Substance Name: Phenol, alkylation products (mainly in para position) with C12-rich branched alkyl chains from oligomerisation, covering any individual isomers and/ or combinations thereof (PDDP)

EC Number: -

CAS Number: -

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF

PHENOL, ALKYLATION PRODUCTS (MAINLY IN PARA POSITION) WITH C12-RICH BRANCHED ALKYL CHAINS FROM OLIGOMERISATION, COVERING ANY INDIVIDUAL ISOMERS AND/ OR COMBINATIONS THEREOF (PDDP)

AS SUBSTANCES OF VERY HIGH CONCERN BECAUSE OF THEIR TOXIC FOR REPRODUCTION PROPERTIES (ARTICLE 57C), ENDOCRINE DISRUPTING PROPERTIES (ARTICLE 57(F) - ENVIRONMENT) AND ENDOCRINE DISRUPTING PROPERTIES (ARTICLE 57(F) - HUMAN HEALTH)

Adopted on 16 June 2021

CONTENTS

TABLES

Abbreviations

^{3}H-E2	Tritiated 17β-estradiol
4-DP	4-Dodecylphenol
4-DPM	4-Dodecylphenol (mixture of isomers)
4-NP	4-Nonylphenol
4-tOP	4–tert-Octylphenol
AGD(i)	Anogenital Distance (Index)
AO(P)	Adverse Outcome (Pathway)
ALAT	Alanine transaminase
AR	Androgen Receptor
ASAT	Aspartate transaminase
BC/LA	Bulbocavernosus/levator ani muscle
DIO 1, 2, 3	Deiodinases 1, 2, 3
E2	17β-Estradiol
EATS	(O)estrogen, androgen, thyroid and steroidogenic
EAS	(O)estrogen, androgen and steroidogenic
ED	Endocrine disruptor
EE2	17α-Ethinylestradiol
EL50	Median effective loading
ELOC	Equivalent level of concern
ER	(O)estrogen receptor
ERα, ß	(O)estrogen receptor α or ß
EC50	Half-maximal effective concentration
ErC50	Half-maximal effective concentration (growth rate)
GGT	γ-Glutamyltransferase
GLP	Good Laboratory Practice
GSI	Gonadosomatic index
HEK293	Human embryonic kidney 293
hER	Human oestrogen receptor
HPG axis	Hypothalamic–pituitary–gonadal axis
IC50	Half-maximal inhibitory concentration
IYD	Iodotyrosine deiodinase
KE	Key event
LBD	Ligand Binding Domain
LC50	Half-maximal lethal concentration
LH	Luteinising hormone
LOEC	Lowest observed effect concentration
MIE	Molecular initiating event
NOEC	No-observed-effect concentration
OPPTS	Office of Prevention, Pesticides and Toxic Substances (USA)
PDB	Phenol, dodecyl-, branched
PDDP	Phenol, alkylation products (mainly in para position) with C12-rich branched alkyl chains from oligomerisation, covering any individual isomers and/or combinations thereof
PND	Postnatal day
PTPD	Phenol (tetrapropenyl) derivatives
RAC	Risk Assessment Committee
RBA	Relative binding affinity
rT3	Reverse triiodothyronine
rtER	Rainbow trout oestrogen receptor
rtSBP	Sex steroid binding protein of rainbow trout
SBP	Steroid binding protein

T3	Triiodothyronine
T4	Thyroxine
TP	Testosterone propionate
TPP	Tetrapropenyl phenol
TSH	Thyroid-stimulating hormone
VTG	Vitellogenin
WAF	Water accommodation fraction
WHO/ICPS	World Health Organisation/International Programme on Chemical Safety

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

Substance Name: Phenol, alkylation products (mainly in para position) with C12-rich branched alkyl chains from oligomerisation, covering any individual isomers and/ or combinations thereof (PDDP)

EC Number: -

CAS number: -

- The substances are identified as substances meeting the criteria of Article 57 (c) of Regulation (EC) No 1907/2006 (REACH) owing to their classification in the hazard class reproductive toxicity category 1B[1].
- The substances are identified as substances of equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH) according to Article 57(f) of the REACH Regulation.

References to PDDP in this document include all the group members as described in Chapter 1. The substances thus covered include, among others, the registered substance phenol, dodecyl-, branched (PDB, EC 310-154-3, CAS 121158-58-5). Further examples of substances in the scope of this document are given in section 1.2. The conclusions drawn are mainly based on data from PDB but apply to all substances in scope of this document.

Summary of how the substances meet the criteria set out in Article 57 of the REACH Regulation

CMR assessment

PDB is covered by index number 604-092-00-9 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) and it is classified in the hazard class reproductive toxicity category 1B (H360F[2]). In its respective opinion[3] on the dossier proposing harmonised classification and labelling of 5 December 2013 the Risk Assessment Committee (RAC) states that “the harmonised classification will apply to any substance which predominantly contains C12 (branched) alkyl-substituted phenols”. Therefore, it can be concluded that PDDP meets the criteria of Article 57 (c) of Regulation (EC) No 1907/2006 (REACH) owing to its classification in the hazard class reproductive toxicity category 1B[4].

Art 57(f) assessment

[1] Classification in accordance with section 3.7 of Annex I to Regulation (EC) No 1272/2008.
[2] H360F: ‘May damage fertility’
[3] https://echa.europa.eu/documents/10162/7e7b5949-9d0a-2896-fb10-1504496ab2eb
[4] Classification in accordance with section 3.7 of Annex I to Regulation (EC) No 1272/2008.

ED assessment

PDDP are proposed to be identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because of their endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health and the environment which gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

Considering the results of all available studies, there is strong evidence that the adverse effects on fertility and sexual function (which led to classification of the substance as Repr. 1B), particularly in females, are due to the oestrogenic activity of PDDP. Mechanistic in vitro studies demonstrate oestrogen receptor (ER) binding and activation by PDDP (OECD level 2). The increase in 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 were consistently observed in the majority of OECD Level 4 and 5 studies. Reproductive toxicity studies (OECD level 5) further demonstrated impacts on copulation index and apical fertility endpoints (decreased number of implantations and litter size). All of the above-mentioned parameters are considered as either EATS (oestrogeno-, androgeno-, thyroido-, and steroidogenesis)-mediated or sensitive to EATS modalities (OECD, 2018), and the overall observed effect pattern of PDDP is congruent with that of known model oestrogens.

In conclusion, there is strong evidence that the adverse effects on fertility and sexual function, particularly in females, are plausibly linked to the oestrogenic activity of the substance. Therefore, it is concluded based on the weight of evidence that PDDP is an endocrine disruptor with regard to human health according to the WHO/IPCS definition (as interpreted by the JRC Endocrine Disruptor Expert Advisory Group, 2013).

The evaluation of PDDP for the environment is based on mammalian data and supported by available fish *in vitro* tests and adverse outcome pathways. There are no aquatic *in vivo* long term data for fish and other aquatic vertebrates or investigations for ED available. As effects on growth, development and reproduction in single species are generally regarded relevant for the maintenance of wild populations, the observed effects on reproduction and pubertal development in rats are relevant for mammalian populations in the environment. Therefore, it is concluded based on the weight of evidence that PDDP is an endocrine disruptor for the environment.

Equivalent level of concern assessment

Based on the above conclusion, evidence that PDDP is of an equivalent level of concern includes:

The effects of PDDP on mammals are considered to be of equivalent level of concern as those of CMR Cat 1, PBT or vPvB substances due to the severity and irreversibility of the effects on organisms and populations and the difficulties to quantify a safe level of exposure in the environment. Environmental effects observed after exposure to PDDP are considered to impair population stability and recruitment. The effects may influence a wide range of taxa in different ecosystems due to conservation of the reproductive endocrine system. For most species in the environment no data on endocrine effects that are caused by the substance are available. Furthermore, the organisms in the environment are exposed to a mixture of substances. Hence there can be additive or synergistic effects that might enhance the impact. For example, additive effects were seen for mixtures of long chain alkyl phenols and other oestrogenic compounds like E2 and EE2. Increased sensitivity in an *in vitro* test was seen using 4-DPM with co-exposure to E2 indicating

enhanced mixture effects. Between exposure and effects might be a long time, which also hinders the derivation of a safe effect level.

PDDP severely affects reproduction and development-related processes in organisms. Different aspects of reproduction are affected such as decreased copulation index and impairment of fertility which are important for population stability. Furthermore, PDDP caused a developmental effect in term of precocious puberty in females in pubertal assays and in the two-generation study. This is also of environmental importance as a similar effect could appear regarding seasonal maturation of gonads. Many fish species exhibit seasonal maturation and reproduction cycles. Reproduction and rearing of offspring are energy demanding processes where enough food must be available. Mostly it is connected to a short time frame given by environment. If these energy demanding processes fall out of this time frame due to e.g. precocious puberty this could entail adverse effects on populations.

Thus, in summary, effects in mammals are relevant and serious for the environment. They are considered to be of equivalent concern due to the severity of the effects and the difficulties to quantify a safe level of exposure for oestrogen-like endocrine disruptors.

Registration dossiers submitted for substances of the substance group? Yes (phenol, dodecyl-, branched, EC 310-154-3)

Justification

1. Identity of the substances and physical and chemical properties

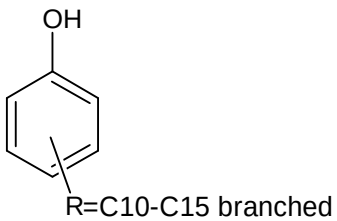
1.1. Name and other identifiers of the substances

Table 1: Substance group identity

EC number:	-
EC name:	-
CAS number (in the EC inventory):	-
CAS number: Deleted CAS numbers:	-
CAS name:	-
IUPAC name:	Phenol, alkylation products (mainly in para position) with C12-rich branched alkyl chains from oligomerisation, covering any individual isomers and/ or combinations thereof (PDDP)
Index number in Annex VI of the CLP Regulation	PDB is covered by Index No. 604-092-00-9
Molecular formula:	n.a. (UVCB[5])
Molecular weight range:	n.a. (UVCB[5])
Synonyms:	Inter alia: DDP; TPP; Phenol, 4-dodecyl, branched; Phenol, (tetrapropenyl), derivatives; Dodecylphenol, mixed isomers; …

Structural formula:

The substances are UVCB substances and therefore cannot be displayed by a single structure. The following structural formula represents the constituents of the substance.



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

1.2. Description of the substances

Name: Phenol, alkylation products (mainly in para position) with C12-rich branched alkyl chains from oligomerisation, covering any individual isomers and/or combinations thereof (PDDP).

The name given above and used for the scope of this document was derived by following the rules set out in the Guidance for identification and naming of substances under REACH and CLP.[6] The term “C12 rich” in this context refers to the fact that C12 alkychains are predominantly present in the UVCB substance. Nevertheless, fractions of C7-C15 constituents can also be present, albeit in lower quantities compared to C12. A substance falling within the scope of this report was registered using the substance name phenol, dodecyl-, branched (EC 310-154-3, CAS 121158-58-5). Next to this substance the following non-exhaustive list of names and numerical identifiers falling into the scope of this report are known:

Table 2: Non-exhaustive list of group members

Substance	EC number	CAS number
Phenol, (tetrapropenyl) derivatives		74499-35-7
Phenol, 4-dodecyl, branched		210555-94-5
Phenol, tetrapropylene		57427-55-1
Phenol, 4-isododecyl; 4-isododecylphenol	-	27459-10-5
Tetrapropenyl phenol		
4-(3,4,5,6-tetramethyloctan-2-yl)phenol		
4-(3,4,5-trimethylheptyl)phenol		
Phenol, alkyl branched (species comprising decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, substituents)		
Phenol, dodecyl-, branched	310-154-3	121158-58-5
Phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation		
Phenol, 4-isododecyl-		27147-75-7

Note: identifiers that formally describe linear structures, e.g. EC N. 248-312-8

[6] https://echa.europa.eu/documents/10162/23036412/substance_id_en.pdf

Dodecylphenol, CAS 27193-86-8 or EC N. 203-202-9, p-dodecylphenol CAS N. 104-43-8, may have been used by certain actors to describe substances falling withing the scope of the proposed entry.

Further considerations on the scope of the document

This support document for SVHC identification covers “Phenol, alkylation products (mainly in para position) with C12-rich branched alkyl chains from oligomerisation, covering any individual isomers and/ or combinations thereof” (PDDP).

Considerations on the test substances

As outlined above, the scope of this document covers substances with different identifiers, CAS and/or EC numbers. Likewise, the database for the description of the hazard properties of PDDP draws from studies using differently named and described test materials. In this document, these test substances are denoted as reported in the respective study and, if applicable, further considerations on their appropriateness or specific relevance is given in the accompanying discussion. Unless specified otherwise, they are considered as relevant for the sake of this document, i.e. for the hazard assessment and identification of PDDP as an SVHC.

Table 3: Test substances

Substance name	Abbreviation	Synonyms	EC No.	CAS No.
Tetrapropenyl phenol	TPP		310-154-3	121158-58-5
Phenol, dodecyl-, branched	PDB	phenol, para alkylation products with C12-rich branched olefins from propene oligomerisation		
phenol with C12 alkylated branched olefin	-	-	-	-
4-dodecylphenol (mixture of isomers)	4-DPM	dodecylphenol	248-312-8	27193-86-8
4-dodecylphenol	4-DP	*p*-dodecylphenol	203-202-9	104-43-8
phenol (tetrapropenyl) derivatives	PTPD		616-100-8	74499-35-7

Substance type: UVCB

Read-across approach for the use of linear p-dodecylphenol (4-DP, CAS No. 104-43-8)

The entry CAS 104-43-8 implies a well-defined substance. As in the case of nonylphenol, this identifier was most likely wrongly used to identify the substance that is manufactured or placed on the market. The structure of this substance is expected to display at least

branched alkyl chains. However, due to the similar structural, physico-chemical and toxicological properties, data from studies based on CAS 104-43-8 are considered as relevant for the sake of this document, i.e. for the hazard assessment and identification of PDDP as an SVHC.

This read-across is based on p-dodecylphenol (4-DP, 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 endocrine activity with regard to the estrogen, androgen, thyroid and steroidogenic (EATS) modalities (see for Table 4 comparison).

- Structure: 4-DP (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. The difference is that 4-DP (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, antiandrogenic, and thyroidal activities of 4-DP (CAS 104-43-8) and PDB are comparable based on in vitro studies and uterotrophic assays.

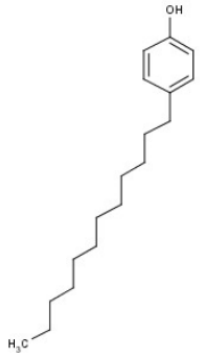
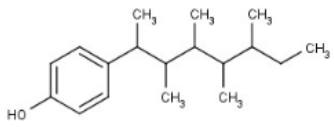
In vitro assays:
The relative binding affinity (RBA) determined in binding assays using mammalian estrogen receptors (ER) are in the same order of magnitude for 4-DP, which is assumed to be linear (IC_{50} not specified, RBA = 0.24 % for human ER (hER)) and PDB (IC_{50} = 1.1 µM, RBA = 0.11 % for rat ER). For the isomeric mixture of p-dodecylphenol (also supposed to be branched like PDB)[7], the RBA is 0.019 % (IC_{50} = 4.85 µM) for rat ER and 0.016 % (IC_{50} = 22 µM) for rainbow trout ER (rtER). For assumed linear 4-DP, a study using rtER is available, without specified effect value or RBA. The estimated RBA is 0.01 %. Androgen receptor (AR) binding studies utilizing rat AR preparations show weak and comparable binding of 4-DP (IC_{50} = 20 µM, RBA = 0.015%) and PDB (IC_{50} = 92 µM, RBA = 0.0016%). Furthermore, for 4-DPM (4-dodecylphenol (mixture of isomers) CAS 27193-86-8) and 4-DP (4-dodecylphenol CAS 104-43-8), the integrative ToxCast ER and AR bioactivity models[8] show very similar scores for ER agonistic and AR antagonistic activity. Regarding the T-modality, 4-DPM and 4-DP inhibit deiodinases (DIO) and iodotyrosine deiodinase (IYD) with very similar potencies.

Uterotrophic assays:
Significant increases in uterus weight occur at a similar dose range for supposedly linear

[7] For p-dodecylphenol which has a linear alkyl chain in para-position of the aromatic ring, there exists only one possible isomer.
[8] 4-DPM: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1027926#bioactivity-toxcast-models.
4-DP: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1022508#bioactivity-toxcast-models.

4-DP (40 mg/kg/day) and for PDB (75 mg/kg bw/d); for the latter it was the lowest tested dose.

Table 4: 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[9]	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)[10]
log P_{OW}[9]	7.91	7.14
Uterotrophic assay, OECD 440, endpoint uterine weights	(Akahori et al., 2008; Yamasaki et al., 2003): 40 mg/kg bw/d onwards	(Edwards, 2010a; Edwards, 2010b): Positive at 75 mg/kg bw/d (lowest dose tested)
Competitive binding assay using human ER or rat ER	Akahori et al. (2008): RBA: 0.24 % (hERα)	Thomas (2012b): IC_{50}: 1.1 µM (ratER) RBA: 0.11 % (ratER) Blair et al. (2000): Isomeric mixture of p-dodecylphenol: IC_{50}: 4.85 µM (ratER) RBA: 0.019 % (ratER)
ToxCast ER agonist model score	0.41	0.392
Competitive binding assay using rainbow trout ER	Knudsen and Pottinger (1999): 10^4-fold more 4-DP was necessary to obtain the	Tollefsen and Nilsen (2008): Isomeric mixture of p-dodecylphenol: IC_{50}: 22 µM

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

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

	same effect as with 17β-estradiol (E2; estimated by author). RBA: 0.01 % (estimated)	RBA: 0.016 %
Competitive binding assays using rat AR	Fang et al. (2003): IC_{50} = 20 µM RBA = 0.015 %	Thomas (2012a): IC_{50} = 92 µM RBA = 0.0016 %
ToxCast AR antagonist model score	0.196	0.2
Inhibition of deiodinases (DIO) and iodotyrosine deiodinase (IYD) (Olker et al., 2018; Olker et al., 2021)	DIO 1: IC_{50}:37.2 µM DIO 2: IC_{50}:74.2 µM DIO 3: IC50 = 11.2 µM IYD: IC50 = 23.1 µM	DIO 1: IC_{50} = 61.4 µM DIO 2: IC_{50} = 84.2 µM DIO 3: IC_{50} = 15.0 µM IYD: IC_{50} = 20.1 µM

1.3. Physicochemical properties

Data from Table 5 is taken from the dissemination site of PDB (EC no 310-154-3). This information is considered applicable for all branched members of the group.

Table 5: Overview of physicochemical properties (as referenced in the ECHA dissemination portal[11])

Property	Description of key information	Value
Physical state at 20°C and 101.3 kPa	*Visual inspection of Tetrapropenyl phenol (TPP)*	*clear bright to amber/brown viscous liquid; test substance: TPP*
Melting/freezing point	*ASTM D 5950, due to the viscous properties of the substance measurement of the pour point was considered more appropriate than measuring a melting point.*	*In a pour point method, the pour point was determined to be -9 °C (± 3 °C); test substance: TPP*
Boiling point	*Thermogravimetric Analysis test (TGA, Chevron ILT Test 10301)*	*189-270°C; test substance: TPP* *Since the substance consists of alkyl phenol analogues that have alkyl side chains that range from C3-C15, it is more appropriate to report a boiling range rather than a single boiling point.*
Vapour pressure	*OECD Method 104 (effusion method, vapour pressure balance)*	*0.011 Pa at 25 °C*
Density	*ASTM D 1298 method*	*Relative density at 20 °C: 0.942, test substance: TPP*
Water solubility	*Method A6 (flask method)*	*1.54 mg/L at 20 °C, (lower alkyl phenols C3 to C9 present in sample); test material: TPP;* *water solubility of "distilled" substance: 0.031 mg/L*
Partition coefficient n-octanol/water (log value)	*slow stirring method*	*log K_{OW} 7.14 at 25 °C (for the main component phenol with C12 alkylated branched olefin),* *(log K_{OW} 6.45 for C9 constituent)*
viscosity	*ASTM D 445 and ASTM D 2161 methods*	*450 cSt at 40 °C and 9 cSt at 100 °C for; Test material: TPP ASTM D 445 and ASTM D 2161 methods*

[11] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14705/4/8

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

Table 6: Structurally related substance(s) identity[12]

EC number:	-
EC name:	-
SMILES:	-
CAS number (in the EC inventory):	-
CAS number:	-
CAS name:	-
IUPAC name:	4-Nonylphenol, branched and linear [substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, covering also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof]
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$C_{15}H_{24}O$
Molecular weight range:	220.35 g/mol
Synonyms:	-

Structurally related substance(s) formula:

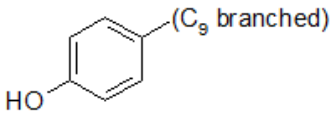


Table 7: Constituents of structurally related substance(s)

Constituents	Typical concentration	Concentration range	Remarks
4-Nonylphenol, branched and linear	100%		

[12] See supporting document for SVHC identification of 4-NP for further information on substance identity (https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807db370). Where 4-nonylphenol is referred to in the document the reference is to 4-NP, branched and linear.

Table 8: Impurities of structurally related substance(s)

Impurities	Typical concentration	Concentration range	Remarks
Not known			

Table 9: Additives of structurally related substance(s)

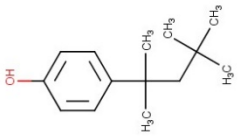
Additives	Typical concentration	Concentration range	Remarks
Not known			

Table 10: Structurally related substance(s) identity[13]

EC number:	205-426-2
EC name:	4-(1,1,3,3-tetramethylbutyl)phenol
SMILES:	CC(C)(C)CC(C)(C)C1=CC=C(O)C=C1
CAS number (in the EC inventory):	140-66-9
CAS number:	140-66-9
CAS name:	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
IUPAC name:	4-(2,4,4-trimethylpentan-2-yl)phenol
Index number in Annex VI of the CLP Regulation	604-075-00-6
Molecular formula:	$C_{14}H_{22}O$
Molecular weight range:	206.32 g/mol
Synonyms:	4-tert-octylphenol

Substance type: mono-constituent

Structurally related substance(s) formula:



[13] See supporting document for SVHC identification of 4-tOP for further information on substance identity (https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807d9e89).

Table 11: Constituents of structurally related substance(s)

Constituents	Typical concentration	Concentration range	Remarks
4-(1,1,3,3-tetramethylbutyl)phenol EC no: 205-426-2	>80%		

Table 12: Impurities of structurally related substance(s)

Impurities	Typical concentration	Concentration range	Remarks
Not known			

Table 13: Additives of structurally related substance(s)

Additives	Typical concentration	Concentration range	Remarks
Not known			

2. Harmonised classification and labelling

Phenol, dodecyl-, branched (PDB) is covered by Index number 604-092-00-9 in part 3 of Annex VI to the CLP Regulation as follows:

Table 14: Classification according to Annex VI, Table 3 (list of harmonised classification and labelling of hazardous substances) of Regulation (EC) No 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 [1]; phenol, 2-dodecyl- [2], branched; phenol, 3-dodecyl-, branched [3]; phenol, 4-dodecyl-, branched [4]; phenol, (tetrapropenyl) derivatives [5]	310-154-3 [1]	121158-58-5 [1], 210555-94-5 [4], 74499-35-7 [5]	Repr. 1B Skin Corr. 1C Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H360F H314 H318 H400 H410	M=10 M(Chronic)=10	

3. Environmental fate properties

The discussion concerns information taken from the dissemination site of the registered substance phenol, dodecyl-, branched (PDB).

3.1. Degradation

3.1.1. Abiotic degradation

3.1.1.1. Hydrolysis

No information available. It is neither technically possible nor scientifically justified to conduct the study. The rationale is based on the water solubility of the substance (1.54 mg/L) and the lack of molecular functional groups which will undergo hydrolysis under environmentally relevant conditions.

3.1.1.2. Oxidation

No information available.

3.1.1.3. Phototransformation/photolysis

3.1.1.3.1. Phototransformation in air

For PDDP a degradation half-life of 2.4 h in the atmosphere can be calculated (Brooke et al., 2007).

3.1.1.3.2. Phototransformation in water

No information available.

3.1.1.3.3. Phototransformation in soil

No information available.

3.1.1.4. Summary on abiotic degradation

The available data shows that abiotic degradation via hydrolysis seems not to be a relevant pathway for removal of the substance from the environment.

3.1.2. Biodegradation

3.1.2.1. Biodegradation in water

3.1.2.1.1. Estimated data

No information available.

3.1.2.1.2. Screening tests

The registration dossiers (accessed via the dissemination site) contain two studies used to inform on this endpoint. A test conducted according to the OECD Test Guideline 301B,with the exception of pre-exposure of the inoculum, is included (Schörberl, 1992). The reference substance showed 95% degradation after 28 days, demonstrating the vitality of the inoculum used. In contrast, the tested material showed limited biodegradation (25% degradation via measured CO_2 evolution at a test concentration of 10 mg/L and 6% at 20 mg/L) over 28 days and it can be concluded that PDDP is not readily biodegradeable. The authors reported a toxicity control which, however, did not comply with the usual provisions of the test guideline because it showed 57% mineralization at day 56 instead of giving details on mineralization at day 14. Thus, it is not possible to compare with the specifications given for toxicity control in the test guideline for screening tests on ready biodegradability.

However, we conclude that degradation was unlikely to be significantly hampered by toxicity because the inoculum was pre-exposed and thus probably adapted prior to use. The inoculum should have at least potentially developed some tolerance against the substance. A test on microbial toxicity in which the EC50 was found to be > 1000 mg/L (nominal) after 3 hours suggests no toxicity at test concentrations of the OECD 301B test and seems to confirm this conclusion (Clarke, 2004).

A second study (Mead and McKenzie, 2005) on inherent biodegradability in an aerobic aqueous medium was conducted according to the draft OECD Test Guideline No. 302D (2001, Inherent Biodegradability: CONCAWE Test). The test concentration utilised was 20 mg C/l and the inoculum was pre-exposed to the test substance. The reference substance showed 98% degradation after 56 days and the toxicity control attained 57% degradation after 56 days, thereby confirming that the test material was not toxic to the inoculum.

Based on the carbon dioxide production the test material attained 10% biodegradation after 56 days (via measured CO_2 evolution) and therefore cannot be considered to be inherently biodegradable from the registrants' perspective. Although this test is not a commonly agreed test design and also not a specified test on inherent biodegradability according to the requirements on screening criteria under the Guidance on information requirements and chemical safety assessment (Chapter R.11: PBT Assessment; Table R. 11-2), it provides strong evidence that the substance will not be degraded fast under environmental conditions.

In summary, the results from these two key studies indicate that PDDP is neither readily biodegradable nor inherently biodegradable. In addition, a lack of degradation (<20% degradation) in an inherent biodegradability test equivalent to the OECD 302 series might be judged as sufficient to confirm persistence without the need for further simulation testing as specified in the Guidance chapter R.11, especially with the enhancements of pre-exposure of the inoculum and prolonged test duration which would have stimulated the degradation performance of the microbes in the test vessels.

3.1.2.1.3. Simulation tests (water and sediments)

No data available.

3.1.2.2. Biodegradation in soil

No data available.

3.1.2.3. Summary and discussion on biodegradation

Based on the available information from the two screening studies on biodegradation (accessed via the dissemination site), it is concluded that PDDP neither fulfills the criteria for being “readily biodegradable” nor “inherently biodegradable”.

3.1.3. Field data

No data available.

3.1.4. Summary and discussion of degradation

Neither available data on abiotic degradability via hydrolysis nor results from two screening tests on ready biodegradation and inherent biodegradation provide indications that the substance PDDP might be degraded easily under environmental conditions. As no environmental simulation studies were provided with the registration dossiers of PDB, no information on degradation half-lives in the three different environmental compartments is available.

3.2. Environmental distribution

3.2.1. Adsorption/desorption

The adsorption coefficient (K_{oc}) of the test item has been determined to be 10.4 to 4.71 x 10^4 L/kg (log K_{oc} 1.02 to 4.67). However, 93.9% of the test item (the dominant components) had a reduced range of 2.49 x 104 to 4.71 x 10^4 L/kg (log K_{oc} 4.40 to 4.67).

3.2.2. Volatilisation

Based on the vapour pressure of the total substance (0.011 Pa at 25°C), PDDP is considered to be of low volatility.

3.2.3. Distribution modelling

No data available.

3.2.4. Field data

Various reports on the occurrence of PDDP in environmental compartments and biota are available. The substance has been detected in various studies on birds contaminated by non-mineral oils including unknown substances (Janssen and Roose, 2011). In a non-target screening study, the substance was identified in all studied levels of the Lake Mjøsa food chain (Thomas and Schlabach, 2015). In a study on oestrogenic endocrine disruptors in surface waters of the Mekong Delta, the substance was also found in addition to a series of other alkylphenols (Nguyen, 2011). Based on the results from a screening study on tertiary butylphenols, methylphenols and long-chain alkylphenols in the Swedish environment, the substance was detected in sludge and sediments and not in water. As the use of PDDP 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 the substance mainly from industrial activity (Remberger, 2003).

PDDP (in the publication: unspecified dodecylphenol, DDP) was detected in several Nordic countries in sewage treatment plant influents and sewage (<125 up to 4096 ng/L) and effluents (<100 up to 2206 ng/L), as well as in landfill effluents (241 – 4902 ng/L). Surface runoff water contained amounts of PDDP from < 100 ng/L up to 1106 ng/L (the highest value of 4280 ng/L was outside the calibration range). Background water samples were in most cases below detection limit for PDDP, though in one case in a freshwater lake in Sweden 88 ng/L was measured. The amounts of PDDP in non-marine and marine sediments were found to be < 25 up to 216 µg/kg dw and < 25 up to 529 µg/kg dw respectively. In biological samples PDDP was detected too: in fish liver of a lacustrine lake (< 100 up to 253 µg/kg ww) and in mussels (< 100 up to 181 µg/kg ww), while levels were low and close to detection limits in both eggs and seals (Hansen and Lassen, 2008).

3.2.5. Summary and discussion of environmental distribution

Based on the log K_{ow} of 7.14, PDDP is expected to partition into the sediment and soil. A rapid decomposition of PDDP is not expected as the substance will not dissociate at environmentally relevant pH and hydrolysis will also not occur. The latter was concluded in the registration dossier as, apart from the phenoxy hydroxyl group, which could be reversibly neutralised with especially strong bases (> 11), there are no functional groups or other structural alerts present in the active organic ingredient that indicate that destructive hydrolysis of the principal active ingredient will occur.
The available data suggest that PDDP is of low volatility and low water solubility (1.54 mg/L, water solubility of distilled substance 0.031 mg/L) and will adsorb strongly to organic matter in soil, sediment and sludge.

3.3. Data indicating potential for long-range transport

The required information on the screening criteria according to Annex D of the Stockholm Convention on Persistent Organic Pollutants for PDDP is not completely available, so that no statement on the long-range transport potential of the substance can be made at this point.

3.4. Bioaccumulation

3.4.1. Bioaccumulation in aquatic organisms (pelagic and sediment organisms)

Blankinship et al. (2006) report BCF 749 – 823 (whole body) for rainbow trout (*Oncorhynchus mykiss*) after an exposure of 27 days followed by a depuration phase of 15 days in OECD 305 (Blankinship et al., 2006). Test concentrations were 1.1 and 11 µg/ L and well within water solubility. Further, they report a steady state reached at day 3 and an elimination of at least 90% by day 11. However, it remains unclear whether or not the BCF are normalised to lipid content or growth corrected. In addition, as the substance is surface active, this raises uncertainties that are still not addressed, e.g. bioavailability issues that could have resulted in a decreased BCF or adsorption to fish surface which could have increased the BCF.

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

No data available.

3.4.3. Field data

No data available.

3.4.4. Summary and discussion of bioaccumulation

While there are some remaining uncertainties regarding the calculation of the BCF, the currently available information suggests that the criterion for bioaccumulation according to Annex XIII of REACH is not met.

4. Human health hazard assessment

4.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

There are no animal studies available addressing toxicokinetics of PDDP after oral exposure. Due to its high lipophilicity and low water solubility, PDDP is expected to be absorbed into and through the cell membrane, resulting in retention and distribution in the body (ECHA, 2013a; ECHA, 2013b). Systemic effects in several toxicity studies confirm absorption and whole-body distribution after oral exposure.

Percutaneous penetration was evaluated *in vitro* using rat or human skin as well as *in vivo* in rats (Bernard, 2012a; Bernard, 2012b). According to these data, about 3% of the applied dose is absorbed. Therefore, bioavailability is expected to be low following human dermal exposure (ECHA, 2013a; ECHA, 2013b).

No inhalative toxicokinetic studies have been performed. Since the vapour pressure of the substance is low (0.011 Pa at 25 °C), inhalative exposure is considered unlikely (ECHA, 2013a; ECHA, 2013b).

4.1.2 Human information (including bioaccumulation in humans)

No data are available.

4.1.3 Conclusion on toxicokinetics (and bioaccumulation in humans)

PDDP is considered to be absorbed in the gastrointestinal tract after oral exposure, and extensive distribution is expected based on the effects observed in several target tissues. After 28 day exposure of rats, effects in some tissues of the high dose group persisted throughout a 14 day recovery period (Harriman, 2004), possibly indicating retention in the body.

Dermal absorption (about 3% of the applied dose) and, therefore, bioavailability is expected to be low following human exposure.

Inhalation exposure is of low relevance due to the low vapour pressure (0.011 Pa at 25 °C) of the substance.

4.2. Acute toxicity

Not relevant for this dossier.

4.3. Irritation

Not relevant for this dossier.

4.4. Corrosivity

Not relevant for this dossier.

4.5. Sensitisation

Not relevant for this dossier.

4.6. Repeated dose toxicity

Repeated dose toxicity studies are considered for hazard assessment regarding endocrine disruption - human health (see 4.11).

4.7. Mutagenicity

Not relevant for this dossier.

4.8. Carcinogenicity

No studies concerning carcinogenicity are available for PDDP.

4.9. Toxicity for reproduction

Reproductive toxicity studies are considered for hazard assessment regarding endocrine disruption - human health (see 4.11).

4.10. Other effects

If relevant, other effects are considered for hazard assessment regarding endocrine disruption - human health (see 4.11).

4.11. Endocrine disruption – human health

4.11.1. General approach – human health

For the assessment of PDDP and for the purpose of this document, all toxicity data regarding human health available from the dissemination site of PDB (EC 310-154-3), 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) were taken into account when weighing the evidence for endocrine disruption. Furthermore, public scientific literature on other test materials as laid out in Table 3 was considered.

4.11.2. *In silico* data (OECD level 1) - QSAR

For PDB, the QSAR Toolbox (Version 3.4.0.17) gives 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 as reproductive and developmental hazards.

4.11.3. Mechanistic *in vitro* studies (OECD level 2)

For an overview of available *in vitro* studies, see Table 15.

An androgen receptor (AR) binding assay (Thomas, 2012a) according to OPPTS (Office of Prevention, Pesticides & Toxic Substances) 890:1150 and an oestrogen receptor (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 PDB (CAS 121158-58-5) to AR with an IC_{50} of 92 µM and a relative binding acitivity (RBA) of 0.0016 % compared to the positive control R1881 (a synthetic AR agonist; IC_{50} = 1.44 nM). The RBA of PDB 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 an 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-DPM (4-dodecylphenol (mixture of isomers), no CAS indicated) was used. The IC_{50} was 4.85 µM corresponding to an 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-DPM 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 4-DP (p-Dodecylphenol, 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 4-DP to the LBD of hERα with an RBA of 0.24 % when compared to the positive control E2 (no IC_{50} values were reported).

4-DP (CAS 104-43-8) was also tested in an AR binding assay based on the LBD and hinge region of rat AR fused to thioredoxin and expressed in E. coli (Fang et al., 2003). This binding assay demonstrated weak competitive binding of 4-DP to AR with an IC_{50} of 20 µM and an RBA of 0.015 % compared to the positive control R1881 (IC_{50} of 3.07 nM)

To further assess endocrine activity specifically with regard to (anti)oestrogenic and (anti)androgenic modalities, the integrative ToxCast ER and AR bioactivity models[14] were consulted for 4-DPM (4-dodecylphenol (mixture of isomers) CAS 27193-86-8) and 4-DP (4-dodecylphenol CAS 104-43-8). For 4-DPM and 4-DP, the scores were 0.392 and 0.41 regarding ER agonistic activity, and 0.2 and 0.196 regarding AR antagonistic activity, respectively. Thus, Toxcast models for 4-DPM and 4-DP predict oestrogenic and antiandrogenic activity.

Regarding the thyroid hormone system, 4-DPM (4-dodecylphenol (mixture of isomers) CAS 27193-86-8) and 4-DP (4-dodecylphenol 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 4-DP and 4-DPM, respectively. IC_{50} values for DIO2 were 74.2 µM and 84.2 µM for 4-DP and 4-DPM, respectively. IC_{50} values for DIO3 were 11.2 µM and 15.0 µM for 4-DP and 4-DPM, respectively. Another high-

[14] 4-DPM: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1027926#bioactivity-toxcast-models.
4-DP: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID1022508#bioactivity-toxcast-models.

throughput screening study by the same group also tested 4-DP and 4-DPM for inhibition of iodotyrosine deiodinase (IYD), an important iodide recycling enzyme also known as dehalogenase (Olker et al., 2021). Human IYD was expressed in SF21 cells and the cell lysate served as the source of the enzyme. Based on the Sandell-Kolthoff reaction, the assay measured IYD-liberated iodide using monoiodotyrosine as substrate. Both test substances, 4-DP and 4-DPM, were identified as inhibitors of IYD with an IC_{50} of 23.1 µM and 20.1 µM, respectively.

In summary, level 2 *in vitro* data indicate interaction with ER, AR, and components of the thyroid hormone system. With regard to ER and AR, the ToxCast bioactivity models reveal oestrogenic and antiandrogenic activity, respectively.

Reliability scores in Table 15 and throughout the dossier relate to the Klimisch scoring system[15].

Table 15: Mechanistic *in vitro* studies (OECD level 2).

Method	Results	Remarks	Reference
Androgen receptor (AR) binding assay (rat prostate cytosol) according to OPPTS (Office of Prevention, Pesticides and Toxic Substances) 890:1150 GLP compliance Competition of PDB with tritiated (^{3}H-) 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 Concentration of test chemicals: 0.1 nM to 1 mM	PDB: IC_{50} = 92 µM RBA = 0.0016 % Positive control (R1881): IC_{50} = 1.44 nM RBA set to 100 % Weak positive control (dexamethasone): IC_{50} = 74 µM RBA = 0.002 %	1 (reliable without restriction) Test material: PDB purity: 100 %	(Thomas, 2012a)
Androgen receptor (AR) binding assay (PanVera AR: rat AR ligand-binding domain	4-DP: IC_{50} = 20 µM	2 (reliable with restriction)	(Fang et al., 2003)

[15] H.-J. Klimisch, M. Andreae, U. Tillmann: A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. In: Regulatory Toxicology and Pharmacology. Band 25, Nummer 1, 1997, S. 1–5, doi:10.1006/rtph.1996.1076, PMID 9056496.

(LBD) and hinge region fused to thioredoxin and expressed in *E. coli*; rat and human LBD are identical) Competition of 4-DP with ^{3}H-R1881 Positive control: R1881 Concentrations of test chemicals: 4.3 nM to 430 µM	RBA = 0.015 % Positive control (R1881): IC_{50} = 3.07 nM RBA set to 100 %	Test material: 4-DP	
ER binding assay (rat uterine cytosol) according to OPPTS 890:1250 GLP compliance Competition of TPP with 3H-17β-estradiol (E2) for ER binding sites in rat uterine cytosol Tissue source: pooled uterine tissue from ovariectomised female Crl:CD(SD) rats Positive control: 17β-estradiol (E2) Weak positive control: 19-norethindrone Negative control: octyltriethoxysilane Concentration of test chemicals: 0.1 nM to 100 µM	PDB: IC_{50} = 1.1 µM RBA = 0.11 % Positive control **(17β-estradiol** (E2)): IC_{50} = 1.2 nM RBA set to 100 % Weak positive control (19-norethindrone): IC_{50} = 3.46 µM RBA = 0.034 % Negative control (octyltriethoxysilane): No binding	1 (reliable without restriction) Test material: PDB (wrong CAS given in study) CAS of test material is not correctly provided in the registration. The CAS given for the test material erroneously corresponds to the positive control E2 (CAS 50-28-2).	(Thomas, 2012b)
ERα receptor binding assay (LBD of hERα fused with GST and expressed in E.coli) Competition of 4-DP with tritiated E2 (^{3}H-E2) Concentrations of test chemicals: 10 pM to 100 µM	4-DP: IC_{50} values not given RBA: 0.24 % Positive control E2: IC_{50} values not given RBA set to 100 %	2 (reliable with restriction) Test material: 4-DP	(Akahori et al., 2008)

Estrogen receptor (ER) binding assay (rat uterine cytosol) Competition of 4-DPM with ^{3}H-E2 Concentrations: 1 nM to 100 µM, Positive control: E2 (33 pM – 100 nM)	4-DPM: IC_{50}: 4.85 µM RBA: 0.019 % Positive control E2 IC_{50} = 0.899 nM RBA set to 100 %	2 (reliable with restriction) Test material: 4-DPM (purity 99.7 %) CAS not provided	(Blair et al., 2000)
Deiodinase (DIO) inhibition assays Source of human DIO 1, 2, 3: Cell lysate of adenovirally expressed enzyme in HEK293 cells Sandell-Kolthoff reaction to measure deiodinase-liberated iodide; substrates for DIO 1, 2, 3 were reverse triiodothyronine (rT3), thyroxine (T4), and triiodothyronine (T3), respectively	4-DPM and 4-DP: Reported as inhibitors of DIO 1, 2, and 3 DIO 1 IC_{50}:37.2 µM (4-DP); 61.4 µM (4-DPM) DIO 2 IC_{50}:74.2 µM (4-DP); 84.2 µM (4-DPM) DIO 3 IC_{50}:11.2 µM (4-DP); 15.0 µM (4-DPM) Positive controls: 6-Propyl-2-thiouracil (IC_{50} of 5.4 µM for DIO 1) Xanthohumol (IC_{50} of 0.8 µM and 0.3 µM for DIO 2 and DIO 3, respectively)	2 (reliable with restriction) Test materials: 4-DPM and 4-DP	(Olker et al., 2018)
Iodotyrosine deiodinase (IYD) inhibition assay Source of human IYD: cell lysate of baculovirally expressed enzyme in SF21 cells Sandell-Kolthoff reaction to measure IYD-liberated iodide with monoiodotyrosine as substrate	4-DPM and 4-DP: Reported as inhibitors of IYD IC_{50}: 20.1 µM (4-DPM); 23.1 µM (4-DP) Positive control: 3-Nitro-L-tyrosine (IC_{50}: 0.04 µM)	2 (reliable with restriction) Test materials: 4-DPM and 4-DP	(Olker et al., 2021)

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

For an overview of mechanistic *in vivo* studies (OECD level 3) see Table 16.

Data from two uterotrophic assays (according to OECD TG 440; reliability 1) each using six ovariectomised female Crl:CD(SD) rats per treatment group are available (Edwards, 2010a; Edwards, 2010b). These assays tested PTPD (CAS 74499-35-7) (Edwards, 2010a) or purified PTPD (impurities more polar than PTPD were removed chromatographically) (Edwards, 2010b), respectively. Rats were exposed in both studies to doses of 0, 75, 125, 250, or 500 mg/kg bw/d PTPD (actually ingested) and positive control groups received 0.2 mg/kg bw/d 17α-ethinylestradiol (EE2). Wet and blotted mean uterine weights were dose-dependently increased by PTPD and both assays demonstrated oestrogenic activity of PTPD already at the lowest dose tested (75 mg/kg bw/d). 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 bw/d of EE2) would correspond to a dose of approximately 400 mg/kg bw/d PTPD. Thus, based on this assay, the oestrogenic activity of PTPD is about 2000 times (400/0.2) lower than that of EE2.

General toxicity consisted of mortality (one animal at 500 mg/kg bw/d PTPD (Edwards, 2010b)) and lower body weights compared to the control were observed in all dose groups. At 500 mg/kg bw/d PTPD, 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 PTPD 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 4-DP (4-dodecylphenol CAS 104-43-8). In the immature rat uterotrophic assay by Akahori et al. (2008) (reliability 2), 65 chemicals were tested. 20-day old female rats were assigned to six rats per group. Three doses (single doses not specified) of 4-DP (examining the oestrogenic effect) alone or in combination with 0.6 µg/kg bw/d EE2 (examining the antioestrogenic effect) were administered subcutaneously on three consecutive days. The vehicle control group received olive oil and the positive control group received 0.6 µg/kg bw/d of EE2. The LED (lowest effective dose) of 4-DP inducing a significant increase in uterine weight was 40 mg/kg bw/d. 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 according to OECD TG 440 and TG 441, respectively (reliability 1). In the uterotrophic assay the dosage was 8, 40 and 200 mg/kg bw/d 4-DP (examining the oestrogenic effect) or 8, 40, 200 mg/kg bw/d 4-DP and 0.6 µg/kg bw/d EE2 (subcutaneous; examining the antioestrogenic effect). The test substance was administered subcutaneously. Compared to the control, the absolute and relative uterine weight was significantly increased at ≥ 40 mg/kg bw/d 4-DP. 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 4-DP at the highest dose.

In the Hershberger assay from Yamasaki et al. (2003), the dosage was 10, 30, 100 mg/kg bw/d 4-DP by gavage (examining the androgenic effect) or 10, 30, 100 mg/kg bw/d 4-DP (gavage) and 0.2 mg/kg bw/d testosterone propionate (TP, subcutaneous; 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 bw/d 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 4-DP at the highest dose.

In summary, mechanistic *in vivo* studies (OECD level 3) clearly indicate oestrogenicity but no antioestrogenic, androgenic, or antiandrogenic activity.

Table 16: 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 bw/d Positive control: 0.2 mg/kg bw/d 17α-ethinylestradiol (EE2) Exposure: oral gavage; once daily during study days 0-2 (three doses)	Endocrine mediated toxicity PTPD: Dose-dependent increases in wet (↑181-739 %) and blotted (↑183-275 %) mean uterine weights at all dose levels Positive control **17α-**ethinylestradiol (EE2): Increase in wet (↑545 %) and blotted (↑264 %) mean uterine weight (magnitude of increase corresponding to approx. 400 mg/kg bw/d PTPD). No macroscopic internal findings in the uterus at any dose General toxicity PTPD: Compared to the controls, mean body weights at day 3 were 5.5 %, 6.6 %, 7.0 %, and 11.1 % lower in the 75, 125, 250, and 500 mg/kg bw/d groups, respectively EE2: 10.7 % lower mean body weight compared to the controls	1 (reliable without restriction) Test material: PTPD	(Edwards, 2010a)
Uterotrophic assay (OECD TG 440) GLP compliance Six ovariectomised female Crl:CD(SD) rats per group 0, 75, 125, 250, 500 mg/kg bw/d Positive control: 0.2 mg/kg bw/d EE2 Exposure: oral gavage; once daily during study days 0-2 (three doses)	Endocrine mediated toxicity PTPD: 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 bw/d group died Lower mean body weight in the 500 mg/kg bw/d group (↓12.9 %; sign.) compared to the controls EE2: ↓9.9 % lower mean body weight compared to the controls	1 (reliable without restriction) Test material: PTPD (CAS most likely the same as in Edwards et al., 2010a) not as manufactured: impurities more polar than PTPD were removed chromatographically.	(Edwards, 2010b)

Uterotrophic assay (according to OECD TG 440) GLP compliance Six immature female Crl:CD (SD) IGS rats (19 days old) per group Three doses (not specified) or three doses (not specified) + EE2 (0.6 µg/kg bw/d) Exposure: subcutaneous injections of the test chemical into the back for three consecutive days (4 mL/kg bw)	Endocrine mediated toxicity 4-DP: Sign. uterine weight increase No antiestrogenic activity of 4-DP was detected when co-exposed with EE2 Lowest effective dose (LED): 151 µmol/kg bw/d (40 mg/kg bw/d); logLED: 2.18 µmol/kg bw/d General toxicity Not reported	2 (reliable with restriction) test material: 4-DP Purity > 95 % 65 chemicals were tested in three doses. The single doses were not specified. According to the authors, dose-range finding studies were performed before with each chemical up to 1000 mg/kg bw/d	(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 bw/d or 0, 8, 40, 200 mg/kg bw/d + EE2 (0.6 µg/kg bw/d) Exposure: subcutaneous injections of the test chemical into the back for three consecutive days (2 mL/kg bw)	Endocrine mediated toxicity 4-DP: Dose-dependent increases in blotted uterine weight at 40 and 200 mg/kg bw/d (absolute and relative) No antiestrogenic activity of 4-DP was detected when co-exposed with EE2 Positive control (EE2) 0.6 mg/kg bw/d: increase in blotted uterine weight (absolute and relative) Tamoxifen (1 mg/kg bw/d): Mitigation of EE2-induced increase in uterine weight General toxicity No clinical abnormalities observed Lower mean body weight in the 200 mg/kg bw/d group (↓3.9 %; not sign.) compared to the controls EE2: Lower mean body weight (↓4.6 %) compared to the controls	1 (reliable without restriction) test material: 4-DP purity unknown	(Yamasaki et al., 2003)

Hershberger assay (according to OECD TG 441) GLP compliance Six castrated male Brl Han: WIST Jcl (GALAS) rats per group 0, 10, 30, 100 mg/kg bw/d or 0, 10, 30, 100 mg/kg bw/d + testosterone propionate (TP; 0.2 mg/kg bw/d) Test substance orally administered by gavage for 10 consecutive days beginning at PND 56 TP was administered by subcutaneous injections into the back	4-DP: No androgenic or antiandrogenic effects Decreased weight of the bulbocavernosus/levator ani muscle (BC/LA) at 100 mg/kg bw/d of 4-DP; toxicological relevance unclear since no other androgen-dependent organ weights were affected. Test considered negative Positive control (TP) 0.2 mg/kg bw/d: increased weight of ventral prostate, seminal vesicle, BC/LA, glans penis, cowper's gland Flutamide (10 mg/kg bw/d): 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 bw/d group (↓8.8 %; not sign.) compared to the controls	1 (reliable without restriction) 4-DP purity unknown	(Yamasaki et al., 2003)

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

Four female pubertal assays (according to or comparable to OPPTS 890.1450; see Table 17) were performed using 15 immature female rats per dose (3-4 doses per experiment; reliability 1) each. One study deviated from the guideline as no measurements of thyroid-stimulating hormone (TSH) and thyroxine (T4), and no histopathology were performed (Knapp, 2007a). Test substances were either PTPD (Knapp, 2009b), distilled PTPD (enriched C12 homologues > 85 %) (Knapp, 2009a) or calcium salt of PTPD (Knapp, 2007a; Knapp, 2007b). Doses tested in these studies ranged from 10 to 1000 mg/kg bw/d. Combined, the results of the four studies indicate oestrogenic activity of PTPD starting at doses of 50 mg/kg bw/d. The most diagnostic parameters for an oestrogenic mode of action were an earlier attainment of vaginal patency (≥ 50 mg/kg bw/d), earlier first oestrus (≥ 60 mg/kg bw/d), oestrus cycle disturbances (≥ 50 mg/kg bw/d), reduced ovary weight (≥ 50 mg/kg bw/d) and absence or reduction of the number of corpora lutea (≥ 20mg/kg bw/d). Two studies reported granulosa cell necrosis and oocyte degeneration (severity dose dependent; ≥ 200 mg/kg bw/d) (Knapp, 2009a; Knapp, 2009b). Luteinising hormone (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 bw/d 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 oestrous 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 LH, animals at the same oestrous stage should be compared, preferably in dioestrus (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 PTPD with the thyroid hormone system was evident. Knapp (2007b) reported a dose-dependent increase in the incidence of thyroid hypertrophy at ≥ 20 mg/kg bw/d. Thyroid hypertrophy and colloid depletion was also noted at 800 mg/kg bw/d (3/15 animals) in Knapp (2009b) and at ≥ 200 mg/kg bw/d (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 bw/d in one out of three pubertal assays which measured this parameter (Knapp, 2009a).

General toxicity was observed starting at 200 mg/kg bw/d as indicated by lower body weights, and mortality occurred at ≥ 800 mg/kg bw/d (Knapp, 2007a; Knapp, 2009a; Knapp, 2009b). Increased weights (absolute and relative to body weight) of liver (≥ 250 mg/kg bw/d) and decreased weights (absolute and relative to body weight) of thymus (≥ 200 mg/kg bw/d) and spleen (800 mg/kg bw/d) 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 bw/d (Knapp, 2007a).

In summary, mechanistic *in vivo* studies (OECD level 4) clearly show oestrogenic activity for PDDP, but also interaction with the thyroid hormone system.

Table 17: Mechanistic *in vivo* studies (OECD level 4) - female pubertal assays.

Method	Results	Remarks	Reference
Female puberty assay comparable to OPPTS 890.1450 15 immature female Crl:CD (SD) rats per group; 0, 60, 250, and 1000 mg/kg bw/d Exposure: oral gavage once daily for 20 consecutive days, postnatal days 22-41	Endocrine mediated toxicity Earlier vaginal opening and lower body weight at time of vaginal opening compared to controls (≥ 60 mg/kg bw/d; sign.); 34.5, 28.3, 27.9, 27.6 days at a body weight of 105.9, 75.4, 75.2, and 67.4 g at 0, 60, 250, and 1000 mg/kg bw/d, respectively Younger age at first oestrus (≥ 60 mg/kg bw/d) No difference in oestrous cycle length but cycle length at this age highly variable Decreased absolute ovary weight (≥ 60 mg/kg bw/d) Decreased uterine weight (relative and absolute; wet and blotted) (≥ 250 mg/kg bw/d)	1 (reliable without restriction) Test material: Calcium salt of PTPD (No CAS given) No histology and no hormone measurements were performed	(Knapp, 2007a)

	General toxicity Two animals in the 1000 mg/kg bw/d group found dead after two days of dosing No effects on mean body weights or body weight gains were observed in the 60 and 250 mg/kg bw/d groups. Mean body weight gain during the first three dosing intervals was lower in the 1000 mg/kg bw/d group but no effect on mean body weight over the entire treatment period was observed Increased mean absolute and relative liver weight (≥ 250 mg/kg bw/d) Increased mean absolute and relative adrenal gland weights (≥ 60 mg/kg bw/d)		
Female puberty assay according to OPPTS 890.1450 15 immature female Crl:CD (SD) rats per group 0, 5, 20, and 60 mg/kg bw/d 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 bw/d; sign.); 33.2, 33.3, 32.7, and 29.1 days at a body weight of 110.9, 108.2, 109.5, and 89.2 g at 0, 5, 20, and 60 mg/kg bw/d, respectively. Decreased number of corpora lutea (≥ 20 mg/kg bw/d) Uterine hypoplasia (60 mg/kg bw/d) Thyroid hypertrophy (≥ 20 mg/kg bw/d) 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)	1 (reliable without restriction) Test material: Calcium salt of PTPD (no CAS given)	(Knapp, 2007b)
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 (≥ 50 mg/kg bw/d;	1 (reliable without restriction)	(Knapp, 2009a)

15 immature female Crl:CD (SD) rats per group 0, 10, 50, 200, and 800 mg/kg bw/d Exposure: oral gavage once daily for 20 consecutive days, postnatal days 22-41	sign.); 32.5, 32.3, 29.3, 29.2, and 29.4 days at a body weight of 111.9, 87.3, 86.1, and 71.6 g at 0, 10, 50, 200, and 800 mg/kg bw/d, respectively (historical control range: 31,8 – 36.5 days) Younger age at first oestrus (dose-dependent; sign. at 800 mg/kg bw/d) Increased number of females in permanent dioestrus or oestrus (≥ 50 mg/kg bw/d) Oestrous 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 bw/d) Uterus atrophy (severity dose dependent; ≥ 200 mg/kg bw/d) Cervix atrophy (800 mg/kg bw/d) Decreased absolute and/or relative ovarian/oviduct weight (sig. ≥ 10 mg/kg bw/d) Decreased number of corpora lutea (dose-dependent; ≥ 200 mg/kg bw/d); complete absence in all animals at 800 mg/kg bw/d Granulosa cell necrosis and oocyte degeneration (severity dose dependent; ≥ 200 mg/kg bw/d) Decreased absolute pituitary weight (dose dependent; sign. at 800 mg/kg bw/d) E2 and TSH levels sign. increased at 800 mg/kg bw/d Luteinising hormone(LH) and T4 levels unchanged Colloid depletion and hypertrophy of the thyroid at ≥ 200 mg/kg bw/d (lower dose groups not investigated); ectopic thymus-tissue (1/15 animals at 200 mg/kg bw/d and 8/15 animals at 800 mg/kg bw/d) General toxicity	Test material: PTPD Purity: 100 % distilled, laboratory-enriched C12 homologs > 85 %; not as commercially manufactured 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. Analysis of LH and E2 levels does not take oestrus stage into account.	

	Clinical signs: Brown material on various body surfaces (800 mg/kg bw/d). Clear material around the mouth; and salivation (≥ 200 mg/kg bw/d) 11/15 females in the 800 mg/kg bw/d 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 bw/d 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 bw/d group Increased absolute and relative liver weights (800 mg/kg bw/d) Decreased absolute and/or relative weights of spleen (dose-dependent; sign. at 200 mg/kg bw/d) and thymus (dose-dependent; sign. at ≥ 200 mg/kg bw/d)		
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 bw/d 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 (dose-dependent; ≥ 50 mg/kg bw/d; sign.); 32.5, 33.3, 28.3, 28.2, and 28.9 days at 111.9, 85.4, 83.4, and 73.9 g at 0, 10, 50, 200, and 800 mg/kg bw/d, respectively (historical control range: 31,8 – 36.5 days) Younger age at first oestrus (dose-dependent; sign. ≥ 200 mg/kg bw/d) Increased number of females in permanent oestrus (≥ 200 mg/kg bw/d) Oestrous 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 bw/d; sign.) Uterus atrophy (800 mg/kg bw/d; only in animals found dead)	1 (reliable without restriction) Test material: PTPD 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. Due to high mortality in the 800 mg/kg bw/d group, no statistical analysis	(Knapp, 2009b) Same control group as in (Knapp, 2009a)

	Decreased absolute and relative ovarian/oviduct weight (dose-dependent; sig. ≥ 50 mg/kg bw/d) Absence of corpora lutea (dose-dependent; ≥ 200 mg/kg bw/d) Granulosa cell necrosis and oocyte degeneration (severity dose dependent; ≥ 200 mg/kg bw/d) E2, LH, T4, and TSH levels unchanged Thyroid hypertrophy at 800 mg/kg bw/d (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 bw/d). Clear material around the mouth; and salivation (≥ 200 mg/kg bw/d) 8/15 females in the 800 mg/kg bw/d group died after one to four days of exposure. 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 bw/d group Lower body weight gain (PND 27-28; sign.) and body weight (↓9.7-16.1 %; PND 24-40; 800 mg/kg bw/d; sign.) Increased absolute and relative liver weights (800 mg/kg bw/d) Decreased absolute and relative weights of spleen (dose-dependent; sign. at 800 mg/kg bw/d) and thymus (dose-dependent; sign. ≥ 200 mg/kg bw/d)	Histology was done only for the two highest dose groups Analysis of LH and E2 levels does not take oestrus stage into account.	

4.11.6. Repeated-dose toxicity studies (OECD level 4)

Several guideline and non-guideline studies investigating repeated-dose toxicity in rats (Haas, 2012; Harriman, 2004; Reyna and Thake, 1988; Vogin, 1970) and one study in dogs (Vogin, 1970) are available for PTPD (CAS 74499-35-7) or 4-DPM (CAS 27193-86-8) (reliability 1 or 2; see Table 18). 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 the test substance. In two out of three studies which investigated this parameter, decreased ovarian weight starting at doses of 100 - 200 mg/kg bw/d was observed (Haas, 2012; Harriman, 2004). Reduced numbers of corpora lutea were reported at doses ≥ 150 mg/kg bw/d in Haas (2012) and at 180 mg/kg bw/d in Harriman (2004). Decreased uterus weight without histological changes was reported by Haas (2012) at doses ≥ 150 mg/kg bw/d.

In male rats, decreased weight of testes and sexual accessory organs were evident in several studies at doses ≥ 180 mg/kg bw/d (Haas, 2012; Harriman, 2004; Reyna and Thake, 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 bw/d (Harriman, 2004; Vogin, 1970)) and/or interstitial atrophy (≥ 180 mg/kg bw/d (Harriman, 2004)), and tubular hypoplasia (300 mg/kg bw/d (Reyna and Thake, 1988; Vogin, 1970)). Histology of male sexual accessory glands revealed decreased secretion of seminal vesicles and prostate (≥ 150 mg/kg bw/d (Haas, 2012; Harriman, 2004; Reyna and Thake, 1988)), hypoplasia/atrophy of the coagulating glands and prostate (≥ 200 mg/kg bw/d (Haas, 2012; Reyna and Thake, 1988)), and hypoplasia and hypospermia in the epididymis (300 mg/kg bw/d (Harriman, 2004; Reyna and Thake, 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)).

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 bw/d (Vogin, 1970) was observed. Thus, this study might indicate a species-specific sensitivity of rat towards this substance. However, the focus of this study was on the male reproductive tract and only three dogs per sex were used in each treatment group.

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 bw/d. Increased liver weights were observed at ≥ 200 mg/kg bw/d (Harriman, 2004; Reyna and Thake, 1988; Vogin, 1970), accompanied by vacuolisation 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 (Alanine transaminase (ALAT), Aspartate transaminase (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 increased in 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 and Thake, 1988). Adrenal cortical hypertrophy was reported in males in one study (Haas, 2012).

In summary, the results of repeated-dose toxicity studies (OECD level 4) in rats demonstrate changes of EAS mediated parameters but also other toxic effects at the same dose levels. In addition, in one study, thyroid hypertrophy was evident in males.

Table 18: Repeated-dose toxicity studies (OECD level 4).

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 bw/d groups; 5/sex/group in other dose groups From the 10 animals/sex in the control and 300 mg/kg bw/d group, 5 animals/sex/group were terminated at 28 days whereas 5 animals/sex/group were assigned to a 14-day recovery period; 0, 5, 20, 60, 180 and 300 mg/kg bw/d Exposure: oral (gavage) 7 days a week for 28 days	Endocrine mediated toxicity Females Reduced ovarian weight (≥ 180 mg/kg bw/d; dose-dependent) Reduced corpora lutea (≥ 180 mg/kg bw/d) Males Decreased absolute testes weight and ratios relative to brain or body weights (300 mg/kg bw/d) Germ cell depletion (300 mg/kg bw/d) and/or interstitial cell atrophy (≥ 180 mg/kg bw/d) Decreased absolute weight and ratios relative to brain or body weights of coagulating gland, epididymidis, prostate, seminal vesicles (dose-dependent; ≥180 mg/kg bw/d; sign.); substantially more affected than terminal body weight Hypospermia and cellular luminal debris in the epididymides (300 mg/kg bw/d) Decreased secretion of prostate and seminal vesicles (≥ 180 mg/kg bw/d; sign.) Thyroid hypertrophy (≥ 5 mg/kg bw/d; incidence dose-dependent) General toxicity Females Reduced body weight gain and lower body weight (not sign.) Increased liver weight (300 mg/kg bw/d; sign.) Centrilobular hepatocellular hypertrophy and periportal hepatocellular vacuolisation (≥ 180 mg/kg bw/d) ALP and ASAT unchanged, gamma-glutamyltransferase (GGT) slightly elevated at ≥ 180 mg/kg bw/d Decreased haematocrit and haemoglobin (≥ 180 mg/kg bw/d, dose-dependent, females only) Decreased serum cholesterol, and increased serum triglycerides (≥ 180 mg/kg bw/d; dose-dependent) Males	1 (reliable without restriction) Test material: PTPD 100 % purity Old OECD TG407 protocol; contains none of the endocrine parameters which have been added in the revised versions from 1998 and 2008.	(Harriman, 2004)

	Reduced food consumption, body weight gain, and lower body weight (↓10 % and ↓13 % at 180 and 300 mg/kg bw/d, sign. respectively) Increased liver weight (300 mg/kg bw/d; sign.) Centrilobular hepatocellular hypertrophy and periportal hepatocellular vacuolisation (≥ 180 mg/kg bw/d) ALP and GGT unchanged; ASAT decreased at 300 mg/kg bw/d Increased absolute and relative adrenal weight (≥ 180 mg/kg bw/d) Decreased absolute weight of the heart and ratio relative to brain (≥ 180 mg/kg bw/d) Recovery: microscopic findings in male and female reproductive organs persisted; thyroid and liver findings resolved		
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/d Exposure: 28 day (7 days/week) Organ weights: adrenals, brain, kidneys, liver, spleen, testes with epididymides; no ovarian weight was determined Laboratory only reported those findings where organs were	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 associated with histological findings 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 consumption (≥ 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) 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)	2 (reliable with restrictions) Test material: PTPD Purity unknown Old OECD TG407 protocol; contains none of the endocrine parameters which have been added in the revised	(Reyna and Thake, 1988)

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	 Males Reduced food consumption (≥ 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)	versions from 1998 and 2008	
90-day repeated dose toxicity study according to OECD TG 408 GLP compliance Dose-range finding study for a two-generation study (Edwards et al., 2012); not all parameters as in OECD TG408 were examined SD rats (10 animals/sex/dose) 0, 50, 100, 150 and 200 mg/kg bw/d Exposure: oral (feed) for 91-92 consecutive days	Endocrine mediated toxicity Females Higher number of females in oestrus (200 mg/kg bw/d; not sign.) Reduced absolute and relative weights (rel. to body and/or brain weight) of ovaries/oviducts (dose dependent; ≥ 100 mg/kg bw/d; sign.) Lower number of corpora lutea (≥ 150 mg/kg bw/d) Reduced uterine weight (≥ 150 mg/kg bw/d; not sign.) without macroscopic or microscopic findings Males Reduced testes weight (200 mg/kg bw/d) Lower weight of prostate (≥ 100 mg/kg bw/d) and prostate atrophy (200 mg/kg bw/d) Lower seminal vesicles weight (≥ 100 mg/kg bw/d) and decreased seminal vesicle secretion (≥ 150 mg/kg bw/d) Atrophy of coagulating glands (200 mg/kg bw/d) General toxicity Females Lower food consumption (≥ 100 mg/kg bw/d) Reduced body weight gain and lower body weight in all dose groups (sign. in all dose groups; ↓10.4-19.4%)	1 (reliable without restriction) Test material: PTPD No analysis of sperm or oestrous cyclicity Old OECD TG 408 protocol; contains none of the endocrine parameters which have been added in	(Haas, 2012)

	Reductions in white blood cells and lymphocytes at 200 mg/kg bw/d Reductions in serum cholesterol (≥ 100 mg/kg bw/d) Lower ASAT (200 mg/kg bw/d) Males Lower food consumption (≥ 100 mg/kg bw/d) Reduced body weight gain and lower body weight in all dose groups (sign. in all dose groups;↓11.7-35.6%) Increased absolute and relative weights (rel. to body and/or brain weight) of the adrenals (≥ 100 mg/kg bw/d) and adrenal cortex hypertrophy (200 mg/kg bw/d) Kidney mineralisation (≥ 50 mg/kg bw/d) Liver vacuolisation (≥ 150 mg/kg bw/d) Lower ALAT (≥ 150 mg/kg bw/d) Lower red blood cell count and lower haemoglobin (200 mg/kg bw/d) Reductions in white blood cells and lymphocytes at 200 mg/kg bw/d	the revised version from 2018.	
90-day repeated dose toxicity study (non-guideline) 20 Albino FDRL rats per sex and group 0, 25, 100 and 200 mg/kg bw/d 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 bw/d (↓23 %) Testicular hypospermia (6/20 animals) at 200 mg/kg bw/d General toxicity Females Reduced food consumption, body weight gain and lower body weight at 200 mg/kg bw/d (↓11 %; not sign.) Increased liver weight relative to body weight at 200 mg/kg bw/d; no histological findings Males Reduced food consumption, body weight gain and lower body weight at 200 mg/kg bw/d (↓18.4 %; not sign.) Increased liver weight relative to body weight at 200 mg/kg bw/d; no histological findings	2 (reliable with restrictions) Test material: 4-DPM Purity unknown No analytical confirmation of dietary concentrations Chronic	(Vogin, 1970)

		bronchitis and chronic inflammation of kidneys in all dose groups	
90-day repeated dose toxicity study (non-guideline) Three Beagle dogs per sex and group 0, 18, 71 and 143 mg/kg bw/d Exposure: oral, treated feed was available 1 h/day, 6 days/week for 13 weeks	No significant general toxicity and no effects on reproductive organs observed	2 (reliable with restrictions) Test material: 4-DPM Purity unknown No analytical confirmation of dietary concentrations	(Vogin, 1970)

4.11.7. Reproductive and developmental toxicity studies (OECD level 4 and 5)

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 (see Table 19).

One-generation reproductive toxicity study (Knapp, 2006)

In a one-generation reproductive toxicity study (OECD TG 415; reliability 1) by Knapp (2006), PTPD 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 bw/d 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 bw/d. Only 4/30 females with evidence of copulation became pregnant (control 28/30). Mean litter size at 125 mg/kg bw/d was 1.7 pups per litter compared to 13 pups per litter in controls. Furthermore, several effects of PTPD 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 oestrous 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 in number of ovarian cysts at 125 mg/kg bw/d. Absolute uterus weight was not significantly changed at any dose level. At 125 mg/kg bw/d, uterus weight relative to brain weight was significantly increased and an increase in number of endometrial cysts was detected microscopically. Oestrous cycle length tended to be longer with increasing dose and a higher number of females were in permanent dioestrus or oestrus at termination (125 mg/kg bw/d). 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 bw/d significant for testes). Further findings in males included reduced epididymal sperm concentrations (≥ 25 mg/kg bw/d), and decreased secretion of prostate (≥ 5 mg/kg bw/d), coagulating glands (≥ 25 mg/kg bw/d), and seminal vesicles (125 mg/kg bw/d). 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 bw/d). However, balano-preputial separation was associated with lower mean body weight, and the timing was within the historical control range of the lab.

Signs of general toxicity were evident in both sexes, characterized by lower food consumption, lower pre-mating weight gain and lower pre-mating body weight (↓9.7% in females and ↓20.6% in males at 125 mg/kg bw/d) as well as lower terminal body weight (↓18.5% in females and ↓28.4% in males at 125 mg/kg bw/d). Kidney weight relative to body weight was increased in males (≥ 25 mg/kg bw/d) and females (≥ 125 mg/kg bw/d) accompanied by kidney mineralisation (≥ 25 mg/kg bw/d in males and at 125 mg/kg bw/d in females). In males only, increased liver weight (relative to body weight) without histological findings (125 mg/kg bw/d), and increased weight of the adrenals (relative to body weight and brain weight; ≥ 25 mg/kg bw/d) with adrenocortical hypertrophy (125 mg/kg bw/d) was reported. F1 offspring at 25 mg/kg bw/d 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 bw/d group due to the small sample size. Effects on body weight were the most significant findings of general toxicity in both sexes and occurred often at similar doses than the effects on reproductive parameters. However, in particular in females, the effects on reproductive parameters cannot be explained only by reduced food consumption and reduced body weight. This is in agreement with the conclusion of RAC with regard to classification of the substance as Repr. 1B (ECHA, 2013a; ECHA, 2013b; ECHA, 2013c; ECHA, 2013d).

Two-generation reproductive toxicity study (Edwards, 2012)

In a two-generation reproductive toxicity study (OECD TG 416; 2001; reliability 1) by Edwards (2012), PTPD 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 bw/d by dietary exposure. Premating exposure lasted for a minimum of 70 consecutive days. F0 and F1 males were dosed throughout mating and through to the day of euthanasia. F0 and F1 females were dosed throughout mating, gestation, and lactation through to 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 generation at 75 mg/kg bw/d compared to the controls, but this did not reach statistical significance. On the other hand, in the F2a generation, the number of pups born (10.1 vs 13.4 in controls) and live litter size (9.5 vs 13.4 in controls) was significantly reduced at 75 mg/kg bw/d. The number of implantation sites was significantly reduced in the F0 adults at 75 mg/kg bw/d (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 bw/d 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 bw/d (27.4 days versus 32.4 days in controls).

Epididymal sperm count was significantly reduced at 75 mg/kg bw/d 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 bw/d. 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 bw/d) 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 F1 and F2a).

General toxicity was evident in both sexes of the F0 and F1 generations. Food consumption, pre-mating weight gain, pre-mating body weight (↓12.6 % in females and ↓17.3 % in males) as well as terminal body weight (↓12% in females and ↓18.5% in males) was lower at 75 mg/kg bw/d. Pup body weight gain and pup body weight was lower in the F1 and F2/F2a generation (mostly at 75 mg/kg bw/d). Body weight effects were also evident in the F1 adult females (1st mating: ↓12.5%, 2nd mating ↓18.8%, termination ↓24% vs control) and males (1st mating: ↓22.5 %, 2nd mating ↓25.9%, termination ↓28.4% vs control) at 75 mg/kg bw/d. Adrenal weight relative to body weight was slightly but significantly increased in both sexes of the F0 and F1 generation (75 mg/kg bw/d) but no histology was performed. F0 and F1 males showed increased incidences of kidney mineralisation (at 75 mg/kg bw/d in F0 and at ≥ 15 mg/kg bw /d in F1). Effects on body weight were the most significant finding of general toxicity occurring at similar doses than reproductive toxicity. In males, body weight effects explained most of the weight changes observed in reproductive organs. In females however, the body weight effects are not considered severe enough to explain the observed effects on endocrine sensitive reproductive parameters which is in agreement with the conclusion of RAC with regard to classification of the substance as Repr. 1B (ECHA, 2013a; ECHA, 2013b; ECHA, 2013c; ECHA, 2013d). In addition, precocious puberty in F1 occurred despite lower body weights, clearly indicating oestrogenic activity and being adverse.

Prenatal developmental toxicity study according to OECD TG 414 (Schroeder, 1987)

A prenatal developmental toxicity study (reliability 1) by Schroeder (1987) was performed according to the outdated protocol of OECD TG 414 from 1981, and no specific parameters diagnostic for endocrine disruption (regarding EATS modalities) were examined. In this study, 4-DPM (CAS 27193-86-8) was administered to 24 female pregnant Sprague-Dawley rats by oral gavage in a dose of 0 (corn oil vehicle control), 20, 100 and 300 mg/kg bw/day on days 6 – 15 of gestation (an additional group receiving 500 mg/kg bw/d was terminated early due to excessive mortality). Dams were sacrificed on day 20 of gestation. Uterine contents were evaluated and fetuses were examined for external, visceral, and skeletal alterations. At 300 mg/kg bw/d, increased resorptions (4.0 compared to 0.8 in the controls) and reduced litter size (8.9 compared to 12.5 in the controls) were reported. Numbers of corpora lutea and implantations remained unchanged in all treatment group. Pre-implantation loss was higher at 300 mg/kg bw/d (0.132 vs 0.007 in controls; not significant). Foetal weight was significantly lower at 300 mg/kg bw/d (↓12% compared to the controls). An increased incidence of foetuses with ossification variations was observed at 300 mg/kg bw/d, indicating a general developmental delay.

General toxicity in dams was limited to soft stool and significantly reduced bodyweight gain and reduced food consumption at 300 mg/kg bw/d. Body weight gain over the whole pregnancy was significantly lower (↓30%) and in particular lower during the dosing period on days 6- 15 of gestation (↓62%). At day 20, body weight was 8 % lower than the controls (unclear whether this is corrected body weight). Although, increased resorptions and reduced litter size could be due to an endocrine mode of action of the substance affecting the reproductive hormone axis, the substancially lower body weight gain of dams during pregnancy days 6-15 indicates also unspecific toxicity. It should be however noted in the context of this evaluation that the study design involving exposure of already pregnant animals during the time of organogenesis is not dedicated to specifically detect effects related to fertility and sexual function.

In summary, the results of reproductive toxicity studies (OECD level 4 and 5) demonstrate changes in EAS mediated parameters, and adversity related to fertility and reproductive function. Pubertal development in females was accelerated pointing to oestrogenic activity of the test substance. Particularly in females, the body weight effects cannot explain the consistent findings on reproductive parameters.

Table 19: Reproductive and developmental toxicity studies (OECD level 4 and 5).

Method	Results	Remarks	Reference
One-generation reproduction toxicity study according to OECD TG 415 (1983) with some extension GLP compliance 0, 5, 25 or 125 mg/kg bw/d SD Crl:CD rats (30/sex/group) Exposure: oral (gavage) daily Premating exposure: both sexes for 73 days prior to mating F0 males: dosed throughout mating and through the day prior of euthanasia for a total of 138-143 doses F0 females: dosed throughout mating,	Endocrine mediated toxicity (P) Females (P) Reduced live litter size at 125 mg/kg bw/d (sign.) Reduced fertility index at 125 mg/kg bw/d (sign.) Reduced number of implantation sites (125 mg/kg bw/d) Reduced number of corpora lutea (125 mg/kg bw/d) Increased number of females in permanent oestrus or dioestrus (125 mg/kg bw/d); more females in oestrus at termination Dose-dependent increase of oestrous cycle length (≥25 mg/kg bw/d; not sign.) Dose-dependently decreased ovary/oviduct weight (≥25 mg/kg bw/d; absolute and rel to brain weight; at 125 mg/kg bw/d; rel. to body weight; sign.) Increased uterus weight (at 125 mg/kg bw/d; absolute; not sign.; rel. to brain weight; not sign.; rel. to body weight; sign.) Increased number of ovarian and endometrial gland cysts (125 mg/kg bw/d; sign.) Histological findings in vagina and mammary gland (type of findings not reported) Males (P) Reduced copulation index at 125 mg/kg bw/d (sign.) Reduced weight of seminal vesicles/coagulating gland (≥5 mg/kg bw/d; sign. rel. to brain weight; sign. at 5 and 125 mg/kg bw/d rel. to body weight) Reduced weight of cauda epididymis (≥25 mg/kg bw/d; sign. rel. to brain weight) Reduced weight of epididymis, prostate, and right testis (125 mg/kg bw/d; sign. rel. to brain weight) Reduced epididymal sperm concentrations (≥25 mg/kg bw/d)	1 (reliable without restriction) Test material: PTPD Purity 100% Initial body weights were lower than recommended in the guideline No statistical evaluation of pups in the 125 mg/kg bw/d group due to insufficient litters No exposure post-weaning	(Knapp, 2006)

gestation, lactation and through the day prior of euthanasia for a total of 115-128 doses	Decreased secretion of prostate (dose dependent; sign. at ≥25 mg/kg bw/d), coagulating glands (dose-dependent; sign. at ≥25 mg/kg bw/d), and seminal vesicles (125 mg/kg bw/d) General toxicity (P) Females (P) Lower food consumption and lower pre-mating weight gain and lower pre-mating body weight (↓9.7% at 125 mg/kg bw/d; sign.); Lower body weight during gestation (↓17.3% at 125 mg/kg bw/d; sign.) and at termination (↓18.5% at 125 mg/kg bw/d; sign.) Increased kidney weight (rel. to body weight; 125 mg/kg bw/d) Mineralisation in kidneys (125 mg/kg bw/d) Males (P) Lower food consumption and lower pre-mating weight gain and lower pre-mating body weight (↓20.6%; at 125 mg/kg bw/d; sign.); Lower body weight at termination (↓12.9 at 25 mg/kg bw/d and ↓28.4% at 125 mg/kg bw/d; sign.) increased liver weight (rel. to body weight; 125 mg/kg bw/d); no histological findings Increased kidney weight (rel. to body weight; ≥25 mg/kg bw/d) Mineralisation in kidneys (≥ 25 mg/kg bw/d) Increased weight of the adrenal glands with hypertrophy of the adrenal cortex (males at 125 mg/kg bw/d) Endocrine mediated toxicity (F1) Note: pups were not exposed post-weaning No effect on time to vaginal opening in females Delayed balano-preputial separation in males (sign. at 25 mg/kg bw/d; 125 mg/kg bw/d was not statistically evaluated). However, delayed balano-preputial separation was associated with lower mean body weight, and the value was within the historical control range of the lab General toxicity (F1)		

	Decreased postnatal survival (125 mg/kg bw/d) and lower body weight and body weight gain (≥25 mg/kg bw/d)		
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 bw/d 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 bw/d) but not sign. and close to historical control range Reduced number of implantation sites (↓12 %; 75 mg/kg bw/d; sig.) Reduced ovary weight at 75 mg/kg bw/d (↓29.6 %; absolute; sign.). Decreased ovary weight rel. to body weight (↓21.6 %) and rel. to brain weight (↓26 %; 75 mg/kg bw/d; sig.) Decreased presence of corpora lutea at 75 mg/kg bw/d (6/28 vs 1/30 in controls; sign.) Increased length of oestrus cycle (75 mg/kg bw/d; sign.) Increased number of females in permanent dioestrus (75 mg/kg bw/d) Increased ovarian cysts (≥ 15 mg/kg bw/d; not sign.) Males (P) Decreased epididymal sperm concentration (↓26 %; 75 mg/kg bw/d; sig.) Decreased absolute seminal vesicle weight (↓30 %; 75 mg/kg bw/d; sig.). Decreased seminal vesicle weight rel. to body weight (↓14.4 %) and rel. to brain weight (↓29 %; 75 mg/kg bw/d; sign.) Decreased absolute prostate weight (↓22 %; 75 mg/kg bw/d; sig.). Decreased prostate weight rel. to body weight (↓4.3 %; 75 mg/kg bw/d; not sign.) and rel. to brain weight (↓21 %; 75 mg/kg bw/d; 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 bw/d; sign.) General toxicity (P)	1 (reliable without restriction) Test material: PTPD purity 100 % 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”. No thyroid histology performed.	(Edwards, 2012)

	Females P Decreased consumption and food utilisation efficiency at premating (75 mg/kg bw/d) Decreased body weight at initiation of mating (↓12.6 %) and at termination (↓12 % at 75 mg/kg bw/d; sign.) Increased relative adrenal weight at 75 mg/kg bw/d; (sign.) Males P Decreased consumption and food utilisation efficiency at premating (75 mg/kg bw/d) Decreased body weight at initiation of mating (↓17.3 %) and at termination (↓18.5 %; 75 mg/kg bw/d; sign.) Increased relative adrenal weight (75 mg/kg bw/d; sign.) Renal mineralisation (75 mg/kg bw/d) Endocrine mediated toxicity (F1) Females F1 Reduced number of pups born and decreased live litter size at 75 mg/kg bw/d; sign. in F2a, not sign. in F2 Reduced ovary weight at 75 mg/kg bw/d (↓38 %; absolute; sign.). Decreased ovary weight rel. to body weight (↓19.2 %) and rel. to brain weight (↓35.7 %; 75 mg/kg bw/d; sign.) Decreased presence of corpora lutea at 75 mg/kg bw/d (16/26 vs 6/28 in controls; sign.) Increased length of oestrous cycle (75 mg/kg bw/d; sign.) Increased number of females in permanent oestrus or dioestrus (75 mg/kg bw/d) Earlier vaginal patency (75 mg/kg bw/d; sign.; 27.4 days at 112 g vs 32.4 days at 60.8g in controls) 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 bw/d; sign.) Delayed balano-preputial separation (75 mg/kg bw/d) 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 adrenal weights absolute, relative to body weight and brain weight (75 mg/kg bw/d; sign.) 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 bw/d (sign.) Renal mineralisation (≥ 15 mg/kg bw/d) Increased relative adrenal weight (75 mg/kg bw/d; sign.) Toxicity (F2/F2a pups) Reduced survival from birth to PND4 (75 mg/kg bw/d). Lower birth weights and lower body weight gains (75 mg/kg bw/d) No effects on (AGD 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 bw/day	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	1 (reliable without restriction) Test material: 4-DPM 100 % purity Old OECD TG 414 protocol, no endocrine-related	(Schroeder, 1987)

Exposure: oral: (gavage) once/day, from GD 6 – 15 (females only); termination at GD 20 Foetuses were evaluated for external, visceral, and skeletal alterations	Reduced food consumption and weight gain (300 mg/kg bw/d) during treatment (↓62 %; sign.), and reduced weight gain over the whole pregnancy (↓30 %; sign.). Lower body weight at day 20 (300 mg/kg bw/d; ↓8 %; sign.) 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 (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)	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	

4.11.8. Analysis of the mode of action and the biological plausibility of a link between the adverse effects and endocrine activity

Several assays and studies corresponding to levels 2 – 5 of the OECD conceptual framework for testing and assessment of endocrine disruptors (OECD, 2018) are available for PDDP. The substance (as TPP) is classified as Repr. 1B (H360F) as included in the ninth ATP of the CLP Regulation (data on TPP EC 310-154-3 and CAS 121158-58-5 as well as 7449-35-7 was taken into account by RAC). 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 75 mg/kg with test material PTPD in the two-generation reproductive study in rats (Edwards, 2012).
- Reduced number of pups born in the F2a generation exposed to a dose of 75 mg/kg (Edwards, 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 oestrogenic and androgenic activity."

This classification implies adverse effects on apical toxicity endpoints concerning fertility and sexual function which are not considered to be secondary non-specific consequences of other toxic effects. Therefore, adversity is clearly established, and all of the effects mentioned above are EAS mediated or sensitive to the EAS modalities (OECD, 2018).

Although the available in vitro data show interaction of PDDP with ER as well as with AR, for the present document oestrogenicity is put forward as the most relevant mode of action underlying the observed effects on fertility and sexual function particularly in females due to the following reasons:

1) The RBA of PDDP to the ER was higher than to the AR, and the Toxcast models predict higher scores for oestrogenic than for antiandrogenic activity.

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

3) Particularly in males, antiandrogenic as well as oestrogenic activity could lead to effects similar to the ones observed for PDDP on certain reproductive parameters, and an overlap of both modes of action cannot be completely excluded. However, regarding parameters specific for androgenic/antiandrogenic activity, there was no significant effect on AGD in the two-generation study (Edwards, 2012) with PTPD and a Hershberger assay with 4-DP was negative (Yamasaki et al., 2003).

There are also indications for interaction of PDDP with the thyroid system. *In vitro* assays demonstrated inhibition of DIO and IYD activites by 4-DPM and 4-DP (Olker et al., 2018; Olker et al., 2021). Both test substances (different CAS numbers) also display TSH receptor agonism in an EDSP21 *in vitro* assay (as assessed on 2021-02-22). *In vivo*, thyroid hyperplasia and colloid depletion were reported in some studies, and TSH was increased in one female pubertal assay (at 800 mg/kg bw/d). These findings raise an additional concern for disruption of the thyroid hormone system. However, effects on the

thyroid gland were not consistently observed in all studies in which thyroid histology was performed.

Therefore, a mode of action analysis was performed on the oestrogen modality with focus on the female reproductive system. The following Adverse Outcome Pathways (AOPs) for ER agonism and adverse outcomes in female mammals are under development:

- AOP 167 (Early-life estrogen receptor activity leading to endometrial carcinoma in the mouse; https://aopwiki.org/aops/167).
- AOP 295 (Early-life stromal estrogen receptor activation by endocrine disrupting chemicals in the mammary gland leading to enhanced cancer risk; https://aopwiki.org/aops/295)
- AOP 200 (Estrogen receptor activation leading to breast cancer; https://aopwiki.org/aops/200)
- AOP 146 (Estrogen Receptor Activation and Female Precocious Puberty; https://aopwiki.org/aops/146)

Since all of these AOPs are based on oestrogenic activity, they are in principle relevant for PDDP. However, cancerogenic studies have not been performed with PDDP and mammary gland histology was not investigated or is poorly reported in the available studies. AOP 146 on the other hand is highly relevant for this document since induction of precocious puberty by PDDP was clearly observed in several studies and was considered by RAC as an adverse effect leading to classification. However, this AOP is still under development. Therefore, a mode of action analysis was performed on ER agonism leading to reduced fertility as adverse outcome (AO) via a molecular initiating event (MIE) followed by different key events (KEs) (Table 20).

The hypothesised mode of action and the resulting AO applies not only for female human health but also for mammals in general and has populational relevance in an ecotoxicological context (see chapter Environmental relevance 5.2.5). It should also be noted that this mode of action analysis is simplified and oestrogens exert a variety of effects on reproductive function including feedback on pituitary gonadotropins as well as direct actions on reproductive organs.

Table 20: Analysis of mode of action

		Supporting evidence
MIE	ER activation	Strong evidence: Studies show binding of PDDP to ER (Akahori et al., 2008; Blair et al., 2000; Thomas, 2012b), and the ToxCast ER bioactivity model (status from 2021-02-22) additionally shows ER activation[14]
KE1	Increased oestrogen signaling	Strong evidence: 4/4 uterotrophic assays (Akahori et al., 2008; Edwards, 2010a; Edwards, 2010b; Yamasaki et al., 2003) and 4/4 pubertal assays (Knapp, 2007a; Knapp, 2007b; Knapp, 2009a; Knapp, 2009b) are positive
KE2	Disturbed oestrous cycle	Strong evidence: Of the studies which reliably determined these parameters, 2/3 studies show prolonged (Edwards, 2012; Knapp, 2006) and 5/5 studies showed irregular oestrous cycles (Edwards, 2012; Haas, 2012; Knapp, 2006; Knapp, 2009a; Knapp, 2009b), including both generations in the OECD TG 416 study.
KE3	Decreased ovulation;	Strong evidence: Decreased corpora lutea in 7/10 studies (Edwards, 2012; Haas, 2012; Harriman, 2004; Knapp, 2006; Knapp, 2007b;

	decreased ovary weight	Knapp, 2009a; Knapp, 2009b); consistent between F0 and F1 generations of the OECD TG 416 study. Reduced ovary weight in 7/10 studies (Edwards, 2012; Haas, 2012; Harriman, 2004; Knapp, 2006; Knapp, 2007a; Knapp, 2009a; Knapp, 2009b); also consistent between F0 and F1 generations of the OECD TG 416 study
KE4	Decreased implantations	Strong evidence: Decreased number of implantation sites were detected in 2/2 reproductive toxicity studies (Edwards, 2012; Knapp, 2006).
AO	Decreased fertility	Strong evidence: Decreased litter size was observed in 2/2 reproductive toxicity studies (Edwards, 2012; Knapp, 2006)

There is strong evidence for ER binding and activation by PDDP. Increased oestrogen signaling is confirmed by findings in mechanistic *in vivo* studies (increased uterus weight in uterotrophic assays and accelerated pubertal development in female pubertal assays). Increased oestrogen signaling leads to disturbed oestrous cycles, and decreased ovulation as observed by decreased numbers of corpora lutea and reduced ovarian weight in several studies. This in turn leads to a decreased number of implantation sites and finally results in reduced litter size. Both KE4 (decreased implantation) and the AO (reduced litter size) were observed in reproductive toxicity studies. The biological plausibility of the links between the different KEs and the AO is rated high based on rodent studies with model oestrogens (see 4.11.10 and Table 21), as well as from knowledge on mammalian reproductive endocrinology and human contraception.

4.11.9. Human relevance

Oestrogen signaling plays a key role in mammalian reproduction. There is no reason to assume that the observed adverse effects on fertility by disruption of oestrogen signaling in rats have no human relevance. The lack of effects in the single dog study with PDB which used a low number of animals (Vogin, 1970) gives no reason to question the human relevance of the proposed mode of action and AO. The ToxCast ER bioactivity model in addition shows oestrogenic agonist activity for 4-DPM and 4-DP, and this prediction model is built on a number of assays utilizing human ERs.

4.11.10. Summary and discussion of endocrine human health effects

Considering the results of all available studies, 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 increase in 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 oestrous cycle was consistently observed in the majority of studies with PDDP, 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 21 and Biegel et al. (1998a), Biegel et al. (1998b), NTP (2010a), (Biegel et al., 1998a; Biegel et al., 1998b; NTP, 2010a); NTP (2010b)). In cycling females, PDDP-induced aberrant oestrous cycles and decreased uterus weight (sometimes accompanied by hypoplasia/atrophy) were 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 oestrous 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 PDDP 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, preferably 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 contradict an oestrogenic mode of action of PDDP.

Also, in males, several effects of PDDP on the reproductive system were observed, including lower weight of testes and accessory reproductive organs. However, when organ to body weight ratios were compared, the effects observed in PDDP 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 bw/d). At doses ≥ 180 mg/kg bw/d, 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 PDDP 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 PDDP, in particular on the female reproductive system (e.g. on ovarian weight and histology, oestrous cycle, implantations, etc.), can be plausibly linked to the oestrogenic activity of the substance and explain the adverse impacts seen on apical fertility endpoints such as live litters born or litter size (see Table 20). However, most of the affected reproductive parameters are also sensitive to non-endocrine toxicity, and several findings which can be interpreted as general toxicity were observed at similar doses. These effects include reduced food consumption and body weight gain, 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. Among these, body weight effects associated with reduced food consumption were the most relevant findings which may have an unspecific impact on reproductive function in particular in the males. With regard to females, however, the decrease in body weights are judged not to be severe enough to explain the adverse effects seen on fertility and reproductive function (ECHA, 2013a; ECHA, 2013b; ECHA, 2013c; ECHA, 2013d).

In addition, basically all of these general toxicity parameters are sensitive to (but not diagnostic of) EATS-mediated toxicity (OECD, 2018) (ECHA et al., 2018). In fact, several effects of PDDP, which are usually interpreted as general/unspecific toxicity, are also observed in studies with model oestrogens. Very consistently, reduced body weight gain and lower body weight (often associated with lower food consumption) 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 bw/d E2 (Biegel et al., 1998b); 10-16 % lower body weight after 90 days exposure to 4-6 µg/kg bw/d EE2 (NTP, 2010a)). Furthermore, lower body weights were also seen for EE2 in the uterotrophic assays reported in the present document (Edwards, 2010a; Edwards, 2010b; Yamasaki et al., 2003) Therefore, the lower body weight gains and body weights due to PDDP exposure can be explained, at least in part, by the oestrogenicity of PDDP. Further effects of E2 and EE2 include increased weights of liver

(with centrilobular hypertrophy) and adrenal gland (sometimes with cortical hypertrophy), decreased thymus and spleen weight, kidney mineralisation in males, and effects on blood and serum parameters (Biegel et al., 1998b; Hart, 1990; NTP, 2010a; NTP, 2010b). Thus, it is plausible that some of these effects which also occurred in studies with PDDP are not just unspecific toxicity but could also be mediated by the oestrogenic activity of the substance to some degree (see also Table 21).

In conclusion, the effects on the female reproductive organs and functional parameters are consistent with an oestrogenic mode of action of PDDP and cannot simply be attributed to the observed signs of general, non-specific toxicity. Instead, there is strong evidence that the adverse effects on fertility and sexual function, particularly in females, are plausibly linked to the oestrogenic activity of the substance. PDDP is therefore an endocrine disruptor according to the WHO/IPCS definition (WHO/IPCS, 2002) with regard to human health.

Table 21: Comparison between *in vivo* effects of PDDP female and male rats, and the expected oestrogenic response as inferred from low-dose rodent studies with potent model oestrogens (EE2 and E2). Parameters are additionally grouped in 'EATS-mediated' and 'sensitive to, but not diagnostic, of EATS'

Females		
Effects of PDDP	Response to model oestrogens	Remarks/Uncertainties
EATS-mediated		
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 PDDP at 500 mg/kg bw/d and EE2 (positive control) at 0.2 mg/kg bw/d
Decreased uterus weight in 4/8 studies with cycling females. One study reported an increase and 3 studies reported no effects	Mostly increases but sometimes also decreases in uterus weight are detected in cycling female rats (e.g. in the dose range finding study reported in (NTP, 2010a))	Parameter highly dependent on oestrous stage. Uterus weights in PDDP studies with cycling females were not correlated to oestrous 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 gain usually lead to a delay in vaginal opening.
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
Increased length of oestrous 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 oestrous 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 5/5 studies which investigated/reported this parameter	Irregularities of oestrous 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)	PDDP-induced lower ovary weights cannot be explained by lower body weights or other general toxicity (ECHA, 2013c; ECHA, 2013d)
Sensitive to, but not diagnostic of EATS		
Decreased corpora lutea in 7/10 studies; also consistent between F0 and F1 generations of the OECD TG 416 study	Decreased corpora lutea (Biegel et al., 1998b)	Parameter sensitive to oestrogenicity as well as to general toxicity
Decreased implantation sites	Decreased implantation sites (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 (Biegel et al., 1998b)	Parameter sensitive to oestrogenicity as well as to general toxicity
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 PDDP
Increased relative liver weight reported in 6/11 studies at doses ≥ 200 mg/kg bw/d; sometimes with hepatic centrilobular hypertrophy	Increased relative liver weights with hepatic centrilobular hypertrophy reported (Biegel et al., 1998b)	Parameter sensitive to oestrogenicity as well as to general toxicity
Increased adrenal weight in some studies with/without cortical hypertrophy	Increased adrenal weight sometimes with cortical hypertrophy (Biegel et al., 1998b; Hart, 1990)	Parameter sensitive to oestrogenicity as well as to general toxicity (stress)
Males		
Effects of PDDP	Response to model oestrogens	Remarks/Uncertainties
EATS-mediated		
Delayed balano-preputial separation at the same or at	Delayed balano-preputial separation (Biegel et al.,	No clear evidence for hormonal effect of PDDP due

lower body weight than controls	1998b)	to markedly lower body weights
Decreased weight of testes and sexual accessory organs only at high doses of PDDP. 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)	Effects of PDDP 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 bw/d)	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
Sensitive to, but not diagnostic of EATS		
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 PDDP
Increased relative liver weight reported at doses ≥ 125 mg/kg bw/d; sometimes with hepatic centrilobular hypertrophy	Hepatic centrilobular hypertrophy (Biegel et al., 1998b)	Parameter sensitive to oestrogenicity as well as to general toxicity
Increased adrenal weight in some studies with/without cortical hypertrophy	Increased adrenal weight sometimes with cortical hypertrophy (Biegel et al., 1998b; Hart, 1990)	Parameter sensitive to oestrogenicity as well as to general toxicity (stress)
Kidney mineralisation in 3/6 studies; also observed in F0 and F1 generations of the OECD TG 416 study	Kidney mineralisation observed in males exposed to EE2 at low doses (NTP, 2010a; NTP, 2010b)	PDDP-induced kidney mineralisation likely due to oestrogenic activity

5. Environmental hazard assessment

5.1. Aquatic compartment (including sediment)

The substance has a harmonised classification as Aquatic acute 1 and Aquatic chronic 1.

5.1.1. Fish

The dissemination site contains four acute fish tests. According to the registration dossier, the first three tests were conducted with *Pimephales* promelas according or similar to OECD TG 203. In the first test using PTPD the LC_{50} after 96 h exposure was 40 mg/L (WAF). Sublethal effects such as loss of equilibrium, lethargy and gasping were seen (Klimisch 2). The second test with PTPD had a LC_{50} of 3.2 mg/L (nominal) after 96 h. The test was assigned Klimisch 3 by the registrant, due to inappropriate dispersion of test material in the solutions. The test vessels 10, 35 and 100 mg/L contained insoluble material and were cloudy. The third test with PDB showed a nominal LC_{50} of 24 mg/L (Klimisch 3, due to inappropriate dispersion of test material). The fourth test, which was conducted according to EU Method C.1 (Acute Toxicity for Fish), using *Leuciscus idus melanotus* with PDB showed no toxicity (Klimisch 4). The concentration was analytically measured, but could not be specified, as it was below the limit of detection (0.5 mg/L). The exposure duration was not given. All reliability scores were assigned by the registrant.

There are no tests for long-term toxicity to fish available.

5.1.2. Aquatic invertebrates

The ECHA dissemination site reports that PDB is very acutely toxic to the aquatic compartment with an EC_{50} of 0.037 mg/L for *D. magna* as the most sensitive tested species (Klimisch 1) in a test according to OECD Guideline 202. The test concentrations were confirmed analytically.

There were four other less reliable acute invertebrate studies (number 2 to 5). The second test with *D. magna* exposed to PTPD according to OECD TG 202 with reliability 2 had analysed concentrations. The effect value was EL_{50} 3.4 mg/L, however the effect concentration was nominal and it is not stated, that the measured concentrations were in the range of 80 to 120% of nominal concentrations. The third test with *D. magna* and exposure to PDB was conducted according to Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US EPA, 1975) without analytics. The effect value was EC_{50} 0.072 mg/L and the reliability Klimisch 2 was assigned. In the fourth test *Americamysis bahia* were exposed to PDB. It was conducted according to EPA OTS 797.1930 (Mysid Acute Toxicity Test), (reliability 2) with analytics and the effect value was EL_{50} 0.58 mg/L (nominal). The fifth study according to EU Method C.2 (Acute Toxicity for Daphnia) was without analytics and assigned reliability 4 (EC_{50} 0.093 mg/L PDB). All reliability scores were assigned by the registrant.

The chronic *D. magna* study according to OECD TG 211 with PDB 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 (21 d) for reproduction and immobilisation were 0.0086 mg/L and 0.0079 mg/L, respectively. EC_{10} values were not provided (Klimisch 1, assigned by the registrant).

5.1.3. Algae and aquatic plants

According to the ECHA dissemination site, the ErC_{50} (72 h) of algae was 0.36 mg/L and the NOEC was 0.07 mg/L (the basis for effect was not specified) in a test according to OECD TG 201 and the test material PDB. The test was assessed by the registrant with Klimisch 1. The effect values were given by the registrant as nominal values. Analytical monitoring was conducted. Analysis of the test solutions at t = 0 showed measured concentrations of 19 to 38 % of nominal and at t = 72 h they ranged from 17 to 24 % of nominal. .
By calculating the effect values using the measured concentrations they would be only about 24 % of nominal (ErC50: 0.087 mg/L, NOEC: 0.017 mg/L). The second and third study had no analytics and were less sensitive than the first study.

5.2. Endocrine Disruption – environment

5.2.1. General approach – environment

According to section 4.11, there is strong evidence that the adverse effects on fertility and sexual function in rats, particularly in females, are plausibly linked to the oestrogenic activity of the substance. PDDP is therefore an endocrine disruptor according to the WHO/IPCS definition (WHO/IPCS, 2002) with regard to human health.

The evaluation of PDDP for the environment is based on the mammalian data used for the human health assessment and supported by available fish *in vitro* tests and adverse outcome pathways. There are no aquatic *in vivo* long term data for fish and other aquatic vertebrates or investigations for ED available.

Section 5.2.2 describes why and how results with organisms tested for human health assessment can be used for the assessment for the environment. Endpoints considered for human health and environment assessment differ, i.e. with regard to the environment only endpoints considered to be of population relevance are used. Thus, the relevance of effects observed in the mammalian studies with regard to environmental populations is evaluated in section 5.2.3 when weighing the evidence for endocrine disruption.

5.2.2. Use of human health data for environmental ED assessment

Information on effects on the environment can be drawn from the effects on mammals in the human health assessment, as mammals are also environmental organisms and because the tested organisms are representatives for all other terrestrial and marine mammals.

This is in line with the ECHA-EFSA guidance for the identification of endocrine disruptors in the context of the Biocidal Products Regulation or the Plant Protection Products Regulation (ECHA et al., 2018) which proposes that the same data base can be used to conclude on the endocrine disrupting properties for human health and the environment: *“The information needed to assess ED properties for humans and non-target organisms may overlap. Mammalian data are always relevant for ED assessment on non-target organisms. Furthermore, there may be information on non-target organisms that could be relevant also for the ED assessment for humans.“* and *“[…] it is recommended to strive for a conclusion on the ED properties with regard to humans and in parallel, using the same*

database, to strive for a conclusion on mammals as non-target organisms."[16] Additionally, it is in line with the Revised Guidance Document 150 (OECD, 2018) which states that results from non-human mammalian studies are also highly relevant for mammalian wildlife species.

As further described in the ECHA-EFSA guidance: *"It is sufficient that the substance meets the ED criteria in one taxonomic group in order to conclude that a substance meets the ED criteria for non-target organisms."*

Thus, information on population relevant effects on mammals are sufficient to conclude that a substance is an endocrine disruptor for the environment too.

In addition, with regard to the observed mode of action of PDDP, effects on mammals can also give information on endocrine disruption in non-mammalian vertebrates. Due to the evolutionary conservation of the endocrine system, in particular with regard to ER and AR (Ankley and Gray, 2013), there is strong evidence that PDDP may act as an oestrogenic endocrine disruptor in non-mammalian aquatic vertebrates too:

- Ankley and Gray (2013) compared 21–day reproductive fish assays with mammalian uterotrophic assays and female pubertal 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 utilising either fish or human ER. The reproductive endocrine system in mammals and fish is to a large extent similar (Kime, 1998) and therefore, effects can be expected to be comparable (Wester et al., 2004).

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 estrogen, 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."*

5.2.3. Population relevance of endocrine disruptive effects of PDDP for the environment

As stated e.g. by the European Commission's Endocrine Disrupters Expert Advisory group *"ecotoxicological [ED] assessment relates to impact at the population level rather than the individual level"*, thus "*relevance is applied in the context of identified adverse effects being relevant for the population*" (JRC, 2013). Effects on growth, development and reproduction in single species are generally regarded relevant for the maintenance of the wild populations (e.g. (Kortenkamp et al., 2011).
As described in section 4.11.10, studies in rats clearly demonstrated adverse effects of PDDP on the reproductive function in mammals that can be plausibly linked to the oestrogenic activity of the substance.

[16] Quotes from page 7 and 9 of the ECHA-EFSA guidance for endocrine disruptors.

PDDP caused significant effects on fecundity and fertility (such as reduction in number of corpora lutea, reduced number of implantation sites, reduced fertility index, reduced number of pups, reduced litter size) in reproductive toxicity studies of level 4 and 5 (Edwards, 2012; Knapp, 2006) leading to a reduced number of offspring. Due to reduced copulation index and reduced fertility, a marked number of females did not become pregnant after PDDP exposure.

It is concluded that PDDP fulfills the WHO/IPCS criteria (WHO/IPCS, 2002) of an endocrine disruptor for the environment since the effects observed in mammals are relevant for populations.

5.2.4. Environmental data in non-mammalian species

Available environmental *in vitro* data and AOPs (adverse outcome pathways) further support the statement that PDDP is an endocrine disruptor for the environment (see sections 5.2.4.2. and 5.2.4.3).

5.2.4.1. 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), 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) are already identified as substances of very high concern for being environmental ED due to their oestrogenic properties in fish. In comparison to PDDP, which has a carbon-chain length of 12 carbon atoms, these substances only differ in their alkyl chain length in para-position of the phenol ring (C4 up to C9 branched carbon chains). The high degree of structural similarity suggests that PDDP may also act as an endocrine disruptor via binding to similar molecular targets.

5.2.4.2. *In vitro* information indicative of endocrine activity

In this section, *in vitro* tests with fish cells/tissues are evaluated with 4-DPM and the read-across substance 4-DP. In one study (Knudsen and Pottinger, 1999) it is stated that linear alkyl phenols were tested (p-n-dodecylphenol, 4-DP). The authors of several publications (Tollefsen, 2007; Tollefsen et al., 2008; Tollefsen and Nilsen, 2008) stated that 4-dodecylphenol with the CAS 104-43-8 was used, but they specified that it was an isomeric mixture, hence, it is assumed that they used branched 4-DPM.

In vitro studies with mammalian receptors/enzymes on PDDP (including 4-DPM), as well as on 4-DP (read-across substance with supposed linear dodecyl chain) are evaluated in section 4.11.3.

Fish *in vitro* tests with 4-DPM and 4-DP:
Competitive binding studies are available for rainbow trout (rt) ER and the plasma sex steroid binding protein (SBP) (Knudsen and Pottinger, 1999; Tollefsen, 2007; Tollefsen and Nilsen, 2008). In addition, an oestrogen transactivation assay with rainbow trout hepatocytes (Tollefsen et al., 2008) is available. Results are summarised in Table 22.

For the competitive binding assay (Tollefsen and Nilsen, 2008) with the hepatic rtER, 4-DPM was used. The IC_{50} is 22 µM, comparable to the IC_{50} for 4-t-octylphenol (4-tOP, CAS 140-66-9) of 51 µM. Regarding the RBA (compared to E2), the potency of 4-DPM 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 4-DPM: 0.016 %.

For the competitive binding study by Tollefsen (2007) on the sex steroid binding protein of rainbow trout (rtSBP), 4-DPM was used. The plasma rtSBP binds sex steroids such as E2. The IC_{50} of 4-DPM for competition with the SBP was 320 µM, comparable to the IC_{50} of 4-tOP of 120 µM. The RBA of 0.00049 % for 4-DPM was lower than for 4-tOP (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 4-DPM binds and activates the ER as measured by the expression of vitellogenin (VTG) protein. Exposure of the cells to 4-DPM 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, 4-DPM 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).

In the competitive binding study with the read-across substance 4-DP (Knudsen and Pottinger, 1999) hepatic rtER was used. No effect values were specified but it was stated by the authors 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-nonylphenol, p-n-octylphenol) were in the same range, therefore this also applies for 4-DP. The RBA estimated from this value is 0.01 % compared to E2.

In addition, a graphical description is available. Effect values of three concentrations were depicted in a diagram: maximum displacement of tritiated E2 (^{3}H-E2) (approx. 60 %, read from graph) by 4-DP 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). 4-DP was used without specified purity.

Table 22: *In vitro* assays based on rainbow trout with 4-DPM and 4-DP.

Mechanistic *in vitro* studies based on rainbow trout (OECD level 2)			
Method	Results	Remarks	Reference
Competitive binding assay, hepatic rainbow trout oestrogen receptor (rtER)	4-DPM: IC_{50}: 22 µM RBA: 0.016 % Positive control (**17β-estradiol** (E2)): IC_{50}: 3.5 nM	4-DPM[17], (purity 96 %) Reliability 2	(Tollefsen and Nilsen, 2008)

[17] CAS 104-43-8 (4-DP) was specified in the publication, however the authors indicated that they used an isomeric mixture

	RBA set to 100 %		
Competitive binding assay rainbow trout plasma sex steroid-binding protein (rtSBP)	4-DPM: IC_{50}: 320 µM RBA: 0.0005 % Positive control (E2): IC_{50}: 1.6 nM RBA set to 100 %	4-DPM[17], (purity 96 %) Reliability 2	(Tollefsen, 2007)
Induction of vitellogenin (VTG) production in rainbow trout hepatocytes	4-DPM: In combination with E2 (0.1 nM): LOEC 10 µM, with E2 (0.3 nM): LOEC 30 µM (values read from graph) LOEC > 300 µM (without combination with E2) Cytotoxicity at 30 µM (25 % decreased viability)	4-DPM[17], (purity 96 %) Reliability 2	(Tollefsen et al., 2008)
Competitive binding assay hepatic rtER	4-DP: 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-estradiol by p-n-Dodecylphenol almost in the same range as 4-n-nonylphenol	4-DP (purity not specified), read-across substance CAS not provided Reliability 2	(Knudsen and Pottinger, 1999)

Although no *in vivo* studies in non-mammalian vertebrates are available, 4-DPM and 4-DP (read-across substance) 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). These results show oestrogenic activity on OECD level 2 for fish and hence support the conclusion that PDDP is an endocrine disruptor not only for for mammalian but as well for non-mammalian vertebrates such as fish.

Comparison of *in vitro* and *in vivo* effects of PDDP with 4-tOP and 4-NP:
The comparison in the table below of the in vitro effects of 4-tOP and 4-NP with PDDP shows similar effects. Additionally, for reasons of structural similarity (para-alkylphenol) and on basis of the AOPs (see below) it is suggested that PDDP causes similar in vivo effects as 4-tOP and 4-NP (see supporting document for SVHC identification of 4-NP[18] and 4-tOP[19] as well as (Demska-Zakęś, 2005)).

Table 23: Comparison of in vitro and in vivo data of 4-tOP, 4-NP and PDDP

Parameter	4-tert-octylphenol	4-nonylphenol	PDDP
In vitro data for estrogen receptor mediated pathway			
Binding to Estrogen Receptors			
Rainbow trout	(Hornung et al., 2014): RBA =9.4 x10^{-5} = 0.0094 %	(Hornung et al., 2014): 5 different isomers tested (1 linear, 4 branched): RBA ranges from 1.6 x 10^{-4} to 4.6 x 10^{-4} = 0.016 to 0.046 %	
Rainbow trout	(Tollefsen and Nilsen, 2008): RBA = 6.9 x 10^{-5} = 0.0069 %	(Tollefsen and Nilsen, 2008): RBA = 1 x 10^{-5} = 0.001 %	(Tollefsen and Nilsen, 2008): 4-DPM: RBA = 1.6 x 10^{-4} = 0.016 %
Rainbow trout	(Knudsen and Pottinger, 1999): 10^4-fold more 4-n-octylphenol than those of E2 was required to obtain similar displacement of specifically bound [^{3}H]E2. RBA: 0.01 % (estimated). Maximum displacement achieved: ca. 45%	(Knudsen and Pottinger, 1999): 10^4-fold more 4-n-nonylphenol than those of E2 was required to obtain similar displacement of specifically bound [^{3}H]E2. RBA: 0.01 % (estimated). Maximum displacement achieved: ca. 50%	(Knudsen and Pottinger, 1999): 10^4-fold more 4-DP (read-across substance) than those of E2 was required to obtain similar displacement of specifically bound [^{3}H]E2. RBA: 0.01 % (estimated). Maximum displacement achieved: ca. 60 %
Binding to sex steroid-binding protein			
Rainbow trout	(Tollefsen, 2007): RBA = 0.0013 %	(Tollefsen, 2007): 4-n-Nonylphenol was only a weak binder here	(Tollefsen, 2007): 4-DPM: RBA = 0.0005 %
Expression of vitellogenin			
Rainbow trout	(Tollefsen et al., 2008): LOEC = 1 µM	(Tollefsen et al., 2008): 4-n-Nonylphenol LOEC = 30 µM	(Tollefsen et al., 2008): 4-DPM LOEC > 300µM; in combination with E2 (0.1 nM): LOEC 10 µM, with E2 (0.3 nM): LOEC 30 µM
In vivo effects in fish (LOECs in mg/L)[18, 19]			
Pimephales promelas			
Reproduction assay or comparable		0.071 fecundity 0.00025 behaviour 0.015 VTG 0.071 secondary sexual characteristics	
Danio rerio			
FSDT or comparable tests		0.01 skewed sex	

		ratio 0.1 Gametogenesis females 0.01 Gametogenesis males 0.03 testis-ova 0.1 Ovarian follicle atresia 0.1 VTG	
FLC	0.035 fertility, time to first spawn, body length		
Oryzias latipes			
FSDT	0.011 VTG 0.023 testis-ova 0.0481 sex ratio	0.0012 VTG and testis-ova 0.024 sex ratio	
Reproduction assay	0.02 VTG < 0.02 fertility	0.005 (VTG) 0.184 Inhibition of spermatogenesis 0.0061 fecundity and fertility	
FLC	0.0099 VTG 0.03 testis-ova < 0.01 fertility	0.0018 testis- ova 0.052 sex ratio based on gonadal histology in F0 0.018 sex ratio based on gonadal histology in F1	
Oncorhynchus mykiss			
FSDT		0.00105 VTG 0.01 Growth	
Reproduction assay & other	0.039 VTG ≤ 0.039 increased percentage of early sperm stages (spermatogonia), reduced GSI in initial experiment	0.01 VTG 0.001 VTG (F1 without exposure) 0.037 Inhibition of spermatogenesis 0.086 non developed ovaries 0.01 sexual steroids in F1	
Sander lucioperca, (Demska-Zakęś, 2005)			
Effects on sex ratio (histological):		0.001 (nom.) Decrease of male fish 0.010 (nom.) Increase of female fish 0.001 (nom.) Intersex	
Chronic toxicity to fish Mortality/length/weight/ condition factor		LOEC: >0.2 (nom.)	

5.2.4.3. Adverse outcome pathways

It is also possible to obtain information from the AOPs 29 and 52. However, it should be noted that they are yet under development.

- AOP29: Estrogen receptor agonism leading to reproductive dysfunction (https://aopwiki.org/aops/29)
- AOP52: Skewed sex ratio (https://aopwiki.org/aops/52)

AOP29: Estrogen receptor agonism leading to reproductive dysfunction

The AOP 29 describes the linkages between agonism of the ER and population relevant impacts on reproductive function in oviparous vertebrates including amphibia, birds and fish.

The molecular initiating event is agonistic binding to and activation of the ER. This was examined for 4-DPM and 4-DP in *in vitro* tests conducted with fish tissues/cells. The tests showed binding and activation of the ER, therefore confirming for fish the molecular initiating event (MIE) of AOP 29. In the AOP, the MIE leads via the different key events (KEs) to the adverse outcome of reproductive dysfunction. This adverse effect could be shown for PDDP in mammalian *in vivo* level 4 and 5 assays. For the various KEs no data for PDDP itself are available, but data on the structurally similar compounds 4-nonylphenol (4-NP) and 4-tert-octylphenol (4-tOP) show that these KEs can be triggerd by long-chain alkylphenols.

Table 24: Analysis of mode of action for AOP 29

	Defined in AOP	Supporting evidence
MIE	Agonism, oestrogen receptor	ER binding and activation: Strong evidence: Studies show binding of PDDP to rainbow trout ER (rtER) (Tollefsen and Nilsen, 2008), (Knudsen and Pottinger, 1999). Induction of VTG production in rainbow trout hepatocytes (Tollefsen et al., 2008)
KE	Reduced cumulative fecundity and spawning	Reduced fecundity observed with4- NP in *P.promelas* (Harries et al., 2000), in *O.latipes* (Ishibashi et al., 2006) and with 4-tOP in *O.latipes* (Gronen et al., 1999)
KE	Increase of plasma vitellogenin concentrations	Increased plasma VTG observed in several fish species with NP (Harries et al., 2000), (Schoenfuss et al., 2008), (Schwaiger et al., 2002), (Jobling et al., 1996) and with 4-tOP (Gronen et al., 1999), (Jobling et al., 1996), (Karels et al., 2003)
KE	Increase of Vitellogenin synthesis in liver	Increased hepatic VTG observed in several fish species with 4-NP (Ackermann et al., 2002; Cardinali et al., 2004; Kang et al., 2003; Seki et

		al., 2003) and with 4-tOP (Seki et al., 2003)
KE	Increase of renal pathology due to VTG deposition	No information
AO	Decreased population trajectory	No information
AO	Altered reproductive behavior	Delayed appearance of courtship behaviour in *P.reticulata* (guppy) observed with 4-NP (Cardinali et al., 2004)
AO	Altered larval development	Time to hatch increased in *O.latipes* observed with 4-NP (Ishibashi et al., 2006)
AO	Impaired development of reproductive organs	Inhibition of testicular growth measured as GSI (Gonadosomatic index) observed with 4-NP and 4-tOP in *O.mykiss* (Jobling et al., 1996)

AOP52: Skewed sex ratio

The MIE in this case is also ER binding and activation. It was observed for 4-DPM and 4-DP.

Table 25: Analysis of mode of action for AOP 52

	Defined in AOP	Supporting evidence
MIE	Agonism, oestrogen receptor	ER binding and activation: Strong evidence: Studies show binding of PDDP to rainbow trout ER (rtER) (Tollefsen and Nilsen, 2008), (Knudsen and Pottinger, 1999). Induction of VTG production in rainbow trout hepatocytes (Tollefsen et al., 2008)
KE	AOP 52 refers to AOP 29 regarding key events	See AOP 29
AO	skewed sex ratio	Sex-ratio skewed towards females observed with 4-NP (Seki et al., 2003), (Yokota et al., 2001) and 4-tOP (Seki et al., 2003) in *O.latipes*

In conclusion the binding and activation of oestrogen receptors leads to population relevant adverse effects, which is described in both AOPs.

Information on effects of 4-nonylphenol (4-NP) and 4-tert-octylphenol show that the effects as described in the AOPs can occur by exposure of fish to long-chain alkylphenols. This information, as listed in the table, is described in detail in the SVHC Support Documents for 4-nonylphenol (4-NP)[18] and 4-tert-octylphenol (4-tOP)[19] and e.g. in the publication by (Seki et al., 2003).

[18] See supporting document for SVHC identification of 4-NP (https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807db370)
[19] See supporting document for SVHC identification of 4-tOP (https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807d9e89)

For both AOPs: The available *in vitro* data confirm that the MIE in fish, common for both AOPs, is triggerd by 4-DPM and 4-DP. Analogous long chain alkylphenols like 4-NP were found to trigger the KEs of both AOPs linking ER activation with adverse outcomes on reproductive function. Hence, it is likely that PDDP exposure leads to the adverse outcomes in fish as specified in the AOPs.

Hence, the information from the AOPs cited above support the conclusion that PDDP exerts oestrogenic effects on environmental organisms other than mammals.

5.2.5. Environmental relevance

In section 5.2.3. it is shown that PDDP causes adverse effects in mammals, which are relevant at the populational level. These effects of PDDP are endocrine mediated via an oestrogenic mode of action. As a consequence of the oestrogen-like action, the reproduction of mammals is impaired in several ways leading to decreased fertility. For more information see the evaluation of effects regarding human health (section 4.11) or the summary and discussion of human health effects (section 4.11.10).

As mammals also are environmental organisms, these effects are relevant for the environment. Additionally, due to the conservation of the endocrine system, the endocrine disruptive (oestrogenic) mode of action of PDDP is similarly relevant for non-mammalian vertebrates such as fish (see section 5.2.2 Use of human health data for environmental ED assessment).

Other long chain alkylphenol substances that are already identified as SVHC (4-tert-octylphenol and 4-nonylphenol) showed indicative and adverse effects on fish linked to an oestrogenic mode of action. Diagnostic effects were e.g. increase of plasma and liver vitellogenin. Adverse effects were e.g. skewed sex ratio, decreased fecundity and fertility.

5.2.6. Summary and discussion of endocrine environmental effects

Mammalian data show endocrine effects that are population relevant. These data on human health (see section 4.11.10) are also used for assessment of endocrine disruption for the environment: There is strong evidence that the adverse effects on fertility and sexual function particularly in females are due to the oestrogenic activity of PDB.

In section 5.2.5 above it was shown that these effects are relevant for the assessment of effects on the environment. Hence these effects that show an impairment of reproduction in mammals endanger population stability in the environment. In conclusion the effects seen in mammals also give a strong concern for endocrine effects on organisms (mammals and non-mammalian vertebrates) in the environment. Information from section 5.2.4 supports this conclusion.

6. For the environment *in vitro* data are available that support the conclusion drawn in the human health endocrine assessment section. No environmental aquatic in vivo data for fish or other vertebrates are available. Conclusions on the SVHC Properties

6.1. CMR assessment

PDB is covered by index number 604-092-00-9 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) and it is classified in the hazard class reproductive toxicity category 1B (H360F[20]). In its respective opinion[21] on the dossier proposing harmonised classification and labelling of 5 December 2013 RAC states that "the harmonised classification will apply to any substance which predominantly contains C12 (branched) alkyl-substituted phenols.

Therefore, it can be concluded that PDDP meets the criteria of Article 57 (c) of Regulation (EC) No 1907/2006 (REACH) owing to its classification in the hazard class reproductive toxicity category 1B (ECHA, 2013c; ECHA, 2013d)[22].

6.2. PBT and vPvB assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (c) and (f) REACH.

6.3. Art 57(f) assessment

6.3.1. Summary of the data provided

There is strong evidence for ER binding and activation by PDDP in mechanistic *in vitro* studies (OECD level 2). Mechanistic *in vivo* studies (OECD level 3 and 4) show oestrogenic activity of PDDP: increased uterus weight in uterotrophic assays and accelerated pubertal development in female pubertal assays (see sections 4.11.3, 4.11.4, 4.11.5).

The results of repeated dose as well as reproductive toxicity studies (OECD level 4 and 5) demonstrate changes in EAS-sensitive as well as EAS-mediated parameters, and adversity related to fertility and reproductive function. Female pubertal development in the two-generation reproductive toxicity study was similarly accelerated as in pubertal assays pointing to and confirming the oestrogenic activity of the test substance (see section 4.11.7).

In the evaluation of endocrine effects on human health it was concluded that there is strong evidence that the adverse effects on fertility and sexual function, particularly in females, are plausibly linked to the oestrogenic activity of the substance. PDDP is therefore an endocrine disruptor according to the WHO/IPCS definition (WHO/IPCS, 2002) with regard to human health.

The evaluation of PDDP for environment is based on mammalian data and supported by

[20] H360F: 'May damage fertility'
[21] https://echa.europa.eu/documents/10162/7e7b5949-9d0a-2896-fb10-1504496ab2eb
[22] Classification in accordance with section 3.7 of Annex I to Regulation (EC) No 1272/2008.

available fish *in vitro* tests and adverse outcome pathways. There are no aquatic *in vivo* long term data for fish and other aquatic vertebrates or investigations for ED available.

As effects on growth, development and reproduction in single species are generally regarded relevant for the maintenance of the wild populations (Kortenkamp et al., 2011) the above listed effects on reproduction and development in rats are relevant for populations in the environment (see section 5.2.3).

6.3.2. Equivalent level of concern assessment for human health

The observed effects on fertility and sexual function are of equivalent level of concern to substances classified as CMR and/or PBT/vPvB substances. This is due to the following reasons specified below.

Potential severity: The observed effects on fertility and sexual function have led to the harmonised classification Repr. 1B – a CMR classification to which Art. 57 (f) directly refers.

Irreversiblility: No data are available for PDDP to conclude on the reversibility of the observed effects. Some effects such as disturbed oestrous cycles and reduced fertility might be reversible when adults are exposed. However, effects resulting from exposure during sensitive developmental windows do not fully manifest until reproductive age (delay of effects) and can be permanent as has been shown for other oestrogenic substances. Based on the available data on PDDP, this is specifically the case for accelaterated female puberty which can lead to a variety of adverse health outcomes later in life (Day et al., 2015; Golub et al., 2008).

Quality of life: A reduced ability to reproduce considerably affects the quality of life negatively for the individuals affected as well as for their partners and families.

Negative impact on society: A reduced ability to reproduce negatively affects society as it contributes to an increased financial burden e.g. on the health care sector, both providing assisted fertilisation treatments and clinical treatment for individuals with adverse reproductive effects. Reduced fertility is of general concern in the EU countries.

Additionally, the findings for an interaction of PDDP with the thyroid hormone system and a lack of appropriate characterization of corresponding endpoints raises further concern for developmental neurotoxicity resulting from exposure during sensitive life stages.

6.3.3. Equivalent level of concern assessment for the environment

The effects of PDDP on mammals are considered to be of equivalent level of concern as those of CMR and/or PBT/vPvB substances. This is due to the severity and irreversibility of the effects on organisms and populations and the difficulties to quantify a safe level of exposure in the environment.

PDDP severely affects reproduction and development-related processes in organisms

PDDP adversely affects normal development (puberty) and reproductive ability (fertility) in rats. Due to the general conservation of hormone systems between different mammalian species, rats are an appropriate animal model for other mammalian wildlife species. The observed reproductive effects are considered an adverse and serious effect with population level relevance.

PDDP can affect a large variety of species in different ecosystems

The reproductive endocrine system is highly conserved not only between mammals, but also between mammals and oviparous vertebrates such as fish. Hence, adverse effects on reproduction as observed for rats are expected to occur in other mammals, and in addition, it is highly likely that PDDP also leads to adverse effects (such as reproductive dysfunction and skewed sex ratios) in oviparous vertebrates. Furthermore, the organisms in the environment are exposed to a mixture of substances. Hence there can be additive or synergistic effects that might enhance the impact. For example, additive effects were seen for mixtures of long chain alkyl phenols and other oestrogenic compounds like E2 and EE2 (Brian et al., 2005), (Sumpter et al. 2006). Increased sensitivity in an *in vitro* test was seen using 4-DPM with co-exposure to E2 indicating enhanced mixture effects (Tollefsen et al., 2008).

Irreversibility of effects:
In a one-generation study the copulation index was decreased. Furthermore, due to PDDP-induced (125 mg/kg bw/d) impairment of fertility, only 13 % (4/30) of females with evidence of copulation became pregnant (in controls 93 %, 28/30). Also, litter size was decreased (PDDP exposure: 1.7 pups per litter, compared to control 13 pups per litter). Different aspects of reproduction which are important for population stability are affected. Consequently, the population stability can be irreversibly endangered.

PDDP caused a developmental effect in terms of precocious puberty in females in pubertal assays and in the two-generation study. A similar effect could appear regarding seasonal maturation of gonads. For successful reproduction, conditions like appropriate energy input must be fulfilled. Energy achievement is mostly season dependent. Encina and Granado-Lorencio (1997) showed seasonal changes in condition, nutrition, gonad maturation and energy content in the fish species barbel (*Barbus sclateri*): "Most fish species exhibit seasonal dynamic growth and reproductive styles" that are adapted to environmental conditions. A study by Meier et al. showed that after exposure to a mixture of alkylphenols (C4 to C7) dose-dependently the number of immature fish was decreased and instead more mature female fish (atlantic cod) were seen (Meier et al., 2011). Animals are adapted to brief windows of opportunity, which need precise timing of breeding (Varpe, 2017). Hence too early gonad maturation could lead to an adverse effect in seasonal breeding species. If reproduction occurs too early, the parents or the offspring may suffer from nutritional deficiencies. In this case the parents may not be able to care for their offspring or the offspring will not develop in a normal way due to nutritional deficiencies. It is conceivable that due to earlier gonad maturation, recruitment is endangered and therefore irreversible adverse effects on populations may occur.

In general, impaired reproduction, as was seen in the *in vivo* studies with mammals (see above), can cause a decline of population and contribute to species extinction.

Broad environmental relevance:
Effects on reproduction exerted via an oestrogenic mode of action have a broad environmental relevance. In mammalian studies, clear adverse effects on sexual development and fertility were observed. Due to the conservation of ERs and the function of the HPG axis, mammals and oviparous animals in the terrestrial and aquatic environment could be affected. Hence, oestrogenic effects in a wide range of wildlife species with different functions in the ecosystems can occur.

Impairment of reproduction can have severe and irreversible consequences for populations or subpopulations under environmental conditions (e.g. push out of the populations' normal operation range, caused by stress. One example is, if organsims are no longer adapted to the environmental conditions, like enough food, due to precocious

puberty and reproduction). Especially vulnerable populations that are e.g. weakened by other environmental stressors are endangered.

Reproductive effects as caused by PDDP are of particular concern for mammalian wildlife including top predator species and endangered species where the described reproductive effects may lead to serious effects for populations because of a naturally low reproductive rate of such species.

Difficulties to derive and quantify a safe level of exposure for PDDP
Endocrine regulation is a very complex feedback process that is set up during critical life stages in vertebrates. As summarised in disturbance of this regulation during transient but vulnerable life stages can result in irreversible effects during the entire life-time.

It is difficult to derive a safe level of exposure that could be regarded as sufficiently protective for all species and ecosystems, based on the following uncertainties:

- Regarding invertebrates, there is still no agreed guidance which clearly defines biologically plausible links between endocrine modes of action and adverse effects for invertebrate taxa and knowledge is still scarce in light of the large number and variety of invertebrates and their endocrine systems.
- Even transient exposure to endocrine disruptors during sensitive life stages can have severe consequences in later life stages or even following generations. This decoupling between exposure and adverse effects in time is difficult to assess with the available ED specific test guidelines and hence, predictions of future effects and safe exposure levels for the environment are not possible based on our current knowledge.
- Validated test guidelines exist so far only for a limited number of adverse ED specific endpoints in various species. For example, thyroid related adversity cannot be assessed for fish species at the moment.

Since no example from PDDP testing with fish is available, an example of testing of another long chain alkylphenol (4–tert-octylphenol, 4-tOP, CAS 140-66-9) is used to illustrate this. For 4-tOP, two tests with a different start of exposure are available. One test (Seki et al., 2003) started with fertilised eggs, in the other test (Gray et al., 1999) exposure was initiated one day post hatch. In the first test significant effects were seen on sex ratio and occurrence of testis-ova, while in the second case no or no significant effects were seen. Hence it was concluded that the exposure that began at the first sensitive life stage is important for the appearance of the effect.

6.3.4. Conclusion on the Art 57(f) assessment

ED assessment

PDDP are proposed to be identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because of their endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health and the environment which gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

Considering the results of all available studies, there is strong evidence that the adverse effects on fertility and sexual function (which led to classification of the substance as Repr. 1B), particularly in females, are due to the oestrogenic activity of PDDP. Mechanistic in vitro studies demonstrate oestrogen receptor (ER) binding and activation by PDDP (OECD level 2). The increase in 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 oestrous cycle were consistently observed in the majority of OECD Level 4 and 5 studies. Reproductive toxicity studies (OECD level 5) further demonstrated impacts on copulation index and apical fertility endpoints (decreased number of implantations and litter size). All of the above-mentioned parameters are considered as either EATS (oestrogeno-, androgeno-, thyroido-, and steroidogenesis)-mediated or sensitive to EATS modalities (OECD, 2018), and the overall observed effect pattern of PDDP is congruent with that of known model oestrogens.

In conclusion, there is strong evidence that the adverse effects on fertility and sexual function, particularly in females, are plausibly linked to the oestrogenic activity of the substance. Therefore, it is concluded based on the weight of evidence that PDDP is an endocrine disruptor with regard to human health according to the WHO/IPCS definition (as interpreted by the JRC Endocrine Disruptor Expert Advisory Group, 2013).

The evaluation of PDDP for the environment is based on mammalian data and supported by available fish *in vitro* tests and adverse outcome pathways. There are no aquatic *in vivo* long term data for fish and other aquatic vertebrates or investigations for ED available. As effects on growth, development and reproduction in single species are generally regarded relevant for the maintenance of wild populations, the observed effects on reproduction and pubertal development in rats are relevant for mammalian populations in the environment. Therefore, it is concluded based on the weight of evidence that PDDP is an endocrine disruptor for the environment.

Equivalent level of concern assessment

Based on the above conclusion, evidence that PDDP is of an equivalent level of concern includes:

The effects of PDDP on mammals are considered to be of equivalent level of concern as those of CMR Cat 1, PBT or vPvB substances due to the severity and irreversibility of the effects on organisms and populations and the difficulties to quantify a safe level of exposure in the environment. Environmental effects observed after exposure to PDDP are considered to impair population stability and recruitment. The effects may influence a wide range of taxa in different ecosystems due to conservation of the reproductive endocrine system. For most species in the environment no data on endocrine effects that are caused by the substance are available. Furthermore, the organisms in the environment are exposed to a mixture of substances. Hence there can be additive or synergistic effects that might enhance the impact. For example, additive effects were seen for mixtures of long chain alkyl phenols and other oestrogenic compounds like E2 and EE2. Increased sensitivity in an *in vitro* test was seen using 4-DPM with co-exposure to E2 indicating enhanced mixture effects. Between exposure and effects might be a long time, which also hinders the derivation of a safe effect level.

PDDP severely affects reproduction and development-related processes in organisms. Different aspects of reproduction are affected such as decreased copulation index and impairment of fertility which are important for population stability. Furthermore, PDDP caused a developmental effect in term of precocious puberty in females in pubertal assays and in the two-generation study. This is also of environmental importance as a similar effect could appear regarding seasonal maturation of gonads. Many fish species exhibit seasonal maturation and reproduction cycles. Reproduction and rearing of offspring are energy demanding processes where enough food must be available. Mostly it is connected to a short time frame given by environment. If these energy demanding processes fall out

of this time frame due to e.g. precocious puberty this could entail adverse effects on populations.

Thus, in summary, effects in mammals are relevant and serious for the environment. They are considered to be of equivalent concern due to the severity of the effects and the difficulties to quantify a safe level of exposure for oestrogen-like endocrine disruptors.

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