the foetuses as have been added in the revised version from 2018 An additional group administered 500 mg/kg bw/d was terminated early due to excessive mortality

	Soft stool observed during and after the dosing period (300 mg/kg bw/d) **F1 Toxicity** Lower fetal weight (300 mg/kg bw/d) increased incidence of foetuses with ossification variations at (300 mg/kg bw/d) wavy ribs (22 % at 300 mg/kg bw/d) curved scapula and/or scapular spine; and abnormally shaped long bones (humerus, ulna, radius and femur) (26.1 % at 300 mg/kg bw/d)		

6.11.2. Endocrine disruption – Environment

6.11.2.1. Structural alerts

The alkyl phenols 4-nonylphenol, branched and linear (no EC and CAS number is available for this isomeric mixture), 4-tert-octylphenol (EC 205-426-2) and 4-tert-pentylphenol (EC 201-280-9), 4-tert-butylphenol (EC 202-679-0), as well as phenol, heptyl derivs. (RP-HP, EC not available) were already identified as substances of very high concern for being environmental ED due to their oestrogenic properties. In comparison to PDB, these substances only differ in their alkyl chain length in para-position of the phenol ring. The very similar structure suggests that PDB also acts as an endocrine disruptor.

6.11.2.2. *In vitro* studies

In this section, *in vitro* tests with fish cells/tissues are evaluated with the substance p-dodecylphenol (CAS 104-43-8). In contrast to phenol, dodecyl-, branched (PDB), p-dodecylphenol is supposed to have a linear dodecyl chain. However, the authors of the publications (Tollefsen, 2007; Tollefsen et al., 2008; Tollefsen and Nilsen, 2008) stated that an isomeric mixture of p-dodecylphenol was used. An isomeric mixture of a linear dodecylphenol is supposed to contain also branched dodecylphenol. Therefore, it is assumed that these studies were conducted with PBD.

Read-across:
Only in the study of Knudsen and Pottinger (1999) it is stated that linear alkyl phenols were tested. Therefore, it is assumed that p-n-dodecylphenol was used.

In vitro studies on PDB (including isomeric mixture of p-dodecylphenol), as well as on the read-across substance p-dodecylphenol (with supposed linear dodecyl chain) with mammalian receptors/enzymes are evaluated in section 6.11.1 Endocrine disruption - human health.

Fish *in vitro* tests with isomeric mixture of p-dodecylphenol, (CAS 104-43-8):
Competitive binding studies are available for ER and the plasma sex steroid binding protein (SBP), (Knudsen and Pottinger, 1999; Tollefsen, 2007; Tollefsen and Nilsen,

2008). In addition, there is an oestrogen transactivation assay with rainbow trout hepatocytes (Tollefsen et al., 2008). Results are summarised in Table 11.
For the competitive binding assay (Tollefsen and Nilsen, 2008) with the hepatic rtER an isomeric mixture of p-dodecylphenol was used. The IC_{50} is 22 µM, comparable to the IC_{50} for 4-t-octylphenol (CAS 140-66-9) of 51 µM. Regarding the RBA (compared to 17β-oestradiol (E2)), the potency of p-dodecylphenol is two and 16 times higher than the potencies of 4-tOP and 4-NP, respectively:
RBA of 4-tOP: 0.0069 %
RBA of 4-nP: 0.001 %
RBA of p-dodecylphenol, isomeric mixture: 0.016 %.

For the competitive binding study by (Tollefsen, 2007), on the sex steroid binding protein from rainbow trout (rtSBP), an isomeric mixture of p-dodecylphenol was used. The plasma rtSBP binds sex steroids such as E2 with high affinity. The IC_{50} for competition with the SBP was 320 µM, comparable to the IC_{50} of 4tOP of 120 µM. The RBA of 0.00049 % for p-dodecylphenol was lower than for 4tOP (0.0013 %) but higher compared to 4-NP.

In the transactivation assay by Tollefsen et al. (2008), rainbow trout hepatocytes were used to examine whether an isomeric mixture of p-dodecylphenol binds and activates the ER as measured by the expression of vitellogenin (VTG) protein. Exposure of the cells to p-dodecylphenol (isomeric mixture) alone did not cause VTG elevation up to 300 µM. However, co-exposure with E2 increased the sensitivity of the assay and caused enhanced oestrogenicity of alkyl phenols. Hence, p-dodecylphenol (isomeric mixture) was found to increase VTG production at a LOEC of 10 µM when co-exposed to 0.1 nM E2, the LOEC was 30 µM when co-exposed to 0.3 nM E2. All values were read from graph. Viability of cells was slightly decreased at 10 µM (about 90 % viability of cells) and was further decreased at 30 µM to about 75 % (values read from graph).

Fish *in vitro* tests with the read-across source substance: linear p-dodecylphenol (CAS 104-43-8):
In the competitive binding study with p-n-dodecylphenol (Knudsen and Pottinger, 1999) , where hepatic rtER is used, no effect values were specified but it was stated that 10^4-fold more of the tested alkyl phenols were necessary to obtain the same effect as with E2. All alkyl phenols (amongst others also p-n-dodecylphenol, p-n-nonylphenol, p-n-octylphenol) were in the same range, therefore this also applies for p-dodecylphenol. The RBA estimated from this value is 0.01 %.
In addition, a graphical description is available. Effect values of three concentrations were depicted in a diagram: maximum displacement of 3H-E2 (approx. 60 %, read from graph) by p-n-dodecylphenol appeared at 165 µM (highest concentration tested). Displacement of E2 from rtER was slightly less compared to 4-NP at the two lower concentrations (1.65 and 16.5 µM) and slightly higher at the highest concentration (165 µM). Altogether, displacement was in the same range as for 4-NP and 4-n-octylphenol (CAS 1806-26-4). p-n-dodecylphenol was used without specified purity.

Table 16: *In vitro* assays based on rainbow trout with p-dodecylphenol (CAS 104-43-8).

Mechanistic *in vitro* studies based on rainbow trout (OECD level 2)			
Method	**Results**	**Remarks**	**Reference**
Competitive binding assay, hepatic rainbow trout oestrogen receptor (rtER)	**p-Dodecylphenol (isomeric mixture):** IC_{50}: 22 µM RBA: 0.016 %	p-Dodecylphenol (isomeric mixture) CAS 104-43-8, (purity 96 %)	(Tollefsen and Nilsen, 2008)

	Positive control (17β-oestradiol (E2)): IC_{50}: 3.5 nM RBA set to 100 %	Reliability 2	
Competitive binding assay rainbow trout plasma sex steroid-binding protein (rtSBP)	**p-Dodecylphenol (isomeric mixture):** IC_{50}: 320 µM RBA: 0.0005 % **Positive control (E2):** IC_{50}: 1.6 nM RBA set to 100 %	p-Dodecylphenol (isomeric mixture), (purity 96 %) Reliability 2	(Tollefsen, 2007)
Induction of vitellogenin (VTG) production in rainbow trout hepatocytes	**p-Dodecylphenol (isomeric mixture):** LOEC > 300 µM (without combination with E2) In combination with E2 (0.1 nM): LOEC 10 µM, with E2 (0.3 nM): LOEC 30 µM (values read from graph) Cytotoxicity at 30 µM (25 % decreased viability)	p-Dodecylphenol (isomeric mixture) CAS 104-43-8, (purity 96 %) Reliability 2	(Tollefsen et al., 2008)
Competitive binding assay hepatic rtER	**p-Dodecylphenol (linear):** No effect values given, only graphical description. RBA: 0.01% (estimated), 10^4-fold more p-dodecylphenol was necessary to obtain the same effect as with E2 (estimated by author) Displacement of ^{3}H-oestradiol by p-n-Dodecylphenol almost in the same range as 4-n-nonylphenol	p-n-Dodecylphenol (purity not specified) CAS not provided read-across substance Reliability 2	(Knudsen and Pottinger, 1999)

6.11.3. Conclusion on endocrine disrupting properties

6.11.3.1. Discussion and conclusion on endocrine disruptive properties of PDB regarding human health

Several assays and studies corresponding to levels 2 – 5 of the OECD conceptual framework for testing and assessment of EDs (OECD, 2018) are available for PDB. PDB is classified as Repr. 1B (H360F) as included in the ninth ATP of the CLP Regulation. This

classification implies adverse effects on apical toxicity endpoints concerning fertility. Specifically, according to RAC, the classification is based on following effects observed in experimental studies (ECHA, 2013c; ECHA, 2013d):

- *"Reduced epididymal sperm count and prolongation of oestrous cycle at a dose of 75mg/kg in the two-generation reproductive study in rats (Edwards et al., 2012).*
- *Reduced number of pups born in the F2a generation exposed to a dose of 75mg/kg (Edwards et al., 2012).*
- *Reduced proportion of animals copulating when cohabited, reduced litter size, alterations in number of corpora lutea, prolongation of oestrous cycle and reduced epididymal sperm count in animals exposed at 125 mg/kg in the one-generation study in rats (Knapp, 2006).*
- *Acceleration of sexual maturation in female animals that is reported in the two-generation study and in the female pubertal assays.*
- *The mechanistic information further suggests that TPP has weak estrogenic and androgenic activity."*

RAC further concluded: "*The effects observed in the two-generation and one-generation studies with TPP may be related to an estrogenic action of TPP which has been shown in uterotrophic bioassays in rats (Edwards et al., 2010a and 2010b), and in female pubertal assays in rats (Knapp, 2007a, 2007b, 2009a and 2009b). TPP is also considered as a substance interacting with the ER based on results of the in vitro rat uterine estrogen receptor competitive binding assay (Thomas et al., 2012b). Based on the in vitro rat prostate androgen receptor competitive binding assay (Thomas et al., 2012a) TPP is considered an AR binder. The binding affinity of TPP was similar to the weak positive control, dexamethasone.*"

Considering the results of all available studies, the eMSCA concludes there is strong evidence that the adverse effects on fertility and sexual function (which lead to classification of PDB as Repr. 1B) particularly in females are due to the oestrogenic activity of PDB. The increases of uterus weight (as seen in two uterotrophic assays) and accelerated vaginal opening (as seen in four female pubertal assays and a two-generation study) are highly diagnostic parameters for oestrogenicity. Furthermore, reduced ovary weight, decreased corpora lutea and prolongation of the oestrus cycle was consistently observed in the majority of studies with PDB, and the number of implantations was decreased in both, the one- and the two-generation study. All of these parameters are considered as either EATS-mediated or sensitive to EATS modalities (OECD, 2018), and the direction of their changes is congruent to the effects seen with model oestrogens such as E2 and EE2 at low doses (see Table 17 and (Biegel et al., 1998a; Biegel et al., 1998b; NTP, 2010a; NTP, 2010b)). In cycling females, PDB-induced aberrant oestrus cycles, and decreased uterus weight (sometimes accompanied by hypoplasia/atrophy) was reported in several studies (whereas two studies reported no effect and one study reported increased uterus weight). Reduced uterus weight seems contrary to an oestrogenic response. However, uterus weights and histology have to be considered in the context of the cycling status (Goldman et al., 2000). Since at termination, rats were not matched by oestrus stage, a different distribution of oestrous stages between control and treatment groups leads to high variability and might explain the observed lower uterus weights in some cases. Similarly, oestrous stage at the time of blood sampling has significant influence on the levels of reproductive hormones. This might explain the lack of effects of PDB on LH as determined in two pubertal assays. In order to detect substance-induced changes in LH, generally a high number of animals needs to be investigated and comparison should be done between animals at the same oestrous stage, preferable in dioestrus (Biegel et al., 1998a; Goldman et al., 2000). Since hormone levels were not grouped according to oestrous stage, the data regarding LH (and E2) are not informative. Thus, the absence of any effects on LH does not speak against an oestrogenic mode of action of PDB. Also in males, several effects of PDB on fertility and sexual function were observed, including lower weight of testes and accessory reproductive organs. However, when organ to body weight

ratios were compared, the effects observed in PDB exposed male rats can be in many cases attributed to the lower body weight. Histological findings in males included decreased secretion of seminal vesicles and prostate as well hypoplasia/atrophy of coagulating glands, epididymis and prostate (most of these findings occurred at doses ≥ 150 mg/kg/day). At doses ≥ 180 mg/kg/day, histology of testes revealed germ cell depletion, hypospermia, tubular hypoplasia, and interstitial atrophy. Decreased epididymal sperm count was reported but this parameter was inconsistent between F0 and F1 males in the two-generation study. Nonetheless, the effects of PDB seen on male fertility and sexual function were in general consistent with the effects of model oestrogens where the clear findings similarly occurred at higher doses in males than in females (Biegel et al., 1998b; Cook et al., 1998; NTP, 2010a).

The multitude of effects of PDB in particular on the female reproductive system (e.g. on ovarian weight and histology, oestrous cycle, implantations, etc.), plausibly explain the adverse impacts seen on apical fertility endpoints such as live litters born or litter size. However, most of the affected parameters are also sensitive to non-endocrine toxicity. Several findings were observed which can be interpreted as general toxicity, including reduced body weight gain and lower body weight, increased liver weight (sometimes accompanied by vacuolisation and centrilobular hypertrophy), increased weight of the adrenals (sometimes accompanied by cortical hypertrophy) or changed blood and serum parameters in repeated dose toxicity studies. These findings alone do not indicate an endocrine mode of action of the test substance. However, all of these parameters are sensitive to (but no diagnostic of) EATS-mediated toxicity (OECD, 2018). In fact, several effects of PDB, which are usually interpreted as general toxicity, are also observed in studies with model oestrogens. Very consistently, reduced body weight gain and lower body weight has been reported in rats exposed to E2 or EE2 (e.g. 20-35 % lower body weight after 90 day exposure to 0.5-0.7 mg/kg/day E2 (Biegel et al., 1998b); 10-16 % lower body weight after 90 days exposure to 4-6 µg/kg bw/day EE2 (NTP, 2010a)). Therefore, the lower body weight gains and body weights due to PDB exposure can be explained, at least in part, by the oestrogenicity of PDB. Further effects of E2 and EE2 included increased weights of liver (with centrilobular hypertrophy) and adrenal glands (sometimes with cortical hypertrophy), kidney mineralisation in males and effects on blood and serum parameters (Biegel et al., 1998b; NTP, 2010a). Thus, it is plausible that some of these effects, which also occurred in studies with PDB, are similarly mediated by its oestrogenic activity.

Although receptor binding studies showed weak ER as well as AR binding of PDB, it is concluded by the eMSCA that oestrogenicity is the main endocrine mode of action underlying the observed effects on fertility and sexual function particularly in females due to the following reasons:

1) The RBA of PDB to the ER was higher than to the AR.

2) There are clear and consistent oestrogenic responses in uterotrophic assays and female pubertal assays and changes of further EATS-mediated as well as EATS-sensitive parameters (OECD, 2018) in several studies congruent with the effect pattern of known oestrogens.

3) Antiandrogenic as well as oestrogenic activity could lead to effects similar to the ones observed for PDB on certain reproductive parameters (particularly in males). However, regarding parameters specific for androgenic/antiandrogenic activity, there was no significant effect on AGD in the two-generation study (Edwards, 2012) with phenol (tetrapropenyl) derivatives and a Hershberger assay (with CAS 104-43-8, read-across) was negative (Yamasaki et al., 2003).

There are also indications for interaction of PDB with the thyroid system. *In vitro* assays demonstrated inhibition of deiodinase activity by dodecylphenol (CAS 27193-86-6) and 4-dodecylphenol (CAS 104-43-8, read-across) (Olker et al., 2018). *In vivo*, thyroid hyperplasia and colloid depletion was reported in some studies and TSH was

increased in one female pubertal assay (at 800 mg/kg/day). However, these findings were not consistently observed in all studies which performed thyroid histology. Thus, a potential interaction of PDB with the thyroid system is of lower concern than the oestrogenic activity of PDB.

In summary, there is strong evidence that the adverse effects on fertility and sexual function (which lead to classification of PDB as Repr. 1B) particularly in females are due to the oestrogenic activity of PDB. The eMSCA comes to the conclusion that PDB fulfills the WHO definition (WHO/IPCS, 2002) of an endocrine disruptor (for human health).

Table 17: Comparison between *in vivo* effects of PDB and the expected oestrogenic response as inferred from low-dose studies with potent model oestrogens (EE2 and E2).

Females		
Effects of PDB	**Expected oestrogenic response**	**Remarks**
Increased uterus weight in 4/4 uterotrophic assays	Increased uterus weight in uterotrophic assays (Kanno et al., 2001)	Potency about 2000-fold lower than EE2. Effects on body weights comparable between PDB at 500 mg/kg/day and EE2 (positive control) at 0.2 mg/kg/day
Decreased uterus weight in 4/7 studies with cycling females. One study reported an increase and one study reported no effects	Mostly increases but sometimes also decreases in uterus weight are detected (e.g. in the dose range finding study reported in (NTP, 2010a))	Parameter highly dependent on oestrus stage. Uterus weights in PDB studies with cycling females were not correlated to oestrus stage at termination
Accelerated vaginal opening in females (at lower body weight) in 4/4 female pubertal assays and in the OECD TG 416 study	Accelerated vaginal opening in females at lower body weight (Biegel et al., 1998b; NTP, 2010a)	Parameter highly diagnostic for oestrogenicity. General toxicity and lower body weight usually lead to a delay in vaginal opening.
Increased length of oestrus cycle in 2/3 studies which reliably determined this parameter; observed in the F0 and F1 generations of the OECD TG 416 study	Increased length of oestrus cycle (Biegel et al., 1998b; NTP, 2010a)	Parameter sensitive to oestrogenicity as well as to general toxicity
Irregularities of oestrus cycle (increased number of females in permanent dioestrus or oestrus) in 6/6 studies which investigated/reported this parameter	Irregularities of oestrus cycle; increased number of females in permanent oestrus (most studies) or dioestrus (some studies) (Biegel et al., 1998b; NTP, 2010a)	Parameter sensitive to oestrogenicity as well as to general toxicity

Reduced ovary weight in 7/10 studies; also consistent between F0 and F1 generations of the OECD TG 416 study	Reduced ovary weight (Biegel et al., 1998b; NTP, 2010a)	PDB-induced lower ovary weights cannot be explained by lower body weights or other general toxicity (ECHA, 2013c; ECHA, 2013d)
Decreased corpora lutea in 7/9 studies; also consistent between F0 and F1 generations of the OECD TG 416 study	Decreased corpora lutea (E2 ≥ 0.14-0.17 mg/kg/day; (Biegel et al., 1998b))	Parameter sensitive to oestrogenicity as well as to general toxicity
Decreased implantation sites	Decreased implantation sites (E2 ≥ 0.14-0.17 mg/kg/day; (Biegel et al., 1998b))	Parameter sensitive to oestrogenicity as well as to general toxicity
Decreased fertility/number of pups born/litter size in the one-generation study; weak effect in the two-generation study	Decreased fertility/number of pups born/litter size (E2 ≥ 0.14-0.17 mg/kg/day; (Biegel et al., 1998b))	Parameter sensitive to oestrogenicity as well as to general toxicity
No effect on AGD in the OECD TG 416 study	No clear response in studies with E2 and EE2 (Biegel et al., 1998b; NTP, 2010a)	Lack of effect not contrary to oestrogenic activity
Lower body weight and body weight gain	E2 and EE2 consistently induced lower body weight gain and lower body weight even in trace amounts (Biegel et al., 1998b; NTP, 2010a; NTP, 2010b)	Lower body weight gain and body weight can, at least in part, be explained by the oestrogenic activity of PDB
Increased relative liver weight reported in 6/11 studies at doses ≥ 200 mg/kg/day; sometimes with hepatic centrilobular hypertrophy	Increased relative liver weights with hepatic centrilobular hypertrophy reported (E2: 0.5-0.7 mg/kg bw/day (Biegel et al., 1998b))	Parameter sensitive to oestrogenicity as well as to general toxicity
Males		
Delayed balano-preputial separation at the same or at lower body weight than controls	Delayed balano-preputial separation (Biegel et al., 1998b)	No clear evidence for hormonal effect of PDB due to markedly lower body weights

Decreased weight of testes and sexual accessory organs only at high doses of PDB. Seminal vesicles most sensitive	Depending on dose, decreased weight of testes and accessory reproductive organs (Biegel et al., 1998b; Cook et al., 1998; NTP, 2010a; NTP, 2010b) 50 ppm in NTP	Effects of PDB due to lower body weights or oestrogenicity
Reduced epididymal sperm count in OECD TG 415. Inconsistent between F0 and F1 generations of the OECD TG 416 study	Depending on dose, reduced epididymal sperm count (Cook et al., 1998)	Parameter sensitive to oestrogenicity as well as to general toxicity
Decreased secretions and/or hypoplasia of reproductive accessory organs; histological effects on testes (≥ 200 mg/kg/day)	Depending on dose, decreased secretions and histological effects on reproductive/ reproductive accessory organs (Biegel et al., 1998b; Cook et al., 1998; NTP, 2010a; NTP, 2010b)	Parameter sensitive to oestrogenicity as well as to general toxicity
No effect on AGD in the OECD TG 416 study	No clear response in studies with EE2 (Biegel et al., 1998b; NTP, 2010a)	Lack of effect not contrary to oestrogenic activity
Lower body weight and body weight gain	E2 and EE2 consistently induced lower body weight gain and lower body weight even in trace amounts (Biegel et al., 1998b; NTP, 2010a; NTP, 2010b)	Lower body weight gain and body weight can, at least in part, be explained by the oestrogenic activity of PDB
Increased relative liver weight reported at doses ≥ 125 mg/kg/day; sometimes with hepatic centrilobular hypertrophy	Hepatic centrilobular hypertrophy (E2: 0.5-0.7 mg/kg/day(Biegel et al., 1998b))	Parameter sensitive to oestrogenicity as well as to general toxicity
Kidney mineralization in 3/6 studies; also observed in F0 and F1 generations of the OECD TG 416 study	Kidney mineralization observed in males exposed to EE2 at low doses ((NTP, 2010a; NTP, 2010b))	PDB-induced kidney mineralization likely due to oestrogenic activity

6.11.3.2. Discussion and conclusion on endocrine disruptive properties of PDB for the environment

Based on studies in rats, there is strong evidence that the adverse effects on fertility and sexual function (which led to classification of PDB as Repr. 1B) particularly in females are due to the oestrogenic activity of PDB. These effects are, in the view of the eMSCA, adverse and population relevant. Thus, the eMSCA comes to the conclusion that PDB

fulfills the WHO/IPCS criteria (WHO/IPCS, 2002) of an endocrine disruptor for the environment. For specific information, please see section 6.11.3.1.

Rats are a representative species in human health assessment. In addition, they are representative for terrestrial and marine wildlife mammals, as well as for non-mammalian vertebrates (see below). Therefore and since the effects observed in rat studies are relevant for population stability, PDB has to be considered as an endocrine disruptor for the environment as well.

This is in line with the Revised Guidance Document 150 (OECD, 2018) which states that: "*Cross-species extrapolations should be considered during data assessment. Endocrine systems with respect to hormone structure, receptors, synthesis pathways, hormonal axes and degradation pathways are well conserved across vertebrate taxa especially in the case of oestrogen, androgen and thyroid hormones and steroidogenesis*." And: "*When interpreting data for endocrine assessment, this conservation should be borne in mind as results from tests using human in vitro or non-human mammalian (in vitro and in vivo) systems may be highly relevant for vertebrate wildlife species and vice versa. In addition, results from non-human mammalian studies are also highly relevant for mammalian wildlife species.*"

Furthermore, PDB is considered as an endocrine disruptor for non-mammalian vertebrates in the environment based on the following reasoning:

Due to the evolutionary conservation of the endocrine system, in particular with regard to ER and AR (Ankley and Gray, 2013), there is strong concern that PDB similarly acts as an oestrogenic endocrine disruptor in aquatic vertebrates as it was shown for mammals. Ankley and Gray compared 21–day reproductive fish assays with mammalian uterotrophic assays and female pupertal assays for oestrogenic chemicals. The results showed a high correlation between findings in mammalian and fish assays.

McRobb et al. (2014) demonstrated by in silico analysis that the ligand-binding sites of ERα and ERß are 92−100 % conserved in *D. rerio* and *P. promelas* in comparison to humans. Dang et al. (2011) reviewed data from transcriptional activation assays covering 90 chemicals and found a good concordance between studies utilizing either fish or human ER. The reproductive endocrine system is to a large extent similar in mammals and fish (Kime, 1998) and therefore, effects can be expected to be comparable (Wester et al., 2004).

Although no *in vivo* studies in fish are available, p-dodecylphenol (isomeric mixture) showed oestrogenic activity in two *in vitro* competitive binding assays using rtER, and VTG was induced in rainbow trout hepatocytes (after co-exposure with E2). Furthermore, competitive binding to the hepatic rtER was shown by the read-across substance p-dodecylphenol with supposed linear alkyl chain. These results show oestrogenic activity on OECD level 2 for fish and hence support to adopt the conclusion for PDB as an endocrine disruptor for mammalian as well as for non-mammalian vertebrates such as fish.

In the *in vivo* rat tests for PDB, the oral exposure route was used. In comparison, the fish in aquatic endocrine tests and fish in the environment are directly exposed in water that contains the endocrine acting chemical. Exposure occurs through the skin, by uptake over the gills and the digestive tract. Therefore, it might be possible that fish are more sensitive to endocrine substances than mammals in tests via the oral route.

To summarise, it is reasonable to conclude from the available data of PDB in rats on the effects of this substance in the environment. The effects observed in rats are adverse and population relevant and are evoked by an endocrine mode of action via the HPG axis. Hence, the eMSCA concludes that there is strong evidence that PDB acts as an endocrine disruptor in the environment.

6.12. Exposure assessment

6.13. Environment

PDB is mainly used for the preparation of a variety of lubricant additive materials and for fuel system cleaners. These additives are metal salts (especially calcium salts) of alkyl phenol sulfides ("dodecylphenates"). The dodecylphenates contain PDB as an impurity. Depending on the starting materials and the process conditions, different levels of unreacted PDB are found in the additives. For the production of fuel and lubricant oils the amount of PDB is further diluted and typical levels in the finished products are in the range of 0.1 up to 2.0 %. To this end, the eMSCA carried out a web-based research and evaluated a large number of safety data sheets for these preparations.

Automobile lubricants are designed to minimize wear, improve efficiency, and hence prolong the life of an engine. Dodecylphenates are detergents/dispersants, which can react with metal surfaces to form a protective film, which keeps metal surfaces of an engine clean. In addition to cleaning, they also neutralize acidic combustion and oxidation products, thereby minimizing corrosion and deposit formation in the engine (Nassar, 2017). Taking into account a combustion temperature of approximate 2,000 °C for the diesel and petrol engine, an almost complete combustion of the dodecylphenates in the engine would be expected. It is concluded by SIAM/UK that during the use of engine oils up to 95 % of the residual PDB is oxidized (UK/ICCA, 2006).

PDB is further used as an intermediate for the production of special resins, paints, varnishes and coating resins (PCC Synteza, 2019) as well as a monomer for phenol/formaldehyde resins and ink resins (SI Group, 2018) for industrial, professional and consumer applications. In addition, a smaller use of PDB in the manufacture of tires is mentioned in literature (Brooke, 2007). High PDB contents of 2.5 – 5 % are found in products such as paints and coating resins (Sika, 2019). In certain resins and hardeners, the content of PDB increases up to 50 %.

Emissions to the Environment

Emissions to the environment will occur from industrial manufacture and formulation of lubricant and fuel additives, blending into finished oils as well as from the use and disposal of used lubricants. A provisional UK environment (Brooke et al., 2007) risk assessment of para-C12-alkylphenols (dodecylphenol and tetrapropenylphenol) identifies potential environmental risks from production, use as an intermediate and most end-uses of the derivatives and resins (which all contain some para-C12-alkylphenols as impurities). In the production of PDB, high exceedances of the risk quotients resp. risk characterisation ratios (RCR) for surface water and sediment were determined. The results further suggested that the substance might pose a significant risk to marine waters and sediments from all stages of the substance life cycle. RCRs for the soil compartment were also found orders of magnitude above the trigger level. The evaluation was based on generic exposure scenarios, which are refined in the registration dossier.
Considering the intended use of PDB described in the registration, the exposure estimation and environmental risk assessment is based on the scenario manufacture of the substance (ERC 1) and use of monomer in polymerization processes at industrial site (ERC 6C).

In both scenarios from the registration dossier, a number of risk mitigation measures are considered to reduce emissions into the environment. Manufacturing of PDB and polymerization processes are carried out in closed vessels minimizing emissions. Finished products are handled in a closed system of pipes and thus exposure is reduced during product transfers. Beside other technical onsite conditions and measures any process water will be treated in a waste water treatment plant.

The exposure assessment for manufacture of the substance (ERC1) is based on the maximum tonnage at a site without an existing wastewater treatment plant (worst case). In the registration dossier, the registrant has further calculated the maximum allowable emissions assuming that an external wastewater treatment plant with a capacity of at least 2000 m³/d is available. The resulting concentration limits in the effluent of the wastewater treatment plant are based on compliance with the risk reduction measures at plant site.

For the use of PDB as a monomer in polymerization processes at industrial site (ERC 6C), the exposure assessment is based on measurements made at two sites during periods when the substance manufacturing and polymerization reaction were active. A specific environmental release category (ESVOC SpERC 4.20.vl – 43) has been applied for exposure estimation.

There is one open point in the exposure assessment in the chemical safety report provided with the registration. In contrast to the production of additives for engine and lubricating oils, no concrete data on the amount of PDB for the formulation of other polymers are available (see above). The uses of these polymers by downstream users are not considered in the CSR, as this is not obligatory for polymers under REACH. Therefore, it is unlikely that further information can be gained during substance evaluation in this case.

The eMSCA has reviewed the literature for information on the amount of PDB used in production of epoxy and phenolic resins. Brooke et al. (2007) concluded that the overall level of production and import quantities of para-C12-alkylphenols in Europe is ca. 50,000 t/year. Around 99 % of the consumption volume is used in the production of oil and lubricant additives. Thus, a maximum of 500 t/year of PDB could be considered for the above-mentioned uses.

For the reasons given above, the registrant has not carried out an environmental risk assessment for PDB for the life-cycle of engine and lubricating oils and their use and subsequent disposal. This also applies to the use of PDB in the manufacture and use of other polymer types such as epoxy and phenolic resins, paints, varnishes and coating resins.

Occurrence of PDB in the environment

There are findings on the occurrence of PDB in various environmental compartments and biota. The presence of PDB in seawater and its involvement in the death of stranded seabirds has been described in literature. Among other hazardous chemicals, PDB has been detected in various studies on contaminated birds by non-mineral oils including unknown substances (Janssen C., 2011). In the winter of 1990, PDB together with lubricating oil has been identified as one of the toxic substances isolated from feathers of gannets washed ashore on the Frisian Islands. The authors of the study concluded that PDB probably had markedly contributed to the sickness and mortality among the gannets (Zoun and Boshuizen, 1992). Camphuysen (1991) reported on several cases of seabird mortality due to spillage of oils and phenolic compounds (e.g. nonylphenol, dodecylphenol) in the North Sea as well as in the Wadden Sea.

In a non-target screening study, PDB was identified in all studied levels of the Lake Mjøsa food chain (Thomas K., 2015). In a study on oestrogenic endocrine disruptors in surface waters of the Mekong Delta, PDB was also found in addition to numerous other alkyl phenols (Nguyen, 2011). Following the results from a screening on tertiary butylphenols, methylphenols and long chain alkylphenols in the Swedish environment, PDB was less frequently detected in sludge and sediments and not in water. As the use of PDB is fairly limited in Sweden and the solubility of the substance is low, it was concluded that the most likely source for releases is spills and leakage of oils and fuels containing PDB mainly from industrial activity (Remberger, 2003).

Branched dodecylphenol (mixed isomers) has also been identified in a screening project on phenolic substances in various environmental compartments (soil, water, sediment, surface run-off) as well as influent/effluent water from sewage treatment plants and sewage sludge. The sludge samples had the highest content of 4-dodecylphenol (8,463 - 47,396 ng/L), and for the sewage water samples 4-dodecylphenol (< 125 - 4,096 ng/L) was detected in high concentrations. The amounts of 4-dodecylphenol in non-marine and marine sediments were found to be < 25-216 µg/kg dw and <25-529 µg/kg dw respectively. 4-dodecylphenol was further detected in fish (< 100 µg/kg ww) and mussels (<100-181 µg/kg ww), while levels were low and close to detection limits in both eggs and seals (Hansen, 2008). In a survey of alkylphenols and alkylphenol ethoxylates, the authors concluded that dodecylphenols and their ethoxylates compared to other alkylphenols are released in lower quantities, but are of concern due to their environmental and health properties. For substances used in lubricants and oils like dodecylphenols, it is stated that data on potential releases from spill and waste oil are scarce or missing (Lassen, 2013).

6.13.1. Combined exposure assessment

The exposure assessment conducted in the registration applies to the local scale. No combined exposure assessment has been carried out.

6.14. Risk characterisation

Notwithstanding the demonstrated safe use of PDB by the registrant during manufacture and processing as an isolated intermediate, the substance has endocrine disrupting properties and therefore, its emissions into the environment should be minimised. Although PDB is primarily used as an additive in lubricants and fuels, it is released into the environment during its life cycle. This is proven by the findings of PDB in the influent and effluent of sewage treatment plants, sludge, fresh and sea water and their sediments as well as in biota. PDB will also be released into the environment from other sources as a component of epoxy and phenol resins, paints and special coatings, from oil field applications. Due to its persistence, PDB remains in the environment for a very long time, mainly in the sediments of surface waters and seawater, where it can exert its endocrine disrupting properties on wildlife.

6.15. Abbreviations

The following abbreviations are used in the dossier:

4-NP	4-nonylphenol
4tOP	4-tert-octylphenol
AGD	anogenital distance
AGDi	anogenital distance index
AR	androgen receptor
BC/LA	bulbocavernosus/levator ani muscle
E2	17β-oestradiol
EATS	oestrogenic, androgenic, thyroid, steroidogenic
ED	endocrine disrupting /endocrine disruptor

EE2 17α-ethinyloestradiol

ER oestrogen receptor

ERα oestrogen receptor alpha

IPCS International Programme on Chemical Safety

F2 transgenerational foetus development

hER human oestrogen receptor

LBD ligand-binding domain

LED lowest effective dose

LH luteinizing hormone

OPPTS Office of Prevention, Pesticides and Toxic Substances

PDB phenol, dodecyl-, branched

RBA relative binding affinity

RCR risk characterisation ratios

rtER rainbow trout oestrogen receptor

rtSBP rainbow trout sex steroid binding protein

SBP sex steroid binding protein

SVHC substance of very high concern

T4 thyroxine

TP testosterone propionate

TPP tetrapropenyl phenol

TSH thyroid-stimulating hormone

UVCB substance of unknown or variable composition, complex reaction products or biological materials

VTG vitellogenin

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