Annex XV report

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

Substance Name(s)[1]: 4-Heptylphenol, branched and linear (4-HPbl)

EC Number(s): -

CAS Number(s): -

Submitted by: Environment Agency Austria on behalf of the Austrian Competent Authority (Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management)

Date: August 2016

[1] Please note that the full name of the entry as it is proposed for the Candidate List is: 4-Heptylphenol, branched and linear [substances with a linear and/or branched alkyl chain with a carbon number of 7 covalently bound predominantly in position 4 to phenol, covering also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof]

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ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCF	Bioconcentration Factor
B/vB	Bioaccumulating/very bioaccumulating
BW	Body weight
CF	Condition factor
C&L	Classification & Labelling
4',7-DHI	4',7-dihydroxyisoflavone
dph	days post hatch
DWC	Dilution water control
E_2	17 ß - Estradiol
ED	Endocrine disruptor
ER	Estrogen receptor
FSDT	Fish sexual development test
GC/MS	Gas chromatography–mass spectrometry
GLP	Good laboratory practice
4-HPbl	4-Heptylphenol, branched and linear
Koc	Organic carbon normalized adsorption coefficient
LC_{50}	Lethal Concentration: concentration of a substance that will kill 50% of organisms exposed to it
LOAEL	Lowest observed adverse effect level
LOEC	Lowest observed effect concentration
log Kow	Logarithm of the n-Octanol-Water partition coefficient
4nHOP	4-n-Heptyloxyphenol
4nHP	4-n-Heptylphenol
4nNP	4-n-Nonylphenol
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
IPCS	International Programme on Chemical Safety

OECD	Organisation for Economic Co-operation and Development
PND	Postnatal day
QSAR	Quantitative structure–activity relationship
RAR	Retinoic acid receptor
RBA	Relative binding affinity
REP	Relative estrogenic potency
SBP	Sex-steroid binding protein
SVHC	Substance of very high concern
4tBP	4-*tert*-butylphenol
4tHP	4-*tert*-Heptylphenol
TL	Total length
UVCB	Substances of Unknown or Variable composition, Complex reaction products or Biological materials
VTG	Vitellogenin
WAF	Water Accommodated Fraction
WHO	World Health Organization
YES	Yeast estrogen screen

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

Substance Name(s): 4-Heptylphenol, branched and linear (4-HPbl)[2]

EC Number(s): -

CAS number(s): -

- It is proposed to identify the substances as a substance of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is a group of substances with endocrine disrupting properties for which there is scientific evidence of probable serious effects to 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 of REACH.

Note-the full name of the entry as it is proposed for the Candidate List is: 4-Heptylphenol, branched and linear [substances with a linear and/or branched alkyl chain with a carbon number of 7 covalently bound predominantly in position 4 to phenol, covering also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof]. However the abbreviation 4HPbl is used throughout the text in this report.

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

4-HPbl is proposed to be identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is a group of substances with endocrine disrupting properties for which there is scientific evidence of probable serious effects to 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.

Based on the available mechanistic information from *in silico* and *in vitro* studies with 4-heptylphenol isomers it can be unambiguously concluded that 4-HPbl is able to bind to the estrogen receptors of fish, humans and rats and to activate these receptors.

In a reliable *in vivo* study with *Sander lucioperca* (Demska-Zakęś, 2005) the ratio of male fish (according to histological determination) was significantly decreased at the lowest used 4-n-heptylphenol (4nHP) concentration (1 µg/L) after 28 days of exposure. The shift in sex ratio was dose-dependent, leading to 98 and 100% female fish at 88 and 144 days post hatch (dph), respectively, indicating that the observed effects on the sex characteristics were irreversible.

The appearance of intersex species comprising sex characteristics from both sexes, e.g. testis-ova / ovotestis and formation of an oviduct (with regressed spermatogenic lobules in the same fish), was significant at 4nHP concentrations of at least 1 µg/L.

[2] The full name of the entry as it is proposed for the Candidate List is: 4-Heptylphenol, branched and linear [substances with a linear and/or branched alkyl chain with a carbon number of 7 covalently bound predominantly in position 4 to phenol, covering also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof]

4-HPbl belongs to a group of structurally similar alkylphenols monoalkylated predominantly in 4-position with different alkyl chain lengths. To substantiate the findings for 4-HPbl, a read across approach is applied using the following source alkylphenols:

- 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
- 4-*tert*-octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol, EC number: 205-426-2)
- 4-*tert*-pentylphenol (*p*-(1,1-dimethylpropyl)phenol, EC number: 201-280-9)
- 4-*tert*-butylphenol (4-(1,1-dimethylethyl) phenol, EC number: 202-679-0)

Regarding chain length, 4-HPbl is in the middle of 4-nonylphenol, branched and linear and 4-*tert*-octylphenol on the one side and 4-*tert*-pentylphenol and 4-*tert*-butylphenol on the other side. The findings for 4-HPbl were substantiated by the effects seen also in tests performed with the source substances.

- *In vitro* data confirm that all four source substances and the target substance do interact with the estrogen receptors.
- As for 4-HPbl it was demonstrated that exposure to 4-nonylphenol and 4-butylphenol (branched and linear forms) lead to a female biased sex ratio in *Sander lucioperca* at low concentration (effects seen at lowest dose of 1 µg/L).
- Substantial effects were also seen in other fish species (*Pimephales promelas*, *Danio rerio*, *Oryzias latipes*, *Cyprinus carpio*, *Oncorhynchus mykiss*) for the source chemicals. These include effect data like a female biased sex ratio and indicative effects like feminisation of gonadal ducts, testis-ova and effects on secondary sex characteristics.

4-Nonylphenol and 4-*tert*-octylphenol are already identified as substances of very high concern due to their endocrine disrupting properties for the environment, which are considered to give rise to an equivalent level of concern. The effects observed for 4-HPbl are similar to those for 4-*tert*-octylphenol and 4-nonylphenol and occur in similar concentration ranges (ECHA, 2011 and ECHA, 2012).

In summary, it is demonstrated that endocrine disrupting properties for the environment occur for alkylphenols with alkyl chain lengths of 4,5,7,8 and 9 C-atoms.

Taking all the evidence into consideration 4-HPbl is proposed to be identified as an endocrine disruptor for the environment according to the OECD guidance document (OECD, 2012) and the WHO/IPCS definition for endocrine disrupters.

4-HPbl is assessed as substances giving rise to an equivalent level of concern due to their estrogenic mode of action and the type of effects caused by this mode of action (e.g. shift in sex ratio).

- At 1 µg/L the ratio of male fish was significantly decreased and intersex fish appeared. At 10 µg/L the ratio of female fish was significantly increased to approximately 75% while at 200 µg/L approximately 100% fish were female. These effects remained manifest even after the exposure had ceased underlining that exposure during sensitive life stages may change the endocrine feedback system for the entire life.

- A read-across from 4-*tert*-octylphenol and 4-nonylphenol indicates that although a safe level of exposure for 4-HPbl may exist, it is difficult to establish it.

    - Effects on non-traditional endpoints may start at much lower

concentrations than those considered in the OECD test guidelines.

- Although it is not possible to unambiguously conclude that the adverse effects on other organisms such as invertebrates and amphibians are endocrine mediated, these effects are in accordance with the fact that steroids play an important role in both invertebrates (Kendall et al., 1998) and amphibians (Kortenkamp et al., 2012). Owing to the lack of in depth knowledge of their endocrine system and the lack of test systems, it is currently nearly impossible to estimate which species are the most sensitive and which concentration should be regarded as safe for the environment.

- Read across of the effects observed for the similar alkylphenols 4-nonylphenol and 4-tert-octylphenol shows that a transient exposure during sensitive life stages may result in effects that remain during the entire life and even in the following generations. Thus local exposure of migratory species might not only locally affect population stability but also in other areas.

In summary, the effects observed due to 4-HPbl exposure, and confirmed by data on other alkylphenols, are considered to impair population stability and recruitment. They may occur even after short term exposure and thus may have impact in regions other than those where the exposure occurred. The effects persist even after the exposure has ceased and may have long-lasting influence on population level e.g. due to transgenerational effects or changes in the gene pool. Effects may influence a wide range of taxa. A safe level of exposure is difficult to derive although it may exist.

Consequently, 4-HPbl is considered to give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

Registration dossiers submitted for the substance?

Yes, for the UVCB substance phenol, heptyl derivs. (EC No. 276-743-1), that is part of the group entry 4-HPbl.

PART I

Justification

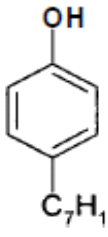
1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	-
EC name:	-
CAS number (in the EC inventory):	-
CAS number: **Deleted CAS numbers:**	-
CAS name:	-
IUPAC name:	4-Heptylphenol, branched and linear *[substances with a linear and/or branched alkyl chain with a carbon number of 7 covalently bound predominantly 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:	$C_{13}H_{20}O$
Molecular weight range:	192,3 g/mol
Synonyms:	-

Structural formula for 4-HPbl:



(branched and linear)

1.2 Composition of the substance

Name: 4-Heptylphenol, branched and linear *[substances with a linear and/or branched alkyl chain with a carbon number of 7 covalently bound predominantly in position 4 to phenol, covering also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof]*

Description: group entry

Substance type: mono-constituent / multi-constituent / UVCB[3]

Table 2: Constituents other than impurities/additives

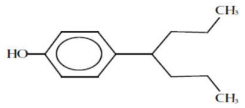
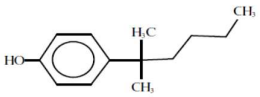
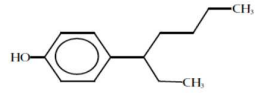
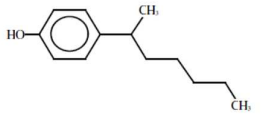
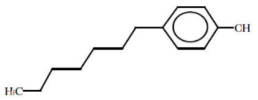
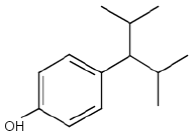
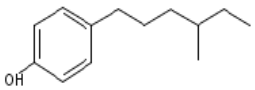
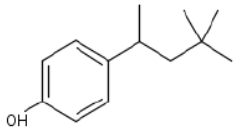
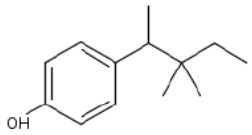
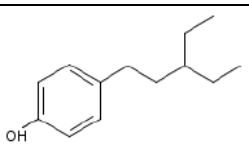
Constituents	Typical concentration	Concentration range	Remarks
-			

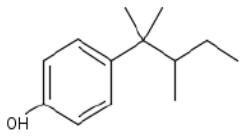
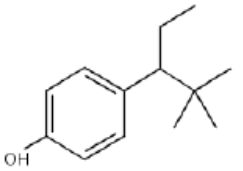
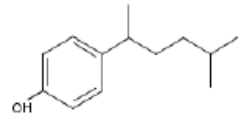
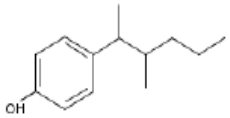
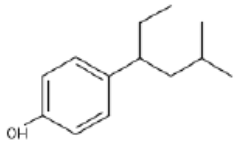
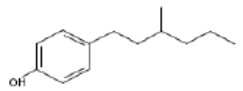
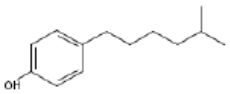
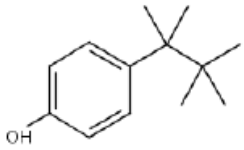
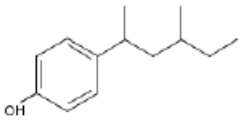
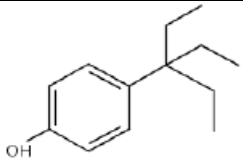
The given identity 4-heptylphenol, branched and linear *[substances with a linear and/or branched alkyl chain with a carbon number of 7 covalently bound predominantly in position 4 to phenol, covering also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof]* shall cover the group of p-heptylphenols with linear or branched alkyl chain. These substances may include, in addition to mono-, para-substituted isomers also mono- and multi-substituted isomers in ortho, meta and para position at lesser concentration levels. In Table 3 a list of example substances is given which are covered by the group entry.

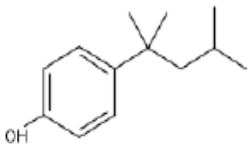
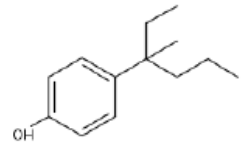
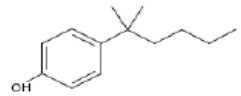
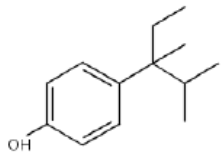
The UVCB substance phenol, heptyl derivs. (EC-No. 276-743-1) is registered and for 4-heptylphenol (EC-No. 217-862-0) C&L notifications are submitted. None of the other substances is registered or has a C&L notification at the time of the submission of this Annex XV report.

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

Table 3: Non-exhaustive list of examples of substances covered by the group name 4-heptylphenol, branched and linear

Name	CAS No.	EC No.	Remarks	Smiles	Structure
phenol, 4-(1-propylbutyl)-	6465-71-0		Not registered	c1(C(CCC)CCC)ccc(O)cc1	
p-*tert*-heptylphenol			Not registered	C(C)(C)(c1ccc(O)cc1)CCCC	
phenol, 4-(1-ethylpentyl)-	6465-74-3		Not registered	c1(C(CCCC)CC)ccc(O)cc1	
phenol, 4-(1-methylhexyl)-	6863-24-7		Not registered	c1(C(C)CCCCC)ccc(O)cc1	
p-n-heptylphenol 4-heptylphenol phenol, 4-heptyl- phenol, p-heptyl-	1987-50-4	217-862-0	Pre-registered C&L Notification	c1(O)ccc(CCCCCCC)cc1	
Phenol, heptyl derivs.	72624-02-3	276-743-1	Registered C&L Notification	n/a, UVCB	n/a, UVCB
Phenol, 4-[2-methyl-1-(1-methylethyl)propyl]-	1824346-00-0		Not registered	Oc1ccc(cc1)C(C(C)C)C(C)C	
Phenol, 4-(4-methylhexyl)-	1139800-98-8		Not registered	Oc1ccc(CCCC(C)CC)cc1	
Phenol, 4-(1,3,3-trimethylbutyl)-	911371-07-8		Not registered	Oc1ccc(cc1)C(C)CC(C)(C)C	
Phenol, 4-(1,2,2-trimethylbutyl)-	911371-06-7		Not registered	Oc1ccc(cc1)C(C)C(C)(C)CC	
Phenol, 4-(3-ethylpentyl)-	911370-98-4		Not registered	Oc1ccc(CCC(CC)CC)cc1	

Phenol, 4-(1,1,2-trimethylbutyl)-	861011-60-1		Not registered	Oc1ccc(cc1)C(C)(C)C(C)CC	
Phenol, 4-(1-ethyl-2,2-dimethylpropyl)-	861010-65-3		Not registered	Oc1ccc(cc1)C(CC)C(C)(C)C	
Phenol, 4-(1,4-dimethylpentyl)-	857629-71-1		Not registered	Oc1ccc(cc1)C(C)CCC(C)C	
Phenol, 4-(1,2-dimethylpentyl)-	854904-93-1		Not registered	Oc1ccc(cc1)C(C)C(C)CCC	
Phenol, 4-(1-ethyl-3-methylbutyl)-	854904-92-0		Not registered	Oc1ccc(cc1)C(CC(C)C)CC	
Phenol, 4-(3-methylhexyl)-	102570-52-5		Not registered	Oc1ccc(CCC(C)CCC)cc1	
Phenol, 4-(5-methylhexyl)-	100532-36-3		Not registered	Oc1ccc(CCCCC(C)C)cc1	
Phenol, 4-(1,1,2,2-tetramethylpropyl)-	72861-06-4		Not registered	Oc1ccc(cc1)C(C)(C)C(C)(C)C	
Phenol, 4-(1,3-dimethylpentyl)-	71945-81-8		Not registered	Oc1ccc(cc1)C(C)CC(C)CC	
Phenol, 4-(1,1-diethylpropyl)-	37872-24-5		Not registered	Oc1ccc(cc1)C(CC)(CC)CC	

Phenol, 4-(1,1,3-trimethylbutyl)-	33104-11-9		Not registered	Oc1ccc(cc1)C(C)(C)CC(C)C	
Phenol, 4-(1-ethyl-1-methylbutyl)-	30784-32-8		Not registered	Oc1ccc(cc1)C(C)(CCC)CC	
Phenol, 4-(1,1-dimethylpentyl)-	30784-31-7		Not registered	Oc1ccc(cc1)C(C)(C)CCCC	
Phenol, 4-(1-ethyl-1,2-dimethylpropyl)-	30784-27-1		Not registered	Oc1ccc(cc1)C(C)(CC)C(C)C	

Further information on the registered UVCB substance phenol, heptyl derivs.

Manufacture:

Phenol, heptyl derivs. is made through the acid-catalyzed alkylation of phenol with industrial grade heptenes. The heptenes used to make phenol, heptyl derivs. are a complex mixture of branched isomers obtained from the acid catalyzed polymerization of propylene–butylene mixtures (US EPA HPV Challenge Program, 2006a).

No information on the production process is available from the registration dossiers.

Composition:
Based on the manufacture of the substance by alkylation of phenol, mono-substitutions at the ortho-, meta and para- position of the phenol ring are possible in theory. Industrial grade alkenes are used as alkylation-agents containing 7 carbons (UVCB-substances themselves). 117 mono isomers are possible in theory based on the different potential variations of the alkyl groups and the location of the group at the ring. Branched and linear alkylgroups are possible based on the wide range of potential structures. For branched structures type and degree of branching may vary. The substance is chemically described as 4-heptylphenol, branched by the registrant.

Multiple substitutions of the same ring are also possible. The number of substitutions per ring may be influenced by the reaction conditions used e.g. relative ratio of alkylating agent used, agitation. Referring to the manufacturing process of this substance, formation of mono-isomers is most relevant. Di- and tri-isomers are also formed to a lower degree. Thousands of potential di-isomers are predicted taking two alkylgroups and all possible structures of the alkyl groups into account.

Based on the chemistry of the formation of this substance, substitution of the para-position is preferred (+M-effect of hydroxylgroup). Substitution of the ortho-position is also relevant, but less likely (+M-effect of hydroxylgroup as well but steric hindrance in comparison to the para-position). Whereas, substitution of the meta-position is considered to be unlikely based on the lack of the +M-effect.

According to the registrant, only phenols with branched alkyl chains are present in the UVCB, predominantly bound at para (4-) position.

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

Not applicable.

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

4-HPbl can be considered as part of a group of alkylphenols with a linear or branched alkyl chain in para-position. The substances differ in the length of the alkyl chain and the degree of branching. The following substances can be considered as part of this group:

- **4-Nonylphenol, branched and linear:** Nonylphenols have previously been identified as SVHC due to equivalent level of concern having probable serious effects to environment (Article 57(f), due to endocrine disrupting properties in the environment)[4].
- **4-*tert*-octylphenol**: This substance has previously been identified as SVHC due to equivalent level of concern having probable serious effects to environment (Article 57(f), due to endocrine disrupting properties in the environment)[5].
- **4-*tert*-pentylphenol:** A substance evaluation under REACH for this substance was performed in 2014. Submission of an SVHC Dossier due to equivalent level of concern having probable serious effects to environment (Article 57(f), due to endocrine disrupting properties in the environment) has been announced in the Registry of Intentions (August 2016)[6].
- **4-*tert*-butylphenol:** A substance evaluation under REACH for this substance was performed in 2014. Submission of an SVHC Dossier due to equivalent level of concern having probable serious effects to environment (Article 57(f), due to endocrine disrupting properties in the environment) has been announced in the Registry of Intentions (August 2016)[7].

[4] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807db370
[5] https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807d9e89
[6] https://echa.europa.eu/registry-of-current-svhc-intentions/-/substance-rev/13943/term
[7] https://echa.europa.eu/registry-of-current-svhc-intentions/-/substance-rev/13942/term

Table 4: Structurally related substance(s) identity for 4-Nonylphenol, branched and linear

EC number:	-
EC name:	-
SMILES:	Covers UVCB as well as well-defined substances (see chemical name)
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 (for EC number 246-672-0):	601-053-00-8
Molecular formula:	$C_{15}H_{24}O$
Molecular weight range:	220.35 g/mol

Structurally related substance(s) formula:

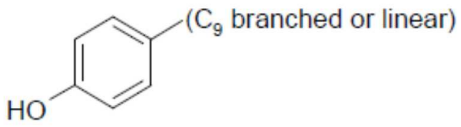


Table 5: Structurally related substance(s) identity for 4-*tert*-octylphenol

EC number:	205-426-2
EC name:	4-(1,1,3,3-tetramethylbutyl)phenol
SMILES:	Oc(ccc(c1)C(CC(C)(C)C)(C)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:	601-053-00-8
Molecular formula:	$C_{14}H_{22}O$
Molecular weight range:	206.32 g/mol

Substance type: mono-constituent

Structurally related substance(s) formula:

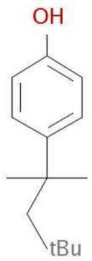


Table 6: Structurally related substance(s) identity for 4-*tert*-pentylphenol

EC number:	201-280-9
EC name:	p-(1,1-dimethylpropyl)phenol
SMILES:	CCC(C)(C)c1ccc(O)cc1
CAS number (in the EC inventory):	80-46-6
CAS number:	80-46-6
CAS name:	Phenol, 4-(1,1,3,3-tetramethylbutyl)-
IUPAC name:	4-(1,1-dimethylpropyl)phenol
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	$C_{11}H_{16}O$
Molecular weight range:	164.24 g/mol

Substance type: mono-constituent

Structurally related substance(s) formula:

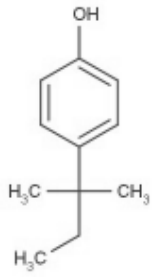
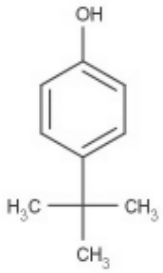


Table 7: Structurally related substance(s) identity for 4-*tert*-butylphenol

EC number:	202-679-0
EC name:	4-*tert*-butylphenol
SMILES:	CC(C)(C)c1ccc(O)cc1
CAS number (in the EC inventory):	98-54-4
CAS number:	98-54-4
CAS name:	Phenol, 4-(1,1-dimethylethyl)-
IUPAC name:	4-(1,1-dimethylethyl)-phenol
Index number in Annex VI of the CLP Regulation:	604-090-00-8
Molecular formula:	$C_{10}H_{14}O$
Molecular weight range:	150.22 g/mol

Substance type: mono-constituent

Structurally related substance(s) formula:



1.5 Physicochemical properties

For the registered UVCB substance phenol, heptyl derivs., which is covered by the group entry, physical chemical properties are given. These data are shown in

Table 8. For the other heptylphenol isomers covered by the group entry reliable comprehensive physical chemical data could not be found.

Table 8: Overview of physical chemical properties for phenol, heptyl derivs. (If not indicated otherwise, data are taken from the ECHA dissemination site (CAS No 72624-02-3))

Property	Description of key information	Value	Reference/source of information
Physical state at 20°C and 101.3 kPa	The substance is an amber coloured viscous liquid with no reported odour	liquid at 20°C and 101.3 kPa	Dissemination site*
Melting/freezing point	The pour point of the test item has been determined to be -9°C	pour point -9°C.	Dissemination site
Boiling point	The test item boiled partially from approximately 544 K (270°C) at 100.6 kPa. The remaining amber coloured residue found on completion of each determination confirmed the presence of a fraction of test item with a boiling temperature greater than 673 K (400°C).	270 °C at 101.3 kPa	Dissemination site
Vapour pressure	The vapour pressure was determined using a vapour pressure balance with measurements being made at several temperatures and linear regression analysis used to calculate the vapour pressure at 25°C. Testing was conducted using a procedure designed to be compatible with Method A4 Vapour Pressure of Commission Regulation (EC) No 440/2208 of 30 May 2008. The vapour pressure of the test item has been determined to be 2.6×10^{-1}Pa at 25ºC.	0.26 Pa at 25 °C	Dissemination site

Density	The relative density of the test item has been determined to be 0.965 at 15.6°C.	0.965 at 15.6°C	Dissemination site
Water solubility	The water solubility of the test item has been determined to be 4.21 x 10^{-2} g/l of solution at 20.0 ± 0.5°C.	0.0421 g/L at 20 °C	Dissemination site
Partition coefficient n-octanol/water (log value)	log Kow values for 117 heptylphenol isomers range from 4.78 to 5.01	4.78 - 5.01	EPISUITE v4.11
Surface tension	The surface tension has been determined to be 43.0 mN/m at 21.5 ± 0.5°C.	43.0 mN/m at 21.5 ± 0.5°C. The test item is considered to be a surface-active material.	Dissemination site

*https://echa.europa.eu/information-on-chemicals/registered-substances. Site was accessed at 21.07.2016

Using the OECD-toolbox for the 117 potential mono-substituted isomers of this substance, the following QSAR-prediction ranges are predicted for the properties listed in

Table 9 below. The properties of the "real" UVCB-substance depend on the composition of the constituents. The order of magnitude of these predictions is in accordance with the values in **Table 8** taken from the dissemination site. Nevertheless, it needs to be taken into account that any contained fractions of di-substituted isomers, other constituents or impurities lead to shifts of these QSAR predicted ranges.

Table 9: Estimated physical chemical data for the 117 monosubstituted heptylphenol isomers

Property	QSAR	Range
Boiling point	EPISUITE v4.11	265 – 296°C
Log Kow	EPISUITE v4.11	4.78 – 5.01
Selected vapour pressure	EPISUITE v4.11	0.0372 – 0.321 Pa
Water solubility	EPISUITE v4.11	9.65 – 14.9 mg/L

2. Harmonised classification and labelling

No harmonised classification and labelling is available for any of the substances in the group of 4-Heptylphenols with branched or linear alkyl chain.

3. Environmental fate properties

3.1 Degradation

3.1.1 Abiotic degradation

According to a recent OECD Guideline 111 study (Hydrolysis as a function of pH) the half-life of phenol, heptyl derivs. is more than one year at pH 4, 7 and 9 (Study report, 2012a; Klimisch 1).

3.1.2 Biodegradation

According to the available screening data the substance is not readily biodegradable: phenol, heptyl derivs. showed 1.6% degradation after 28 d (O_2 consumption) in an OECD Guideline 301 D study (Ready Biodegradability: Closed Bottle Test; Study report, 2012b; Klimisch 1).

In an OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test) using preadapted mixed culture inoculum derived from activated sewage sludge/soil 25.4% degradation (CO2 evolution) was observed for phenol, heptyl derivs. after 29 days (Study report,1998; Klimisch 2).

3.1.3 Field data

No data.

3.1.4 Summary and discussion of degradation

According to the available screening data and QSAR data the substance is not readily biodegradable and estimated hydrolysis half-life is more than one year.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

According to an OECD Guideline 121 study (Estimation of the Adsorption Coefficient (Koc) on soil and on sewage sludge using High Performance Liquid Chromatography a very broad adsorption coefficient range of 91.8 to 5790 was determined (Study report 2012a; Klimisch 1). The dominant component of the test item (90.0% by percentage area normalisation) resulted in an adsorption coefficient value of 2430.

The dissociation constants of certain functional groups in the test item made it impossible to measure the ionised and the unionised form separately. From structural information estimations of the phenol functional group dissociation constants of 10.3 to 11.8 were derived using predictive computer modelling (SPARC version 4.6, October 2011, w4.6.1691-s4.6.1687).

3.2.2 Volatilisation

Based on HENRYWIN v3.20 predictions, the predicted range of the Henry's law constants of the monosubstituted heptylphenol isomers is 0.411 – 0.9 Pa*m^3/mol. The main fraction of the UVCB-substance consists of these isomers. Nevertheless, it needs to be taken into account that it also contains fractions of dialkylated phenols, starting materials and

impurities having impact on the measured volatility of the UVCB-substance taking all constituents into account. Therefore, the predicted and the measured volatilities can differ in principle.

3.2.3 Distribution modelling

3.2.4 Field data

No data.

3.2.5 Summary and discussion of environmental distribution

According to an OECD Guideline 121 test (Estimation of the Adsorption Coefficient (Koc) on soil and on sewage sludge using High Performance Liquid Chromatography a very broad adsorption coefficient range of 91.8 to 5790 was determined.

Based on HENRYWIN v3.20 predictions, the predicted range of the Henry's law constants of the monosubstituted heptylphenol isomers is 0.411 – 0.9 Pa*m^3/mol.

3.3 Data indicating potential for long-range transport

No data.

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

3.4.1.1 Estimated and experimental log Kow values

The current test to determine log Kow values are unsuitable for surface active chemicals, not least because of the tendency for surfactant molecules to accumulate at phase interfaces or form emulsions, thereby giving unreliable results. Nevertheless, estimated log Kow values for 117 heptylphenol isomers range from 4.78 to 5.01 (EPISUITE, 2012; version 1.68), which is above the screening criterion of 4.5.

3.4.1.2. Estimated BCFs for selected p-heptylphenols

BCFs were estimated using the BCFBAF v3.01 program within EPISUITE v4.11 (see Table 10). The predictions have been carried out using the log Kow value estimated by the program.

Table 10: Bioaccumulation and log Kow estimates (EPISUITE, 2012) for selected examples of 4-HPbl

Substance Name	CAS No. or Smiles Code	Bioaccumulation estimates (BCFBAF v3.01), BCF (regression based method)	log Kow (version 1.68 estimate)
phenol, 4-(1-propylbutyl)-	6465-71-0	835.5	4.93
p-*tert*-heptylphenol	C(C)(C)(c1ccc(O)cc1)CCCC	789.1	4.90
phenol, 4-(1-ethylpentyl)-	6465-74-3	835.5	4.93
phenol, 4-(1-methylhexyl)-	6863-24-7	835.5	4.93
p-n-heptylphenol 4-heptylphenol phenol, 4-heptyl- phenol, p-heptyl-	1987-50-4	934.2	5.01

3.4.1.3 Experimentally determined BCFs

A flow-through study conducted by Tollefsen et al. (1998) used juvenile Atlantic Cod from Norway (rated Klimisch 3). The test was conducted according to OECD 305 (1981, 1996) using 4-[14C]heptylphenol (1 Ci/mol), [14C-4-HP]. GC/MS analyses of the product revealed that the product contained 2 para-substituted isomers of heptylphenol: a branched heptylphenol and n-heptylphenol. The exposure period was 192 hours and the elimination period 192 hours. Fish sampling took place at 192, 292 and 388 hours. Mean steady-state BCF (BCFss) and kinetic-BCF (BCFk) values were calculated. The mean BCFss for samples collected at 96 and 192 hrs was 555 ± 16. BCFk was 578 ± 127 (k1 = 19.94 ± 1.83/h, k2 = 0.052 ± 0.011/h). The elimination rate constant, k2 of the substances comprises elimination via gills, metabolic transformation, growth dilution and elimination via faeces. Growth dilution has bigger influence in early life stages. Elimination of 4-heptylphenol occurred rapidly following first order kinetics with an estimated biological half-life of 13 hours. During the initial part of the elimination period substantial radioactivity was detected in seawater, but no radioactivity was detected in water after more than 96 hours recovery in clean seawater. Tollefsen et al. (1998) reported preferential distribution of 4-14C-heptylphenol to bile, liver, intestines, kidney and heart compared to blood. Therefore, it can be assumed that excretion is primarily via bile and faeces. In addition, Cravedi and Zalko (2005) concluded that excretion of nonylphenol and other alkylphenols occurred predominantly in faeces and bile. Tollefsen et al. (1998) identified high residues

of 4-14C-heptylphenol after 192 h seawater exposure in kidneys (comparable to concentrations in the liver) and indicated also excretion via urine. Excretion via gills was also suggested.

In tissues of cod no radioactivity was detected at the end of the elimination period. Radioactivity in autoradiograms extracted with nonpolar and polar solvents was not observed, supporting the conclusion that 4-heptylphenol and metabolites do not bind to specific macromolecules or tissue structures in cod.

The study by Tollefsen et al. (1998) has some deficiencies. The fish were exposed only to 1 radioactive labelled concentration, whereas the OECD method 305 requires two concentrations. Radiolabelled purity of the ^{14}C-4-HP was not indicated, fish weight was not measured at the end of the study, and fish growth could not be calculated. No lipid and growth corrections were performed on the calculated BCF values.

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

No data.

3.4.3 Field data

No data.

3.4.4 Summary and discussion of bioaccumulation

Based on the established screening criterion (log Kow > 4.5) for bioaccumulation, the selected p-heptylphenols potentially fulfil the B/vB criterion. Estimated BCF-values are < 2000, which indicate that p-heptylphenols do not fulfil the B- or vB criterion.

4. Human health hazard assessment

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

4.1.1 Non-human information

No experimentally derived ADME (absorption, distribution, metabolism, and elimination) data and toxicokinetic studies with mammalian species are available for 4-HPbl. Therefore, data on physical chemical properties, QSAR, toxicity and toxicokinetics in fish were used. Although some toxicokinetics models have been proposed for fish (e.g. Nichols et al., 1990 & 2004; cited in ECHA, 2014), direct quantitative extrapolation between fish and other vertebrates is not currently possible, because of the substantial differences in their physiology (e.g. respiration via gills rather than lungs) and metabolic rates. However, rough comparisons can be made on a case-by-case basis (ECHA, 2014).

Absorption and distribution

Properties of the UVCB substance that affect absorption include octanol/water partition coefficient (log Kow) and water solubility. The log Kow value of 4-HPbl is above the range of -1 to 4 that is proposed to be favourable for absorption (ECHA, 2014). However, its water solubility of 42.1 mg/L is moderate. According to the estimations based on the Danish QSAR Database (SMILES input: 4-n-heptylphenol, c1(O)ccc(CCCCCCC)cc1) oral absorption is likely. The bioavailability score (Lipinski's Rule-of-five score) is zero, which indicates that the substance may be bioavailable. Absorption from the gastrointestinal tract for a 1 mg dose was estimated to be 100% with this tool. Oral absorption of a similar alkylphenol, 4-*tert*-octylphenol is rapid and the compound is quickly released into the blood. Within 10 min 4-*tert*-octylphenol is present in blood and C_{max} is reached between 20 min (male Wistar rats) and 2 h (Sprague Dawley rats) after administration (ECHA, 2011). Also nonylphenol is initially rapidly absorbed from the gastrointestinal tract after oral exposure according to EC (2002).

Systemic effects including organ weight and histopathological changes in rats at 150 and 160 mg/kg bw/day indicate further bioavailability and distribution of 4-HPbl, and/or metabolites in a 28-day repeated dose and reproduction/developmental toxicity screening studies (see Chapters 4.6 and 4.9, tested substance: phenol, heptyl derivs.).

Toxicokinetic studies with dermal application are not available. In an acute dermal toxicity study with phenol, heptyl derivs. no clinical signs were observed after application of 2000 mg/kg bw. However necrosis and severe edema of the skin followed by body weight loss in some animals occurred. One animal that exhibited body weight loss died at day 12 following exposure (Study report, 1985). Based on the water solubility, the partition coefficient of 4.5 of phenol, heptyl derivs. and the molecular weight of phenol, heptyl derivs. of 192.3 g/mol a high dermal bioavailability can be assumed.

Once the substance is absorbed, it is expected to be distributed via the blood to the liver and other tissues (cf. 28-day repeat dose and reproduction/developmental screening studies, Chapters 4.6 and 4.9).

Metabolism and elimination

Based on the structural similarity information on metabolism of 4-*tert*-octylphenol was considered: "From experiments using rat liver perfusion or primary rat hepatocytes it can be concluded that 4-*tert*-octylphenol undergoes a rapid first pass metabolism by phase I and phase II enzymes in the liver. Detoxification pathways include hydroxylation, glucuronidation and sulfation. Enzymes involved in phase II metabolism include rat and human UGT2B1 and human SULT 1E1 and 2A1, as shown in *in vitro* experiments. In an *in vitro* test with untreated rat liver microsomes up to 94% of 4-*tert*-octylphenol was

metabolized within 15 min. In a liver perfusion assay 38% of the applied 4-*tert*-octylphenol dose was directly excreted into the bile of Sprague Dawley rats as glucuronide" (ECHA, 2011). Data on nonylphenol confirmed that major metabolic pathways are likely to involve glucuronide and sulphate conjugation with evidence of extensive first pass metabolism. The bioavailability of unconjugated nonylphenol is probably limited to 10-20% of the administered dose following oral exposure (EC, 2002).

Glucuronidation of other alkylphenols (4-nonylphenol, hexylphenol, butylphenol, and ethylphenol) in the rat liver (from Sprague Dawley rats) was observed using a liver perfusion assay. Alkylphenols (nonylphenol, hexylphenol, butylphenol, and ethylphenol) were glucuronidated by rat liver microsomes and alkylphenol glucuronide conjugates (except 4-nonylphenol) were excreted to bile. The excretion by MRP2 (multi resistant protein) was found to decrease with longer alkyl chain (Daidoji, 2003).

Elimination of this substance and metabolites is expected to occur by biliary and/or renal excretion (cf. also toxicokinetic data on fish). Measured half-lives for 4-*tert*-octylphenol after oral application of Sprague-Dawley rats ranged between 5 and 38 hours depending on the dose and sex subject to some uncertainties associated with high blood concentrations at the last sampling time point (ECHA, 2011). Also for nonylphenol major routes of excretion are via faeces and urine (EC, 2002).

For 4-HPbl similar metabolism and elimination can be expected as seen for the referenced alkylphenols.

Toxicokinetic data on fish

Atlantic cod (*Gadus morhua*) was exposed in the laboratory to tritium labelled 4-*tert*-butylphenol, 4-n-pentylphenol, 4-n-hexylphenol, and 4-n-heptylphenol via seawater (8 ng/L) and via contaminated feed (5 µg/kg fish per day) (Klimisch 2). Measurements of different fish tissues during eight days of exposure and eight subsequent days of recovery revealed that alkylphenols administered via spiked seawater were readily taken up during the first two days of exposure. Steady state for spiked feed was also reached after day 2 but uptake was far less efficient when alkylphenols were administered in spiked feed. Approximately 10% of the alkylphenol administered (4-n-pentylphenol 8%, all other 12-14%) via spiked feed was accounted for in the tissues analysed (excluding the intestine) (Sundt et al. 2009). These values are comparable to results of feeding study in flounder (8% 4-*tert*-octylphenol residues in liver and muscle tissue) by Madson et al. (2003). However, lower values are reported for ^{3}H-4-n-nonylphenol (Cravedi and Zalko, 2005).

Elimination half-lives for ^{3}H labelled 4-*tert*-butylphenol, 4-n-pentylphenol, 4-n-hexylphenol, and 4-n-heptylphenol independent of the exposure route (seawater or feed) range between 10 to 20 hours in cod (Sundt et al., 2009). This finding is consistent with the study (Klimisch 2) performed by Tollefsen et al. (1998). Steady state in Atlantic cod exposed via seawater to 4-[^{14}C]-heptylphenol (2 para substituted isomers: one branched and one linear) was reached by 58 hours with an elimination of 13 hours. [^{14}C]-Environment and Health Canada (2001) reported half-lives for nonylphenol in fish of 0.8 days in rainbow trout, 1.2 to 1.4 days in fathead minnow and 4 days in Atlantic salmon.

According to Sundt et al. (2009) tissue distribution of ^{3}H labelled 4-*tert*-butylphenol, 4-n-pentylphenol, 4-n-hexylphenol, and 4-n-heptylphenol in large cod showed high residues particularly in the bile fluid as well as in the intestine, intestine content and stomach content. Also with spiked feed the bile fluid showed the highest concentrations. After 8 days of recovery, still bile had the highest residues. The liver and other tissues studied (muscle, pooled sample of spleen/heart/kidney/brain and gonads) contributed only little to the total radioactivity detected. Tollefsen et al. (1998) reported preferential distribution of 4-[^{14}C]-heptylphenol to bile, liver, intestines, kidney and heart compared to blood. Therefore, it can be assumed that excretion is primarily via bile and faeces. In addition, Cravedi and Zalko (2005) concluded in their review paper on the metabolic fate of

nonylphenols and related alkylphenols in fish that excretion of nonylphenol and other alkylphenols occurred predominantly in faeces and bile. Tollefsen et al. (1998) identified high residues of 4-^{14}C-heptylphenol after 192 h seawater exposure in kidneys (comparable to concentrations in the liver) and indicated also excretion via urine. Excretion via gills was also suggested.

Metabolism in fish was investigated for nine individual alkylphenols including 4-*tert*-butylphenol, 4-n-pentylphenol and 4-n-heptylphenol after intermuscular injection in Atlantic cod (Jonsson et al. 2008). The glucuronic acid conjugate was the most abundant metabolite in cod bile (approx. 84%, 87% and 90% relative concentration for 4-*tert*-butylphenol, 4-n-pentylphenol and 4-n-heptylphenol, respectively). After 4-n-heptylphenol administration, also glucosides, sulfates and unchanged parent were detected in the bile with 6.1%, approx. 5% and approx. 4%, respectively (Jonsson et al. 2008). Biotransformation pathways were also investigated in different fish species and with different alkylphenols (4-n-nonylphenol, branched nonylphenols, and 4-*tert*-octylphenol) according to Cravedi and Zalko (2005). Also here the predominant metabolic pathway for alkylphenols was the conjugation of the phenol group to glucuronic acid and secondly the oxidative biotransformation of the alkyl side-chain (subsequent or prior to glucuronidation). The terminal and sub-terminal oxidative biotransformation might be responsible for more hydroxylated metabolites from branched alkylphenols. Alkylphenol sulfation is poorly demonstrated in fish compared to rat according to Cravedi and Zalko (2005). Linear side chain alkylphenols may enter the ß-oxidation pathway thereby producing shorter side-chain carboxylic acid metabolites. This pathway was established and extensively characterized *in vivo* for 4-*n*-nonylphenol. In addition, a ring-hydroxylated pathway was demonstrated for 4-*tert*-octylphenol yielding catechol metabolites and reactive intermediates (Cravedi and Zalko, 2005).

In summary, it can be concluded, that the referenced alkylphenols do have similar toxicokinetic patterns.

4.1.2 Human information (including bioaccumulation in humans)

No data.

4.1.3 Conclusion on toxicokinetics (and bioaccumulation in humans)

No experimental toxicokinetic data in mammalian species for 4-HPbl are available. Therefore, data on physical chemical properties, QSAR, structurally similar alkylphenols, toxicity of phenol, heptyl derivs., and toxicokinetics in fish were used.

4-HPbl is assumed to be rapidly absorbed after oral exposure and distributed via blood to different tissues including the liver, kidney, muscle and fat. For fish the uptake was higher from spiked seawater compared to that from spiked food. From studies with structurally similar alkylphenols and 4-n-heptylphenol it is concluded that 4-HPbl is biotransformed in both fish and rats mainly to glucuronide conjugates. Also hydroxylated metabolites are likely both on the alkyl chain and the ring structure, where in the latter case reactive intermediates are formed. Linear side chain alkylphenols may enter the ß-oxidation pathway thereby producing shorter side-chain carboxylic acid metabolites. Sulfation was demonstrated primarily in rats and to a lesser extent in fish. To a minor degree also the formation of glucosides was shown in fish. Efficient metabolism in fish (and rats) as well as excretion lead to short half-lives of exposed tested organisms (fish and for 4-*tert*-octylphenol rats). In fish alkylphenol residues elimination occurs predominantly in faeces and bile with similar half-lives that range from 10 to 20 hours (for water or feed exposure).

4.2 Acute toxicity

Not relevant.

4.3 Irritation

Not relevant.

4.4 Corrosivity

Not relevant.

4.5 Sensitisation

Not relevant.

4.6 Repeated dose toxicity

4.6.1 Non-human information

4.6.1.1 Repeated dose toxicity: oral

14-day study

In a GLP conforming 14-day range finding study (Klimisch 2) phenol, heptyl derivs. (purity 99.62%) was administered by gavage for 14-days to Sprague-Dawley (Crl:CD IGS BR) rats. Dose levels were 50, 100, 200, 300 and 450 mg/kg/day (including a vehicle corn oil control). Three animals/sex were assigned per group. The main results were as follows. Body weights were not affected, and there were no macroscopic findings at study termination. Higher absolute and mean relative liver and kidney weights were observed in the 300 and 450 mg/kg/day group, males and females. Clinical observations (including excessive salivation and yellow material on various body surfaces) were noticed in males and females at 300 and 450 mg/kg/day and occasionally at 200 mg/kg/day. The dosage level of 450 mg/kg/day was considered as maximum tolerated dose and was recommended for the high dose group for a 28-day study (Study report, 2006b).

28-day study

In a GLP conforming 28-day oral feeding study according to OECD Test Guideline 407 (Klimisch 1) in Sprague-Dawley (Crl:CD IGS BR) rats toxicity of phenol, heptyl derivs. (CAS No. 72624-02-3) after repeated exposure was investigated (Study Report, 2006a). In this study doses of 50, 150, or 450 mg/kg/day phenol, heptyl derivs. in corn oil (purity 99.62%) and a vehicle control were administered by gavage daily to 10 rats/sex/group in the control and high dose group and 5 rats/sex in the low and mid dose group. The post exposure observation period was 14-day recovery in the control and high dose group. Two deaths occurred in the high dose groups (1 male and 1 female) at day 27, most likely treatment related, observed clinical signs noticed prior to death include decreased defecation, dermal atonia, hypothermia and thinness. Another animal died on day 28 in the mid dose group and one female at the high dose group at day 4, but these deaths were not attributed to treatments. Test article related clinical observations in the surviving animals in the high dose group included clear material around the mouth and/or ventral neck and forelimbs, signs of unkempt appearance and occasionally dermal atonia and thinness.

The mean body weight was up to 14% lower in males receiving 450 mg/kg/day compared to the control group. The absolute organ weights decreased for heart, thymus and seminal vesicle (450 mg/kg/day, males). Organ weights relative to the final body weight (see Table 11) were significantly increased in the highest dose group in male animals (liver, epididymides, testes, brain, liver, thyroids, adrenal glands) and female animals (liver

(increase in a dose dependant manner also significantly different from control at 150 mg/kg/day) and kidneys).

Concurrent absolute and relative organ weight changes were observed in males for seminal vesicles (decrease) and liver (increase) and in females for liver and kidney (increases).

Histopathological investigations were carried out on organs of five male and five female animals both in the control and the highest dose group. Histopatological findings included vacuolation of the hepatocytes (450 mg/kg/day male/female, 150 mg/kg/bw males), squamous hyperplasia of the non-glandular stomach (high dose group, males; assumed to be an local irritating effect), depletion of secretion of the seminal vesicles, lymphoid depletion of the thymus (high dose group, males and females), haemorrhage of the thymus (50 and 450 mg/kg/day, males) and renal lesions compatible with tubular nephropathy (450 mg/kg/day males and females, 150 mg/kg/day males). There was a dose dependent and treatment related increase in the incidence of medullary plasmacytosis and lymphoid hyperplasia of the mandibular lymph nodes in males. The above described observed histopathological changes partly recovered after the 14-day post exposure period with total recovery from vacuolation of the hepatocytes (females) and squamous hyperplasia (males and females).

The test article related effects on seminal vesicles (pronounced relative and absolute weight decrease including depletion of secretion of the seminal vesicles) at the highest dose might indicate an estrogen mode of action of phenol, heptyl derivs. in mammals. Nevertheless, at this concentration systemic effects including one death and reduced mean body weights >10% (males) were observed.

Table 11: Statistically significant organ weight changes at study termination according to the draft study report (un-audited[8]) (draft study report, 2006)

Organ	Absolute organ weights changes in %	Relative organ weights changes in %
Females	450 mg/kg bw/day	
Liver	47.6	59.7
Kidney	102.2	121.1
Males		
Liver	22.6	52.4
Seminal vesicle	-51.1	-40.6
Heart	-26.7	*
Thymus	-41.8	*
Adrenal glands	*	35.3
Thyroids/Para	*	33.3
Epididymides	*	17.3
Testes	*	28.6
Brain	*	25.6

* weight change is not statistically significant compared to control

Moreover, high variance and high standard deviations were reported in this study for uterine weight, although no significant change was observed: the mean weight was 0.48, 0.58, 0.76 and 0.33 g in the control, 50 mg/kg/d, 150 mg/kg/d and 450 mg/kg/d group, respectively. The highest mean weight was measured in the 150 mg/kg/d group, whereas the lowest was in the 450 mg/kg/d group. (draft study report, 2006[8]).

[8] https://java.epa.gov/oppt_chemical_search/proxy?filename=2006-1-8EHQ-06-16349A_8ehq-0106-16349a_88060000121s.pdf

There were no test article related effects on functional observation battery assessments.

Clinical chemistry parameters were investigated at the end of the study. An increase in the 450 mg/kg/day dose group was observed in urea, nitrogen and creatinine (females only),in alanine aminotransferase (ALT)(both sexes) and in aspartate aminotransferase (AST) (males only). During recovery, these values returned to normal, except for ALT and AST in males, which remained statistically significantly elevated compared to the control group. These changes were compatible with the test-article related histopathologic lesions in the kidneys and liver. Higher urine volume was noted in the 450 mg/kg/day males following treatment. The study summary in the registration dossier reported no treatment related effect on haematology parameters.

According to the study summary the NOAEL is 150 mg/kg/day based on lethality, clinical observations (decreased defecation, dermal atonia, hypothermia), lower body weights, serum chemistry changes and several histological changes (tubular nephropathy in the kidneys, fatty change of the liver, stratified squamous hyperplasia of the nonglandular stomach, thymic lymphoid depletion and haemorrhage and depletion of secretion of seminal vesicles). However, based on observed histopathological findings in liver and kidney and statistically significant increased liver weights (>10%, females) already reported at 150 mg/kg/day, this dose level can be considered as LOAEL.

4.6.2 Human information

No data available.

4.6.3 Summary and discussion of repeated dose toxicity

There is one GLP conforming study available with repeated administration of phenol, heptyl derivs. to adult rats of both sexes and with the oral route of administration.

During the study with repeated oral administration for 28 days systemically toxic effects (e.g. reductions in body weights and body weight gain in males, changes in liver and kidney organ weights in males and females) were observed at dosages of 150 and 450 mg/kg bw/day. Histopathological changes included vacuolation of the liver and renal lesions compatible with tubular nephropathy that correspond well to changes in clinical chemistry parameters.

Effects on male reproductive organ weights (pronounced decrease in absolute and relative weight of seminal vesicles, depletion of secretion of the seminal vesicles), and other endocrine organs like increased relative thyroid/parathyroid and adrenal glands weight in males were reported. In addition, depletion of lymphoid of the thymus occurred in males. These effects were observed at the highest dosage of 450 mg/kg bw/day with occurring systemically toxic side effects (reduced body weights, clinical signs and 1 death). The LOAEL was determined to be 150 mg/kg bw/day based on observed histopathological findings in liver (females) and kidney (males) and increased liver weights (>10% compared to control, females).
The test article related effects on seminal vesicles (pronounced relative and absolute weight decrease including depletion of secretion of the seminal vesicles) at the highest dose might indicate an estrogen mode of action of phenol, heptyl derivs. in male mammals. Nevertheless, at this concentration systemic effects including one death and reduced mean body weights >10% (males) occurred.

4.7 Mutagenicity

Not relevant.

4.8 Carcinogenicity

Not relevant.

4.9 Toxicity for reproduction

4.9.1 Effects on fertility

4.9.1.1 Non-human information

Animal study with oral administration

A GLP conforming reproduction toxicity screening test (Klimisch 1) was conducted according to OECD test guideline 421 (Study report, 2012c). In the study doses of 0, 20, 40, 80 and 160 mg/kg bw/day phenol, heptyl derivs. in corn oil (purity 100.0%) were administered daily by gavage to 12 Sprague-Dawley (Crl:CD IGS BR) rats /sex/day per dose group. Males received 14 daily doses prior to mating and during the mating period for a total of 31 doses. Females were dosed 14 days prior to pairing, during pregnancy and until 4 days post partum for 39 (females that failed to deliver) to 51 doses.

Parameters examined in the parental male generations include testis weight, epididymides weight, seminal vesicle weight, microscopic examinations of testes, epididymides, seminal vesicles, coagulating glands and prostate gland.

At the highest dose group one female was found dead on lactation day 0 following observations of dystocia. The cause of death could not be determined microscopically. This female had 15 late resorptions in utero and delivered 1 dead pup that had been partially cannibalized on PND 0. Dystocia was also noted in the concurrent control group. Historical control data indicated 4 cases out of 4033 animals.

Body weight gain (statistically significant, -6.3% compared to control) and food intake were slightly decreased in parental males in the high dose group. No effects on reproductive performances were noted. Liver weights in males (17% relative weight) and females (16 and 19.8% absolute and relative weights) were significantly increased. Relative kidney weight was increased only in males (12.7%). No gross pathology changes in parental animals were observed. Histopathological changes in male parental animals were detected in liver (minimal to mild vacuolation) and kidney (increase in basophilic tubules and increase in minimal tubular dilatation) at 160 mg/kg bw/day. No effects on reproductive organs (e.g. seminal vesicles) were seen at any dose level.

Offsprings were not affected concerning viability, body weights and gross pathology (histopathology and clinical biochemistry was not examined). Concerning live litter size (11.9 and 11.3 pups per dam) lower mean number of pups born occurred at doses of 80 and 160 mg/kg bw/day, respectively (mean number in the control group was 14.5 pups per dam). The result from the 80 mg/kg/bw/day group is within to the historical minimum values observed at the study laboratory historical control data, 11.6 pups per dam (mean 14.1). The lower mean number of pups born in the high dose group was primarily attributed to one female (single pup born). Excluding this female – mean number of pups per dam was 12.0, which is within the historical data ranges. Mean viable litter size at 20 and 40 mg/kg bw/day was unaffected. Live litter size decreased in a dose dependent manner, but the changes were not statistically significant compared to the control group.

Due to the screening character of this study only limited information was obtained concerning the effects of phenol, heptyl derivs. on male and female reproductive performances. Effects on reproduction/development cannot be excluded based on reduced live litter size at the two highest dose levels. Therefore the actual NOAEL for reproduction

and developmental toxicity might be lower than the reported 160 mg/kg bw/day. The NOAEL systemic for the parental animals is 80 mg/kg bw/day

4.9.1.2 Human information

No data.

4.9.2 Developmental toxicity

4.9.2.1 Non-human information

Please see section 4.9.1.1

4.9.2.2 Human information

No data.

4.9.3 Summary and discussion of reproductive toxicity

Effects on fertility resulting from oral treatment with phenol, heptyl derivs. were investigated in a screening study in accordance with OECD TG 421. In this reproduction/developmental toxicity screening study evidence of fertility impairment in terms of reduced live litter size compared to the control was observed in the two highest dose levels (80 and 160 mg/kg bw/day). These effects, though statistically not significant, were seen in the dosing group where no parental systemic toxicity occurred (NOAEL 80 mg/kg bw/day). Also slightly lower mean numbers of corpora lutea were detected in the test substance treated groups.

Due to the screening character of this study only limited information was obtained concerning the effects of phenol, heptyl derivs. on male and female reproductive performances, and thus effects on reproduction/development cannot be fully excluded.

4.10 Other effects: *Endocrine Disruption*

4.10.1 Non-human information

4.10.2 Human information toxicity

4.10.3 Endocrine Disruption

Results of *in vitro* data regarding human and rat estrogen and other endocrine mode of action pathways are reported in chapter 5.6.2.2.

The test article related effects on seminal vesicles (pronounced relative and absolute weight decrease including depletion of secretion of the seminal vesicles) at the highest dose in the 28 day repeated dose study might indicate an estrogen mode of action of phenol, heptyl derivs. in male mammals. Nevertheless, at this concentration systemic effects including one death and reduced mean body weights >10% (males) were observed. In *in vitro* tests 4-HPbl showed an interaction with human and rat estrogen receptors (see chapter 5.6.2.2 on *in vitro* data).

Moreover, high variance and high standard deviations were reported in this study for uterine weight, although no significant change was observed: the mean weight was 0.48, 0.58, 0.76 and 0.33 g in the control, 50 mg/kg/d, 150 mg/kg/d and 450 mg/kg/d group,

respectively. However, systemic toxicity was observed.

In the reproductive screening assay phenol, heptyl derivs. effects on male and female reproduction cannot be excluded due to the reduced live litter size at the two highest dose levels.

No other long term reproductive or repeated dose studies are available. Also no carcinogenicity study is available.

Due to the scarce data set, no conclusions regarding endocrine disruptive effects can be drawn for human health assessment at the moment.

4.10.4 Summary and discussion of other effects – human health

Due to the scarce data set, no conclusions regarding endocrine disruptive effects can be drawn for human health assessment at the moment.

4.11 Summary and discussion of human health hazard assessment

Due to the scarce data set, no conclusions regarding endocrine disruptive effects can be drawn for human health assessment at the moment.

5. Environmental hazard assessment

5.1 Aquatic compartment (including sediment)

5.1.1 Fish

5.1.1.1 Short-term toxicity to fish

The short-term toxicity of phenol, heptyl derivs. was tested in a semi-static study according to OECD Guideline 203 (Fish Acute Toxicity Test) with juvenile *Oncorhynchus mykiss* (Study report, 2012c; Klimisch 2). The fish were exposed, in groups of seven, to Water Accommodated Fractions (WAFs) of the test item over a range of nominal loading rates of 1.0, 1.8, 3.2, 5.6 and 10 mg/L for a period of 96 hours at a temperature of approximately 15°C. The mortality and any sub-lethal effects were determined at 2, 6, 24, 48, 72 and 96 hours after the start of exposure.

The results were based on nominal loading rates (n).

There was 100% mortality at concentrations ≥3.2 (n) mg/L and 0% mortality at ≤ 1.8 (n) mg/L. Therefore, the 96h-LC_{50} was calculated using the geometric mean of 3.2 (n) and 1.8 (n) mg/L resulting in 2.4 (n) mg/L.

The short-term toxicity of 4-n-heptylphenol (97% purity) was tested under flow-through conditions according to OECD Guideline 203 (Fish Acute Toxicity Test) with juvenile *Gadus morhua* (Tollefsen et al., 1998; Klimisch 2).

The fish (weighing 4.6 ± 0.51 g) were exposed for 168 h to nominal concentrations of 4-n-heptylphenol in sea water of 0.5, 1, 2.1, 4.2 µmol/L corresponding to 0.10, 0.19, 0.40 and 0.81 mg/L, respectively. The test temperature was 9.7 ± 0.1°C. Methanol was used as the solvent.

The 96h-LC_{50} was 0.56 mg/L (2.9 µmol/L) and the 168h-LC_{50} was 0.52 mg/L (2.7 µmol/L). No mortality was found in the control groups exposed to methanol alone.

5.1.1.2 Long-term toxicity to fish

In a long term study with pikeperch (*Sander lucioperca*) the effects of 4-n-heptylphenol on mortality, development (weight, length, condition factor, gonads) and sex ratio (based on histological examination) were investigated (Demska-Zakęś, 2005).

For details on the test design and a detailed study description please see Chapter 5.6.2.3.2 and Annex II, respectively.

In this study no statistically significant effects on mortality, total length, body weight and condition factor of the fish were observed. However, 4-n-heptylphenol had significant effects on the gonads, starting from the lowest test concentration of 1 µg/L (see Chapter 5.6.2.3.2).

5.1.2 Aquatic invertebrates

5.1.2.1 Short-term toxicity to aquatic invertebrates

The short-term toxicity of phenol, heptyl derivs. was tested in a static study according to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) with *Daphnia magna* (Study Report, 2005a; Klimisch 2). For phenol, heptyl derivs a 48h-EC_{50} of 0.38 mg/L (0.32-0.46,

95% CI) and a NOEC of 0.19 mg/L for immobilization were observed. Both effect concentrations were based on the measured concentrations.

5.1.2.2 Long-term toxicity to aquatic invertebrates

No data.

5.1.3 Algae and aquatic plants

In an OECD Guideline 201 study (Alga, Growth Inhibition Test) with *Desmodesmus subspicatus* 72h-EC_{50} value of 1.2 mg/L (nominal) based on growth rate was determined for phenol, heptyl derivs. (Study Report, 2005b; Klimisch 2). The determined 72h-NOEC values were 0.048 mg/L (nominal) and 0.028 mg/L (geometric mean measured concentration).

5.1.4 Sediment organisms

No data.

5.1.5 Other aquatic organisms

No data.

5.2 Terrestrial compartment

No data other than the rodent data described in the human health section are available.

5.3 Atmospheric compartment

No data.

5.4 Microbiological activity in sewage treatment systems

In an OECD Guideline 209 test (Activated Sludge, Respiration Inhibition Test) with phenol, heptyl derivs. by Roulstone P (2012), an EC_{50} (3 h) of 58 mg/L was determined.

5.5 Toxicity to birds

No data.

5.6 Other effects: Endocrine disruption

In the registration dossier for phenol, heptyl derivs. the potential endocrine disrupting properties were not assessed. No long-term toxicity test on aquatic species was included. Moreover, no *in vitro* studies on potential endocrine disrupting mode of action were mentioned.

This chapter summarises the available information on the potential endocrine disrupting

properties of 4-HPbl.

5.6.1 Adverse effects (non-ED)

Not relevant.

5.6.2 Endocrine Disruption

5.6.2.1 General approach – environment

5.6.2.1.1. Relevance of different 4-heptylphenol compounds for 4-HPbl

The substance group 4-HPbl contains different heptylphenol compounds with a linear and/or branched alkyl chain of 7 carbon atoms covalently bound to a phenol ring, predominantly to position 4. This covers also UVCB- and well-defined substances which include any of the individual isomers or a combination thereof. Data are available for the UVCB substance phenol, heptyl derivs. as well as for specific isomers.

The registered UVCB substance phenol, heptyl derivs. consists of branched and predominantly monoalkylated 4-heptylphenol isomers. The registration dossier includes studies with phenol, heptyl derivs., as well as studies with 4-n-heptylphenol (e.g for acute fish toxicity) and a mixture of two 4-heptylphenol isomers (one linear, one branched; used for assessment of bioconcentration).

In vitro data are available for the isomers 4-n-heptylphenol (linear) and 4-*tert*-heptylphenol (branched), which show estrogenic activity in a comparable concentration range (see *in vitro* data).

Apart from the alkyl chain length 4-HPbl is comparable to the Candidate list entry 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].* According to the SVHC dossier for 4-nonylphenol, branched and linear, the estrogen activity of branched and linear 4-nonylphenol needs to be considered as similar (see SVHC-dossier (supporting document: ECHA, 2011)). In analogy it can be assumed that with regard to 4-heptylphenol linear and branched isomers should be considered to show similar estrogenic activity. This is also underlined by QSAR data (OECD Toolbox, 3.1.0.21): Data for all 117 possible monosubstituted heptylphenols show moderate estrogen binding activity according to the applied profiler. Also the physical chemical properties of these 117 isomers are predicted to have similar properties in a narrow range, see **Table 12** below. Therefore, data from single isomers are expected to be applicable for various compositions of the isomers such as the UVCB substance phenol, heptyl derivs.

Table 12: Estimated Data for the 117 monosubstituted heptylphenol isomers

Property	QSAR	Range
Boiling point	EPISUITE	265 – 296°C
Log KOW	EPISUITE	4.78 – 5.01
Selected vapour pressure	EPISUITE	0.0372 – 0.321 Pa
Water solubility	EPISUITE	9.65 – 14.9 mg/L
Estrogen receptor binding	OECD Toolbox 3.1.0.21	Moderate binder, OH group (see explanation below)

In the concept of the OECD toolbox a strong estrogen receptor binder has a molecular weight (MW) between 200 and 500, and a non-impaired OH group attached to a ring of 5

or 6 carbon atoms. A moderate binder has a MW between 170 and 200 with a non-impaired OH group attached to a ring of 5 or 6 carbon atoms.

According to this profiler chemicals with a single 5 or 6 carbon ring structure and an unhindered OH-group (one in the para- or meta-position of the ring) bind to the estrogen receptor (ER). This holds true for all 117 possible isomers of heptylphenol as well as the other alkylphenols used for read-across.

A schematic representation of a hydroxylated ligand interacting at site A of the estrogen receptor binding pocket is shown in **Figure 1**.

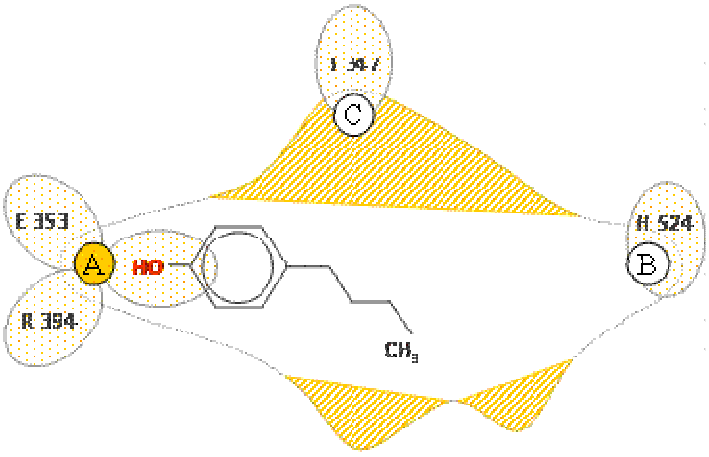


Figure 1: Schematic representation of a hydroxylated ligand interacting at site A of the estrogen receptor binding pocket (picture from OECD Toolbox, references for this concept are Schultz et al. 2006, Cronin and Worth, 2008, Tong et al. 2004, Schmieder et al. 2003 and Schultz et al. 2002).

5.6.2.1.2. Relevance of other structurally similar alkyl phenols

Similar branched alkylphenols such as the SVHC substances 4-nonylphenol, branched and linear and 4-*tert*-octylphenol and the substances 4-*tert*-pentylphenol and 4-*tert*-butylphenol are known to have endocrine disrupting properties. The two latter substances were in the substance evaluation under REACH in 2014 with potential endocrine disruption as a concern. The analysis of the data revealed endocrine disruptive properties for the environment, and Germany is submitting, in parallel with this dossier, SVHC dossiers to identify these substances as endocrine disruptors for the environment.

Also toxicokinetic data on branched and linear alkylphenols show similarities as described in section 4.1 (toxicokinetics) and in Annex I (read across).

Relevant data on the substances 4-nonylphenol, branched and linear, 4-*tert*-octylphenol, 4-*tert*-pentylphenol and 4-*tert*-butylphenol as well as a read across justification are included in the read across chapter 5.6.2.4. The available in vitro and *in vivo* read across data show that although the length of the chain differs between these substances, the binding to the estrogen receptors is not affected. Moreover, QSAR data on 4-HPbl, as well as *in vitro* and *in vivo* data for 4-HPbl, 4-nonylphenols and 4-butylphenols demonstrate that both linear and branched isomers are able to affect estrogen receptors.

5.6.2.2 *In vitro* information indicative of endocrine activity

In vitro data are analysed with respect to the potential endocrine disrupting mode of action of 4-HPbl.

Estrogen activity of 4-HPbl was assessed *in vitro* utilizing assays for both binding and receptor activation. These are described in Chapter 5.6.2.2.1 and summarised in **Table 13**.

The data regarding other endocrine modes of action are described in Chapter 5.6.2.2.2 and summarised in **Table 14**.

5.6.2.2.1 Data regarding Estrogen Mode of Action

Competitive ligand-binding assays

Binding to ER

Competitive ligand-binding assays are used to assess whether or not a test chemical is able to specifically bind to a given receptor. Typically, preparations of estrogen receptors (ER) are incubated with radioactively labelled ligand (e.g. 17ß-estradiol) at a concentration that results in saturation of the receptors ligand-binding site. Then, unlabelled test chemicals or unlabelled model ligands are added at increasing concentrations. Depending on the binding affinity of the test chemical to the receptor lower or higher concentrations are needed to displace a certain percentage of the radiolabelled ligand from the ER. From the established binding curves the relative binding affinity of the test chemicals compared to a model ligand can be calculated.

There are three studies available assessing whether 4-heptylphenols are able to specifically bind to the estrogen receptors of fish (Hornung et al. 2014, Tollefsen and Nilsen, 2008, and Knudsen and Pottinger, 1999). In all three studies, heptylphenol was demonstrated to displace specifically bound 17ß-estradiol (E2) from the estrogen receptors (ER).

Hornung et al. (2014) conducted competitive binding assays using liver cytosolic preparations (cyto rtERαß) from immature rainbow trout. The preparations contained all ER receptors found in trout liver. The two results relevant for 4-HPbl showed similar values. The relative binding affinity (RBA[9]) for 4-n-heptylphenol was 2.1×10^{-4}, whereas the value for 4-*tert*-heptylphenol was 1.4×10^{-4}. These values were similar to those gained for the known endocrine disruptors nonylphenol and 4-*tert*-octyphenol. The RBAs for various nonylphenol isomers were from 1.6×10^{-4} to 4.6×10^{-4}. The RBA for 4-*tert*-octylphenol was 9.4×10^{-4}, while 4-n-octylphenol had a similar RBA of 9.1×10^{-4}.

RBA reported by Tollefsen and Nilsen (2008) for 4-n-heptylphenol (3.2×10^{-5}) was also in the same order of magnitude as those for the known endocrine disruptors nonylphenol (1.0×10^{-5}) and 4-*tert*-octylphenol (6.9×10^{-5}). For 4-n-octylphenol the RBA was also in the same range (7.8×10^{-5}).

In Knudsen and Pottinger (1999), the maximum displacement achieved by heptylphenol (substance identity not specified in more detail) was ca. 60 %, which is even higher than those of octylphenol (ca. 45%) or nonylphenol (ca. 50%).

In two further studies the binding affinities of 4-n-heptylphenol to human ER were investigated. Satoh and Nagai (2002) reported a rather high RBA of 0.00163 for hERα, whereas no binding was observed for hERß. In another study Akahori et al. (2005) reported RBA of 8.5×10^{-6}.

A study (Laws et al. 2006) using rat uterine cytosol confirmed 4-n-heptylphenol to be a true competitive binder (by Lineweaver-Burk plots and slope replots) with an RBA of 1.24×10^{-5}.

In summary, it has been shown that 4-heptylphenol is able to bind to estrogen receptors of fish, humans and rats.

[9] RBA: calculated as IC50(E2)/IC50(4-t-BP). The IC50 in binding studies is the equilibrium inhibitory concentration, calculated as the concentration causing 50% inhibition of [3H]-E2 binding.

Binding to sex steroid binding proteins

The binding of alkylphenols to sex steroid binding proteins of rainbow trout (rtSBP) under competitive conditions was examined by Tollefsen (2007). Plasma samples of female rainbow trout were used and incubated with [^{3}H]E_2 with increasing concentrations of test compounds.

The RBA for displacement of [^{3}H]E_2 in rainbow trout plasma was 6.6 x 10^{-6} which is in the same order of magnitude as the RBA for 4-*tert*-octylphenol (1.3 x 10^{-5}; 4-n-octylphenol and 4-n-nonylphenol were only weak binders in this assay).

Expression of estrogen-responsive genes

Expression profiling of estrogen-responsive genes
Terasaka et al. (2006) used a cDNA microarray assay with breast cancer cells (MCF-7) for after treatment with 4-n-heptylphenol and observed high correlation coefficients between the profiles for E_2 and 4-n-heptylphenol (R-value for 4-n-heptylphenol is 0.82). This value is in the same range for 4-*tert*-octylphenol (R-value = 0.75) and 4-nonylphenol (R-value =0.90).

Vitellogenin expression

Tollefsen et al. (2008) investigated the effect of 4-*n*-heptylphenol on vitellogenin (VTG) expression in primary fish hepatocytes.Primary hepatocytes were derived from male and/or immature rainbow trout (*Oncorhynchus mykiss*). In this assay 4-n-heptylphenol did not show effects under the employed conditions.

Reporter gene assays

Transcriptional activation in recombinant yeast (Yeast estrogen screen, YES)

The potential of 4-n-heptylphenol and 4-*tert*-heptylphenol to act as agonists of the ER was also investigated by means of a reporter gene assay based on recombinant yeast cells. The DNA sequence of the human estrogen receptor was integrated into the yeast genome, which also contained expression plasmids carrying estrogen-responsive sequences controlling the expression of the reporter gene Lac-Z (encoding the enzyme β-galactosidase). Thus, due to estrogens β-galactosidase is synthesized and causes a change of colour that is measurable. Not only binding but also activation of the receptor is measured. Routledge & Sumpter (1997) determined the relative estrogenic potency (REP) to be 3 x 10^{-3} for 4-*tert*-heptylphenol (80% pure, 15% 2-*tert*-heptylphenol) and 7.5 x 10^{-4} for 4-n-heptylphenol (98% pure). EC_{50} values were not reported. In this assay 4-*tert*-heptylphenol was 3000 times less potent compared to E_2. 4-n-heptylphenol was 25 times less potent than 4-*tert*-heptylphenol. These values are similar to those of nonylphenol (4-nonylphenol) with a REP of 3 x 10^{-4} and 4-*tert*-octylphenol with a REP of 1 x 10^{-3}.

Yeast Two-hybrid Assay

Nishihara at al. (2000) used a yeast two-hybrid assay system where the estrogen receptor ERα and the coactivator TIF2 two expression plasmids, which carry a β-galactosidase reporter gene and require tryptophan and leucine for growth, were introduced into yeast cells. 4-n-Heptylphenol was reported to be negative in this assay. Also 4-n-nonylphenol and 4-n-octylphenol were negative in this assay, while 4-*tert*-octylphenol was positive.

Summary of data on estrogen mode of action

The competitive ligand-binding studies clearly demonstrate that 4-HPbl is able to displace specifically bound E_2 from the ER ligand-binding pocket and act as a ligand for the ER. The RBA of 4-*n*-heptylphenol for the ER of rainbow trout, human or rat ranged from 0.163 to

1.24×10^{-5}.

Binding of 4-HPbl to sex-steroid binding protein (SBP) was also studied. In a study with plasma preparation of rainbow trout the binding to the SBP was comparable to the binding to the ER.

Modulation of ER-mediated gene expression was also evidenced in an assay with expression profiling of estrogen-responsive genes, where high correlation values with E2 were found. In an assay testing vitellogenin expression in rainbow trout hepatocytes 4-n-heptylphenol did not show effects under the employed conditions.

The relative estrogenic potency (REP) obtained in the transcriptional activation assay using recombinant yeast (yeast estrogen screen, YES) was 3×10^{-3} for 4-*tert*-heptylphenols and 7.5×10^{-4} for 4-n-heptylphenol.

In a yeast two-hybrid assay 4-n-heptylphenol was negative.

Based on the available mechanistic information on 4-n-heptylphenol and 4-*tert*-heptylphenol it can be concluded that 4-HPbl is also able to bind to the estrogen receptors of fish, humans and rats and to activate these receptors.

Table 13: Summary of *in vitro* studies assessing the potential of 4-heptylphenol isomers grouped under 4-HPbl, to interact with the ER-mediated pathway

Endpoint: Competitive ligand-binding (IC_{50} is the concentration displacing 50% of [^{3}H]E_2 from ER ligand binding pocket).						
Binding to ER						
Species	**Reference**	**Receptor origin and preparation**	**Test conditions**	**Endocrine mediated measurement parameters**	**Relative binding affinity (RBA) compared to 17β-estradiol**	**Comment**
Oncorhynchus mykiss rainbow trout	Hornung et al. (2014)	Cytosolic liver preparations (cyto rtERαß) from immature rainbow trout. Preparations contained the ER receptors α1, α2, ß1, ß2.	Testing in triplicate at a minimum of six concentrations covering four to six log intervals (maximum concentration 0.01 M), together with [3H]-E_2. 4 n-heptylphenol and 4-*tert*-heptylphenol were tested solvent: ethanol	No IC_{50} value given	4-n-Heptylphenol RBA (cyto rtERαß binding) = 2.1 x 10^{-4} 4-*tert*-Heptylphenol RBA (cyto rtERαß binding) =1.4 x 10^{-4} RBA= IC_{50} (E_2)/ IC_{50} (4HP)	Similar (rather high) RBAs were found for 4-n-heptylphenol and 4-*tert*-heptylphenol Klimisch 2
Oncorhynchus mykiss rainbow trout	Tollefsen and Nilsen (2008)	Cytosolic preparation of female trout liver homogenates	Pooled liver homogenates (2.5 mg/ml protein) were incubated with 2.5nM [^{3}H]E_2 for 16h at 4 °C) in the absence or presence of different concentrations of 4nHP (0.25 x 10^{-6} M to 7.5 x 10^{-3} M) or E_2 (75 x 10^{-12} M to 75 x 10^{-9} M)	IC_{50} (E_2) = 3.5 x 10^{-9} M (0.95 µg/L) IC_{50} (4nHP) = 1.1 x 10^{-4} M	RBA = 3.2 x 10^{-5} RBA was calculated as IC_{50}(E_2)/IC_{50}(4-nHP)	Klimisch 2

			Solvent: Methanol, c_{max} =1.25% (v/v) / n=3			
Oncorhynchus mykiss rainbow trout	Knudsen and Pottinger (1999)	Cytosolic preparation of mixed sex trout liver	Cytosol was incubated with 1 pM [2,4,6,7-^{3}H]E_2 in the absence or presence of different concentrations of heptylphenol (500-, 5000- and 50000-fold the concentration of [^{3}H]E_2).	Concentrations of alkylphenols including heptylphenol 10^4-fold > those of the maxium displacement achieved was E_2 required to produce similar amounts of displacement of specifically bound - [^{3}H]E_2	The maximum displacement achieved was ca. 60% at the highest concentration	Klimisch 2
Rat	Laws et al. (2006)	Rat uterine cytosol containing ERα and ß	Cytosol was exposed to concentrations from 0.1 to 100µM of 4-n-heptylphenol for 18 h (two replicates) at 4°C.	IC_{50} (E_2) = 0.00052µM IC_{50} (4nHP) = 42µM	RBA = 1.24 x 10^{-5} RBA was calculated as $IC_{50}(E_2)/IC_{50}$(4-HP)	4-n-Heptylphenol was confirmed to be a true competitive binder (by Lineweaver-Burk plots and slope replots). It showed partial competitive binding curves. It was assigned to a group of chemicals that displaced ^{3}H-E_2 by 50 – 74% Klimisch 2
Human	Akahori et al. (2005)	Recombinant hERα-ligand binding domain fusion protein	Varied concentrations of 1 x 10^{-11} to 1 x 10^{-4} were used. Incubation time was 1 hour.	No details provided	RBA = 8.5 x 10^{-6}	Klimisch 2

		expressed in E. coli				

Human	Satoh and Nagai (2002)	ERα, other details not available	No details available, data cited from Dang (2010)		RBA =0.00163	Klimisch 4
Human	Satoh and Nagai (2002)	ER ß , other details not available	No details available, data cited from Dang (2010)		No binding	Klimisch 4
Endpoint: Binding to sex steroid-binding protein						
Species	**Reference**	**Receptor origin and preparation**	**Test conditions**	**Endocrine mediated measurement parameters**	**Relative binding affinity (RBA) compared to 17β-estradiol**	**Comment**
Oncorhynchus mykiss rainbow trout	Tollefsen (2007)	Plasma preparation of female rainbow trout	Plasma samples were incubated with $[^3H]E_2$ for 16h at 4°C in the absence or presence of different concentrations of 4nHP (25 nM-250 mM) or E_2. solvent: methanol, c_{max} = 2.5%	IC50 (E2) = 1.6 x 10^{-9} M IC50 (4nHP) = 2.4 10^{-4} M	RBA = 6.6 x 10^{-6} RBA was calculated as $IC_{50}(E_2)/IC_{50}(4nHP)$	Klimisch 2
Endpoint: Expression of estrogen-sensitive genes						
Expression of vitellogenin						
Species	**Reference**	**Cell type and origin**	**Test conditions**	**Endocrine mediated measurement parameters**	**Potency (relative to 17 β-estradiol=1)**	**Comment**
Oncorhynchus mykiss rainbow trout	Tollefsen et al. (2008)	Primary hepatocytes derived from male, immature fish	Cells were exposed to serial dilutions of 4nHP for 96h. The exposure medium was renewed after two days.	Expression of vitellogenin protein (rtVTG) LOEC (E_2) = 1 x 10^{-10} M (2.7 x 10^{-2} µg/L) LOEC (4nHP)>300	REP = no effect under condition employed.	Klimisch 2

			Solvent: DMSO, c_{max} < 0.3% (v/v) / n=3, i=3 (Cells from different isolations were used to perform replicates.)			
Expression profiling of estrogen-responsive genes						
Species	**Reference**	**Cell type**	**Test conditions**	**Endocrine mediated measurement parameters**	**Potency (relative to 17ß-estradiol=1)**	**Comment**
Human	Terasaka et al. (2006)	MCF-7 cells cDNA microarray assay	MCF-7 cells were exposed for 3 days to 4-n-heptylphenol (10 µM), then cDNA microarray (EstrArray) performed (average of nine assays for 120 genes)	R-value (statistical correlation) for EstrArray: 17ß-estradiol: 0.91 4-n-heptylphenol: 0.82		Klimisch 2
Endpoint: Transcriptional activation of reporter genes under the control of the ER						
Transcriptional activation assay using recombinant yeast (yeast estrogen screen, YES)						
Species	**Reference**	**Cell type**	**Test conditions**	**Endocrine mediated measurement parameters**	**Potency (relative to 17ß-estradiol=1)**	**Comment**
Human	Routledge and Sumpter (1997)	YES assay Recombinant yeast expressing human ER (hER).	4-*tert*-heptylphenol (80% pure, 15% 2-*tert*-heptylphenol) and 4-n-heptylphenol (98% pure) Yeast cells were exposed to increasing concentrations of 4tHP or E_2 for 84h at 32 °C	Induction of β-galactosidase activity No EC_{50} values reported given	4-*tert*-heptylphenol: REP = 3 x 10^{-3} (3000-fold less potent than 17ß-estradiol) 4-n-heptylphenol: REP = 7.5 x 10^{-4} = 25-fold less potent than 4-*tert*-heptylphenols	4-*tert*-heptylphenol is 25-fold more potent than 4-n-heptylphenol Klimisch 2

			(1 x 10^{-8} to 5 x 10^{-12} M). solvent: ethanol			
Human	Nishihara et al. (2000)	Yeast Two-Hybrid Assay Recombinant yeast expressing human ER (hER).	Yeast cells were exposed to 4-n-heptylphenol for 4 h at 30°C. solvent: DMSO	REC10 > 1 x 10^{-3} correlating to a negative test result		

ER =estrogen receptor, E_2 = 17 β-estradiol, n = number of independent experiments, I = number of replicates within each experiment, ECmax = concentration, at which highest response was observed, LOEC = lowest observed effect concentration, cmax = maximal concentration of test chemical or solvent in the assay
RBA = relative binding affinity.
REP = relative estrogen potency, calculated as $EC_{50}(E_2)/EC_{50}$(heptylphenol)
REC10: The concentration showing 10% activity of 10^{-7}M 17β-estradiol (relative activity), quantified by the activity of ß-galactosidase in the reporter gene system

5.6.2.2.2 *In vitro* data regarding endocrine modes of action other than estrogenic

Some data regarding potential endocrine modes of action other than estrogenic are available regarding retinoic acid, androgen and cortisol receptors. These data are summarised in Table 14.

Retinoic Acid Receptor Transactivation Assay

Kamata et al. (2008) investigated the binding of 543 chemicals to the RARγ in a yeast two-hybrid system to detect transcriptional activation via the human RARγ. The results show that monoalkylphenols having an alkyl chain of 6 to 9 carbons in para position to the hydroxylated phenolic group possess high affinity for the RARγ. Of the tested monoalkylphenols 4-n-heptylphenol was the most potent activator of the RARγ with 1.363% activity compared to that of all trans-Retinoic acid (RA). REC20 (20% of the activity of 10^{-8}M all trans RA) and ECx10 (concentration of a test solution producing luminescence intensity 10 times that of the blank control) were 0.49± 0.26 x10^{-6}M and 0.21± 0.11 x10^{-6}M.

Androgen Receptor Binding

In a study by Knudsen and Pottinger (1999) rainbow trout liver cytosol was incubated with [^{3}H]testosterone in the absence or presence of increasing concentrations of heptylphenol (substance identity not further specified). No activity was observed.

Corticosteroid Receptor Binding

In a study by Knudsen and Pottinger (1999) rainbow trout liver cytosol was incubated with [^{3}H]cortisol in the absence or presence of increasing concentrations of heptylphenol (substance identity not further specified). No activity was observed.

Table 14: Summary of *in vitro* studies assessing the potential of 4-heptylphenol isomers grouped under 4-HPbl to interact with endocrine pathway other than estrogenic

Endpoint: Retinoic Acid Receptor						
Species	**Reference**	**Cell type**	**Test conditions**	**Endocrine mediated measurement parameters**	**Activity (relative to retinoic acid) in %**	**Comment**
Human	Kamata et al. (2008)	Yeast two-hybrid assay expressing human RARγ (retinoic acid receptor)	543 chemicals tested amongst others 4-n-heptylphenol Yeast cells were exposed to seven concentrations in the range of 10µM to 156 nM for 4 h 30 °C. Solvent: DMSO	Induction of β-galactosidase activity ECx10 (x10^{-6}M)values: 4-n-heptylphenol: 0.49±0.26 (al trans-retinoic acid:0.00541±0.00173) 4-*tert*-octylphenol: 0.78±0.41 4-n-octylphenol: 1.7±0.56 4-nonylphenol (mixed isomers): 1.36±0.7 4-n nonylphenol: 4.61±1.01 4-*tert*-pentylphenol: 9.92±2.29	4-n-heptylphenol: 1.363% (100% al trans-retinoic acid) 4-*tert*-octylphenol: 0.997% 4-n-octylphenol: 0.446% 4-nonylphenol (mixed isomers): 0.476% 4-n-nonylphenol: 0.1% 4-*tert*-pentylphenol: 0.056%	4-n-heptylphenol was the most potent binder compared to other monoalkylphenols and other tested compounds Klimisch 2
Endpoint: Androgen Receptor Binding (competitive)						
Species	**Reference**	**Cell type**	**Test conditions**	**Endocrine mediated measurement parameters**	**Potency**	**Comment**
Oncorhynchus mykiss rainbow trout	Knudsen and Pottinger (1999)	Cytosolic preparation of mixed sex trout liver	Cytosol was incubated with [^{3}H]testosterone in the absence or presence of increasing concentrations of heptylphenol	No displacement of specifically bound [^{3}H]testosterone		Klimisch 2
Endpoint: Corticosteroid Receptor Binding (Cortisol, competitive)						

Species	**Reference**	**Cell type**	**Test conditions**	**Endocrine mediated measurement parameters**	**Potency**	**Comment**
Oncorhynchus mykiss rainbow trout	Knudsen and Pottinger (1999)	Cytosolic preparation of mixed sex trout liver	Cytosol was incubated with [^{3}H]cortisol in the absence or presence of increasing concentrations of heptylphenol	No displacement of specifically bound [^{3}H]cortisol		Klimisch 2

5.6.2.3 *In vivo* effects with regard to an endocrine mode of action

In the following chapter scientific data will be evaluated, showing that effects of 4-HPbl are caused by its endocrine disrupting properties.

5.6.2.3.1. Approach used for assessing the endocrine activity in vertebrates

The WHO/IPCS definition for endocrine disruptors (2002) is used as a basis for identification of 4-HPbl as an endocrine disruptor for the environment. It is noted that the recent European Commission's proposal for scientific criteria for endocrine disruptors for the Biocidal Products Regulation and the Plant Protection Products Regulation (European Commission, 201610) is also based on the WHO/IPCS definition.

The assessment of the endocrine properties of 4-HPbl in fish was mainly based on the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (OECD, 2012b) and the OECD Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption (OECD, 2012).

Information provided in these documents is supplemented by information from other guidance documents (e.g. OECD 123 Guidance document on the diagnosis of endocrine related histopathology in fish gonads (OECD, 2010)) and information from literature (e.g.(IPCS, 2002; Kendall et al., 1998; OECD, 2004)).

In general two different types of effects are considered and analysed separately:

- Indicators for an endocrine mode of action and
- Adverse effects on apical endpoints that are considered to be caused by the substance via an endocrine mode of action.

Change of sex ratio towards females is a known result of estrogen exposure during sexual development (IPCS, 2002; Kendall et al., 1998; OECD, 2004).
Female biased sex ratio is an apical endpoint that is considered to be estrogen or anti-androgen specific.

Moreover, to substantiate the findings for 4-HPbl, a read across from structurally similar alkyphenols is applied, as explained in more detail in Chapter 5.6.2.4.

5.6.2.3.2. Analysis of available vertebrate data

Analysis of available data for fish species

In one available long term study with fish (pikeperch, *Sander lucioperca*) the effects of 4-n-heptylphenol and other substances on mortality, development (weight, length, condition factor[11], gonads) and sex ratio (based on histological examination) were investigated (Demska-Zakęś, 2005). Sexually undifferentiated fish from artificial spawning were exposed to 4-n-heptylphenol from 60 days post hatch (dph) until 88 dph. These 28 days of exposure were followed by 56 days of rearing without test substance (until 144 dph). The test included a dilution water control, a solvent control (ethanol, 10 µL/L) and four treatment concentrations of 1, 10, 100, 200 µg/L (nominal) for 4-n-heptylphenol and as well for the positive controls (17 ß-estradiol and 4',7-

[10] European Commission (2016): Draft Commission Delegated Regulation (EU) …/... of XXX setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 (Text with EEA relevance); see http://ec.europa.eu/health/endocrine_disruptors/docs/2016_bpcriteria_en.pdf

[11] Condition factor =100*bodyweight*length-3

dihydroxyisoflavone) and the other tested substances: 4-n-heptyloxyphenol, 4-n-nonylphenol, 4-n-butylphenol, 4-sec-butylphenol, 4-*tert*-butylphenol, phenol, 1,6-dihydroxynaphthalene and 1,5-dihydroxynaphthalene. Per treatment 80 fish per tank were tested in three replicates. The fish were kept in tanks with a water volume of 80 L under semi-static conditions (approximately 50% water exchange per 24h) and permanent lighting (50-60 lux). Each tank was separately filtered by a biological filter (filter performance was 4 L/min corresponding to the 3-fold tank volume per hour). The test temperature was 22.0±0.5°C.

The fish were examined before the start of the test (59 dph), on 88 dph (after 28 days exposure) and on 144 dph at the test end.

For a detailed description of the test design please see Annex II.

Results for 4-n-heptylphenol

No statistically significant effects of 4-n-heptylphenol on mortality, total length, body weight, and condition factor of the fish were observed.

4-n-Heptylphenol had significant effects on the gonads, starting from the lowest test concentration (Table 15, Figure 2 and Figure 3). In neither of the investigated endpoints a statistically significant difference between the dilution water control and the solvent control was encountered.

Table 15: Sex structure of pikeperch after 28 days of exposure to 4-n-heptylphenol (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated[1] from a graph (Fig. 17 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	48^{a}	52^{a}	0^{a}	0^{a}
Solvent control	53^{ab}	47^{a}	0^{a}	0^{a}
1	59^{b}	24^{b}	18^{b}	0^{a}
10	75^{c}	8^{c}	16^{b}	0^{a}
100	85^{d}	0^{d}	15^{b}	0^{a}
200	98^{e}	0^{d}	2^{a}	0^{a}
D144				
Dilution water control	50^{a}	50^{a}	0^{a}	0^{a}
Solvent control	50^{a}	50^{a}	0^{a}	0^{a}
1	59^{a}	20^{b}	21^{b}	0^{a}
10	75^{b}	5^{c}	20^{b}	0^{a}
100	87^{bc}	0^{c}	13^{ab}	0^{a}
200	100^{c}	0^{c}	0^{a}	0^{a}

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

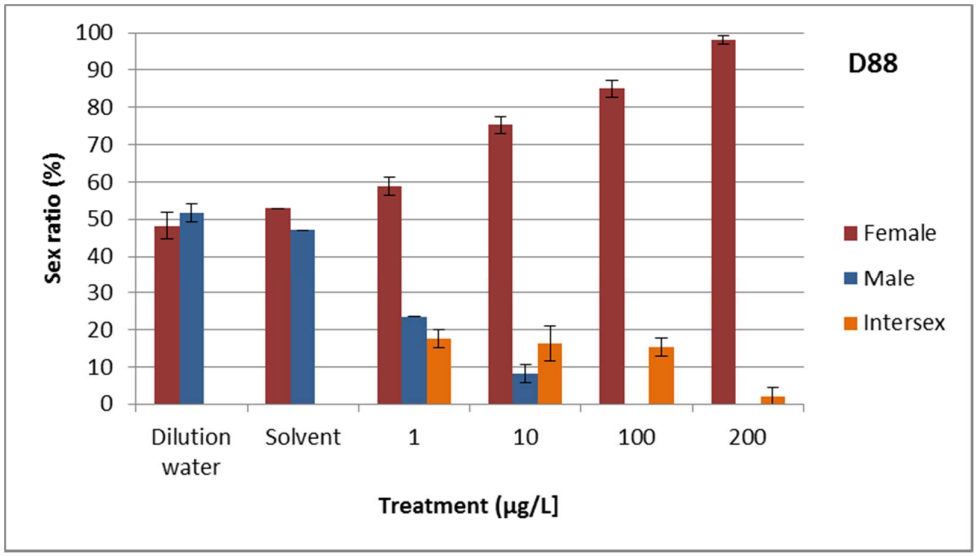


Figure 2: Sex structure of pikeperch after 28 days of exposure to 4-n-heptylphenol (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 17 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

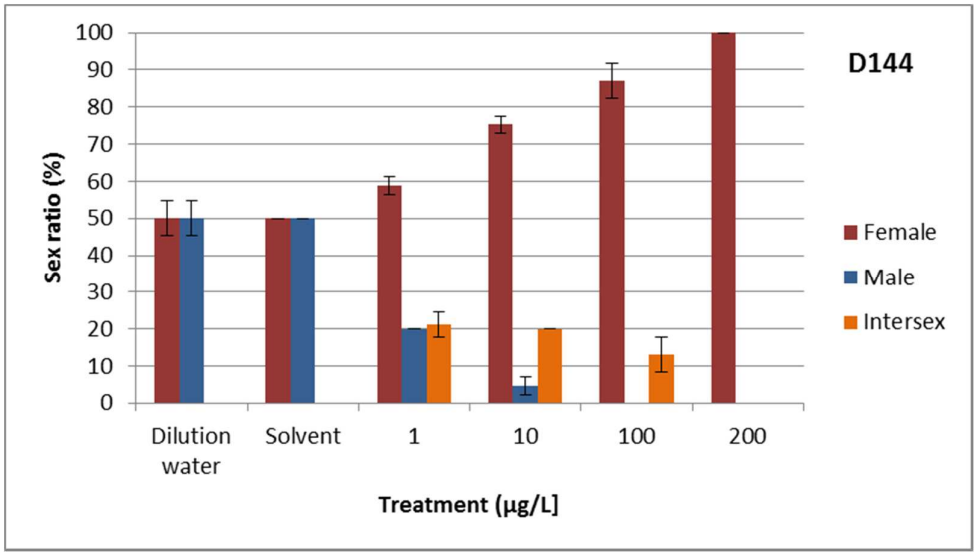


Figure 3: Sex structure of pikeperch after 28 days of exposure to 4-n-heptylphenol and a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 17 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

After 28 days of exposure the ratio of male fish (according to histological determination) was significantly decreased at the lowest test concentration (1 µg/L). Compared to the dilution water control the ratio of female fish is increased in the lowest test concentration, but compared to the more relevant solvent control this effect is significant from 10 µg/L on (Table 15). The appearance of intersex species (comprising sex characteristics from both sexes e.g. testis-ova/ ovotestis, formation of an oviduct, with regressed spermatogenic lobules in the same fish) was significant starting from 1 µg/L and was not observed in the controls.

The shift in sex ratio was dose-dependent, leading to approximately 98 and 100% female fish at 88 dph (Figure 2) and 144 dph (Figure 3), respectively. The values of the respective effect concentrations do not differ between 88 dph and 144 dhp (Table 16). This indicates that the observed effects on the sex characteristics were irreversible, also during the 56 days of rearing without exposure to the test substance.

Table 16: Effect concentrations for the investigated endpoints in juvenile pikeperch after 28 days of exposure to 4-n-heptylphenol. Concerning these effect concentrations there was no difference between the two sample points on 88 dph and 144 dph. Therefore, the values are listed combined in one table.

	Mortality ↑	TL ↓	BW ↓	CF ↓	Female ↑	Male ↓	Intersex	Sterile
NOEC (µg/L)	>200	>200	>200	>200	1	<1	<1	>200
LOEC (µg/L)	>200	>200	>200	>200	10	1	1	>200

BW ↓ decrease of of body weight, CF ↓ decrease of condition factor, Female ↑ increase of female sex characteristics, Intersex appearance of intersex species, Male ↓ decrease of male sex characteristics, Mortality ↑ increase of mortality, Sterile appearance of sterile species, TL ↓decrease of total length

Analysis of available mammalian data

In the *in vitro* tests an interaction with human and rat estrogen receptors was demonstrated (see Chapter 5.6.2.2. on *in vitro* data).

The test article related effects on seminal vesicles (pronounced relative and absolute weight decrease including depletion of secretion of the seminal vesicles) at the highest dose in the 28 day repeated dose study might indicate an estrogen mode of action of phenol, heptyl derivs. in male mammals. Nevertheless, at this concentration systemic effects including one death and reduced mean body weights >10% (males) were observed. In *in vitro* tests 4-HPbl showed an interaction with human and rat estrogen receptors (see chapter 5.6.2.2 on *in vitro* data).

Moreover, high variance and high standard deviations were reported in this study for uterine weight, although no significant change was observed: the mean weight was 0.48, 0.58, 0.76 and 0.33 g in the control, 50 mg/kg/d, 150 mg/kg/d and 450 mg/kg/d group, respectively. However, systemic toxicity was observed.

In a 28 day repeated dose study phenol, heptyl derivs. related effects on seminal vesicles (pronounced relative and absolute weight decrease including depletion of secretion of the seminal vesicles) at the highest dose might indicate an estrogen mode of action in male mammals. Nevertheless, at this concentration systemic effects including one death and reduced mean body weights >10% (males) were observed.

Moreover, high variance and high standard deviations were reported in this study for uterine weight, although no significant change was observed: the mean weight was 0.48, 0.58, 0.76 and 0.33 g in the control, 50 mg/kg/d, 150 mg/kg/d and 450 mg/kg/d group, respectively. However, systemic toxicity was observed.

In the reproductive screening assay phenol, heptyl derivs. effects on male and female reproduction cannot be excluded due to reduced live litter size at the two highest dose levels.

No other long term reproductive or repeated dose studies are available. Also no carcinogenicity study is available.

Due to the scarce data set, no conclusions regarding endocrine disruptive effects can be drawn for human health assessment at the moment.

5.6.2.4. Read across approach from structurally similar alkylphenols

Read across is carried out to support the hazard assessment of the estrogenic mediated endocrine disrupting properties. The substances in this group (4-*tert*-butylphenol, 4-*tert*-pentylphenol, 4-HPbl, 4-*tert*-octylphenol and 4-Nonylphenol, branched and linear) do have similar structures, similar physical-chemical properties or have expected differences as predicted by the difference in molecular weight and length of the alkyl chain (e.g. regarding water solubility).

The read across is based on the hypothesis that all these alkylphenols share the same structural moieties (phenol with alkyl chain (predominantly) in para position) responsible for the estrogen mode of action. Regarding the length of the alkyl chain, 4- heptylphenol, branched and linear, is between the source substances. Details on the read across approach can be found in Annex I.

Available *in vitro* and *in vivo* studies in fish show that, although substances differ in the length of the alkylchain they show similar endocrine disrupting properties and thus results from the other alkylphenols can be used to substantiate the effects observed for 4-HPbl in a weight of evidence approach.

Based on the data provided for 4-*tert*-octylphenol and 4-nonylphenol, branched and linear which are provided in the support documents for SVHC identification (ECHA, 2011 and ECHA, 2012) as well as data summarized for 4-*tert*-pentylphenol in an accompanying SVHC dossier it can be concluded that:

- Data for other alkylphenols strengthen the reliability of results for 4-HPbl: effects observed in *Sander lucioperca* for 4-HPbl and other alkylphenols including 4-n-nonylphenol are very similar. They are in line with results observed for other fish species with other alkylphenols, supporting the reliability of these effects.
- Data for other alkyphenols support the conclusion that effects observed for 4-HPbl in fish are estrogen mediated.
- For other alkylphenols a much broader variety of fish species was tested. For many of these species the data clearly show that the alkylphenols act as endocrine disruptors. Based on the read across approach, similar effects for 4-HPbl can be anticipated, indicating that 4-HPbl acts as an endocrine disruptor in a variety of fish species including seasonal breeders.
- Although only limited data are available for 4-HPbl results for other alkylphenols indicate that short term exposure of fish during sensitive life stages may result in adverse effects in the entire life cycle and also in following generations.

In summary, all the available information indicates that all these alkylphenols share the same mode of action and cause estrogenic mediated adverse effects at similar test concentrations. Thus, these data substantiate that 4-heptylphenol, branched and linear, is an endocrine disruptor comparable to 4-*tert*-octylphenol and 4-nonylphenol.

Based on information available for other alkylphenols it is very plausible, that 4- heptylphenol, branched and linear, acts as an endocrine disruptor in other fish species too and that effects observed in standard tests might underestimate its endocrine disrupting properties.

5.6.2.5. Summary of the plausible link between adverse effects and endocrine mode of action

Indicative effects for 4-HPbl supporting the mode of action:

- *In vitro* and QSAR data showing an interaction with the estrogen receptors
- Intersex: LOEC 1 µg/L *(Sander lucioperca)*
- Female biased sex ratio according to histological examinations: LOEC 1 µg/L *(Sander lucioperca)*

Adverse effects for 4-HPbl:

- Histological female biased sex ratio: LOEC 1 µg/L *(Sander lucioperca)*

From effects observed in the *in vitro* tests, the female-biased sex ratio in fish and data regarding the structure of the substance it can be concluded that 4-HPbl is able to interact with and activate estrogen receptors.

The female biased histological sex ratio needs also to be considered as a serious adverse effect with potential consequences on the population level.

There is a high probability that the shift in sex ratio is mediated via an estrogen mode of action. These findings are substantiated by a read across from structurally similar alkylphenols.

- 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
- 4-*tert*-octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol)
- 4-*tert*-pentylphenol (*p*-(1,1-dimethylpropyl)phenol)
- 4-*tert*-butylphenol (4-(1,1-dimethylethyl) phenol)

It is demonstrated that although the alkyl chain lengths vary, the four source substances and the target substance 4-HPbl have endocrine disrupting properties for the environment.

5.6.2.6 Environmental relevance

The effects observed for 4-HPbl are indicative as well as adverse. In pikeperch 4-HPbl was observed to induce female biased sex ratio. In addition, relevant read across source substances have been shown to cause female biased sex ratio as well as several other adverse effects (, effects on reproduction and growth) in other fish species. These effects may impair population stability and thus are considered to be relevant on the population level and have environmental relevance.

5.7 Summary and discussion of the environmental hazard assessment

Based on the available mechanistic information from QSAR data (moderate estrogen binding activity for all 117 possible monosubstituted heptylphenols according to OECD Toolbox) and *in vitro* studies on 4-heptylphenol isomers it can be concluded that 4-HPbl is able to bind to the estrogen receptors of fish, humans and rats and to activate these receptors.

In a reliable long term study using *Sander lucioperca* the ratio of male fish (according to

histological determination) was significantly decreased at the lowest 4-n-heptylphenol concentration (1 µg/L) after 28 days of exposure. The shift in sex ratio was dose-dependent, leading to 98 and 100% fish with female sex characteristics at 88 dph and 144 dph, respectively, indicating that the observed effects on the sex characteristics were irreversible. The appearance of intersex species comprising sex characteristics from both sexes e.g. testis-ova/ ovotestis, formation of an oviduct (with regressed spermatogenic lobules in the same fish) significantly appeared also at concentrations of at least 1 µg/L.

To substantiate the findings for 4-HPbl, a read across approach (see Annex I) was applied using the following source alkylphenols:

- 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
- 4-*tert*-octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol)
- 4-*tert*-pentylphenol (*p*-(1,1-dimethylpropyl)phenol)
- 4-*tert*-butylphenol (4-(1,1-dimethylethyl) phenol)

Regarding chain length, 4-HPbl is between 4-Nonylphenol, branched and linear and 4-*tert*-octylphenol on the one side and 4-*tert*-pentylphenol and 4-*tert*-butylphenol on the other side. The findings for 4-HPbl were substantiated by the effects seen also for the source substances.

- *In vitro* data confirm that all four source substances and the target substance do interact with estrogen receptors
- As for 4-n-heptylphenol it was demonstrated that exposure to 4-n-nonylphenol and 4- -butylphenol (branched and linear forms) lead to a female biased sex ratio in *Sander lucioperca* at a low concentration (effects seen at lowest dose of 1 µg/L).

Substantial effects were also seen in other fish species (*Pimephales promelas*, *Danio rerio*, *Oryzias latipes*, *Cyprinus carpio*, *Oncorhynchus mykiss*) for the source chemicals. These include female biased sex ratio and indicative effects such as feminisation of gonadal ducts, testis-ova and changes in secondary sex characteristics. Some of the effects occurred at the same concentration.In summary, it is demonstrated that endocrine disrupting properties for the environment occur for alkylphenyls with alkyl chain lengths of 4,5,7,8 and 9 C-atoms.

In an oral 28-day repeated dose study with rats effects on male reproductive organ weights were observed. These were most pronounced by a decrease in absolute and relative weights of the seminal vesicles by 51 and 41%, respectively. In the same study increase in relative thyroid/parathyroid and adrenal glands weights and decrease in absolute thymus weight in males were reported in the highest dose group (450 mg/kg bw/day). Other increases in absolute and relative organ weights were also observed at 450 mg/kg/day dose group including liver (both sexes by 22 to 60%, females also in the 150 mg/kg/day dose group) and kidney (females only, 102 to 122%). In males reduced body weights (-19%) and body weight gains were reported. In the highest dosage group both sexes suffered from systemical toxic side effects (clinical signs and deaths). Histopathological changes included vacuolation of the liver and renal lesions compatible with tubular nephropathy that corresponds well to changes in clinical chemistry parameters. In males depletion of secretion of the seminal vesicles and of lymphoid of the thymus occurred in the highest dose group.

The observed significant relative and absolute weight decrease in male mammals, together with the observed depletion of secretion of the seminal vesicles at the highest dose, might indicate an estrogen mode of action for phenol, heptyl derivs.. However, at this concentration systemic effects including one death and reduced mean body weights >10% were observed.

In a reproductive/developmental screening assay with phenol, heptyl derivs. effects on male and

female reproduction cannot be excluded due to the reduced live litter size at the two highest dose levels.

In summary, taking all the evidence into consideration it is concluded that 4-HPbl fulfils the WHO/IPCS definition for endocrine disrupters for the environment.

It is also noted that 4-HPbl fulfils the European Commission's draft scientific criteria for endocrine disruptors, which were recently published in the context of the Biocidal Products Regulation and the Plant Protection Products Regulation (European Commission, 2016[12]).

[12] European Commission (2016): Draft Commission Delegated Regulation (EU) …/... of XXX setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 (Text with EEA relevance); see http://ec.europa.eu/health/endocrine_disruptors/docs/2016_bpcriteria_en.pdf

6. Conclusions on the SVHC Properties

6.1 CMR assessment

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

6.2 PBT and vPvB assessment

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

6.3 Assessment under Article 57(f)

4-Heptylphenol, branched and linear is identified as a substance of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) due to its endocrine disrupting properties for which there is scientific evidence of probable serious effects to 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.

6.3.1 Summary of the data on the hazardous properties

A detailed description of the hazardous properties (endocrine disruption for the environment) of 4-HPbl is provided in chapter 5.6. and a summary is given in chapter 5.7.

6.3.2 Equivalent level of concern assessment

6.3.2.1 Human health

Not relevant at present, as this SVHC proposal focuses on the endocrine disruptive effects on the environment.

6.3.2.2 Environment

The summary provided in Chapter 5.7 shows that 4-HPbl leads to severe adverse effects such as skewed sex ratio: according to histological determination the ratio of male fish was significantly decreased already at the lowest studied concentration (1 µg/L) after 28 days of exposure. The shift in sex ratio was dose-dependent, leading to 98 and 100% female fish at 88 and 144 dph, respectively. This indicates that the observed effects on the sex ratio were irreversible.

These results underline the potential of 4-HPbl to impair population structure and ecosystem function and stability.

No data are available to assess whether short-term exposure at particular sensitive life stages of 4-HPbl results in delayed long-term, or even intergenerational effects. However, effects observed for 4-HPbl are similar to those for the read across source substances 4-nonylphenol and for 4-*tert*-octylphenol and occur at comparable concentrations. Therefore, as described in Annex I, due to similarities in structure, physico-chemical and endocrine disrupting properties, it is possible to read across lacking information for 4-tert-butylphenol from studies available for

4-tert-octylphenol and 4-nonylphenol.

For 4-nonylphenol and 4-*tert*-octylphenol several studies show that they may cause long lasting effects which persist even after the exposure has ceased (see SVHC dossiers on 4-nonylphenol and 4-tert-octylphenol, (ECHA, 2012) and (ECHA, 2011) for details):

- Studies in several fish species show that transient exposure during sensitive life stages may cause effects that not only remain irreversible during the entire life of the exposed individuals but also in the following generations. Thus effects persist after exposure has ceased and exposure of migrating fish might not only adversely affect population stability locally but also in other areas (both 4-nonylphenol and 4-*tert*-octylphenol).
- Exposure of male fish to 4-nonylphenol results in reduced reproduction even if females are not exposed (see ECHA, 2012, for details).
- Prolonged exposure may result in more pronounced effects, which are not covered in one generation tests (4-nonylphenol)

Due to the similar properties of 4-HPbl, 4-*tert*-octylphenol and 4-nonylphenol regarding endocrine disruption, it can be assumed, that such effects would also occur after exposure to 4-HPbl.

These observations are in line with the knowledge about the endocrine system. Endocrine modulation is a very complex feedback process that is set up during sensitive life stages. As summarized in IPCS (2002) disturbance of this set up may result in effects during the entire lifetime.

In addition, results for 4-nonylphenol and 4-*tert*-octylphenol indicate that it is difficult to quantify safe levels of exposure for substances with endocrine activity. The results indicate that other species might be affected too:

- Studies on non-traditional endpoints indicate that effects may start at much lower concentrations than those considered in the OECD test guidelines.
- Although it is not possible to unambiguously conclude that the adverse effects on other organisms such as invertebrates, amphibians and rodents are endocrine mediated, these effects are in accordance with the fact that steroids play an important role in both vertebrates and invertebrates (Kortenkamp et al., 2012, Kendall et al., 1998). Owing to the lack of in depth knowledge of their endocrine system and the lack of test systems, it is currently nearly impossible to estimate which species are most sensitive and which concentration should be regarded as safe for the environment.

In summary, effects due to 4-HPbl exposure are likely to impair population stability and recruitment. They may occur even after short term exposure and thus result in impairment in regions other than those where the exposure occurred. Effects persist even after the exposure has ceased and may have influence on population level on a long term basis e.g. due to transgenerational effects and/or changes in the gene pool. The effects may influence a wide range of taxa. It cannot be excluded that a safe level of exposure exists, but it is difficult to estimate this level.

Consequently, for the observations and reasons listed above, the serious effects in the environment, that 4-HPbl has the potential to cause, are considered to be of an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

6.3.3 Conclusion on the hazard properties and equivalent level of concern assessment

4-HPbl is proposed to be identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is a group of substances with endocrine disrupting properties for which there is scientific evidence of probable serious effects to 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.

Based on the available mechanistic information from *in silico* and *in vitro* studies with 4-heptylphenol isomers it can be unambiguously concluded that 4-HPbl is able to bind to the estrogen receptors of fish, humans and rats and to activate these receptors.

In a reliable *in vivo* study with *Sander lucioperca* (Demska-Zakęś, 2005) the ratio of male fish (according to histological determination) was significantly decreased at the lowest used 4-n-heptylphenol (4nHP) concentration (1 µg/L) after 28 days of exposure. The shift in sex ratio was dose-dependent, leading to 98 and 100% female fish at 88 and 144 days post hatch (dph), respectively, indicating that the observed effects on the sex characteristics were irreversible.

The appearance of intersex species comprising sex characteristics from both sexes, e.g. testis-ova / ovotestis and formation of an oviduct (with regressed spermatogenic lobules in the same fish), was significant at 4nHP concentrations of at least 1 µg/L.

4-HPbl belongs to a group of structurally similar alkylphenols monoalkylated predominantly in 4-position with different alkyl chain lengths. To substantiate the findings for 4-HPbl, a read across approach is applied using the following source alkylphenols:

- 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
- 4-*tert*-octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol, EC number: 205-426-2)
- 4-*tert*-pentylphenol (*p*-(1,1-dimethylpropyl)phenol, EC number: 201-280-9)
- 4-*tert*-butylphenol (4-(1,1-dimethylethyl) phenol, EC number: 202-679-0)

Regarding chain length, 4-HPbl is in the middle of 4-nonylphenol, branched and linear and 4-*tert*-octylphenol on the one side and 4-*tert*-pentylphenol and 4-*tert*-butylphenol on the other side. The findings for 4-HPbl were substantiated by the effects seen also in tests performed with the source substances.

- *In vitro* data confirm that all four source substances and the target substance do interact with the estrogen receptors.
- As for 4-HPbl it was demonstrated that exposure to 4-nonylphenol and 4- butylphenol (branched and linear forms) lead to a female biased sex ratio in *Sander lucioperca* at low concentration (effects seen at lowest dose of 1 µg/L).
- Substantial effects were also seen in other fish species (*Pimephales promelas*, *Danio rerio*, *Oryzias latipes*, *Cyprinus carpio*, *Oncorhynchus mykiss*) for the source chemicals. These include effect data like a female biased sex ratio and indicative effects like feminisation of gonadal ducts, testis-ova and effects on secondary sex characteristics.

4-Nonylphenol and 4-*tert*-octylphenol are already identified as substances of very high concern due to their endocrine disrupting properties for the environment, which are considered to give rise to an equivalent level of concern. The effects observed for 4-HPbl are similar to those for 4-*tert*-octylphenol and 4-nonylphenol and occur in similar concentration ranges (ECHA, 2011 and ECHA, 2012).

In summary, it is demonstrated that endocrine disrupting properties for the environment occur for alkylphenols with alkyl chain lengths of 4,5,7,8 and 9 C-atoms.

Taking all the evidence into consideration 4-HPbl is proposed to be identified as an endocrine

disruptor for the environment according to the OECD guidance document (OECD, 2012) and the WHO/IPCS definition for endocrine disrupters.

4-HPbl is assessed as substances giving rise to an equivalent level of concern due to their estrogenic mode of action and the type of effects caused by this mode of action (e.g. shift in sex ratio).

- At 1 µg/L the ratio of male fish was significantly decreased and intersex fish appeared. At 10 µg/L the ratio of female fish was significantly increased to approximately 75% while at 200 µg/L approximately 100% fish were female. These effects remained manifest even after the exposure had ceased underlining that exposure during sensitive life stages may change the endocrine feedback system for the entire life.

- A read-across from 4-*tert*-octylphenol and 4-nonylphenol indicates that although a safe level of exposure for 4-HPbl may exist, it is difficult to establish it.

    - Effects on non-traditional endpoints may start at much lower concentrations than those considered in the OECD test guidelines.

    - Although it is not possible to unambiguously conclude that the adverse effects on other organisms such as invertebrates and amphibians are endocrine mediated, these effects are in accordance with the fact that steroids play an important role in both invertebrates (Kendall et al., 1998) and amphibians (Kortenkamp et al., 2012). Owing to the lack of in depth knowledge of their endocrine system and the lack of test systems, it is currently nearly impossible to estimate which species are the most sensitive and which concentration should be regarded as safe for the environment.

- Read across of the effects observed for the similar alkylphenols 4-nonylphenol and 4-tert-octylphenol shows that a transient exposure during sensitive life stages may result in effects that remain during the entire life and even in the following generations. Thus local exposure of migratory species might not only locally affect population stability but also in other areas.

In summary, the effects observed due to 4-HPbl exposure, and confirmed by data on other alkylphenols, are considered to impair population stability and recruitment. They may occur even after short term exposure and thus may have impact in regions other than those where the exposure occurred. The effects persist even after the exposure has ceased and may have long-lasting influence on population level e.g. due to transgenerational effects or changes in the gene pool. Effects may influence a wide range of taxa. A safe level of exposure is difficult to derive although it may exist.

Consequently, 4-HPbl is considered to give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

Part II

7. Registration and C&L notification status

The UVCB substance phenol, heptyl derivs. is the only representative of the group entry 4-HPbl that has been registered under REACH.

7.1 Registration status

Table 17 Registration status for phenol, heptyl derivs.

From the ECHA dissemination site[13]	
Registrations	☒ Full registration(s) (Art. 10) ☐ Intermediate registration(s) (Art. 17 and/or 18)

7.2 CLP notification status

CLP notifications are available for phenol, heptyl derivs. and 4-heptylphenol.

Table 18: CLP notifications for phenol, heptyl derivs. (EC No. 276-743-1)

	CLP Notifications[14]
Number of aggregated notifications	3
Total number of notifiers	362

Table 19: CLP notifications for 4-heptylphenol (EC No. 217-862-0)

	CLP Notifications[15]
Number of aggregated notifications	3
Total number of notifiers	358

8. Total tonnage of the substance

The total tonnage band is given for the registered UVCB substance phenol, heptyl derivs.

Table 20: Tonnage status

[13] *http://echa.europa.eu/registration-dossier/-/registered-dossier/14111* (accessed 02 July 2016)
[14] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 02 July 2016)
[15] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 02 July 2016)

Total tonnage band for the registered UVCB substance phenol, heptyl derivs. (excluding the volume registered under Art 17 or Art 18)[16]	100-1.000 t/pa

9. Information on uses of the substance

The UVCB substance phenol, heptyl derivs. is registered as a monomer. Uses of the resulting polymers are not considered in the registration dossier as there is no formal obligation to cover downstream uses of polymers in the monomer registration dossier.

Polymers derived from phenol, heptyl derivs are usually used in lubricant additives. According to the registrant(s) information, phenol, heptyl derivs. is imported in the EU only in polymerised form and as formulated in commercial mixtures. On the basis of current knowledge it can be expected that these mixtures have industrial, professional and consumer uses in lubricants and greases in vehicles or machinery, which will result in wide dispersive indoor and outdoor use in closed and open systems. The residual content of unreacted monomer (phenol, heptyl derivs.) in the polymer material which is imported, is assumed to be well below 0,1%.

4-heptylphenol has not been registered in the EU yet. Indications from safety data sheets point to uses in scientific research and development.

Table 21: Uses of registered UVCB substance phenol, heptyl derivs.

	Use(s)	Registered use	Use in the scope of Authorisation
Uses as intermediate	Imported in the EU in polymerised form	No	No

10. Information on structure of the supply chain

No information is available in addition to that in Chapter 9.

11. Additional information

11.1 Substances with similar hazard and use profiles on the Candidate List

As listed in Chapter 1.4 the similar substances 4-nonylphenol, branched and linear and 4-*tert*-octylphenol are already included in the candidate list due to their endocrine disrupting properties in the environment (Art. 57(f)).

In parallel to this dossier, SVHC dossiers have been prepared for the similar substances 4-*tert*-butylphenol and 4-*tert*-pentylphenol.
Currently no factual information exists that phenol, heptyl derivs. might be used as substitute for other alkylphenols already identified (or in the process of identification) as SVHCs. However,

[16] http://echa.europa.eu/registration-dossier/-/registered-dossier/14111 *(accessed at 2 July 2016)*

based on its similar structure and physical chemical properties, it is likely that phenol, heptyl derivs. has the potential of being used as substitute.

11.2 Alternatives

The additives derived from phenol, heptyl derivs. are used as detergents and metal deactivators in a wide variety of lubricating applications including industrial and automotive gear oils, automatic transmission formulations, and small engine applications (HPV programm, 2006a).

Alternative chemicals for the manufacture of detergents, metal deactivators or corrosion inhibitors are available and in use. Their suitability to substitute phenol, heptyl derivs. depends on the properties needed for specific applications.

11.3 Existing EU legislation

No existing other relevant EU legislation has been identified.

11.4 Previous assessments by other authorities

Phenol, heptyl derivs. is part of the US EPA HPV Challenge program. Evaluation has started in 2003. Since then several data gaps have been closed by additional tests by industry and finalised health and environmental effect data have been made available in 2006.[17]

Additionally in a second approach alkyl phenols (as a whole group, including phenol, heptyl derivs.) have been evaluated by another industry "sponsor" in the HPV Challenge program. No testing for phenol, heptyl derivs. was proposed as data gaps had been closed by calculated data and read-across. Starting in 2001 hazard data have been collected and evaluated in a second step by the Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) in 2009.[18]

[17] https://java.epa.gov/oppt_chemical_search/ (search term 72624-02-3)
[18] https://java.epa.gov/oppt_chemical_search/ (search term alkylphenols)

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Annex I - Additional information on read across approach

Substantiation of the available data with a read across approach to structurally similar alkylphenols

Hypothesis for the analogue approach

To substantiate the findings for 4-HPbl, a read across approach is applied using the following source alkylphenols:

- 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
- 4-*tert*-octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol)
- 4-*tert*-pentylphenol (p-(1,1-dimethylpropyl)phenol)
- 4-*tert*-butylphenol (4-(1,1-dimethylethyl) phenol)

This group of substances including 4-HPbl are all alkylphenols with a carbon chain length from 4 to 9 and can be considered to be of a similar structure – they have an aromatic ring and a sterically unhindered hydroxyl-group (-OH), which is considered relevant for interaction with the estrogen receptors (see chapter 5.6.2.1.1) regarding OECD Toolbox 3.1.0.21). They differ in chain length and branching of the alkyl chain only.

4-Nonylphenol, branched and linear as well as 4-*tert*-octylphenol are already on the candidate list due to their endocrine disrupting properties for the environment. 4-*tert*-pentylphenol (p-(1,1-dimethylpropyl)phenol) and 4-*tert*-butylphenol (4-(1,1-dimethylethyl) phenol) have been in substance evaluation in 2014. Analysis of the available data revealed endocrine disrupting properties for the environment also for these substances.

It can be anticipated that physical chemical properties of the substances in this group such as log Kow, water solubility and bioaccumulation follow a linear trend due to increasing lipophilicity with increasing alkyl chain length.

With regard to endocrine disruption it is anticipated that all substances of the group activate the estrogen receptor as they all share structural moieties responsible for binding (i.e. a sterically unhindered hydroxyl group attached to an aromatic ring; OECD, 2009). Binding of the hydroxyl group to the A site of the receptor pocket can be increased through hydrophobic forces in the center of the ER subpocket (OECD, 2009). Thus it could be anticipated that estrogen binding affinity increases with increasing chain length. However, *in vitro* data are not consistent (see below).

For 4-HPbl one fish study is available, which is clearly demonstrating estrogenic mediated adverse effects (female biased sex ratio). Via this read across approach it can be shown that data from 4-nonylphenol, branched and linear, 4-*tert*-octylphenol, 4-*tert*-pentylphenol and 4-*tert*-butylphenol can be used to substantiate the evidence for the endocrine disrupting effects of 4-HPbl for the environment.

Information on substance identity, physical chemical properties, toxiokinetics/bioconcentration in fish and environmental toxicity (including *in vitro* and *in vivo* data) of 4-*tert*-butylphenol, 4-*tert*-pentylphenol, 4-HPbl, 4-*tert*-octyphenol and 4-nonylphenol, branched and linear are summarized in Table 22 below. Of the large amount of information available for 4-*tert*-octylphenol and 4-nonylphenol, branched and linear only a selection of available fish data is

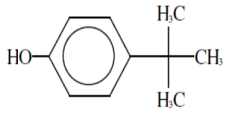
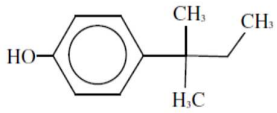
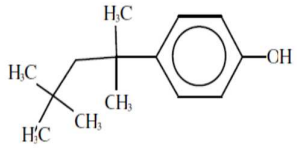
provided due to abundancy.

Data used for 4-HPbl are data described in the previous sections including those on single isomers of 4-heptylphenol (4-n-heptylphenol and 4-*tert*-heptylphenol), a mixture comprising one linear and one branched isomer (for bioconcentration) and phenol, heptyl derivs..

For 4-nonylphenol and 4-*tert*-octylphenol data are taken from the their SVHC dossiers (see ECHA, 2011 and ECHA, 2012) with the exception of 4-nonylphenol toxicokinetics data (also other sources used) and additional 4-nonylphenol data on *Sander lucioperca* from Demska-Zakęś (2005).

Only data from studies rated Klimisch 1 or 2 are included in the section for *in vivo* data for endocrine disruption in fish.

Table 22: Summary data on identification, physical and chemical properties, environmental fate/behaviour and environmental toxicity data of 4-*tert*-butylphenol, 4-*tert*-pentylphenol, 4-HPbl, 4-*tert*-octyphenol and 4-nonylphenol, branched and linear

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
IDENTITY					
Chemical name	4-*tert*-butylphenol IUPAC4-(1,1-dimethylethyl) phenol	p-(1,1-dimethylpropyl)phenol	4-heptylphenol, branched and linear	4-(1,1,3,3-tetramethylbutyl)phenol, 4-*tert*-octylphenol	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
CAS no.	98-54-4	80-46-6	-	140-66-9	-
EC no.	202-679-0	201-280-9	-	205-426-2	-
Chemical structure			group		group
SMILES	CC(C)(C)c1ccc(O)cc1	CCC(C)(C)c1ccc(O)cc1	group	Oc(ccc(c1)C(CC(C)(C)C)(C)C)c1	Covers UVCB as well as well-defined substances (see chemical name)
Molecular formula	$C_{10}H_{14}O$	$C_{11}H_{16}O$	$C_{13}H_{20}O$ (mono-subst.) $C_{20}H_{34}O$ (di-subst.)	$C_{14}H_{22}O$	$C_{15}H_{24}O$

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
Molecular weight (g/mol)	150.2176	164.244	192.3 (mono-subst.) 290.5 (di-subst.)	206.32	220.35
PHYSICAL CHEMICAL PROPERTIES					
Physical state at 20°C and 101.3 kPa	Solid (flakes)	Solid (flakes)	liquid at 20°C and 101.3 kPa	Solid	pale yellow viscous liquid
Water solubility (mg/L, 20 °C)	610 mg/L at 25 °C, pH = 6 – 7	190 mg/L at 21 °C, pH 6 - 7	42.1 mg/L at 20 °C	19 mg/L at 22 °C	Ca. 5.7 mg/L at 25°C
Partition coefficient n-octanol/water (log Kow)	3.0 at 23 °C, pH = 5.7	3.6 at 22 °C, pH 6 – 7	4.78 – 5.01 EPISUITE v4.11 for 117 heptylphenol isomers	4.12 at 20.5°C (OECD 107, shake flask method) 3.7, temperature not indicated	5.4 at 23°C, pH 5.7
Dissociation constant (pKa)	10.13 - 10.23 at 25 °C	10.4 (Crane et al., 2008)	---	pKa 10.33 at 25 °C (calculated)	pK ca. 10
***IN VITRO* DATA FOR ESTROGEN RECEPTOR MEDIATED PATHWAY**					
Binding to Estrogen Receptors					
Rainbow trout	Hornung et al. (2014): RBA = 1.4 $x10^{-5}$	Hornung et al. (2014): RBA = 4 $x10^{-5}$ RBA for 4-n-Pentylphenol = 5.3 $x10^{-5}$	Hornung et al. (2014): RBA for 4-n-Heptylphenol 2.1 $x10^{-4}$ RBA for 4-*tert*-Heptylphenol: 1.4 $x10^{-4}$	Hornung et al. (2014): RBA =9.4 $x10^{-5}$	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}

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
	Tollefsen and Nilsen (2008): RBA = 4 x 10^{-5}	Tollefsen and Nilsen (2008): RBA = 7 x 10^{-5}	Tollefsen and Nilsen (2008): RBA = 3.2 x 10^{-5}	Tollefsen and Nilsen (2008): RBA = 6.9 x 10^{-5}	Tollefsen and Nilsen (2008): RBA = 1 x 10^{-5}
	Olsen et al. (2005): RBA =7.7 x 10^{-5}			Olsen et al. 2005: RBA = 7.6 x 10^{-5}	
			Knudsen and Pottinger (1999): Concentrations of alkylphenols including heptylphenol 10^4-fold > those of the maxium displacement achieved was E_2 required to produce similar amounts of displacement of specifically bound - [^{3}H]E_2 Maximum displacement achieved: ca. 60%	Knudsen and Pottinger (1999): Concentrations of alkylphenols including octylphenol 10^4-fold > those of the maxium displacement achieved was E_2 required to produce similar amounts of displacement of specifically bound - [^{3}H]E_2 Maximum displacement achieved: ca. 45%	Knudsen and Pottinger (1999): Concentrations of alkylphenols including nonylphenol 10^4-fold > those of the maxium displacement achieved was E_2 required to produce similar amounts of displacement of specifically bound - [^{3}H]E_2 Maximum displacement achieved: ca. 50%
Human			Satoh and Nagai (2002): ERα-RBA =0.00163; ERß no binding	Satoh and Nagai (2002): ERα-RBA =0.008; ERß-RBA =0.00708;	Satoh and Nagai (2002): ERα-RBA = 0.0222; ERß-RBA = 0.0213
	Akahori et al. (2005): RBA = 2.3 x 10^{-5}	Akahori et al. (2005): RBA = 1.7 x 10^{-4}	Akahori et al. (2005): RBA = 8.5 x 10^{-6}	Akahori et al. (2005): RBA = 0.00123	
	Olsen et al. (2005): RBA 2.1 x 10-^{6}			Olsen et al. (2005): RBA 6.4 x 10-^{5}	

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
Rat	Blairs et al. (2000): RBA = 2.4x 10^{-6}	Blairs et al. (2000): RBA = 5 x 10^{-6}	Laws et al. (2006): RBA = 1.24 x 10^{-5}	Blairs et al. (2000): RBA 1.4 x 10^{-4}	Blairs et al. (2000): RBA = 3.7 – 1.9 x 10^{-4} 4-n-Nonylphenol RBA = 3.2 x 10^{-5}
Binding to sex steroid-binding protein					
Rainbow trout	Tollefsen (2007): RBA = 6.1 x 10^{-6}	Tollefsen (2007): RBA = 4.3 x 10^{-5}	Tollefsen (2007): RBA = 6.6 x 10^{-6}	Tollefsen (2007): RBA = 1.3 x 10^{-5}	Tollefsen (2007): 4-n-Nonylphenol was here only a weak binder
Expression of vitellogenin					
Rainbow trout	Tollefsen et al. (2008): LOEC = 3 µM	Tollefsen et al. (2008): LOEC 3 µM	Tollefsen et al. (2008): no effect under condition employed	Tollefsen et al. (2008): LOEC = 1 µM	Tollefsen et al. (2008): LOEC = 30 µM
	Jobling and Sumpter (1993): REP 1.6 x 10^{-4}			Jobling and Sumpter (1993): REP 3.7 x 10^{-5}	
	Olsen et al. (2005): REP 5.6 x 10^{-6}			Olsen et al. (2005): REP 3.2 x 10^{-5}	
Expression profiling of estrogen-responsive genes					
Human			Terasaka et al. (2006): R-value (statistical correlation) for EstrArray: 0.82	Terasaka et al. (2006): R-value (statistical correlation) for EstrArray: 0.75	Terasaka et al. (2006): R-value (statistical correlation) for EstrArray: 0.9
Transcriptional activation assay using recombinant yeast (yeast estrogen screen, YES)					
Human	Routledge and Sumpter (1997): REP = 1.5 x 10^{-6}	Routledge and Sumpter (1997): REP = 1 x 10^{-5}	Routledge and Sumpter (1997):	Routledge and Sumpter (1997): REP = 1 x 10^{-3}	Routledge and Sumpter (1997): REP = 3 x 10^{-4}

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
			4-*tert*-heptylphenols: REP = 3×10^{-3} 4-n-heptylphenol: REP = 7.5×10^{-4} = 25-fold less potent than 4-*tert*-heptylphenol		
		Schultz et al. (2000): EC_{50} = 4.67 µM			Schultz et al. (2000): EC_{50} = 0.177µM
	Nishihara et al. (2000): REC10 3×10^{-5}		Nishihara et al. (2000): negative	Nishihara et al. (2000): REC10 2×10^{-7}(= positive)	Nishihara et al. (2000): negative for 4-n-Nonylphenol
MCF cell proliferation assays (E-Screen)					
Human	Soto et al. (1995): RPE = 0.71 RPP = 3×10^{-6}	Soto et al (1995): RPE = 1.05			Soto et al. (1995): RPE = 1 RPP = 3×10^{-6}
	Körner et al. (1998): RPE= 0.78			Körner et al. (1998): RPE=0.97	Körner et al. (1998): RPE= 1.05
TOXICOKINETICS AND BIOACCUMULATION IN FISH					
Toxico-kinetics in fish Absorption	Rapid uptake via seawater (co-exposure) in Atlantic cod, steady state reached within 24 h (or 48 h exposure via spiked feed) (Sundt et al., 2009).	Rapid uptake of 4-n-pentylphenol via seawater (co-exposure) in Atlantic cod, steady state reached within 24 h (or 48 h exposure via spiked feed) (Sundt et al., 2009).	Rapid uptake via seawater or spiked feed (co-exposure) in Atlantic cod, steady state reached within 48 h. Higher body burden compared to 4-*tert*-	Steady state conditions in the whole fish (*Oncorhynchus mykiss*) were reached after 4 days in a flow-through system (ECHA, 2011).	Steady state reached within 12 h in rainbow trout (Lewis and Lech, 1996)

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
			butylphenol and 4-n-pentylphenol (related to higher log Kow value) (Sundt et al., 2009).		
Distribution	Highest residues after 8 day co-exposure in bile and to a lesser extent in the intestine, intestine/stomach content (12-14% residues of spiked administered feed were recovered in tissue) (Sundt et al., 2009)	Highest residues after 8 day co-exposure in bile and to a lesser extent in the intestine, intestine/stomach content. (8% residues of spiked administered feed were recovered in tissue) (Sundt et al., 2009).	Highest residues after 8 day co-exposure in bile and to a lesser extent in the intestine, intestine/stomach content (12-14% residues of spiked administered feed were recovered in tissue) (Sundt et al., 2009).	In rainbow trout highest residues after 10 days waterborne exposure in bile, followed by faeces, pyloric caeca, liver and intestine, in rudd highest concentrations were in bile and liver (cited in Cravedi and Zalko, 2005). 8% 4-*tert*-octylphenol residues in liver and muscle tissue after 10 day exposure in flounder (Madsen et al., 2003).	[^{14}C]-nonylphenol residues were highest in bile after 14 h waterborne exposure in rainbow trout. [^{3}H]-4-n-nonylphenol residues in Atlantic salmon showed wide tissue distribution with high levels in bile, viscera, liver, fat and kidney (cited in Cravedi and Zalko, 2005).
Metabolism	Predominant metabolic pathway: conjugation to glucuronic acid. (Jonsson et al., 2008)	Predominant metabolic pathway: conjugation to glucuronic acid. (Jonsson et al., 2008)	Predominant metabolic pathway: conjugation to glucuronic acid. (Jonsson et al., 2008)	Predominant metabolic pathway: conjugation to glucuronic acid (Cravedi and Zalko, 2005).	Predominant metabolic pathway: conjugation to glucuronic acid (Cravedi and Zalko, 2005).
Elimination	Half-life 10 hours, rapid excretion via bile and faeces (Sundt et al., 2009).	Half-life 10 – 20 hours, rapid excretion via bile and faeces (Sundt et al., 2009).	Half-life 13 hours (Atlantic cod) (Tollefsen et al., 1998)	Excretion via bile and faeces. Half-live 7.7 h in medaka (Cravedi and Zalko, 2005).	Half-lives of 0.8 days in rainbow trout, 1.2 to 1.4 days in fathead minnow and 4 days in Atlantic salmon. (Environment and

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
			Half-life 10 – 20 hours (Atlantic cod), rapid excretion via bile and faeces (Sundt et al., 2009)		Health Canada 2001)Excretion via bile and faeces. Half-life 9.9 h in medaka, but higher half-lives after i.v. in Atlantic salmon (clearance half-live 4 days) (Cravedi and Zalko, 2005).
Bio-concentration factor (BCF)	125 (calculated based on TGD method) *C. carpio*: 48 -88 *Chlorella. fusca* 34 measured *Lecicus idus*: 120	No experimental data, fish BCF of 501 L/kg may be estimated from the log Kow (4.0) using the QSAR recommended in the TGD11	The bioaccumulation cannot be fully excluded, as the study is not state-of the art, but based on the BCF values < 2000 and the elimination half-live of 0.052 / hour (Tollefsen et al., 1998) the bioaccumulation potential is moderate to low.	The bioaccumulation potential in aquatic organisms is low to moderate. The experimentally determined BCF ranges between 12 and 471	No data in SVHC dossier
ACUTE AQUATIC TOXICITY [mg/L]					
Acute toxicity to fish:	96h-LC_{50}: 5.14 (m)	96h-LC_{50}: 1 (n)	Phenol, heptyl derivs. 96h-LC_{50}: 2.4 (n.) 96h-LC_{50}: 0.41 (m.) 96h-LC_0: 1.8 (n.) 96h-LC_0: 0.066 (m.) *O.mykiss*	LC_{50}: 0.17	LC_{50}: 0.135 mg/L

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
			4-n-heptylphenol 96h-LC_{50}: 0.56 (n.) *Gadus morhua*		
Acute toxicity to invertebrates	96h-LC_{50}: 1.9 (m) (*Crangon septemspinosa*) 48h-EC_{50}: 3.9 (n) *D. magna*	EC_{50}: 1.7 96h-EC_{50}: 1.7 (m) *C.septemspinosa* 48h-EC_{50}: 2.7 (n) *D. magna*	Phenol, heptyl derivs. 48h-EC_{50}: 0.38 (m)	EC_{50}: 0.013	EC_{50}: 0.085
Acute toxicity to algae	72h-ErC_{50}: 14 (n)	72h-EC_{50}: 4.2 (n)	Phenol, heptyl derivs. 72h-ErC_{50}: 1.2 (n)	EC_{50}: 0.300	ErC_{50}: 0.027
ENDOCRINE EFFECTS IN FISH (NOECs/LOECs in mg/L if not stated otherwise)					
Sander lucioperca					
Effects on sex ratio (histological)					
Decrease of male fish	28d-NOEC: <0.001(nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)		28d-NOEC: <0.001(nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)		28d-NOEC: <0.001(nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)
Increase of female fish	28d-NOEC: <0.001(nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)		28d-NOEC: 0.001 (nom.) 28d-LOEC: 0.010 (nom.) Demska-Zakęś (2005)		28d-NOEC: 0.001 (nom.) 28d-LOEC: 0.010 (nom.) Demska-Zakęś (2005)

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
Intersex (histological)	28d-NOEC: <0.001(nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)		28d-NOEC: <0.001 (nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)		28d-NOEC: <0.001 (nom.) 28d-LOEC: 0.001 (nom.) Demska-Zakęś (2005)
Chronic toxicity to fish Mortality/length/weight/condition factor	28d-NOEC: >0.2 (nom.) 28d-LOEC: >0.2 (nom.) *S. lucioperca* (Demska-Zakęś, 2005)		28d-NOEC: >0.2 (nom.) 28d-LOEC: >0.2 (nom.) *S. lucioperca* (Demska-Zakęś, 2005)		28d-NOEC: >0.2 (nom.) 28d-LOEC: >0.2 (nom.) *S. lucioperca* (Demska-Zakęś, 2005)
Pimephales promelas					
FSDT or comparable tests	0.225 VTG (increase females) 0.225 feminisation gonadal ducts, higher proportion immature testis stages 0.5 sex ratio (increase females)[19] 0.027 SSC 0.027 growth (m + f)´ 0.255 time to hatch, survival post hatch (Krueger et al., 2008)	0.18 VTG (increase females) (Panter et al, 2006) 0.093 VTG (decrease females) (OECD, 2011a) 0.056 feminisation gonadal duct (Panter et al., 2006) 0.180 testis ova (Panter et al., 2006) 0.093 - 0.195 sex ratio (increase females/decrease males) (OECD, 2011a, Panter et al., 2006) 0.599 SSC (no			

[19] From pilot study

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
		statistics) (Panter et al., 2006) 0.599 growth, time to hatch (Panter et al., 2006) > 0.320 mortality (OECD, 2011a)			
Reproduction assay or comparable		0.270 - 0.560 VTG (increase males) (OECD, 2006, Panter et al., 2010 0.820 - 0.962 higher proportion immature testis stages (OECD, 2006) 0.270 – 0.997 SCC (OECD, 2006) 0.056 Fertility (Panter et al., 2010) (no spawning at 1 mg/L) (OECD, 2006)> 0.560 survival, hatchability (Panter et al., 2010)			0.071 fecundity 0.00025 behaviour 0.015 VTG 0.071 secondary sexual characteristics
Danio rerio					
FSDT or comparable tests		> 0.096 – 0.100 VTG increase males (OECD, 2011a) 0.062 – 0.100 sex ratio (increase females/decrease males) (OECD, 2011a)			0.01 skewed sex ratio 0.1 Gametogenesis females 0.01 Gametogenesis males 0.03 testis-ova

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
					0.1 Ovarian follicle atresia 0.1 VTG
Reproduction Assay		0.022 VTG (increase males) 0.229 higher proportion immature testis stages 0.787 testis-ova 0.721 - > 787 Fertility All (OECD, 2011a)			
FLC				0.035 fertility, time to first spawn, body length	
Oryzias latipes					
FSDT		0.094 – 0.104 VTG (OECD, 2012a) 0.094 testis-ova (OECD, 2012a) 0.010 - 0.318 sex ratio (less males) Hagino et al., 2001, OECD, 2012a) 0.100 SCC (Hagino et al., 2001) > 0.317 hatch, survival		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 $\leq$ 0.02 fertility	0.005 (VTG) 0.184 Inhibition of spermatogenesis 0.0061 fecundity and fertility

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
FLC		0.051 VTG 0.224 testis ova, 0.224 sex ratio 0.224 Fertility 0.224 SSC 0.224 length F1 0.931 growth, mortality (Seki et al., 2003)		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
Cyprinus carpio					
Reproduction Assay	0.690 VTG (up males) 0.690 GSI, HIS, liver degeneration (Barse et al, 2006)	1.00 VTG > 1.00 weight (Gimeno_et al.,_1998b)			
other		0.036 feminisation gonadal ducts (Gimeno et al., 1998a) 0.090 – 1.00 testis-ova (Gimeno et al., 1998a, Gimeno et al., 1996) 0.140 - > 256 growth (Gimeno et al., 1997, Gimeno et al., 1998a			
Oncorhynchus mykiss					
FSDT					0.00105 VTG 0.01 Growth
Reproduction Assay & other				0.039 VTG ≤ 0.039 increased percentage of early sperm stages (spermatogonia),	0.01 VTG 0.001 VTG (F1 without exposure) 0.037 Inhibition of spermatogenesis

Parameters	4-*tert*-Butylphenol	4-*tert*-Pentylphenol	4-HPbl	4-*tert*-Octylphenol	4-Nonylphenol, branched and linear
				reduced GSI in initial experiment	0.086 non developed ovaries 0.01 sexual steroids in F1

Analogue approach justification

The data collected in Table 22 justify the analogue approach.

Physico-chemical data:

The substances in this group (4-*tert*-butylphenol, 4-*tert*-pentylphenol, 4-HPbl, 4-*tert*-octylphenol and 4-nonylphenol, branched and linear) do have similar physical chemical properties or have expected trends as predicted by their molecular weights and carbon chain lengths (e.g. regarding water solubility).

The partition coefficient log Kow (3.0 for 4-*tert*-butylphenol and 5.4 for 4-nonylphenol) increases with increasing molecular weight. Water solubility decreases with increasing molecular weight from 607.2 mg/L for 4-*tert*-butylphenol to ca. 5.7 mg/L for 4-nonylphenol.

Mechanistic *in vitro* data:

Numerous *in vitro* data unambiguously show that all substances in this group interact with estrogen receptors and act as estrogen agonists. Fish data (receptor binding, binding to sex steroid binding protein, VTG expression) does not follow a linear trend with increasing chain length. Data on human receptors are ambiguous with only some of the effects following a linear trend.

Binding to rainbow trout estrogen receptors was shown in several studies for all 5 alkylphenols with similar affinities (1.4×10^{-4} - 7.7×10^{-5}) : 1.4×10^{-5} to 7.7×10^{-5} for 4-*tert*-butylphenol, 4×10^{-5} – 7×10^{-5} for 4-*tert*-pentylphenol, 1.4×10^{-4} to 3.2×10^{-5} for 4-n-heptylphenol, 6.9×10^{-5} to 9.4×10^{-5} for 4-*tert*-octylphenol and 4.6×10^{-4} to 1 for 4-nonylphenol. No linear trend with increasing chain length was observed. This becomes even more obvious if data for different alkylphenols obtained in the same study are compared: Values for all five substances are available in Hornung et al. (2014) and Tollefsen and Nilsen (2008): In the Tollefsen and Nilsen (2008) study the values are very similar ranging from 1×10^{-5} to 7×10^{-5}. In Hornung et al. (2014) the values vary from 1.4×10^{-5} to 4.6×10^{-4}. In both studies no correlation with the length of the alkyl chain was observed.

Binding to human and rat estrogen receptors was seen for all alkylphenols. Some studies indicate a linear trend for binding affinities, while other do not: Satoh and Nagai (2002) report rather high values for 4-nonylphenol (0.0213-0.222), 4-*tert*-octylphenol (0.00708 to 0.008) and 4-n-heptylphenol (0.00163). Generally lower values were reported by Olsen et al. (2005): 6.4×10^{-6} and 2.1×10^{-6} for 4-ter-octylphenol and 4-*tert*-butylphenol. Akahori et al. (2005) reported a high value for 4-*tert*-octylphenol (0.00123), a "medium" value for 4-*tert*-pentylphenol (1.7×10^{-4}) and rather low values for 4-n-heptylphenol and 4-*tert*-butylphenol (8.5×10^{-6} and 2.3×10^{-5}).

With regard to rat estrogen receptors the study by Blairs et al. (2000) tested all alkylphenols of this group. Results indicate that all of them bind to the receptor but affinity increases with increasing chain length by two orders of magnitude.

A study on the binding affinity to sex steroid-binding protein of rainbow trout reports similar values for all alkylphenols (2.4×10^{-6} - 4.3×10^{-5} (no linear trend)). 4-n-nonylphenol was only a weak binder in this assay.

In test systems examining the expression of vitellogenin (rainbow trout) all alkylphenols except 4-n-heptylphenol provided positive results. No linear trend was observed for the binding affinities, which were all within a very narrow range (e.g. LOEC 1 – 30 µM observed by Tollefsen et al., 2008)

Regarding expression profiling of estrogen-responsive genes (human) data are available for the longer chain alkylphenols (heptyl – nonyl): all three tested substances showed high correlation coefficients to the profiles of E_2: The R-value for 4-n-heptylphenol is 0.82, which is in the range as 4-*tert*-octylphenol (R-value = 0.75) and 4-nonylphenol (R-value =0.90). In some of the transcriptional activation assays positive results were obtained for all alkylphenols. Only some of the results followed a linear trend with the chain length.

Two E-Screen assays (MCF cell proliferation assays) are available comparing 4 of the 5 alkylphenols in this group. While relative proliferative effects (RPE) were similar for 4-*tert*-pentylphenol, 4-*tert*-octylphenol and 4-nonylphenol (0.97 – 1.05 with no specific trend), RPE values for 4-*tert*-butylphenol were slightly lower (0.71- 0.78); (Soto et al., 1995; and Körner et al., 1998).

Toxicokinetic data in fish

Uptake and tissue distribution of 4-*tert*-butylphenol, 4-n-pentylphenol, and 4-n-heptylphenol in Atlantic cod (*Gadus morhua*) followed similar patterns: uptake was rapid via seawater. For exposure via feed time to reach steady state was similar for 4-*tert*-butylphenol, 4-n-pentylphenol and 4-n-heptylphenol. Slightly higher body burdens were found for 4-n-heptylphenol compared to 4-*tert*-butylphenol and 4-n-pentylphenol. This correlates well with the increasing log Kow value with increasing chain length. Distribution in Atlantic cod of 4-*tert*-butylphenol, 4-n-pentylphenol, and 4-n-heptylphenol residues was also similar upon seawater or feed exposure. Highest alkyphenol residue concentrations after 8 day co-exposure were detected in bile and to a lesser extent in the intestine, intestine content and stomach content (Sundt et al., 2009). Also for 4-*tert*-octylphenol and nonylphenols the highest residues were detected in bile. The predominant metabolic pathway for alkylphenols is conjugation of the phenol group to glucuronic acid. Alkyphenols were mainly excreted via bile and faeces with similar half-lives that range from 10 to 20 hours (for water or feed exposure).

Acute aquatic toxicity

Acute fish toxicity data show also that all five alkylphenols have very similar values with the lowest acute toxicity values ranging from 0.135 to 1 mg/L.

For acute algae and acute aquatic invertebrate data, there seem to be tendencies of higher toxicity with a longer chain length. For aquatic invertebrates the acute toxicity values range from 0.013 to 1.9 mg/L (invertebrate data like sea urchin which are available for 4-nonylphenol are not included here), for algae the acute toxicity the values range from 0.027 (4-Nonylphenol) to 4 mg/L.

Endocrine disrupting properties in fish

All the alkylphenols in this group exert similar endocrine disrupting effects: A number of indicative as well as adverse effects were seen in several fish species (*Sander lucioperca, Pimephales promelas, Danio rerio, Oryzias latipes, Cyprinus carpio* and *Oncorhynchus mykiss*). In several studies female biased sex ratio was observed for all the five alkylphenols, which is an adverse effect indicative for an endocrine mode of action. Moreover, several indicative effects such as feminisation of gonadal ducts, testis-ova and effects on secondary sex characteristics were demonstrated. Effect concentrations for all alkylphenols are in a similar range or in most cases not differing in more than factor of 10 based on comparable studies with regard to the most relevant adverse endpoints.

In the study by Demska-Zakęś (2005), using *Sander lucioperca* 3 of the 5 alkylphenols were tested (4-*tert*-butylphenol, 4-n-heptylphenol and 4-n-nonylphenol). They all resulted in a biased a sex ratio towards females at very similar test concentrations. The LOEC for a decrease of male fish ratio (histologically determined) was 0.001 mg/L for all three substances – no NOEC could be established. The LOEC for the increase of female fish (histologically determined) was slightly different due to very small but statistically significant divergences: after 28 days of exposure the LOEC for 4-*tert*-butylphenol was 0.001 mg/L and for 4-n-heptylphenol and 4-n-nonylphenol 0.01 mg/L, resulting in NOECs of 0.001 mg/L for 4-n-heptylphenol and 4-n-nonylphenol. However, after the subsequent 56 days of rearing without exposure to the test substances the LOEC and NOEC for all the three substances were the same (0.01 mg/L and 0.001 mg/L, respectively). The LOEC for the appearance of intersex species (also histologically determined) was also 0.001

mg/L for all three substances with no established NOECs. No effects were seen on mortality, length, weight or condition factor at any concentration tested (highest concentration tested 0.2 mg/L) for any of the three substances.

Furthermore, there are several studies with *Pimephales promelas* with different endpoints showing effects for 4-*tert*-butylphenol, 4-*tert*-pentylphenol and 4-nonylphenol. For Vitellogenin induction the effect concentrations range from 0.015 to 0.56 mg/L (LOEC).For secondary sex characteristics the effect concentrations vary from 0.027mg/L for 4-*tert*-butylphenol to 0.071mg/L for 4-nonylphenol and 0.599 mg/L for 4-*tert*-pentylphenol. Shift in sex ratio was observed at 0.5 mg/L for 4-*tert*-butylphenol in a pilot study of one FSDT. For 4-*tert*-pentylphenol effects on sex ratio were observed in the range from 0.093 to 0.195mg/L.

Very low effect concentration values were also found for fecundity and behaviour: 0.071 mg/L and 0.00025 mg/L respectively (values only available for 4-nonylphenol).

For *Danio rerio* data are available for 4-*tert*-pentylphenol, 4-*tert*-octylphenol and 4-nonylphenol. Values for vitellogenin induction vary from 0.022 to 0.1 mg/L, for testis-ova from 0.03 to 0.787mg/L (4-*tert*-pentylphenol and 4-nonylphenol). Fertility was affected by 4-*tert*-pentylphenol and 4-*tert*-octylphenol. Effects on sex ratio were observed for 4-*tert*-pentylphenol and 4-nonylphenol with values between 0.062 and 0.1 mg/L. For 4-nonylphenol also effects on gametogenesis and ovarian follicle atresia were observed.

For *Oryzias latipes* there are data for 4-*tert*-pentylphenol, 4-*tert*-octylphenol and 4-nonylphenol available: Values for vitellogenin induction vary from 0.0012 to 0.104 mg/L, for testis-ova from 0.0012 to 0.225mg/L and for effects on sex ratio from 0.01 to 0.318 mg/L for the three substances.

For *Cyprinus carpio* data on vitellogenin induction are available for 4-*tert*-butylphenol and 4-*tert*-pentylphenol with proximate values of 0.69 and 1 mg/L, respectively. Additional data are available for 4-*tert*-pentylphenol showing effects at 0.036 mg/L for feminisation of gonadal ducts and at 0.09 -1 mg/L for testis-ova.

For *Oncorhynchus mykiss* data are available for 4-*tert*-octylphenol and 4-nonylphenol: Values for vitellogenin induction vary from 0.001 to 0.039 mg/L. For 4-nonylphenol data on unexposed F1 generation are also available: 0.001 for vitellogenin induction and 0.01 for sexual steroids seen in F1 generation. Effects on sperm stages and spermatogenesis were observed at $\leq$0.039 to 0.37 mg/L for the two substances, respectively.

Conclusion on Read across for environmental hazard assessment

In vitro data as well as *in vivo* data show that a read across to the target chemical 4-HPbl from source alkylphenols with longer chain length is justified:

- All *in vitro* data for fish estrogen receptors unambiguously show binding without major differences in binding affinities among the group. Activation of ER was seen in most studies. All *in vitro* data for rat and human estrogen receptors unambiguously show binding and activation. Some of the tests indicate a correlation of the binding affinity with the alkylchain length, but the differences were small (maximum two orders of magnitude) and others did not find such pattern.
- Only few studies where different alkylphenols are compared are available. However an analysis of all available data show that all alkylphenols show similar effects (histological changes, changes in sex ratio and secondary sex characteristics) which fit to the anticipated mode of action. Test concentrations differ between the studies, and no systematic pattern could be observed.

Thus the data on the structurally related alkylphenols support the findings for 4-HPbl and can be used to substantiate the conclusions made for 4-HPbl in a weight of evidence approach.

It can be concluded, that although the carbon chains of these 5 alkylphenols differ, endocrine disrupting properties for the environment are induced by all the four source substances as well as the target substance 4-HPbl. Data are available for the *in vitro* endocrine mode of action (from fish, rats, humans) as well as for *in vivo* endocrine effects in several fish species (*Sander lucioperca*, *Pimephales promelas*, *Danio rerio*, *Oryzias latipes*, *Cyprinus carpio*, *Oncorhynchus mykiss*). These include effect data such as a female biased sex ratio (shown for all 5 alkylphenols), which is considered to be adverse as well as indicative for an estrogenic mode of action. Also numerous other effects were seen in different studies with different fish species regarding indicative effects such as feminisation of gonadal ducts, testis-ova and changes in secondary sex characteristics.

Annex II – Detailed description of long term study with *Sander lucioperca* (Demska-Zakęś, 2005)

In one available long term study with fish (pikeperch, *Sander lucioperca*) the effects of 4-n-heptylphenol and other substances on mortality, development (weight, length, condition factor[20], gonads) and sex ratio (based on histological examination) were investigated (Demska-Zakęś, 2005). Sexually undifferentiated fish from artificial spawning were exposed to 4-n-heptylphenol from 60 days post hatch (dph) until 88 dph. These 28 days of exposure were followed by 56 days of rearing without test substance (until 144 dph). The test included a dilution water control, a solvent control (ethanol, 10 µL/L) and four treatment concentrations of 1, 10, 100, 200 µg/L (nominal) for 4-n-heptylphenol and as well for the positive controls (17 ß-estradiol and 4',7-dihydroxyisoflavone) and the other tested substances: 4-n-heptyloxyphenol, 4-n-nonylphenol, 4-n-butylphenol, 4-*sec*-butylphenol, 4-*tert*-butylphenol, phenol, 1,6-dihydroxynaphthalene and 1,5-dihydroxynaphthalene. Per treatment 80 fish per tank were tested in three replicates. The fish were kept in tanks with a water volume of 80 L under semi-static conditions (approximately 50% water exchange per 24h) and permanent lighting (50-60 lux). Each tank was separately filtered by a biological filter (filter performance was 4 L/min corresponding to the 3-fold tank volume per hour). The test temperature was 22.0±0.5°C.

The fish were examined before the start of the test (59 dph), on 88 dph (after 28 days exposure) and on 144 dph at the test end. In Table 23 details of the study design are provided.

Table 23: Overview study design for study from Demska-Zakęś (2005).

Parameter	Value	Unit	Remarks
Tank volume (nominal)	100	L	
Tank volume (actual)	80	L	
Loading	80	fish/tank	
Selection of fish from whole batch	7	days before test begin	Too small or too large fish were excluded from testing.
Determination of total length to	± 0.1	cm	
Determination of body weight to	0.01	g	
Range of body weight before test begin	1.6 - 2.1	g	
Narcotic treatment before manipulation of fish	1.5	ml/L	Propiscin solution (for 5 min); Propiscin contains a 0.2% stabilized solution of etomidate (personal communication with Prof. Demska-Zakęś in April, 2016).
Distribution of fish to test concentrations			Randomized
Number of test concentrations per substance	4	-	1, 10, 100 and 200 µg/L, dilution water control, solvent control
Number of replicates per test	3	-	According to the translation

[20] Condition factor =100*bodyweight*length-3

concentration			all treatments were "repeated twice", which is assumed to correspond to a replication number of in total three.
Solvent			Ethanol
Stock solution of test substances	100	mg/L	
Solvent content in the stock solution	5	ml/L	96% ethanol
Dilution medium in stock solution	995	ml/L	Aqua destillata
Dilution medium for preparation of test concentration			Tap water
Solvent content in the highest test concentration	0.01	ml/L	
Adjustment of solvent concentration in all test concentrations to	0.01	ml/L	The solvent concentration was adjusted to an equal concentration in every test concentration (1, 10, 100, 200 µg/L).
Semi-static conditions			
Volume exchange of the test medium	50	%/day	
Treatment of test medium			Each tank obtained each own recirculation system including a biological filter (filter mats/foam blocks) and an aeration system.
Flow rate in the recirculation system	4	L/min	Filter performance was 4 L/min corresponding to the 3-fold tank volume per hour.
Temperature, pH, dissolved O_2			Measurement daily
NH_4-N, NH_3-N, NO_2-N			Measurement every second day
Total hardness ($CaCO_3$), Fe			Measurement on test day 59, 80 and 100
Control of fish	1	per day	
Feeding amount	6	% of fish biomass per day	
Food for the first three weeks of age	*Artemia salina* naupliae and commercial trout starter		
Food after day 25 dph	Only commercial trout diet, NUTRA (TROUTFIT, Nutreco Aquaculture, France), the pellet size was increased during the test in relation to fish size.		
Food application	Feeding automate 4305 FIAP (Fishtechnik GmbH, Germany)		
Fish origin	Experimental Fish Stocking Centre Dgał, Institute for Inland Fishery, Olsztyn		
Fish age at moving to the recirculation system	4	dph	

Temperature adjustment during rearing	Gradually increased from 15 ± 0.5 to 22.0 ± 0.5°C		
Test temperature	22.0 ± 0.5	°C	
$CaCO_3$	200 ± 10	mg/L	
Fe	0.025 ± 0.005	mg/L	
Histological examination	30	fish	Randomized, at day 59 (before exposure) and each treatment/control at day 88 and day 144
Statistical calculations	Program: STATISTICA®, tests: ANOVA and others, level of significance $p<0.05$, results were provided as mean and standard deviation.		

Results for 4-n-heptylphenol

No statistically significant effects of 4-n-heptylphenol on mortality, total length, body weight, and condition factor of the fish were observed.

Nevertheless, 4-n-heptylphenol significantly affected the gonads starting from the lowest test concentration (Table 24, Figure 4 and Figure 5). In neither of the investigated endpoints a statistically significant difference between the dilution water control and the solvent control was encountered.

Table 24: Sex structure of pikeperch after 28 days of exposure to 4-n-heptylphenol (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated[1] from a graph (Fig. 17 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	48^{a}	52^{a}	0^{a}	0^{a}
Solvent control	53^{ab}	47^{a}	0^{a}	0^{a}
1	59^{b}	24^{b}	18^{b}	0^{a}
10	75^{c}	8^{c}	16^{b}	0^{a}
100	85^{d}	0^{d}	15^{b}	0^{a}
200	98^{e}	0^{d}	2^{a}	0^{a}
D144				
Dilution water control	50^{a}	50^{a}	0^{a}	0^{a}
Solvent control	50^{a}	50^{a}	0^{a}	0^{a}
1	59^{a}	20^{b}	21^{b}	0^{a}
10	75^{b}	5^{c}	20^{b}	0^{a}
100	87^{bc}	0^{c}	13^{ab}	0^{a}
200	100^{c}	0^{c}	0^{a}	0^{a}

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

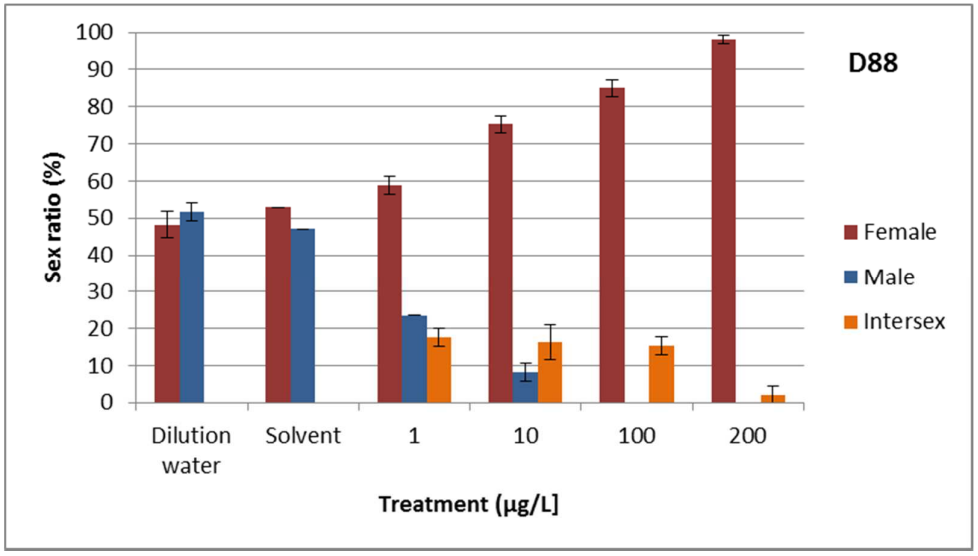


Figure 4: Sex structure of pikeperch after 28 days of exposure to 4-n-heptylphenol (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 17 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation**.**

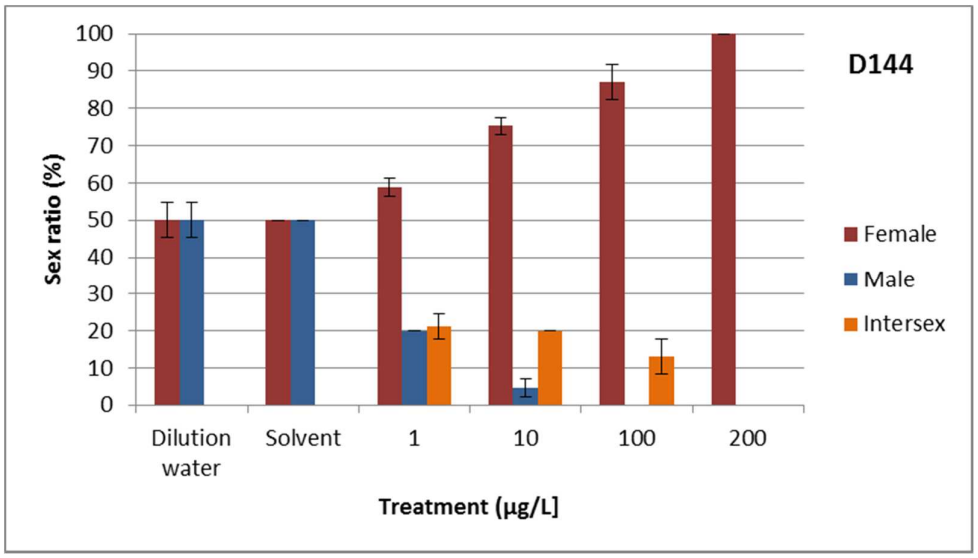


Figure 5: Sex structure of pikeperch after 28 days of exposure to 4-n-heptylphenol and a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 17 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

After 28 days of exposure the ratio of male fish (according to histological determination) was significantly decreased at the lowest test concentration (1 µg/L). Compared to the dilution water control the ratio of female fish is increased in the lowest test concentration, but compared to the more relevant solvent control this effect is significant from 10 µg/L on (Table 24). The appearance of intersex species (comprising sex characteristics from both sexes e.g. testis-ova/ ovotestis, formation of an oviduct, with regressed spermatogenic lobules in the same fish) was significant starting from 1 µg/L and was not observed in the controls. The shift in sex ratio was dose-dependent, leading to

approximately 98 and 100% female fish at 88 dph (Figure 4) and 144 dph (Figure 5), respectively. The values of the respective effect concentrations do not differ between 88 dph and 144 dhp (Table 25). This indicates that the observed effects on the sex characteristics were irreversible, also during the 56 days of rearing without exposure to the test substance.

Table 25: Effect concentrations for the investigated endpoints in juvenile pikeperch after 28 days of exposure to 4-n-heptylphenol. Concerning these effect concentrations there was no difference between the two sample points on 88 dph and 144 dph. Therefore, the values are listed combined in one table.

	Mortality ↑	**TL ↓**	**BW ↓**	**CF ↓**	**Female ↑**	**Male ↓**	**Intersex**	**Sterile**
NOEC (µg/L)	>200	>200	>200	>200	1	<1	<1	>200
LOEC (µg/L)	>200	>200	>200	>200	10	1	1	>200

BW ↓ decrease of of body weight, CF ↓ decrease of condition factor, Female ↑ increase of female sex characteristics, Intersex appearance of intersex species, Male ↓ decrease of male sex characteristics, Mortality ↑ increase of mortality, Sterile appearance of sterile species, TL ↓decrease of total length

Assessment of the reliability of the study by Demska-Zakęś (2005)

The Klimisch score for this study is 2, as it is well conducted although it is not an OECD Guideline study. The number of fish in treatments and controls was high and the results show high consistency. All physical chemical properties regarding testing conditions such as temperature, pH and oxygen concentration (Table 26) were measured and remained consistent during the test duration.

Table 26: Overview abiotic test conditions.

	Temperature °C	pH	Dissolved oxygen mg/L
4-n-heptylphenol	21.7 - 22.2	7.65 - 7.96	7.75 - 7.89
17 ß-estradiol	21.7 - 22.2	7.52 - 7.96	7.74 - 7.90
4',7-dihydroxyisoflavone	21.7 - 22.3	7.54 - 7.99	7.83 - 7.95
4-n-heptyloxyphenol	21.6 - 22.3	7.68 - 7.95	7.75 - 7.85
4-*tert*-butylphenol	21.7 - 22.2	7.55 - 7.95	7.73 - 7.79
4-n-nonylphenol	21.8 - 22.3	7.61 - 7.98	7.78 - 8.01

The data regarding length, weight and mortality (see Table 30) were examined, and male, female, intersex and sterile fish were histologically determined.

The dose response curves of the treatments and the positive controls are explicit and give an unanimous picture of the effects. The tested alkylphenols had estrogenic mediated effects, but did not cause mortality. No systemic or endocrine mediated effects were observed in the negative controls.

The solvent control was adjusted to an equal value in each test concentration. The solvent (ethanol) used is a recommended solvent according to OECD (OECD No. 23 (2000)) and its concentration was below the OECD recommendations of 100 µL/L for chronic studies (OECD No. 23 (2000)) and of 20 µL/L (0.002%) for reproduction studies (Hutchinson et al., 2006, cited in OECD No. 171 (2012d)). The study is compared to OECD Guideline 234 (Fish Sexual Development Test, 2011) in Table 27.

Table 27: Comparison of the study from Demska-Zakęś (2005) with OECD Guideline No. 234 (2011).

	OECD TG No. 234 (2011)	Demska-Zakęś (2005)
Validity criteria		
Dissolved oxygen (% air saturation value)	≥ 60	Fulfilled
Water temperature differences (°C), maximum	± 1.5	Fulfilled
Analysis method (LOD<<lowest test concentration)	Should be available	n.a.
Test concentrations maintained within ± 20% of mean measured values	Evidence required	n.a.
Hatching success (%)	> 80	n.a.
Post hatch survival (%)	≥ 70	Fulfilled
No effects of the solvent on survival		Fulfilled
No endocrine disrupting effects of the solvent		Fulfilled
Test design		
Test substance exposure start (dph)	Newly fertilized eggs (before cleavage of the blastodisc)	60 dph
Test substance exposure duration (dph)	60	28 d (60 dph – 88 dph)
Flow-through or semi-static		
Flow-through: volume exchange (per day)	≥ 5	n.a.
Semi-static: volume exchange (per day)	≥ 66%	50%
Photoperiod (light h / dark h)	12-16 / 8-12	24 / 0
Light intensity (lux)	540-1080	50-60
No. of treatments	≥ 3	4
No. of replicates per treatment	≥ 4	3
No. of animals per treatment	≥ 120 eggs	240 (3*80)
Solvent final concentration, maximum	100 µl/L	10 µl/L
Abiotic monitoring	Temperature[1], dissolved oxygen, salinity (if relevant) as a minimum weekly	Temperature, dissolved oxygen daily
	pH, total hardness, conductivity as a minimum at beginning and end of the test	pH daily, total hardness ($CaCO_3$) at test day 59, 80 and 100.
	Conductivity as a minimum at beginning and end of the test	-
	-	NH_4-N, NH_3-N and NO_2-N every second day
	-	Fe at test day 59, 80 and 100.
Validated test species	*Oryzias latipes, Danio rerio, Gasterosteus aculeatus, (Pimephales promelas)*	*Sander lucioperca*

Endpoints	Sex ratio	Sex ratio
	VTG level	
	Mortality	Mortality
	Standard length	Total length
	Body weight	Body weight
		Condition factor[2]
	Time to start/end of hatching	
	Observed abnormalities (deformation, behaviour)	
	(Genetic sex)	
	(Histopathology)	Histopathology

n.a. not applicable, [1] should preferably be monitored continuously in at least one test chamber, [2] Condition factor = 100*bodyweight*length-^{3}.

The adaptations made were more in accordance with aquaculture practices than with the OECD TG 234 (OECD, 2011). Therefore, the fish were held under constant light of low intensity in a recirculation-system under semi-static conditions, with 50% volume exchange per day and biological filtration of the tank water. The water exchange was slightly less than recommended by OECD TG 234 but was in combination with the biological filtration sufficient to ensure the required water quality.

No measurements of the test concentrations were made. However, the nominal concentrations in semi-static conditions can be considered as the worst case assumptions of real concentrations due to possible degradation and adsorption of the test substance during the test. The study results can be considered valid as the actual concentrations are rather lower than higher compared to the nominal concentrations. The latter does not affect the results demonstrating clear endocrine effects as the NOEC for the change in the sex ratio and the appearance of intersex species is below the lowest nominal test concentration and therefore also below the assumed actual test concentration.

In spite of some differences to OECD Guideline 234 it can be stated, that overall the study design is well reported and reasonable elaborated and fit to reliably assess estrogenic mediated adverse effects. The differences to OECD Guideline 234 are not deemed to invalidate the results of the study.

Although the pikeperch is not a validated OECD species, it is a very important fresh water species for aquaculture and therefore well investigated also by the author of the study (e.g. Zakęś, Z and Demska-Zakęś, K (1998), Wlasow et al. (2010), Kowalska et al. (2012) and Jarmolowicz et al. (2014)).

It is noted, that the sex ratio of approximately 1:1 (male : female fish) measured in the dilution water and solvent controls reported by Demska-Zakęś (2005) is also reported in literature. Data from wildlife indicate that the natural sex ratio is close to 1:1 (e.g. Lappalainen et al. (2003), Ablak and Yilmaz (2004)) with some indication that at higher temperature, males may prevail (Raikova-Petrova and Zivkov (1998)).

The original study is in Polish, but most relevant parts are either available in English or have been discussed with the author. Although the study is not published in an international journal, it needs to be emphasised that Polish habilitation studies undergo a review process including also external reviewers from other universities.

It is emphasised that the results of the study are very consistent with respect to the observed endpoints and different substances investigated. This is also a key -argument for the reliability of the study. An overview of all the results for all the tested substances and the controls is provided in Table 28.

Table 28: Overview of the NOEC and LOEC results[1] of all substances in the study by Demska-Zakęś (2005).

NOEC (µg/L)	**Mortality ↑**	**TL ↓**	**BW ↓**	**CF ↓**	**Female ↑**	**Male ↓**	**Intersex**	**Sterile**
4-n-heptylphenol	>200	>200	>200	>200	1	<1	<1	>200
17 ß-estradiol	10	>200	10	10	<1	<1	<1	100 / 10
4',7-dihydroxy-isoflavone	100	>200	>200	100	<1	<1	<1	>200
1,6-dihydroxy-naphthalene	>200	>200	>200	>200	10	10	10	>200
1,5-dihydroxy-naphthalene	>200	>200	>200	>200	>200	>200	>200	>200
Phenol	>200	>200	>200	>200	>200	>200	100	>200
4-n-heptyloxyphenol	>200	>200	>200	>200	10	<1	<1 / 1	>200
4-n-nonylphenol	>200	>200	>200	>200	1	<1	<1	>200
4-n-butylphenol	>200	>200	>200	>200	1	1	1	>200
4-*sec*-butylphenol	>200	>200	>200	>200	1	1	1	>200
4-*tert*-butylphenol	>200	>200	>200	>200	<1 / 1	<1	1 /<1	>200
LOEC (µg/L)	**Mortality ↑**	**TL ↓**	**BW ↓**	**CF ↓**	**Female ↑**	**Male ↓**	**Intersex**	**Sterile**
4-n-heptylphenol	>200	>200	>200	>200	10	1	1	>200
17 ß-estradiol	100	>200	100	100	1	1	1	200 / 100
4',7-dihydroxy-isoflavone	200	>200	>200	200	1	1	1	>200
1,6-dihydroxy-naphthalene	>200	>200	>200	>200	100	100	100	>200
1,5-dihydroxy-naphthalene	>200	>200	>200	>200	>200	>200	>200	>200
Phenol	>200	>200	>200	>200	>200	>200	200	>200
4-n-heptyloxyphenol	>200	>200	>200	>200	100	1	1 / 10	>200
4-n-nonylphenol	>200	>200	>200	>200	10	1	1	>200
4-n-butylphenol	>200	>200	>200	>200	10	10	10	>200
4-*sec*-butylphenol	>200	>200	>200	>200	10	10	10	>200
4-*tert*-butylphenol	>200	>200	>200	>200	1 / 10	1	10 / 1	>200

[1]The difference between D88 (after 28 days of exposure) and D144 (after 56 days of subsequent rearing without test substance) is indicated as D88 / D144.

BW ↓decrease of of body weight, CF ↓decrease of condition factor, Female ↑ increase of female sex characteristics, Intersex appearance of intersex species, Male ↓ decrease of male sex characteristics, Mortality ↑ increase of mortality, Sterile appearance of sterile species, TL ↓decrease of total length.

No effects on the investigated endpoints were observed for any of the dilution water or

solvent controls.

The positive controls 17 ß-estradiol and 4',7-dihydroxyisoflavone shifted the sex ratio to more female biased. However, it appears that some of the used concentrations were too high, especially in the case of 17 ß-estradiol, as mortality occurred already at 100 µg/L (see Table 29).

Regarding the substances tested a homogenous picture is provided for the tested alkylphenols 4-n-heptylphenol, 4-n-heptyloxyphenol, 4-n-nonylphenol, 4-n-butylphenol, 4-*sec*-butylphenol and 4-*tert*-butylphenol: No mortality was observed, and NOEC values of 1 and < 1 were obtained for female biased sex ratio. It has to be noted that for 4-n-nonylphenol, a known endocrine disruptor, the observed effect concentrations were similar to those for 4-n-heptylphenol. For the different butylphenol isomers 4-n-butylphenol, 4-*sec*-butylphenol and 4-*tert*-butylphenol similar results were obtained, stressing that both branched and linear forms lead to estrogen mediated adverse effects.

No effects (neither systemic nor endocrine mediated) were seen for 1,5-dihydroxynaphthalene. For phenol intersex fish were observed at 200 µg/L, while for 1,6-dihydroxynaphthalene a female biased sex ratio and intersex fish were seen at 100 µg/L.

For the two positive controls 17ß-estradiol and 4',7-dihydroxyisoflavone as well as for 4-n-heptyloxyphenol, 4-*tert*-butylphenol and 4-n-nonylphenol the results are provided in detail.

Table 29: Substances causing significant effects on growth parameters and mortality after 28 days of exposure (D88) and after 56 days of subsequent rearing without test substance (D144).

	Treatment (µg/L)	**Mortality (%)**		**Total length (cm)**		**Body weight (g)**		**Condition factor**	
E_2		D88	D144	D88	D144	D88	D144	D88	D144
	DWC	3.75^a	4.38^a	9.27^a	16.51^a	8.31^a	46.23^a	1.21^a	1.00^a
	SC	3.13^a	3.13^a	9.08^a	16.49^a	8.35^a	45.85^a	1.21^a	1.06^a
	1	2.50^a	3.13^a	9.06^a	16.39^a	8.35^a	45.92^a	1.24^a	1.01^a
	10	3.75^a	3.75^a	9.05^a	16.38^a	8.18^a	45.91^a	1.23^a	0.99^a
	100	14.38^b	17.50^b	9.11^a	16.21^a	4.97^b	38.70^b	0.96^b	0.81^b
	200	20.00^c	25.00^c	8.94^a	15.96^a	4.48^b	34.10^b	0.85^b	0.74^b
4',7-DHI	0	3.13^a	3.13^a	9.19^a	16.52^a	8.23^a	46.05^a	1.26^a	1.07^a
	0*	4.38^a	4.38^a	9.27^a	16.59^a	8.22^a	45.95^a	1.20^a	1.00^a
	1	2.50^a	2.50^a	9.11^a	16.45^a	8.29^a	46.03^a	1.21^a	1.04^a
	10	3.13^a	3.13^a	9.06^a	16.33^a	8.25^a	45.60^a	1.23^a	1.04^a
	100	2.50^a	3.13^a	9.18^a	16.41^a	8.27^a	46.00^a	1.23^a	1.05^a
	200	10.63^b	14.38^b	9.09^a	16.20^a	5.56^a	44.05^a	0.99^b	0.86^b

4',7-DHI 4',7-dihydroxyisoflavone, DWC dilution water control, E2 17ß-estradiol, SC solvent control. Values with the same superscript in the same column are not significantly different (P>0.05).

Table 30: Substances causing no significant effects on growth parameters and mortality after 28 days of exposure (D88) and after 56 days of subsequent rearing without test substance (D144) (data given as range over all tested concentrations including also the values from dilution water and solvent controls).

	Mortality (%)		**Total length (cm)**		**Body weight (g)**		**Condition factor**	
	D 88	D 144	D 88	D 144	D 88	D 144	D 88	D 144
4nHP	0.63 - 4.38	2.50 - 4.38	9.20 - 9.35	16.58 - 16.70	8.30 - 8.40	46.80 - 47.11	1.19 - 1.25	1.00 - 1.05
4nHOP	2.50 - 4.38	2.50 - 4.38	9.19 - 9.30	16.59 - 16.73	8.28 - 8.45	46.79 - 47.05	1.20 - 1.26	0.98 - 1.05
4tBP	2.50 - 4.38	2.50 - 4.38	9.31 - 9.34	16.73 - 16.80	8.20 - 8.30	45.92 - 46.34	1.18 - 1.26	0.99 - 1.10
4nNP	2.50 - 4.38	2.50 - 4.38	9.20 - 9.34	16.60 - 16.72	8.28 - 8.41	46.81 - 47.10	1.20 - 1.25	1.00 - 1.07

4nHP 4-n-heptylphenol, 4nHOP 4-n-heptyloxyphenol, 4nNP 4-n-nonylphenol, 4tBP 4-*tert*-butylphenol.

Results for 17ß-estradiol

17ß-estradiol showed the strongest effects on fish of all testes substances. After 28 days of exposure the ratio of male fish was significantly decreased (Table 31) already at the lowest studied test concentration of 1 µg/L. Intersex species occurred only at 1 µg/L, which was significant compared to the controls. The shift in sex ratio was dose-dependent, leading to 100% of the fish to be female at 10 µg/L, at both 88 dph (Figure 6) and 144 dph (Figure 7).

Table 31: Sex structure of pikeperch after 28 days of exposure to 17ß-estradiol (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated[1] from a graph (Fig. 3 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	48[a]	52[a]	0[a]	0[a]
Solvent control	52[a]	48[a]	0[a]	0[a]
1	78[b]	17[b]	5[b]	0[a]
10	100[c]	0[c]	0[a]	0[a]
100	95[c]	0[c]	0[a]	5[a]
200	80[b]	0[c]	0[a]	20[b]
D144				
Dilution water control	51[a]	49[a]	0[a]	0[a]
Solvent control	52[a]	48[a]	0[a]	0[a]
1	77[b]	14.5[b]	8.5[b]	0[a]
10	100[c]	0[c]	0[a]	0[a]
100	93[c]	0[c]	0[a]	7[b]
200	78[c]	0[c]	0[a]	22[c]

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

At the two highest test concentrations of 17ß-estradiol sterile fish appeared. Sterile fish were not found with any of the other tested substances. The two highest test concentrations were apparently too high for studies on estrogenic mediated effects as an effect on liver (cholestasis), a significant increase in mortality and a significant decrease in body weight and condition factor were reported (Table 31). The effect concentrations were identical at 88 and 144 dhp (Table 28). This indicates that the observed effects on the sex characteristics were irreversible, at least during the test including the 56 days of rearing without exposure to the test substance.

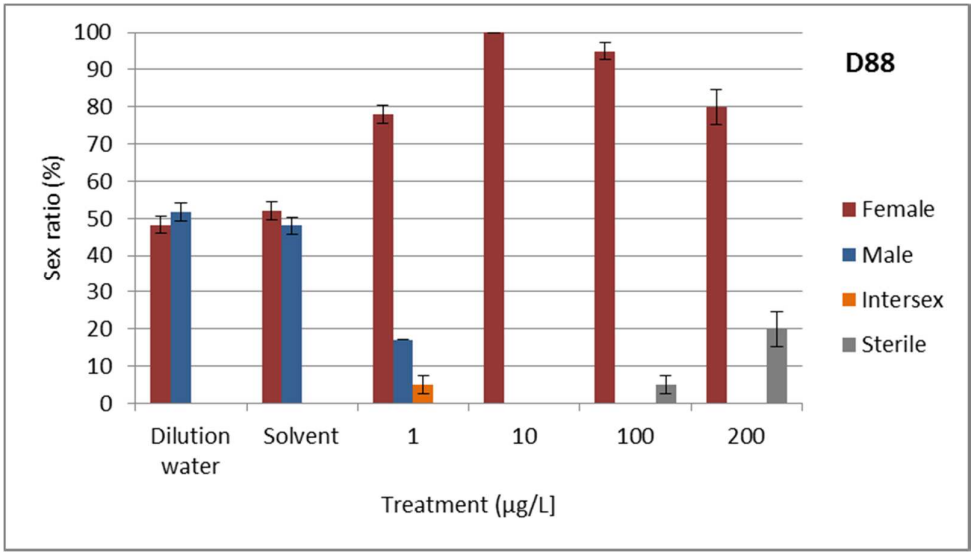


Figure 6: Sex structure of pikeperch after 28 days of exposure to 17ß-estradiol (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 3 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

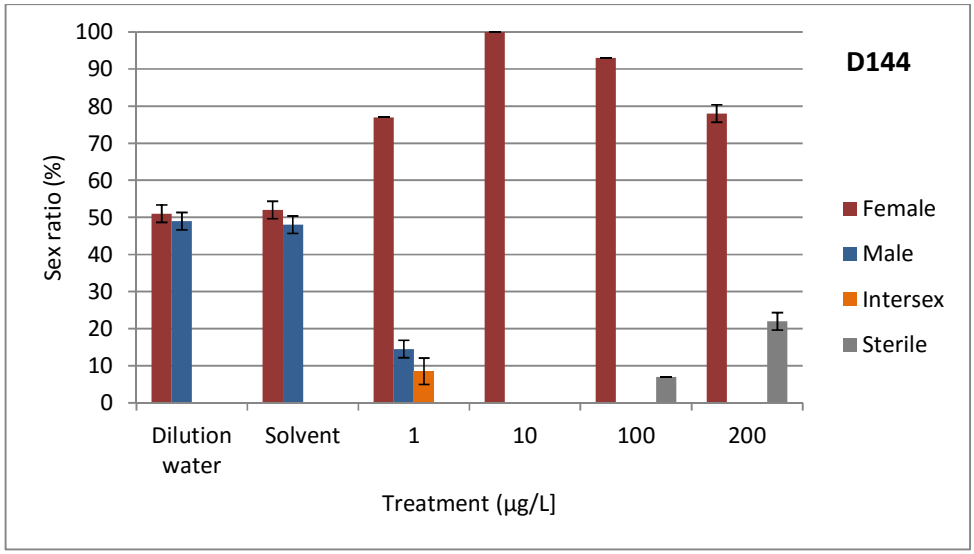


Figure 7: Sex structure of pikeperch after 28 days of exposure to 17ß-estradiol (D144). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 3 in Demska-Zakes, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

Results for 4',7-dihydroxyisoflavone

After 28 days of exposure to 4',7-dihydroxyisoflavone the ratio of male fish was significantly decreased (Table 32) and the ratio of female fish was significantly increased at the lowest test concentration of 1 µg/L. The appearance of intersex species occurred at concentrations from 1 µg/L to 100 µg/L and was significant compared to the controls.

Table 32: Sex structure of pikeperch after 28 days of exposure to 4',7-dihydroxyisoflavone (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated[1] from a graph (Fig. 4 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	48^{a}	52^{a}	0^{a}	0^{a}
Solvent control	50^{a}	50^{a}	0^{a}	0^{a}
1	63^{b}	32^{b}	5^{b}	0^{a}
10	72^{bc}	20^{c}	8^{b}	0^{a}
100	81.5^{c}	3.5^{d}	15^{c}	0^{a}
200	100^{d}	0^{e}	0^{a}	0^{a}
D144				
Dilution water control	52^{a}	48^{a}	0^{a}	0^{a}
Solvent control	52^{a}	48^{a}	0^{a}	0^{a}
1	64^{b}	28^{b}	8^{b}	0^{a}
10	71^{bc}	17^{c}	12^{bc}	0^{a}
100	84^{c}	1.5^{d}	14.5^{c}	0^{a}
200	100^{d}	0^{d}	0^{a}	0^{a}

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

The shift in sex ratio was dose-dependent, leading to 100% of the fish to be female at 200 µg/L, at both 88 dph (Figure 8) and 144 dph (Figure 9). At the highest test concentration an effect on liver (cholestasis), a significant increase in mortality and a significant decrease in condition factor were reported (Table 29). The values of the respective effect concentrations were identical at 88 dph and 144 dhp (Table 28). This indicates that the observed effects on the sex characteristics were irreversible, at least for the duration of the test including the 56 days of rearing without exposure to the test substance.

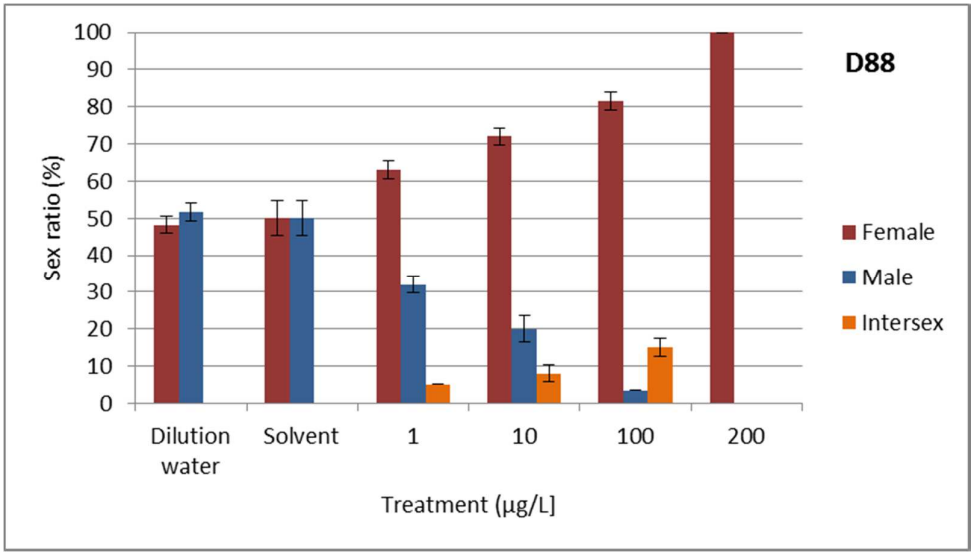


Figure 8: Sex structure of pikeperch after 28 days of exposure to 4',7-dihydroxyisoflavone (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 4 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

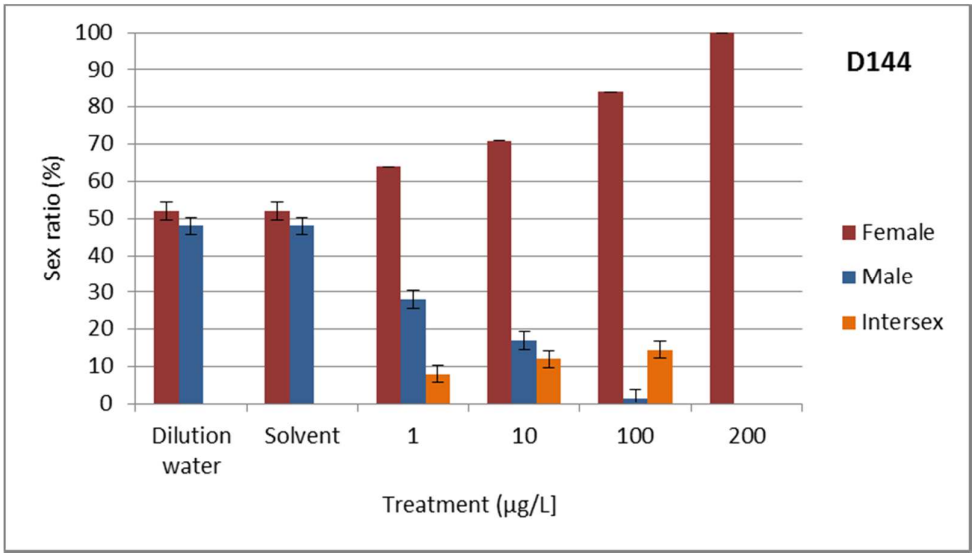


Figure 9: Sex structure of pikeperch after 28 days of exposure to 4',7-dihydroxyisoflavone (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 4 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

Results for 4-n-heptyloxyphenol

The effects of 4-n-heptyloxyphenol on pikeperch were slightly weaker than those observed for 4-n-heptylphenol. After 28 days of exposure the ratio of male fish was significantly decreased at the lowest test concentration of 1 µg/L (Table 33). The ratio of female fish was significantly increased at 100 µg/L. Intersex species appeared significantly at concentrations from 1 to 200 µg/L.

Table 33: Sex structure of pikeperch after 28 days of exposure to 4-n-heptyloxyphenol (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated[1] from a graph (Fig. 16 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	52^{a}	48^{a}	0^{a}	0^{a}
Solvent control	48^{a}	52^{a}	0^{a}	0^{a}
1	55^{a}	33^{b}	12^{b}	0^{a}
10	60.5^{a}	18^{c}	21.5^{b}	0^{a}
100	75.5^{b}	10^{d}	14.5^{b}	0^{a}
200	82^{b}	0^{e}	18^{b}	0^{a}
D144				
Dilution water control	48^{a}	52^{a}	0^{a}	0^{a}
Solvent control	48^{a}	52^{a}	0^{a}	0^{a}
1	59^{a}	32^{b}	9^{ab}	0^{a}
10	59^{a}	21^{c}	20^{b}	0^{a}
100	73^{b}	8^{d}	19^{b}	0^{a}
200	83.5^{b}	0^{d}	16.5^{b}	0^{a}

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

The shift in sex ratio was dose-dependent, leading to approximately 82% and 83.5% female fish at 200 µg/L at 88 dph (Figure 10) and 144 dph (Figure 11), respectively. The values of the respective effect concentrations were identical at 88 and 144 dhp (with the exception of the appearance of intersex species at 1 µg/L, which was only significant at 88 dph, (Table 28). This fact indicates that the observed effects on the sex characteristics were irreversible, at for the duration of the test including the 56 days of rearing without exposure to the test substance.

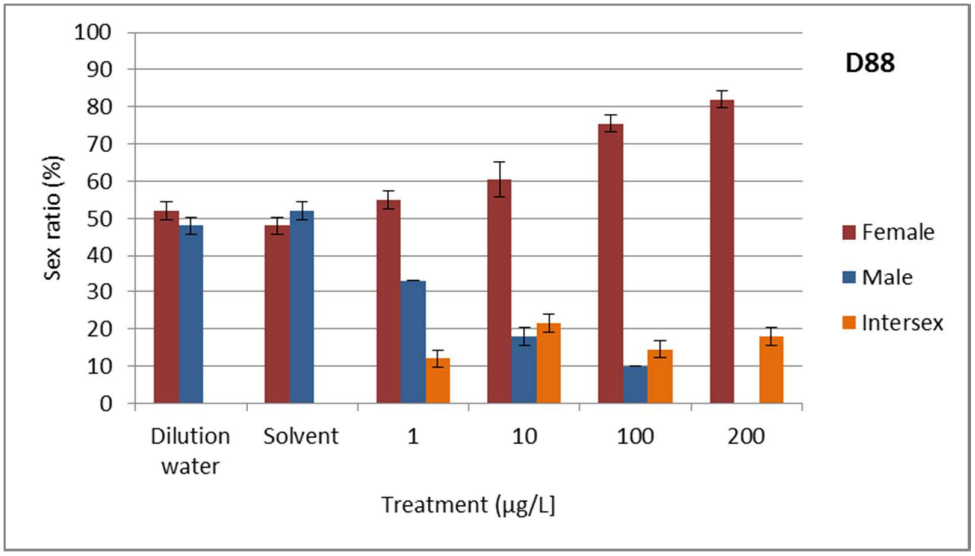


Figure 10: Sex structure of pikeperch after 28 days of exposure to 4-n-heptyloxyphenol (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 16 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

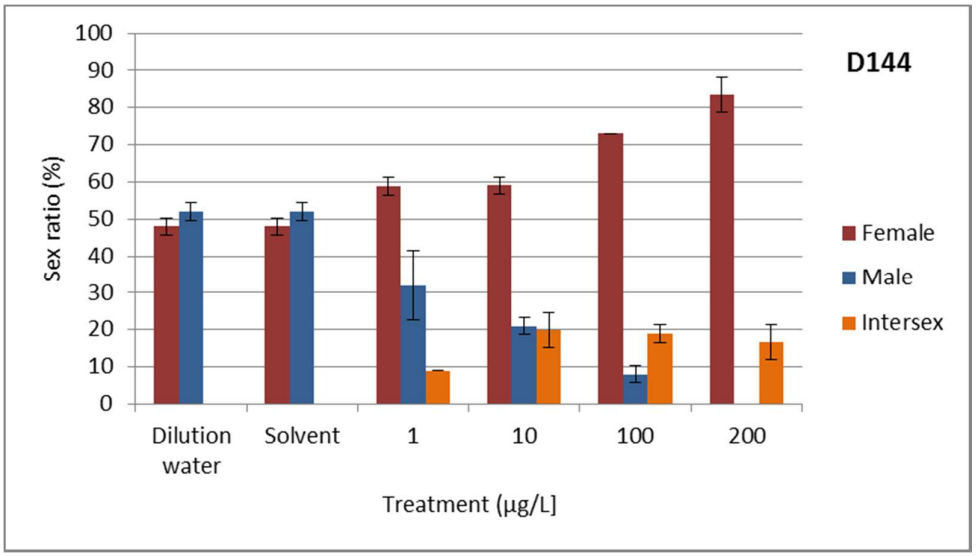


Figure 11: Sex structure of pikeperch after 28 days of exposure to 4-n-heptyloxyphenol (D144). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 16 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

Results for 4-n-nonylphenol

4-n-nonylphenol, a known endocrine disruptor, was also tested in this study and the concentrations where the effects were observed were the same as those stated for 4-n-heptylphenol (Table 28). The dose-response curve of 4-n-nonylphenol was slightly steeper than that for 4-n-heptylphenol leading to approximately 100% female fish at 100 µg/L for 4-n-nonylphenol (Table 34) and at 200 µg/L for 4-n-heptylphenol.

After 28 days of exposure the ratio of male fish was significantly decreased at the lowest test concentration of 1 µg/L (Table 34). The ratio of female fish was significantly increased compared to the solvent control at a test concentration of 10 µg/L. Intersex species appeared from 1 µg/L to 100µg/L, which was significant compared to the controls for 1 µg/L and 10 µg/L.

Table 34: Sex structure of pikeperch after 28 days of exposure to 4-n-nonylphenol (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated[1] from a graph (Fig. 18 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	48^{a}	52^{a}	0^{a}	0^{a}
Solvent control	52^{ab}	48^{a}	0^{a}	0^{a}
1	58.5^{b}	11.5^{b}	30^{b}	0^{a}
10	82^{c}	1^{c}	17^{c}	0^{a}
100	99^{d}	0^{c}	1^{a}	0^{a}
200	100^{d}	0^{c}	0^{a}	0^{a}
D144				
Dilution water control	50^{a}	50^{a}	0^{a}	0^{a}
Solvent control	52^{a}	48^{a}	0^{a}	0^{a}
1	56^{a}	12^{b}	32^{b}	0^{a}
10	82.5^{b}	2.5^{bc}	15^{c}	0^{a}
100	100^{c}	0^{c}	0^{a}	0^{a}
200	100^{c}	0^{c}	0^{a}	0^{a}

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

The shift in sex ratio was dose-dependent, leading to approximately 99% and 100% female fish at 100 µg/L at 88 dph (Figure 12) and 144 dph (Figure 13), respectively. The values of the respective effect concentrations were identical at 88 and 144 dhp (Table 28). This indicates that the observed effects on the sex characteristics were irreversible, at least during the test including the 56 days of rearing without exposure to the test substance.

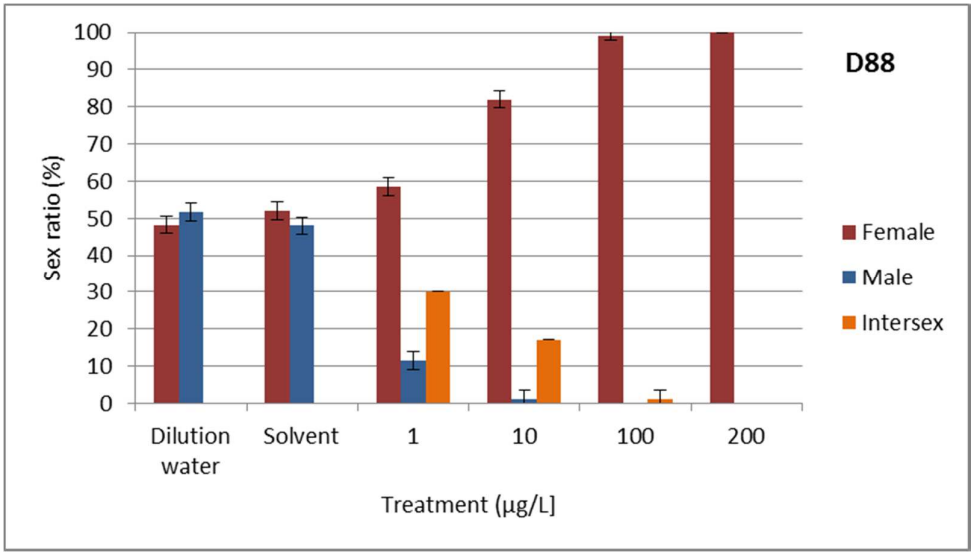


Figure 12: Sex structure of pikeperch after 28 days of exposure to 4-n-nonylphenol (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 18 in Demska-Zakes, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

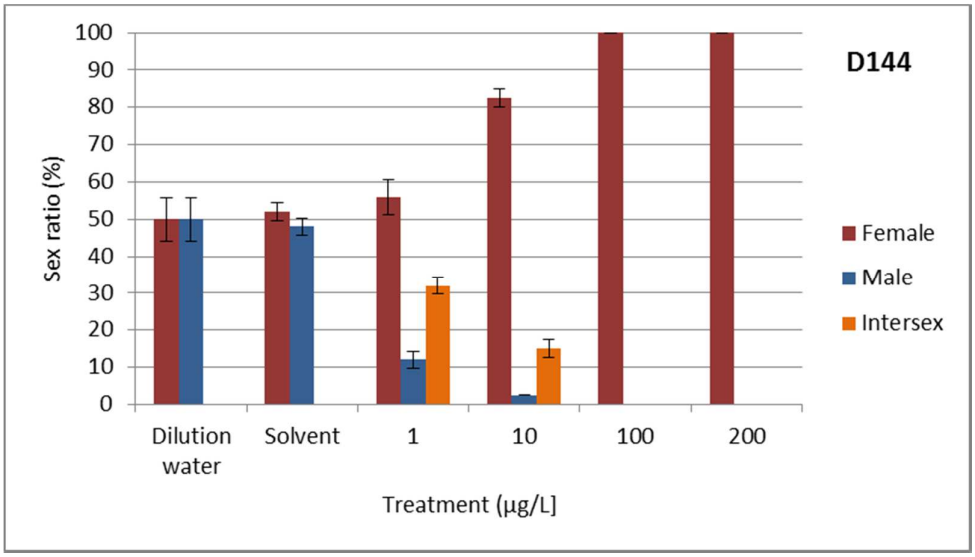


Figure 13: Sex structure of pikeperch after 28 days of exposure to 4-n-nonylphenol (D144). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 18 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

Results for 4-*tert*-butylphenol

After 28 days of exposure to 4-*tert*-butylphenol the ratio of male fish was significantly decreased at the lowest test concentration of 1µg/L. Compared to the solvent control the ratio of female fish is significantly increased at 1µg/L on D88 and at 10 µg/L on D144 (

Table **35**). Intersex species appeared on D88 from 1 µg/L to 200 µg/L, which was significantly compared to the solvent control at 10 µg/L and 100 µg/L. On D144 intersex species appeared from 1 µg/L to 100 µg/L, which was significant compared to the controls.

Table 35: Sex structure of pikeperch after 28 days of exposure to 4-*tert*-butylphenol (D88) and after a subsequent rearing of 56 days without test substance (D144). These values refer to mean numbers of fish in percent and were extrapolated from a graph (Fig. 21 in Demska-Zakęś, 2005).

Treatment (µg/L)	Female	Male	Intersex	Sterile
D88				
Dilution water control	52^{ab}	48^{a}	0^{a}	0^{a}
Solvent control	47^{a}	53^{a}	0^{a}	0^{a}
1	58.5^{bc}	31.5^{b}	10^{ab}	0^{a}
10	68^{c}	15^{c}	17^{b}	0^{a}
100	80^{d}	0^{d}	20^{b}	0^{a}
200	98^{e}	0^{d}	2^{a}	0^{a}
D144				
Dilution water control	48^{a}	52^{a}	0^{a}	0^{a}
Solvent control	52^{ab}	48^{a}	0^{a}	0^{a}
1	57.5^{b}	32^{b}	10.5^{b}	0^{a}
10	68^{c}	16.5^{c}	15.5^{b}	0^{a}
100	78^{d}	0^{d}	22^{c}	0^{a}
200	100^{e}	0^{d}	0^{a}	0^{a}

Values with the same superscript in the same column are not significantly different (P>0.05).
[1] For illustration purposes the values were estimated form the graphs of the study via manual measurement of the height of the bars and transformation via regression function to estimated values in percent. The superscripts indicating the statistical significance were taken from the original graphs.

The shift in sex ratio was dose-dependent, leading to approximately 98 and 100% female fish at 88 dph (Figure 14) and 144 dph (Figure 15), respectively. The observed effects on the sex characteristics were irreversible for the duration of the test including 56 days of rearing without exposure to the test substance.

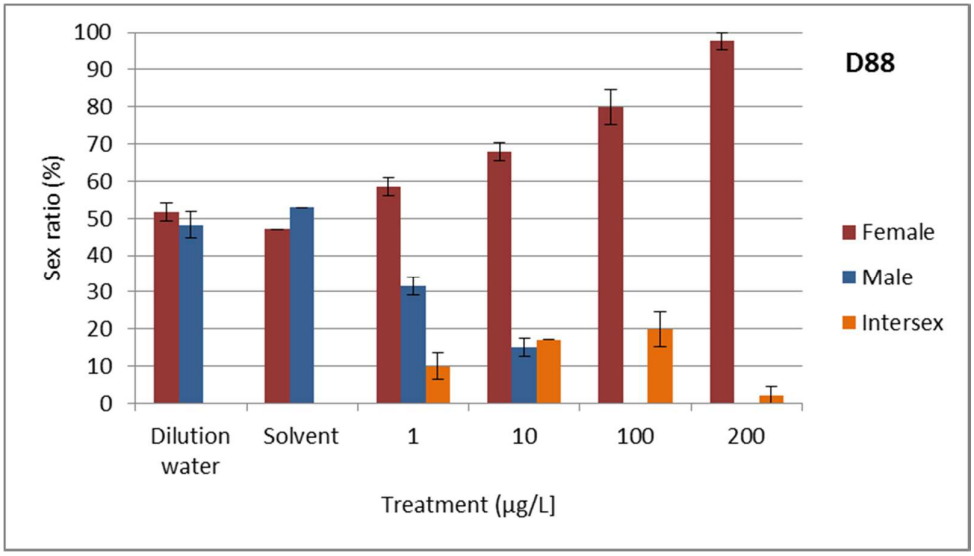


Figure 14: Sex structure of pikeperch after 28 days of exposure to 4-*tert*-butylphenol (D88). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 21 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.

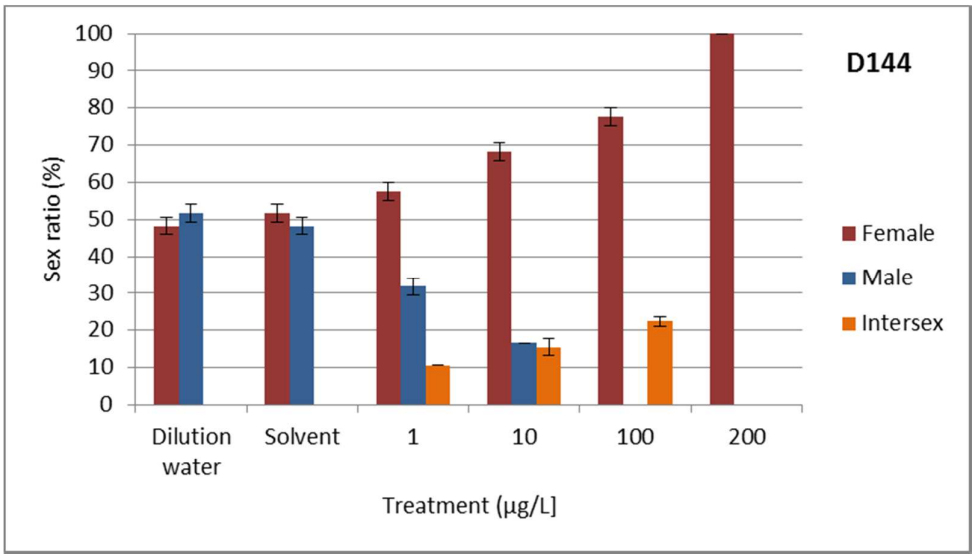


Figure 15: Sex structure of pikeperch after 28 days of exposure to 4-*tert*-butylphenol (D144). These values refer to mean numbers of fish in percent with indication of the standard deviation (n=3) and were extrapolated from a graph (Fig. 21 in Demska-Zakęś, 2005). If no error bar is indicated, the results of the particular replicates (n=3) were either equal or their variance too low for visualisation.