MINISTERIO
PARA LA TRANSICIÓN ECOLÓGICA
Y EL RETO DEMOGRÁFICO

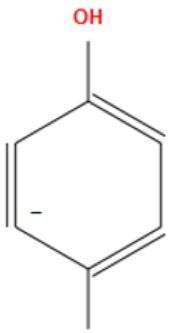
SUBSTANCE EVALUATION CONCLUSION and EVALUATION REPORT

for

4-nonylphenol, branched

EC No 284-325-5

CAS RN 84852-15-3



C_9H_{19}

Evaluating Member State Competent Authority:
Spain, handover from the United Kingdom

Dated: 30 April 2024

Evaluating Member State Competent Authority

Follow up and conclusion of the substance evaluation process

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Year of evaluation in CoRAP: 2014

Further information on registered substances here:

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

Further information on the substance evaluation process here:

https://echa.europa.eu/regulations/reach/evaluation/substance-evaluation

DISCLAIMER

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

Foreword

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the outcome of the Substance Evaluation carried out by the evaluating MSCA. The document consists of two parts i.e. A) the conclusion and B) the evaluation report.

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

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Part A. Conclusion

In the conclusion (part A), the evaluating MSCA considers how the information on the Substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling.

Alternatively, the outcome of the evaluation may be that presently there is no need for regulatory follow-up at EU level if sufficient information on the potential hazards is available and all necessary measures for safe handling of the substance are in place.

1. Scope of the evaluation

4-nonylphenol, branched ("the Substance") was originally selected for substance evaluation to clarify concerns about:

- PBT/vPvB
- Consumer use
- High (aggregated) tonnages
- Wide dispersive use

During the evaluation the following additional concerns were identified:

- Aquatic toxicity (chronic)
- Other environmental hazards(s)
- Exposure of environment
- DNEL derivation and exposure modelling

2. Overview of other processes / EU legislation

Table 2-1 Overview of other processes / EU legislation

No other processes	CCH	TPE	GMT	Previously on CoRAP	Annex VI (CLP)	Annex XVII (Restriction)	Candidate List/Annex XIV (Authorisation)
☐	☒	☒	☒	☐	☒	☒	☒

Other EU legislation	Previous legislation	Stockholm convention	Other
PPP/BPR	NONS/RAR	POP	(e.g., UNEP)
☐	☒	☐	☐

Regulatory obligations exist for this substance under the following EU Legislation:

Chemicals legislation:

- Regulation EC 1272/2008 – Harmonised classification included in the Annex VI with the index number 601-053-00-8.
- Included in the Annex I Part 1 and 2 of the PIC Regulation,
- Included in the Annex XVII (entry 46) of REACH Regulation identified unacceptable environmental risks for some uses,
- Included in the Candidate List of Substances of Very High Concern for Authorisation due to endocrine disrupting properties (Article 57(f) – environment) in 2012.

Environmental protection:

- Directive 2000/60/EC - Included in the Annex X of priority substances of the Water Framework Directive. The annex includes substances targeted for reduction and eventual removal from wastewater discharge. The list includes 'priority hazardous substances' which are subject to special restrictions.

- Directive 2010/75/EC prioritising polluting substance for which emission limit values are assigned under Annex II of the directive on Industrial Emissions (Integrated Pollution Prevention and Control - IPPC)
- Hazardous properties substance included in the Annex III of the Waste Framework Directive (Directive 2008/98/EC)
- Directive 2008/105/EC, Water Environmental Quality Standards Directive (Directive 2008/105/EC). The substance is included in the Annex I – Part A of Priority Substances and Pollutants in Water.

Consumers and workers protection:

- Regulation EC 1223/2009. Prohibited substance in Annex II in the Cosmetic Products Regulation.
- Directive 92/85/EEC, Included in the Annex I+II of the Protection of Pregnant and Breastfeeding Directive,
- Directive 94/33/EC on physical, biological, and chemical agents & processes and work
- Directive 92/58/EC, for the Safety and/or Health Signs at Work Directive (Directive 92/58/EEC). The Substance is not specifically listed in this Directive but is covered as part of the general requirement that all hazardous substances according to Annex VI CLP are signposted at work.
- Directive 98/24/EC, Chemical Agents Directive, Art. 2(b)(i).The Substance does not have a binding occupational exposure or biological limit values in the Directive but falls in the scope of the general requirements for hazardous chemical agents in the Directive.

3. Conclusion and regulatory follow-up action

The evaluation of the available information on the Substance has led the evaluating MSCA to the following conclusions.

Table 3-1 Conclusion and regulatory follow-up action

Initial and additional concern	Conclusion on concern	Regulatory follow-up action
PBT/vPvB	Inconclusive	No need for regulatory follow-up at EU level
Consumer use	Concern confirmed	Need for follow-up regulatory action at EU level
High (aggregated) tonnages	Concern confirmed	Need for follow-up regulatory action at EU level
Wide dispersive use	Concern confirmed	Need for follow-up regulatory action at EU level
Aquatic toxicity (chronic)	Concern confirmed	Need for follow-up regulatory action at EU level
Other environmental hazard	Concern confirmed	Need for follow-up regulatory action at EU level
Exposure of environment	Concern confirmed	Need for follow-up regulatory action at EU level

The Spanish evaluating Member State Competent Authority (ES eMSCA) was not able to clarify the initial concern on potential PBT/vPvB properties of the Substance based on the available information after applying the weight-of-evidence (WoE) approach. At least some of the constituents of the Substance are likely to be B and they are confirmed to be T. No firm conclusion on persistence could be drawn based on the available information. In addition, dinonylphenol, which may be present in the Substance as an impurity, screens potentially PBT/vPvB. However, as the Substance is already identified as SVHC due to its endocrine disrupting properties in the environment, minimisation of emissions and exposure must be ensured for the Substance. Hence, confirmation of the potential PBT/vPvB properties of the constituents or impurities of the Substance would not lead to stricter risk management measures for the Substance. Therefore, it was decided not to request further information to clarify the concern on potential PBT/vPvB properties. However, as based on the available information the PBT/vPvB concern could not be removed, the ES eMSCA recommends that the Substance should be treated as if it is a PBT/vPvB substance.

Regarding chronic aquatic toxicity, the registrants submitted three new long-term tests on aquatic invertebrates (two officially submitted in the registration dossiers to the ECHA database and the third one sent to the ES eMSCA, but not yet included in the registration dossiers[1]) and included further studies found in open literature. Also, reassessment of some of the already existing studies was done. Based on this information the Predicted No effect Concentration (PNEC) values for freshwater and marine water were refined by the eMSCA resulting in lower PNEC values than those previously reported by the registrants. However, these PNEC values are based on "traditional" apical effects seen in the available studies. Endocrine disrupting effects have been observed in some non-standard tests even at lower concentrations. It is considered that it is not possible to define a safe use threshold for endocrine disrupters. Therefore, the PNEC values are not sufficiently protective for the endocrine disrupting effects of the Substance and the registrants of the Substance are required to implement on their sites or to recommend to their downstream users, risk management measures and operational conditions which minimise emissions and exposure of the environment.

New tests on avian toxicity found in the open literature were also included in the registration dossier by the registrants following the Substance Evaluation decision. The PNECoral for birds calculated by the ES eMSCA based on this information was lower than that reported by the registrants. However, as indicated above, the PNEC values are not considered protective enough for the endocrine disrupting properties of the Substance.

Regarding the high tonnage, wide dispersive use, consumer use and exposure of the environment, these are considered as confirmed. Most of the Substance seems to be used as an intermediate in the manufacture of polymers and other derivatives. However, part of the Substance used as intermediate may be present in the manufactured polymers and other derivatives as unreacted impurity. Also, some of these polymers and other derivatives may degrade and form 4-NP when released to the environment. Most of the uses by professional workers and consumers reported in the registration dossiers appear to refer to end uses of the polymers and other derivatives in which the Substance is used as a monomer. However, based on the available information, some of the uses reported as professional or consumer uses in the registration dossier may refer to the use of the Substance in mixtures, e.g. in adhesives. All in all, considering the high aggregated tonnage, even low emissions from industrial uses may add up to significant exposure of the environment. Especially when considering them together with emissions of other substances and polymers that have 4-nonylphenol (4-NP) as a constituent or an impurity or may be precursors of 4-NP in the environment. As 4-NP is identified as an endocrine disrupting substance, emissions and exposure of the environment must be minimised. It

[1] Last checked on 8 February 2024

is noted that the 4-nonylphenol, branched, ethoxylates (a wide group of different UVCBs and polymers) have been identified as SVHC substances and are included in the Authorisation List. The sunset date for these substances was 4 January 2021. Hence, currently, the use of these substances is not allowed without a prior authorisation. This is expected to lead to a decrease in the tonnage of registered 4-NP as well as in its emissions to the environment.

The Risk Characterization Ratios (RCRs) reported by the registrants for freshwater in some of the exposure scenarios are still close to 1. The ES eMSCA acknowledges that RCR close to 1 does not always mean that the exposure in practice is close to that which could lead to effects in the environment as registrants may want to explore the limits of exposure whilst putting more protective measures in place in practice. However, considering that there is still some uncertainty in the efficiency of wastewater treatment plants (WWTP) to remove the Substance, and the Registrants have not used the worst-case estimates for WWTP efficiency, there is a risk that the exposure of the environment is underestimated. Furthermore, the aquatic PNEC value determined by the ES eMSCA is lower than the one reported by the Registrants and, as indicated above, the PNECs are not considered protective enough for the endocrine disrupting properties of the Substance.

In available environmental monitoring studies, 4-NP has been found widely in all environmental compartments (surface water, sediments and soil) and in biota from different trophic levels in Europe and other parts of the world. This indicates extensive exposure of the environment to the Substance. The exposure to 4-NP is likely a sum of emissions from uses of the Substance itself but also of uses of polymers and other substances where 4-NP is present as a constituent or impurity or that may degrade to 4-NP in the environment. It is not clear which uses contribute most to the observed environmental exposure to 4-NP. Further assessment of the current levels of emissions and exposure of total 4-NP, considering also the impact of the new authorisation requirement for 4-NP ethoxylates, is needed.

In addition to being toxic and an endocrine disrupter, based on the evaluation of the ES eMSCA, at least some of the isomers of 4-NP may also be persistent and bioaccumulative. Even though the Substance is already in the Candidate List, it is not expected to be prioritised for the inclusion into Annex XIV, at least not in the near future, as most of the tonnage is used as intermediate and in other uses (*e.g.*, in fuels) that fall outside the scope of Authorisation under REACH. Furthermore, even if the Substance is included in the Authorisation List at some point, the authorisation obligation will only apply to a small number of uses of the Substance with an expected low total volume. Hence, the REACH Authorisation for the Substance would address only a small share of the total emissions leading to exposure of the environment to 4-NP. Therefore, it is considered that further EU regulatory actions are needed to ensure minimisation of the total exposure of the environment to 4-NP, caused by emissions from uses of the Substance itself but also from uses of polymers and other substances that have 4-NP as a constituent or impurity or that may degrade to 4-NP in the environment.

4. Regulatory follow-up actions at EU level

4.1 Harmonised Classification and Labelling

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

4.3 Restriction

As indicated above, the Substance is mainly used as an intermediate in the manufacture of polymers and other derivatives, some of which have wide dispersive uses and high tonnages. The Substance may be present as an unreacted impurity in these polymers and derivatives. Furthermore, some of them may degrade and form 4-NP when released to the environment. Some uses of the Substance in mixtures by professional users and consumers may also take place. Based on the monitoring information, 4-NP is found in the environment, mostly at low concentrations but concentrations above the current PNEC and Environmental Quality Standard (EQL) values have been measured in some locations in the EU. The exposure to 4-NP is likely a sum of emissions from uses of the Substance itself but also of the other substances where 4-NP is present as a constituent or impurity or that may degrade to 4-NP in the environment. It is not clear which uses contribute most to the observed environmental exposure to 4-NP. Therefore, in order to minimise the overall exposure of the environment to 4-NP, a restriction, covering not only the Substance but a wider group of related substances, could potentially be an adequate follow up action. The identified need for restriction to address the wider group of alkylphenols, including substances containing 4-NP, has been included as an entry in the Restrictions Roadmap[2] under the European Commission's Chemicals Strategy for Sustainability. Furthermore, in ECHA's Assessment of regulatory needs report for the substances group "4-hydrocarbylphenols (other than styrenated phenols)" (ECHA 2023), which included the Substance, the following was concluded: *"A restriction of the substances as such, as constituents/impurities in other substances and in mixtures is seen as the most appropriate option as potential for exposure is expected from consumer, professional, and industrial uses (including potentially intermediate, monomer). Moreover, restricting substances in articles should be considered in the context of the restriction as potential exposure from articles needs further investigation first. In addition, inclusion of precursor substances that degrade under environmentally relevant conditions (e.g. 4-NP and 4-OP ethoxylates) or via metabolic transformation to members of this group would be recommended".*

Further assessment is needed to better understand the current emissions and exposure of 4-NP resulting from different uses of the whole group of relevant substances and the impact of the potential restriction on these.

4.4 Other EU-wide regulatory risk management measures

Not applicable.

5. Currently no need for regulatory follow-up at EU level

5.1 No need for regulatory follow-up at EU level

The Substance has already been identified as a SVHC due to endocrine disrupting properties in the environment. Therefore, it is considered to fulfil also the criteria for ED environment Category 1 in the amended CLP Regulation[3]. The registrants should self-classify the Substance accordingly. It should also be assessed whether the Substance may fulfil the CLP criteria for ED for human health, too.

[2] https://ec.europa.eu/docsroom/documents/49734

[3] COMMISSION DELEGATED REGULATION (EU) 2023/707 of 19 December 2022 amending Regulation (EC) No 1272/2008 as regards hazard classes and criteria for the classification, labelling and packaging of substances and mixtures

5.2. Other actions

The EQS under the Water Framework Directive, for 4-nonylphenol branched are set as a maximum allowable concentration (MAC) of 2.0 µg/L and annual average concentration (AAC) of 0.3 µg/L in all Surface Waters. The EQS of the Substance are currently under review and lower values have been proposed.

6. Tentative plan for follow-up actions

As indicated in Tables 3-1 the following regulatory action(s) at EU level are proposed.

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

Table 6-1 Follow-up actions

Follow-up action	Date for intention	Actor
Restriction	to be decided	to be decided

Part B. Substance evaluation report

In the substance evaluation report (part B), the document provides explanation how the evaluating MSCA assessed and drew the conclusions from the information available.

7. Overview of the Substance Evaluation Process

The Substance was placed on CoRAP to be evaluated in year 2014 by the UK as the evaluating Member State Competent Authority (UK eMSCA). Handover of the Substance Evaluation case to the Spanish CA (ES eMSCA) took place on 5 February 2019). In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA evaluated the Substance based on the information in the registration dossier(s) and on other relevant and available information.

There has been several regulatory evaluations of data available for the Substance previously and these were also taken into account in the Substance Evaluation, including:

- An EU risk assessment report (RAR) under the Existing Substances Regulation (ESR) (EC, 2002).
- An ESR RAR addendum by the Environment Agency in the UK which summarized soil organism toxicity data (EA, 2008).
- An unpublished interim Environment Agency report produced during 2009 that summarized fate, ecotoxicity and use scenario data that were not included in EC (2002) (EA, undated).
- A SVHC identification support document for the Substance (ECHA, 2012) and 4-nonylphenol, branched, ethoxylates (NPEOs) (ECHA, 2013).
- A restriction proposal for NPEOs in textiles (KEMI, 2013), including comments received during public consultation[4] and the opinion of ECHA's Risk Assessment Committee (RAC) (ECHA, 2014a) including its background document (ECHA 2014b).

Environment

The initial evaluation by the UK eMSCA under the Substance Evaluation process focussed primarily on environmental exposure but included an assessment of the available environmental fate and ecotoxicity data, particularly in comparison with the evaluations provided in the SVHC dossier and RAC opinion. Relevant recent monitoring data were gathered which included reports from monitoring projects undertaken by the UK eMSCA regarding the potential sources of the registered substance, removal in WWTP and environmental fate.

Human health

Hazard identification – The initial evaluation by the UK eMSCA under the Substance Evaluation process concentrated on any new information that had become available after the conclusion of the ESR RAR (EC, 2002), either in the registration dossier or in the public domain. The UK eMSCA performed a literature search covering the years 2002 – 2014 which revealed several pieces of literature discussing the reproductive effects of the registered substance which were considered in the evaluation. The DNEL values derived by

[4] The RCOM document with all the comments can be found at the following link: https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236e1806b0ddf

the Registrant(s) were also checked by the UK eMSCA to enable an assessment of their risk characterisation to be made.

Additional concerns identified during the initial evaluation.

For the environment, additional concerns were identified by the UK eMSCA about the level of protection against endocrine effects provided by the aquatic PNEC (ECHA, 2014a), the interpretation of environmental half-life data, aquatic risks from degradation of NPEO (as flagged by the RAC opinion (ECHA, 2014a)), and apparent deficiencies in the data sets available for assessing wastewater treatment plant (WWTP) partitioning, bioaccumulation, sediment organism toxicity, soil organism toxicity and secondary poisoning.

For human health, issues were identified regarding the Registrant(s) DNEL derivation and their exposure estimates, particularly for the inhalation route. Additional information was required to ensure that the potential risks to workers and consumers are being sufficiently managed.

The UK eMSCA considered that further information was required to clarify the identified concerns. Therefore, a Decision to request further information was issued according to Article 46 on 29 April 2016.

On 28 July 2016, Registrants appealed some parts of the decision which resulted in two different deadlines:

The non-appealed requirements were:

1. Information for PBT assessment: Analytical information for 2,4-dinonylphenol impurity

2. Information for environmental fate:
   2.1. Surface tension measurement
   2.2. Sewage treatment efficiency assessment
   2.3. Updated robust study summaries for bioaccumulation information

3. Information for human health assessment:
   3.1. Justification for the choice of oral bioavailability value used in the DNEL derivation
   3.2. Further information on exposure

   These data were due to be submitted by 6 November 2017.

On 6 June 2018, the ruling of the BoA was issued, annulling the requirements for the registrants to collect any information on polymers from their downstream users. However, other remaining requests were upheld under this decision:

4. Information for environmental exposure assessment
5. Information for environment PNECs

The revised deadline for these requests was 14 December 2019.

Between 31st October and 22nd November 2017, in accordance with Article 46(2) of REACH, the registrant(s) submitted eleven updates for the different registrations that were received by ECHA. However, as the follow-up part of the evaluation process officially only commences once all the data requests in the decision were received, the 12 months follow-up evaluation period did not start due to the requests delayed by the appeal. The last draft SEv report provided by UK dated 2015.

On 5 February 2019, due to the UK leaving from the European Union, the Spanish Competent Authority took over the environmental part of the Substance Evaluation of the Substance.

Spain as new eMSCA took into account the previous assessment performed by the UK eMSCA, the latest updated registration dossiers and other relevant and available information. ES eMSCA informed registrants on the change of the eMSCA and, on several occasions, reminded them on the need to submit a dossier update including a study on the Long-term and transgenerational effects of nonylphenol exposure via partition-controlled delivery to Eastern oyster (*Crassotrea virginica*) to meet the SEV decision's requests.

On 7 October 2022, Registrants sent directly to the ES eMSCA the information on the oyster study but no definitive registration dossier update including all the requested information has been successfully submitted to ECHA so far[5].

On 15 February 2024, the ES eMSCA concluded the Substance evaluation case even though the 12 months period to conclude the assessment did not start due to lack of official submission of all the information requested in the SEV Decision by the Registrants (Article 46(4) of REACH).

8. Substance identity

The information on the Substance, including identifiers and structural formula, can be found on the cover page. For more details see ECHA: https://echa.europa.eu/home

Synonyms:

Para-nonylphenol

P-nonylphenol

Isononylphenol

8.1. Type of Substance

UVCB.

8.2. Other relevant information

Table 8-1 Other information relevant to the composition of the Substance

Type	Identity	Typical concentration	Concentration range	Remarks
Constituent	*Phenol, 4-nonyl-, branched* *EC 284-325-5*	Confidential information	Confidential information	

4-Nonylphenol, branched, is a substance consisting of a phenol ring and a nine-carbon chain on the para-position. The C_9H_{19} chain may exist as a number of different branched isomers. 4-NP branched, has been registered as a mono-constituent substance even though all the registrations include the EC 284-325-5 corresponding to 4-nonylphenol branched. According to the EU RAR (EC, 2002) for 4-NP branched, given the method of manufacture of nonylphenols, very little if any straight chain nonylphenol is produced. Due to the complexity of the substance including possibly more than hundred branched isomers

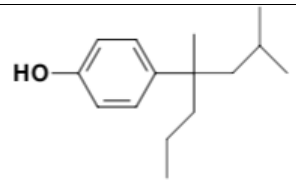
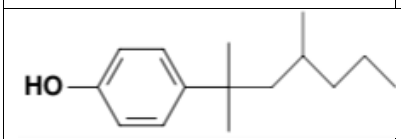
[5] last checked on 8 February 2024.

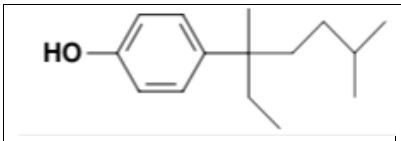
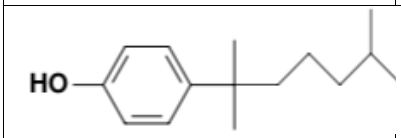
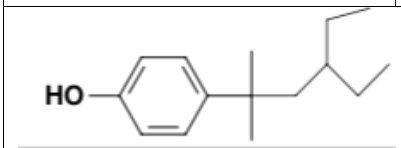
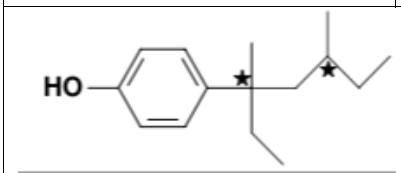
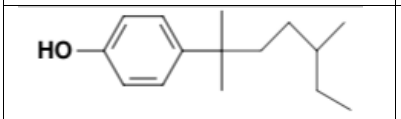
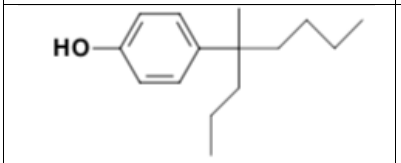
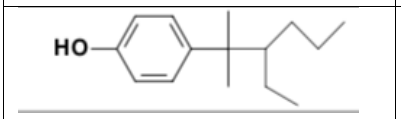
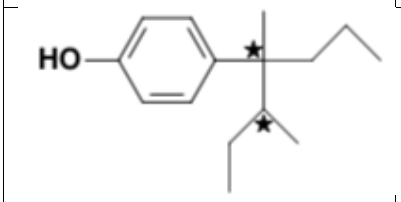
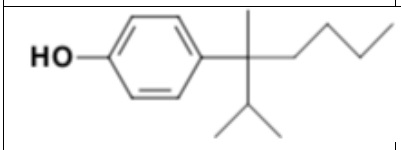
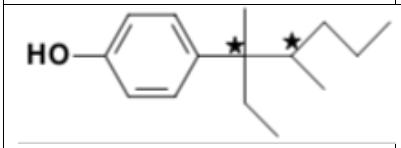
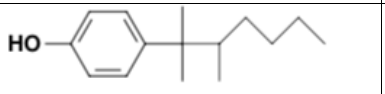
(see below), branched 4-NP is considered by the ES eMSCA as an UVCB substance in this follow up assessment.

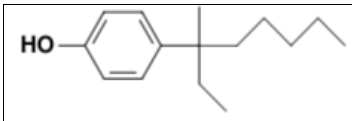
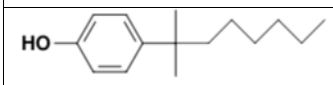
The registration dossier does not contain any information on the degree and type of branching of the alkyl chains. Information on possible isomers was searched from open literature. Based on the available information, the number of possible isomers is high. According to Guenther et al. (2006), theoretically 211 constitutional isomers are possible and when taking into account also the different optical isomers, in total 550 isomers are possible. Wheeler et al. (1997), used high-resolution gas chromatographic (GC) analyses of p-nonylphenol and achieved resolution of 22 major para-isomers that were divided in 5 groups based on the alpha-carbon and beta carbon substitution. The authors propose also structures for these 22 isomers, although it is noted that it was not possible to confirm the exact structure. In other studies, some specific isomers, having the highest concentrations, have been identified. Thiele et al. (2004) separated isothermally p-nonylphenol into 21 isomers that could be classified into six groups with respect to different configurations of the alpha- and beta-C-atoms on the alkyl chains. Based on these basic structures nonylphenol isomers were synthesized and also characterized by gas-chromatography coupled with mass spectrometer (GC-MS). The obtained data elucidated the complete structures of 10 nonylphenol isomers of the technical mixture. It is noted that many isomers may co-elute in chromatographic analyses and hence the peaks may contain more than one isomer. In more recent studies (e.g., Eganhouse et al. 2009, Ieda et al. 2005) with more advanced analytical methods (e.g., two-dimensional GC) further separation of the peaks has been possible. Eganhouse et al. (2009) separated 59-66 peaks assigned to 4-NP isomers and Ieda et al (2005) 102 peaks of NP isomers in technical NP mixtures. Eganhouse et al. (2009) point out that these numbers of isomers should be considered as minimum as it is likely that other isomers, not found in the study, are present in trace amounts. Very closely related isomers can still coelute in the GC × GC analysis. Eganhouse et al. (2009) identified 21 major isomers, 18 of which have known structures. They also detected six significant minor isomers whose exact structure could not be identified. However, some of these seem to have higher degree of branching than the identified 18 major isomers. In conclusion, the number of different branched 4-NP isomers seem to be very high.

Based on the available information on the different structures, the most common isomers have two substitutions (mostly methyl and ethyl, but also longer chains are possible) in the alpha-carbon. The 18 major isomers identified in Eganhouse et al. 2009 are shown in Table 8-2. Information on the concentrations of these isomers in technical NP reported in different studies can be found in the review article by Lu and Gan (2014a).

Table 8-2 Structures of the 18 major 4-NP isomers identified by Eganhouse et al. (2009). Some of the isomers are stereoisomers and the stars indicate the location of asymmetric carbon atoms.

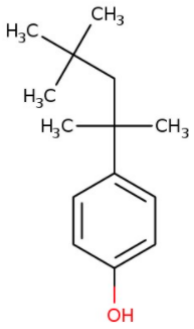
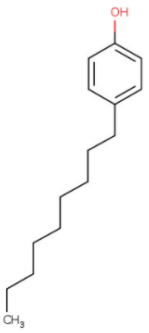
Structure	Short name	IUPAC name	Smiles
	4-NP194	4-[1,3-dimethyl-1-n-propylbutyl]phenol	CCCC(C)(CC(C)C)c1ccc(O)cc1
	4-NP36	4-[1,1,3-trimethylhexyl]phenol	CCCC(C)CC(C)(C)c1ccc(O)cc1

	4-NP112	4-[1-ethyl-1,4-dimethylpentyll]phenol	CCC(C)(CCC(C)C)c1ccc(O)cc1
	4-NP38	4-[1,1,5-trimethylhexyl]phenol	CC(C)CCCC(C)(C)c1ccc(O)cc1
	4-NP128	4-[3-ethyl-1,1-dimethylpentyl]phenol	CCC(CC)CC(C)(C)c1ccc(O)cc1
	4-NP111a,b	4-[1-ethyl-1,3-dimethylpentyl]phenol	CCC(C)CC(C)(CC)c1ccc(O)cc1
	4-NP37	4-[1,1,4-triimethylhexyl]phenol	CCC(C)CCC(C)(C)c1ccc(O)cc1
	4-NP152	4-[1-methyl-1-n-propylpentyl]phenol	CCCCC(C)(CCC)c1ccc(O)cc1
	4-NP119	4-[2-ethyl-1,1-dimethylpentyl]phenol	CCCC(CC)C(C)(C)c1ccc(O)cc1
	4-NP193a,b	4-[1,2-dimethyl-1-n-propylbutyl]phenol	CCCC(C)(c1ccc(O)cc1)C(C)CC
	4-NP143	4-[1-isopropyl-1-methylpentyl]phenol	CCCCC(C)(c1ccc(O)cc1)C(C)C
	4-NP110a,b	4-[1-ethyl-1,2-dimethylpentyl]phenol	CCCC(C)C(C)(CC)c1ccc(O)cc1
	4-NP35	4-[1,1,2-trimethylhexyl]phenol	CCCCC(C)C(C)(C)c1ccc(O)cc1

	4-NP65	4-[1-ethyl-1-methylhexyl]phenol	CCCCCC(C)(CC)c1ccc(O)cc1
	4-NP9	4-[1,1-dimethylheptyl]phenol	CCCCCCC(C)(C)c1ccc(O)cc1

8.3. Analogue substance (read-across)

Table 8-3 Relevant analogue substance(s)

EC/List number	CAS RN	Public Substance name	Chemical structure
205-426-2	140-66-9	4-(1,1,3,3-tetramethylbutyl)phenol	
203-199-4	104-40-5	p-nonylphenol (4-n-NP)	

In order to assess the feasibility of read across from 4-(1,1,3,3-tetramethylbutyl)phenol (EC 205-426-2) (4-t-OP) to 4-NP, information was searched on the presence of 4-NP isomers with similar branching as 4-t-OP, i.e. two quaternary carbons in the alkyl chain, one in the alpha position and another one in the terminal position. This type of isomers for 4-NP were suggested in Wheeler et al. 1997, Acir et al. (2016) (**Error! Reference source not found.**a) and Lu and Gan (2014a), Russ et al. (2005), Shioji et al (2006) (**Error! Reference source not found.**b). The two isomers of 4-NP in **Error! Reference source not found.** are very similar to 4-t-OP. The first one differs only by having one methyl and one ethyl group in the alpha-C instead of two methyls as in the 4-t-OP. The second one differs only by having one carbon more in the linear chain between the two quaternary-Cs. In Russ et al. (2005) and in Shioji et al. (2006), the presence of these isomers in commercial technical NP mixtures was confirmed although based on the results of Russ et al. (2005) the concentration seemed to be low, below 1%. Shioji et al. (2006) synthetised some further similarly branched isomers, but they were not detected in the technical NP mixture in their analysis. However, as indicated before, due to co-elution of similar isomers, detection of some isomers, especially of the ones with low concentration, may be challenging.

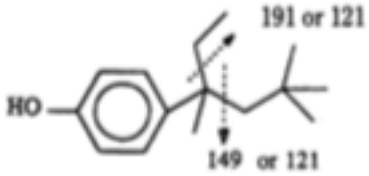
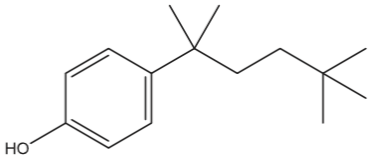
A)	B)
 CCC(C)(CC(C)(C)C)c1ccc(O)cc1	 CC(C)(C)CCC(C)(C)c1ccc(O)cc1

Figure 1 Isomers of 4NP with similar branching as 4-t-OP reported by Wheeler et al. (1997) (a) and Lu and Gan 2014, Russ et al. (2005), Shioji et al. (2006) (b).

Based on the information available in the ECHA dissemination site for 4-t-OP, the physico-chemical properties of 4-t-OP and the similarly branched isomers of 4-NP are expected to be similar. 4-t-OP has an experimental log Kow of 4.8 (HPLC study) while a log Kow of 5.4 is determined for branched 4-NP (mixture of isomers). According to the KOWWIN QSAR models performed for the example constituents of 4-NP, the log Kow values of all isomers are expected to be similar (log Kow values of 5.77-5.88 predicted for the different example isomers). The water solubility of 4-t-OP and branched 4-NP were determined to be 7 and 5.4 mg/L, respectively, in studies following the same standard guideline ASTM E 1148-02. Therefore, the 4-NP isomers with similar branching as 4-t-OP (Figure 1) can be expected to show similar degradation as 4-t-OP and one to one read across can be applied to these isomers. Also it cannot be excluded that degradation of other highly branched isomers of 4-NP could be similar to that of 4-t-OP. However, as the effect of different branching in the degradation is not well known, information on 4-t-OP can be used as supporting evidence in the weight of evidence assessment of the other branched isomers of 4-NP.

Regarding p-nonylphenol (EC 203-199-4), it is registered as a monoconstituent substance consisting of the linear isomer of 4-NP (4-n-NP). Studies with 4-n-NP have been included as read across studies in the registration dossier for different endpoints. However, the effects and environmental fate and behaviour of the linear and branched isomers of 4-NP are expected to differ significantly. Therefore, the eMSCA does not consider a one-to-one read across valid, although for some end points information on 4-n-NP can be used as supporting information.

9. Physicochemical properties

Table 9-1 Overview of physicochemical properties

Property	Value
Molecular weight/weight range	220.35
Physical state at 20°C and 101.3 kPa	Clear yellow liquid with phenolic odour.
Vapour pressure	0.01 mBar (1 Pa) at 38ºC (ASTM-D 2879)
Water solubility	5.7 mg/L at 25ºC (pH 6-7) (ASTM E 1148 – 02, flask method)
Partition coefficient n-octanol/water (Log K_{ow})	5.4 at 23 °C (pH of 5.7) (OECD TG 117) 5 at 40°C (OECD TG 117)
Partition coefficient organic carbon/water (Log K_{oc})	4 (OECD TG 106)
Surface tension	38.9 mN/m at 20.1 °C (OECD TG 115). The test item is regarded as being surface-active

	material according to Regulation (EC) No 440/2008.

According to the results of the OECD TG 115 study, the Substance has surface active properties. This adds some uncertainty to the experimental log Kow value determined using the HPLC method (OECD TG 117). According to the OECD TG 117, the method is not applicable for surface active substances. KOWWIN QSAR model performed by the eMSCA for the example constituents of branched 4-NP predicts log Kow values in the range of 5.77-5.88. In the EU RAR (EC 2002) log Kow values in the range of 3.28-5.76 from different published studies are mentioned and a log Kow of 4.48 was chosen to be used for environmental modelling purposes. This log Kow value comes from a study by Ahel and Giger (1993) that used shake flask method for technical NP. It is noted that shake flask method is not applicable either for surface active substances. Furthermore, the method is recommended to be used only for log Kow values up to 4. Therefore, there are uncertainties in the log Kow value determined in the Ahel and Giger (1993) study.

10. Manufacture and uses

10.1. Quantities

The aggregated tonnage (per year) of the Substance is 10,000 - 50,000 tonnes.

10.2. Overview of uses

Table 10-1 Overview of uses

Main uses	Key information
Formulation	Formulation of adhesives, paints, coatings and inks, fuels, fuel dyes, used as intermediate
Industrial	Used as intermediate e.g. in manufacture of ethoxylates and polymers Use in adhesives, paints, coatings and inks, End-use of paints containing nonylphenol ethoxylates Use in water treatment and oilfield sites Application of tackifier in manufacture of tyres and rubber products Functional fluids Use of ethoxylate in emulsion polymerization
Professional	Use in adhesives, paints, coatings, inks, fuel
Consumer	Use in adhesives, paints, coatings, inks, fuel
Article service life	Articles containing paints, coatings and adhesives, machinery

The Substance appears to be mostly used as intermediate in manufacture of nonylphenol ethoxylates and other derivatives. In some of the end uses in adhesives, paints and coatings, it is indicated that the products contain ethoxylates. Hence, it is not clear whether the Substance as such is present in the mixtures and articles or whether the uses are actually for the ethoxylates.

The SPIN database indicate the following activities for industrial use of 4-NP in 2020 (SPIN and SCIP databases, 2022-accessed): manufacture of chemicals and chemical products, repair and installation of machinery and equipment, machinery and mechanical appliances;

electrical equipment parts thereof; sound recorders and reproducers, television image and sound recorders and reproducers, and parts and accessories of such articles civil engineering, nuclear reactors, boilers, machinery and mechanical appliances; parts thereof > Spark-ignition reciprocating or rotary internal combustion piston engines > Marine propulsion engines > Outboard motors. Other uses also mentioned in the SPIN database before 2018 are manufacture of rubber and plastic products, manufacture of fabricated metal products, except machinery and equipment, specialised construction activities, construction of buildings, undifferentiated goods- and services-producing activities of private households for own use, wholesale and retail trade and maintenance and repair of motor vehicles.

Uses advised against in the registration dossier are those specified in entry 46 of REACH Annex XVII which was a carry-over from previous EU directives and based on protection of the environment. This entry indicates that NP and NPEs shall not be placed on the market, or used, as substances or in mixtures in concentrations equal to or greater than 0,1 % by weight for the following purposes:

1. industrial and institutional cleaning except: — controlled closed dry cleaning systems where the washing liquid is recycled or incinerated,— cleaning systems with special treatment where the washing liquid is recycled or incinerated.
2. domestic cleaning;
3. textiles and leather processing except: — processing with no release into wastewater,— systems with special treatment where the process water is pre-treated to remove the organic fraction completely prior to biological wastewater treatment (degreasing of sheepskin);
4. emulsifier in agricultural teat dips;
5. metal working except: — uses in controlled closed systems where the washing liquid is recycled or incinerated;
6. manufacturing of pulp and paper;
7. cosmetic products;
8. other personal care products except: — spermicides;
9. co-formulants in pesticides and biocides. However national authorisations for pesticides or biocidal products containing nonylphenol ethoxylates as co-formulant, granted before 17 July 2003, shall not be affected by this restriction until their date of expiry.

11. Classification and labelling

Table 11-1 Classification of the Substance

Harmonised classification (Annex VI of CLP)	Self-classification in registrations	Self-classification in C&L notifications
Acute Tox. 4, H302 Skin Corr. 1B, H314 Aquatic Acute 1, H400 Aquatic Chronic 1,H410 Repr. 2, H361fd	Eye Damage 1, H318 Aquatic Acute 1, H400, M(acute)=10 Aquatic Chronic 1, H410, M(chronic)=10	STOT SE 3, H335 (respiratory system)

12. Environmental fate properties

12.1. Degradation

Weight-of-evidence assessment was performed for the persistence assessment of the Substance. An overview of the available (most relevant) studies, the results and conclusions are presented in this section. Further information and assessment of the available studies can be found in the Annex 1.

12.1.1. Abiotic degradation

No experimental data on hydrolysis is available. HYDROWIN cannot be performed as 4-NP does not contain any of the functional groups included in the model. It is noted that in the phototransformation study by Martinez-Zapata et al. (2013), 25% of the test substance 4-n-nonylphenol incubated in ultra-pure water in darkness disappeared at pH 9 while at pH 5 < 10% disappeared. The authors suggested that this could indicate that hydrolysis took place and was higher at pH 9. However, as 4-NP does not contain any readily hydrolysable groups, it is not considered a significant degradation process.

The predicted half-life for phototransformation in air is less than 2 days. Based on the available non-guideline studies (one with branched 4-NP and two with linear 4-NP), 4-NP may be photodegraded in the surface layers of natural water. However, due to the number of factors that affect photodegradation rates in the environment, this process is not generally considered in the persistence assessment under REACH.

Table 12-1 Overview of the available information on abiotic degradation

Type of Evidence	Test material	Test method	Results	Consistency & Specificity	Likelihood/ Biological Plausibility	Confidence / Strength of Evidence	Remaining Uncertainty
AOP Program (v1.92) QSAR model	Example isomers of branched 4-NP	QSAR	half-life of 0.2 days (assuming 12-hr day and a concentration of 1.5 x 106 OH radicals/cm3 in the atmosphere)	Prediction for atmospheric phototransformation	Plausible	Medium	Low
Laboratory and field experiment on photochemical degradation in aqueous solution, Ahel et al (1994)	branched 4-NP	Non standard guideline study	Field experiment-. Half-life of 15-20 h corresponding to a continuous sunlight intensity of 0.700 kW/m2 Laboratory experiment: rate constants for photolysis in distilled water (direct photolysis) 0.20 h-1 filtered lake water (indirect photolysis) 0.92 h-1	Consistent experimental studies indicating that phototransformation in water of 4-NP may occur	Plausible	Medium	Medium
Laboratory experiment on photochemical degradation in aqueous solution, Martinez-Zapata et al (2013)	linear 4-n-NP	Non standard guideline study	Direct photolysis: 98% degradation after 19 h at pH 5 and 97% after 19 h at pH 9 Indirect photolysis: Up to approx. 70% degradation in the natural waters after 5 h	Consistent experimental studies indicating that phototransformation of 4-n-NP and 4-NP in water may occur	Plausible	Medium	Medium
Laboratory experiment on photochemical degradation in aqueous solution, Dulov et al (2013)	linear 4-n-NP	Non standard guideline study	Direct and indirect phototransformation with hydrogen peroxide (H202) and Fe2+as sensitizers: The time taken for 90% conversion of 4n-NP in all the experiments ranged from 12 minutes to > 180 minutes	Consistent experimental studies indicating that phototransformation of 4-n-NP in water may occur	Plausible	Medium	Medium
Conclusion from overall confidence	Based on medium confidence studies (one with branched 4-NP and two with linear 4-NP) 4-NP may be photodegraded in the surface layers of natural water. Based on medium confidence QSAR prediction, branched isomers of 4-NP have phototransformation half-lives in air of less than 2 days.						

12.1.2. Biodegradation

Estimated data and screening tests.

Results of two OECD TG 301B studies show that significant degradation (47.5 -48.2%) of 4-NP (CAS RN 84852-15-3; branched Nonylphenol) occurred (as measured by CO2 evolution) after 28 and 35 days. However, adaptation of the inoculum is indicated by the authors of the studies (Unnamed, 1999a; Staples et al., 2001). In an OECD TG 301F test by Staples et al. (1999) 4-NP (CAS RN 84852-15-3; branched Nonylphenol) reached 62 % degradation after 28 days based on oxygen consumption. However, it is not confirmed whether the inoculum was pre-adapted to the substance. Based on publicly available information on the sewage treatment plant where the activated sludge used as inoculum was collected, pre-adaptation to alkylphenols cannot be excluded. Studies using adapted inoculum are not considered adequate for the P assessment.

Stasinakis et al. (2009) assessed degradation of the linear 4-NP (CAS RN 104-40-5) in a study following OECD TG 301F. However, it should be noted that linear 4-NP may be more easily degraded than branched 4-NP isomers, which may show steric hindrance due to branching. Consequently, the study is not considered relevant for the evaluation of branched 4-NP.

For the read across substance 4-t-OP, OECD 301B study (unnamed 1999b, Staples et al 2001) with the same inoculum adapted to alkylphenols that was used in the OECD 301B test with 4-NP (Unnamed, 1999a; Staples et al, 2001) is available. In this test 62 % degradation of 4-t-OP was observed after 28 days. However, due to the adapted inoculum, the test is not considered adequate for the P assessment. In other two screening tests with 4-t-OP, in a BODIS test and an OECD TG 302C test, 20% and 0 % degradation was observed after 28 days, which indicate potential persistence of 4-t-OP. Some of the isomers of 4-NP are very similar to 4-t-OP and hence similar degradation can be expected for them. It is noted that the test substance concentration (27.5-30 mg/L) in the BODIS and OECD 302C tests were well above the water solubility of 4-t-OP (7 mg/L), and hence, the bioavailability of the test substances could have been reduced. Also, according to ECHA Guidance R.7b, the pre-treatment of the inoculum such as in the MITI test (OECD 301C) may seriously lower the diversity and biodegradation capacity of the microbes. In OECD 302 similar pre-treatment of inoculum is used, and hence, this may cause some uncertainty to the interpretation of the results of the OECD 302C test.

The BIOWIN QSAR models performed for the example constituents of 4-NP support that there may be differences between the degradation of different isomers of 4-NP and that some of them may be potentially P/vP.

Table 12-2 Overview of the available screening information on biodegradation

Type of Evidence	Test material	Test method	Results	Consistency & Specificity	Likelihood/ Biological Plausibility	Confidence / Strength of Evidence	Remaining Uncertainty
Ready biodegradation test, IUCLID dossier (Unnamed 1999a) and Staples et al. (2001)	Branched 4-NP	OECD TG 301B	47.5 % degradation after 28 d 48.2% degradation after 35 d (based on CO2 evolution) Only low concentrations (18 µg/L, initial concentration 13 mg/L) of test substance remained after 28 d and by day 35 4-NP was not detected anymore Pre-exposed inoculum	Consistent with other experimental studies indicating that when pre-exposed inoculum is used, high level of degradation is observed	Plausible	Low	High The degradation may be slower in non-adapted inoculum, The degradation of isomers with different types and degrees of branching may differ
Ready biodegradation test, IUCLID dossier, Staples et al. (1999)	Branched 4-NP	OECD TG 301 F	62 % degradation after 28 days (based on O2 consumption) Pre-exposure of inoculum likely	Consistent with other experimental studies indicating that when pre-exposed inoculum is used, high level of degradation is observed	Plausible	Low	High The degradation may be slower in non-adapted inoculum, The degradation of isomers with different types and degrees of branching may differ
Read across ready biodegradation test, IUCLID dossier, Stasinakis et al. (2008)	linear 4-n-NP.	OECD TG 301 F	61.5 % degradation after 28 days (based on O2 consumption)	Consistent with other experimental studies indicating that linear 4-n-NP is readily degradable	Plausible	Low	High The degradation of the linear 4-n-NP isomer may be faster than that of branched isomers

Read across ready biodegradation test, ECHA dissemination site, Unnamed 1999b, Staples et al. (2001)	4-t-OP	OECD TG 301B	62% degradation after 28 days (based on O2 consumption) Pre-exposed inoculum	Inconsistent with other two screening studies with 4-t-OP showing slow degradation. Consistent with other experimental studies indicating that when pre-exposed inoculum is used, high level of degradation is observed	Plausible	Low	High The degradation may be slower in non-adapted inoculum, The degradation of 4-NP isomers with other type and degree of branching than 4-t-OP may be different
Read across ready biodegradation test (Unnamed, 1991a)	4-t-OP	ISO Draft (BOD Test for Insoluble Substances) guideline (BODIS test)	20 % degradation after 28 days (based on O2 consumption)	Consistent with another experimental study indicating that when non-adapted inoculum is used the degradation may be slower	Plausible	Medium	Medium The degradation of 4-NP isomers with other type and degree of branching than 4-t-OP may be different
Read across inherent degradation test **(unnamed, 1991b)**	4-t-OP	OECD TG 302C	0 % degradation after 28 days	Consistent with another experimental study indicating that when non-adapted inoculum is used the degradation may be slower	Plausible	Medium	Medium The degradation of 4-NP isomers with other type and degree of branching than 4-t-OP may be different
QSAR prediction on ready biodegradation	Example isomers of branched 4-NP	BIOWIN QSAR models	Results for different example constituents: BIOWIN 2: 0.07-0.71 BIOWIN 3: 2.34-2.85 BIOWIN 6: 0.23-0.43	Consistent predictions that there are differences in the degradation of 4-NP isomers and that some of them may degrade slowly	Plausible	Medium	Low
Conclusion from overall confidence	Consistent evidence showing that there may be differences in the degradation of linear and different isomers of branched 4-NP. Consistent evidence showing that when pre-exposed inoculum is used, high level of degradation of the whole 4-NP branched and of the read across substance 4-t-OP may occur in ready biodegradation tests. Consistent, medium confidence evidence showing that when non-adapted inoculum is used, degradation of the read across substance 4-t-OP may be slower. Similar degradation could be expected for 4-NP isomers with similar branching as 4-t-OP.						

In conclusion, based on the available screening information, some branched 4-NP isomers may be inherently or readily biodegradable. However, other more branched isomers, e.g. those with similar branching as 4-t-OP, may degrade slower and are considered to screen potentially P/vP.

Biodegradation in water and sediment

No standard guideline simulation degradation studies are available for 4-NP branched. Several degradation studies in freshwater and seawater as well as in sediment systems from open literature are available for branched 4-NP, linear 4-NP and the similar substance 4-t-OP. An overview of the most relevant studies is shown in the below table. Further studies considered in the weight-of-evidence assessment but considered less relevant can be found in the Annex 1. Based on the available information it can be concluded that there are differences in the degradation of different isomers of 4-NP. Therefore, the results for the linear 4-n-NP isomer or for a mixture of branched 4-NP as a whole cannot be used to conclude on the persistence of all isomers of branched 4-NP. It can be expected that the isomers with the bulkiest alkyl chains, and possibly higher degree and longer branching in the α–carbon, could be the ones that degrade slower in most environmental matrices. 4-t-OP and 4-NP isomers with similar branching are expected to show similar degradation.

Table 12-3 Overview of the most relevant surface water and water-sediment simulation studies

Type of Evidence	Test substance	Test medium	Results	Consistency & Specificity	Likelihood/ Biological Plausibility	Confidence / Strength of Evidence	Remaining Uncertainty
Read across from 4-t-OP, freshwater and freshwater – sediment microcosms study, IUCLID dossier, Johnson et al. (2000)	4-t-OP	Freshwater (aerobic) Freshwater and sediment (anaerobic)	Water: DT50s (at 20°C) zero-order kinetics: 8- 71 d, DT50s (at 12°C) first order kinetics: 17.4 d for Calder river, 23.7, 38.8 and **43.1 d** for Thames river and **109.1 d** for Aire river. Sediment (anaerobic): no degradation	High variation in the reported degradation half-lives. Consistent with Ying and Kookana (2003) and with the OECD TG 302C and BODIS tests indicating that degradation of 4-t-OP may be slow at least in some water samples. Consistent with other studies indicating that degradation in anaerobic sediments is slow.	Plausible	Medium	Medium Not clear what may have caused the high variation in the half-lives and most based on zero-order kinetics, which cannot be used for comparison with A.XIII criteria. High initial concentration 100 µg/L, low test medium volume, static conditions Sampling sites in Calder and Aire rivers have industrial/urban influence, Thames river in more rural area
seawater and seawater-sediment microcosms study IUCLID dossier, Ekelund et al. (1993)	Branched ^{14}C-4-NP	Seawater (aerobic) Seawater and sediment (aerobic and semi-anaerobic)	Water: lag phase first 4 weeks (0.06% day^{-1}), after that degradation increased to 1% day^{-1}, approx. 50 % degradation after 8 weeks (based on	Consistent with other studies indicating that degradation in water may be slower than in aerobic sediments. Consistent with other studies indicating that degradation in	Plausible	Low	High Low recovery of 14C: in experiment with only water 64% of applied radioactivity (AR), in experiment with sediment 49 % of AR.

			14CO2) at 11°C. →DT50 **71 d** at 9°C (based on 14CO2) <u>Sediment (aerobic):</u> faster degradation from the beginning (1.2% day^{-1}), approx. 40 % degradation after 8 weeks <u>Sediment (semi-anaerobic):</u> approx. 20 % degradation after 8 weeks	anaerobic sediments is slow			Results given based on total 14CO2, although it is indicated that all the radioactivity trapped in the CO2 traps did not necessarily represent CO2 but that other volatile metabolites may also have been present. Nevertheless, the primary degradation may have occurred faster that the results based on 14CO2 indicate. Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers.
Read across from linear 4-n-NP and 4-t-OP, seawater and seawater-sediment slurry microcosms study, **IUCLID dossier,** **Ying and Kookana (2003)**	Linear 4-n-NP and 4-t-OP	Seawater (aerobic) Seawater and sediment (aerobic and anaerobic)	<u>Seawater, (without air-bubbling of the vessels):</u> 4-n-NP DT50 5 d at 20°C→ 14 d at 9°C 4-t-OP DT50 60 d at 20 °C→ **171 d** at 9°C (visually determined DT50 ca. 21d at 20 °C→ **60 d** at 9°C)	Consistent with other information indicating that linear 4-NP can degrade faster than 4-t-OP. Consistent with Johnson et al (2000) and with the OECD TG 302C and BODIS tests indicating that degradation of 4-t-OP may be slow at least in some water samples.	Plausible	Medium	Medium Water only: Mixture exposure with the two substances Some uncertainty in the DT50 of 60 d at 20°C reported in the article for 4-t-OP as based on the figure a DT50 of 21 d at 20°C seems more adequate.. Water-sediment slurry: Mixture exposure with 5

			Seawater+sediment: (aerobic): 4-n-NP DT50 5.8 d at 20°C → 16.5 d at 9°C 4-t-OP: slow phase first 21 days, followed by a quick phase where approx. 90% degradation reached within a week Seawater+sediment: (anaerobic): No degradation	Consistent with other studies indicating that degradation in anaerobic sediments is slow			substances
Freshwater sediment slurry microcosm study, IUCLID dossier, De Weert et al. (2010a)	Branched 4-NP present in the sediment, added 4-n-NP and t-NP	Freshwater+ sediment (aerobic)	Branched 4-NP present in the sed: DT50 1.1-1.9 d at 30°C → 5-8 d at 12°C	Consistent with other studies indicating that mixture of branched 4-NP as a whole can degrade rapidly in pre-exposed sediments	Plausible	Low	High Pre-exposed sediment water-sediment slurry. over 50% decrease in 17 days in sterile controls Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers.
Freswater-sediment slurry microcosm desorption-degradation study, IUCLID dossier, De Weert et al (2010b)	Branched 4-NP present in the sediment	Mineral medium (or freshwater)+ sediment (aerobic)	Approx. 50% decrease both in sterile and non-sterile test vessels during first 2 days. After that, 100% decrease after 4 days in non-sterile vessels while in sterile control the concentrations remained stable.	Consistent with other studies indicating that mixture of branched 4-NP as a whole can degrade rapidly in (pre-exposed) sediments.	Plausible	Low	High Pre-exposed sediment. Significant decrease in sterile control. Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers.

Water-sediment microcosm study, IUCLID dossier, Lu and Gan (2014b)	different branched 4-NP isomers	Mineral medium+ sediment (aerobic and anaerobic)	Sediment A (aerobic): DT50 values of different 4-NP isomers approx. 1-13 days (at 21°C). Isomers with short side chain and bulky α-substituents generally were more persistent. Sediment B (aerobic) a lag phase/slower degradation phase during the first 7−14 days followed by a faster degradation phase with half-lives in the range of 15-20 days (at 21°C) Sediment A and B (anaerobic): Negligible degradation	Consistent with other studies indicating that the degradation of different isomers differs and that under anaerobic conditions degradation is slow.	Plausible	Medium	Medium Gently vortexed periodically Sediment B pre-exposed to NP
Freshwater sediment slurry microcosm study, IUCLID dossier, De Weert et al. (2011)	Branched 4-NP present in the sediment, added 4-n-NP and tNP	Freshwater+ sediment (anaerobic)	No degradation of branched 4-NP	Consistent with other studies indicating that degradation in anaerobic sediments is slow	Plausible	Medium	Low Pre-exposed sediment.
Freshwater sediment microcosms with and without	technical NP	Freshwater+ sediment, vegetated and unvegetated	unvegetated sediment without plants: 9.2% of tNP disappeared after 42 days (at 28°C)	Degradation slower in (unvegetated) sediment than in most other available studies	Plausible	Medium	Medium Results for the whole mixture of branched 4-NP cannot be used to

***Phragmites australis* plants, Toyama et al (2011)**			Vegetated sediment: 53 % of tNP disappeared after 42 days (at 28 °C) Both in vegetated and unvegetated sediments, several peaks in GC-MS analysis decreased during the 42d study but other peaks remained almost unchanged.	Consistent with other studies indicating that the degradation of different isomers of 4-NP differs			conclude on all isomers
Freshwater sediment microcosm study, Dutka et al. (1998)	Branched NP	Freshwater sediment (aerobic and anaerobic)	<u>Aerobic non-spiked sediment (NP present in the sediment):</u> NP levels decreased approximately 80-85% in one month and after that remained stable until 6 months. <u>Aerobic spiked sediment:</u> 15 and 30% increase at one and 3 months, after 6 months decreased to approx. 50% <u>Anaerobic sediments:</u> No decrease of NP	Results of the non-spiked sediment consisted with other studies with pre-exposed sediments showing rapid degradation. Results of the spiked sediment inconsistent with the results of the non-spiked sediment. Consistent with other studies indicating that degradation in anaerobic sediments is slow or negligible.	Plausible in general, but not clear what caused the initial increase of NP level in the spiked sediment.	Low (aerobic) Medium (anaerobic)	High Continuous shaking Pre-exposed sediment. Other dissipation processes cannot be excluded
Conclusion from overall confidence	Consistent evidence that the degradation of different isomers may differ. Low to Medium confidence that at least some of the branched isomers of 4-NP may be persistent in freshwater and sea water. Consistent evidence from low to medium confidence studies indicating that at least most of the isomers of 4-NP can be relatively quickly degraded in pre-exposed sediments. However, it cannot be excluded that some of the branched isomers could potentially be persistent in non-adapted sediments. Consistent evidence showing that the degradation of branched 4-NP is very slow under anaerobic conditions.						

Based on available information, it appears that linear 4-n-NP may be quickly degraded in surface water and sediment. However, the degradation of branched 4-NP isomers is expected to be slower. In the study by Ying and Kookana (2003) a half-life of the similar substance 4-t-OP (branched C8 alkyl chain) was 60 days in seawater at 20°C (corresponding to 171 days at the environmental relevant temperature of 9ºC for the marine compartment after Arrhenius transformation) while the half-life for linear 4-n-NP under the same conditions was 5 days. Although also in the study by Ekelund et al. (1993) a slow degradation of branched 4-NP in seawater was observed, reaching approx. 50% degradation (based on $^{14}CO2$ measurements) after 58 days at 11 °C (corresponding to 71 days at 9ºC for the marine compartment after Arrhenius transformation). The degradation in Ekelund et al (1993) was expressed as percent radioactivity in the CO2 fraction per radioactivity of the added 4-NP. However, it is indicated in the study that all the radioactivity trapped in the CO2 traps did not necessarily represent CO2. Other volatile metabolites may also have been present but in every case all the radioactivity should correspond to transformation or degradation products of 4-NP. It is noted that the recovery of radioactivity in the study by Ekelund et al (1993) was low and hence, the results of the study are considered to have high uncertainty. If part of the radioactivity was lost from the system, e.g. by volatilisation of transformation/degradation product, the results may underestimate the real degradation of 4-NP.

In the study by Johnson et al. (2000), degradation of the similar substance 4-t-OP in water sampled from Aire and Calder rivers in the United Kingdom, running through urban/industrial areas and known to be exposed to alkylphenols, as well as the Thames River running through a more rural area, was studied. The initial concentrations used were relatively high (100 µg/L) for determination of environmentally relevant degradation kinetics and most of the reported half-lives followed zero-order kinetics. These cannot be compared with the Annex XIII criteria as half-lives determined based on zero order kinetics depend on the concentration. However, five half-lives obtained with first-order kinetics are also reported in the study and they range between 8 and 51 days. These are considered more relevant for the P assessment. Converting these half-lives measured at 20°C to values at 12 °C using the Arrhenius equation results in half-lives of 17.4 days for Calder river, 23.7, 38.8 and 43.1 days for Thames river and 109.1 days for Aire river. In Aire river, little degradation was observed in the sample from the upland/rural reach during the 26 day incubation period (a half-life of 36 days at 20°C reported based on zero order kinetics), while half-lives of 8 to 13 d (based on zero order kinetics except one that is based on first order kinetics) were seen in the more urban/industrial reaches, which have been highly exposed to alkylphenols. However, it is noted that the study conditions were not fully comparable with OECD TG 309 conditions, e.g. static conditions and lower test water volumes were used, which could possible affect the degradation rates. Also due to the lack of detailed information on the study its reliability cannot be fully assessed. However, the study showed that the degradation of 4-t-OP may be slow in river water even when the microorganisms have been previously exposed to alkylphenols. Furthermore, the comparison between samples collected at different sites of the Aire river indicate that non-adapted micro-organisms degrade 4-t-OP significantly slower than pre-adapted micro-organisms.

Based on the available studies with sediment, no firm conclusion can be drawn. In many of the available studies (Johnson et al. 2000, de Weert et al. 2010a,b, Yuan et al. 2004, Bradley et al. 2008, Writer et al. 2011) the environmental water and/or sediment samples came from sites that were exposed to the substances or their precursors. Furthermore, in several sediment studies continuous shaking was applied leading to sediment slurry conditions which are not comparable with OECD TG 308 conditions. Also the sediment-water volume ratios differed from those indicated in OECD TG 308. Therefore, the results of these studies are not considered relevant for directly comparing with the Annex XIII criteria. Considering all the available information in a Weight-of-Evidence approach, it may be that degradation of at least most of the isomers of 4-NP can occur in the aerobic parts of sediments and it could be faster than in the water phase. But no reliable half-lives are available to compare with the Annex XIII criteria. Based on the available studies, no or very slow degradation of 4-NP in sediments under anaerobic conditions is expected.

Degradation in soil

Degradation of 4-NP was observed in the available non-guideline studies with soil. However, the half-lives calculated in the studies have a lot of uncertainty as other dissipation processes may have also occurred. In addition, in almost all available studies, the tested soils can be considered to have been pre-exposed to NP. Therefore, no conclusion on the persistence in soil can be drawn based on the available information.

Table 12-4 Overview of the available soil simulation studies

Type of Evidence	Test substance	Test medium	Results	Consistency & Specificity	Likelihood/ Biological Plausibility	Confidence / Strength of Evidence	Remaining Uncertainty
Soil microcosm study, IUCLID dossier, Trocmé et al. (1988)	technical NP (85% branched 4-NP and a lesser proportion of 2-nonylphenol and decylphenol	sewage sludge compost (1/3 dry matter) and sandstone (2/3 dry matter)	After 40 days incubation 11% and 38 % nonylphenol remained in the 100 mg/kg and 1,000 mg/kg treatments, respectively. Differences in the disappearance of isomers.	Consistent with other studies indicating that there are differences in the degradation of different isomers Consistent with other experimental studies indicating that mixture of branched 4-NP as a whole degrades rapidly from pre-exposed soils	Plausible	Low	High Pre-exposed soil, NER not considered in the results
Soil microcosm study, IUCLID dossier, Dettenmaier and Doucette (2007)	Nonylphenol (not indicated whether branched or linear)	a mixture of soil/biosolids, some of the microcosms planted with crested wheatgrass (Agropyron cristatum)	Half-lives for NP averaged from 31 to 51 d in the various planted, unplanted, and unplanted aseptic (incomplete sterilisation based on CO2 measurements) systems. Low mineralisation (7 % after 150 days).	Consistent with other experimental studies indicating that mixture of branched 4-NP as a whole disappears rapidly from pre-exposed soils	Plausible	Low	High Soil amended with biosolids that contained 4-NP, NER not considered in the DT50 values Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers
Soil microcosm study, IUCLID dossier, Topp and Starratt (2000)	4-NP and technical NP	three cultivated soils, a noncultivated temperate soil, and two soils from the Canadian Far North, which were presumably pristine with respect to exposure to 4-NP	Mineralisation of 4-NP was biphasic in all soils, with a rapid initial phase lasting about 10 d, (14C-CO2 30% of AR), followed by a much slower second phase with the final amount of 14C-CO2 reaching about 40% of AR. In some of the soils high amounts of bound residues (up to 50% AR). Mineralisation half-lives of 4.5-16.3 d estimated from the initial rapid phase.	Consistent with other experimental studies indicating that mixture of branched 4-NP as a whole disappears rapidly in pre-exposed and not pre-exposed soils	Plausible	Medium	Medium Results based on 14CO2 for the whole mixture of branched 4-NP cannot be used to conclude on all isomers

Greenhouse pot experiment with agricultural plants, IUCLID dossier, Mortensen and Kure (2003)	branched 4-NP and linear 4-NP	Sandy soil, in some of the test pots the soil was amended with different waste products including anaerobic and aerobic sludge, compost, and pig manure	74-91.7% disappearance of the initial amount of branched NP after 30 days in soils treated with sewage sludge or compost and with or without plants. Linear 4-NP disappeared faster (97.8-98.2 % after 30 days).	Consistent with other experimental studies indicating that mixture of branched 4-NP as a whole disappears rapidly in pre-exposed soils	Plausible	Low	High Soil amended with sludge or compost that contained 4-NP, NER not considered in the DT50 values Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers. In some experiments linear 4-NP used as test material, cannot be used to conclude for branched isomers.
Lysimeter study, IUCLID dossier, Jacobsen et al. (2004)	NP present in sewage sludge (expected to be a mixture of branched and possibly also linear 4-NP isomers)	sandy loam soil with anaerobically digested sewage sludge in the top-15-cm soil layer, planted with Spring barley (Hordeum vulgare L.)	DT50 of 37 d was estimated for NP based on the data from samplings in the 15 cm top layer of the soil lysimeters on days 20-110. NP concentrations in the leachate and soil layers below 15 cm were below the detection limits of the analytical methods.	Consistent with other experimental studies indicating that mixture of branched 4-NP as a whole disappears rapidly in pre-exposed soils	Plausible	Low	High Other dissipation processes than degradation may also have caused disappearance Soil amended with sludge so considered to be pre-exposed Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers.
Soil microcosm study, IUCLID dossier, Montgomery-Brown et al. (2008)	Nonylphenol ethoxyacetic acid (NP1EC)	Organic carbon-poor soil from a soil aquifer treatment facility	Nonylphenol ethoxyacetic acid (NP1EC) transformed almost completely to NP after 14 days and the formed NP was quickly transformed to nonylalcohols.	Consistent with other experimental studies indicating that mixture of branched 4-NP as a whole disappears rapidly in pre-exposed soils	Plausible	Low	High Precursor of 4-NP (nonylphenol ethoxyacetic acid) used as test material Pre-exposed soil Other dissipation processes than degradation may also have caused disappearance Results for the whole mixture of branched 4-NP cannot be used to conclude on all isomers.
Conclusion from overall confidence	Low confidence, not possible to conclude on the persistence of isomers of branched 4-NP in soil.						

12.1.3. Conclusion on degradation

In conclusion, based on the available information, the degradation of the different isomers of 4-NP may significantly differ, depending on the position and degree of branching as well as the length of the side chains of the alkyl chain. Therefore, the results for the linear 4-n-NP isomer or for a mixture of branched 4-NP as a whole cannot be used to conclude on the persistence of all isomers of 4-NP. Due to the high number of different possible isomers and limited number of studies where the degradation of different isomers has been studied, it is not possible to firmly conclude on the degradation of different isomers with the available information.

Based on the lag-phase observed in several studies, and the faster degradation observed in pre-exposed than in not pre-exposed environmental matrices, it seems that adapted micro-organisms can degrade rapidly at least most of the 4-NP isomers. However, the similar substance 4-t-OP showed no degradation in an OECD TG 302C study, which suggests persistence of isomers of 4-NP with similar branching. Considering all the available information including the OECD TG 302C study and BODIS test with 4-t-OP, the non-standard degradation studies with 4-t-OP and with the mixture of different branched isomers of 4-NP and the QSAR predictions for example isomers of 4-NP, it appears that at least some of the isomers of branched 4-NP may degrade slowly, especially by non-adapted micro-organisms. Therefore, it cannot be excluded that some of the isomers of 4-NP fulfil the criteria for P and possibly also for vP in freshwater and seawater. Hence, based on the available information it is not possible to conclude that all the isomers are not-P.

12.2. Environmental distribution

Regarding volatilisation, the registrants use a calculated Henry's law constant of 11.02 Pa m^3/mol as the key value for the Substance. This was calculated based on a vapour pressure of 0.3 Pa, a molecular weight of 220.34 g/mol and a water solubility of 6 mg/L. The eMSCA agrees with this value.

The eMSCA performed EPISuite Level III distribution modelling for an example constituent (NP-194) of the Substance using the experimental water solubility, Log Kow and vapour pressure reported by the Registrants as input. This resulted in the following predicted distribution when assuming equal emissions to air, water and soil:

- Air: 0.3 %
- Water: 9 %
- Soil: 77.9 %
- Sediment: 12.8 %

The EPISuite STP Fugacity model predicts for the example constituent (NP-194) of the Substance (using the experimental physico-chemical properties as input) that 85.15 % ends up in sludge, 12.76 % in the effluent water, 1.04 % in air and 0.72 % is biodegraded.

In the EU RAR (EC 2002) the distribution of 4-NP in wastewater treatment plant (WWTP) was calculated using EUSES assuming inherent biodegradation, a log K_{OW} of 4.48 (leading to a K_{OC} of 5,360 L/kg) and the Henry's Law constant as described in Section 12.2. The resulting distribution was as follows:

- *Fraction to air* *0.067*
- *Fraction to surface water* *0.350*
- *Fraction to sludge* *0.344*
- *Fraction degraded* *0.239*

The overall removal efficiency was therefore assumed to be 65%. In the SEV Decision, the Registrants were requested to provide information on the observed variation in actual sewage treatment efficiency (including K_{OC}) to be included in the CSA as part of a sensitivity analysis, in such a way that the conditions of safe use are clearly described in terms of the assumed level of WWTP removal efficiency (affecting both releases to water and to land). This was because in the CSRs it was assumed that the overall removal efficiency is 77.5%

(22.5% release to wastewater). Monitoring evidence from the UK[6] indicates that some WWTPs have removal efficiencies considerably lower than 80% (down to 66%, which is consistent with the reasonable worst-case estimate included in the EU RAR assessment). If the removal efficiency assumed in the EU RAR (EC, 2002) (65%) is used in the modelling included in the CSRs, some local RCRs become greater than 1.

In the updated registration dossier, the Registrants have included more information on the log Koc values and removal efficiency in WWTPs found in the open literature and predicted by them using different models. In the CSR, the removal efficiency in a standard STP is assumed to be 74.2 %. This is still well above the value used in the RAR and the worst case values observed in the UK monitoring data. Also, some of the predicted removal efficiency values reported by the Registrants are below 70%. Therefore, the eMSCA considers that the assumptions on removal in WWTP used by the Registrants in the exposure scenarios do not represent a reasonable worst case scenario for the aquatic environment.

12.3. Mobility

Not evaluated by the eMSCA. However, a log Koc of 4 is reported by the registrants based on the results of an OECD TG 106 study from the open literature (Milinovic et al., 2015). The registration dossier includes several other studies that also support the conclusion that the log Koc is not below 3, and hence, the Substance is not expected to be mobile according to the CLP criteria.

12.4. Bioaccumulation

A weight-of-evidence approach has been taken to the determination of the potential for the Substance to bioaccumulate in aquatic and air-breathing organisms. In this section an overview of the available data, the results and conclusions are presented. Further information and assessment on the studies can be found in the Annex 2. As in the case of persistence, also the bioaccumulation potential of different isomers is expected to vary as the degree and type of branching may affect metabolism and elimination in organisms. However, in the available tests the results are given for the whole substance, and hence, no conclusion can be drawn on individual isomers.

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

Modelling

Korsman et al. (2015) explored the bioconcentration factor (BCF)/bioaccumulation factor (BAF) of nonylphenol and its ethoxylates (NP, NPEO1, NPEO2, NPEO3-16 and total NPEOs (NPEO1-16) in an estuarine-marine food chain, using modelled uptake and elimination rate constants. Modelled uptake and elimination rate constants were compared with empirical rate constants from laboratory studies. Modelled accumulation factors including biota-sediment accumulation factors (BSAF) and biomagnification factors (BMF) were compared to independent field data collected from the Western Scheldt estuary, The Netherlands, and from field data reported in the literature:

BMF for trophic level 1 (primary producers) <1

[6] Comments and response to comments on Annex XV restriction report on Nonylphenol, branched and linear; Nonylphenol, branched and linear, ethoxylated UK CA comment, #313) Available for download at: https://echa.europa.eu/registry-of-restriction-intentions/-/dislist/details/0b0236e1806b0ddf

BMF for trophic level 2 (Herbi-detrivores) <1
BMF for trophic level 3 (primary carnivores) <1
BMF for trophic level 4 (Primary-secondary carnivores) <1
BMF for trophic level 5 (Secondary carnivores, accounting for biotransformation) <0.1
BMF for trophic level 5 (secondary carnivores, without biotransformation) <2.4

Experimental data

In vitro studies

Guomao et al. (2016) assessed the *in vitro* clearance of technical 4-nonylphenol among other chemicals in weever fish and quail bird liver microsomes, providing insight into potential hepatic clearance rates in fish and birds. The incubation of the test chemicals was conducted for 1h and 24 h, with quail microsomes and weever microsomes, respectively.

Benzo[a]pyrene (B[a]P) was added to the incubation mixtures as a benchmark compound to normalize the variation in the transformation rates that occurred in different batches of analyses. B[a]P has high levels of hepatic clearance and does not biomagnify. B[a]P clearance rates of 0.03±0.02 and 1.8±0.61 were determined for weever and quail, respectively.

Significant hepatic clearance demonstrated in the liver microsomes of weever fish and quail bird. Clearance rates of 0.021 mL/h/g of protein, ratio to B[a]P of 0.43 for the weever fish and 0.29 mL/h/g of protein with a ratio to B[a]P of 0.19 for quail. However, this information cannot be used for B assessment.

Bioaccumulation in fish studies

In this section information from bioaccumulation in fish studies has been compiled. No standard guideline studies on fish bioaccumulation are available for 4-NP. Several studies from open literature are available and a weight-of evidence approach is performed. None of the studies is fully reliable and some of them cannot be used in the assessment. In the studies by Spehar et al. (2010), Giesi et al. (2000) and Gautam et al. (2015), test concentrations above NOEC values for fish have been used, and hence, potential adverse effects in the test fish cannot be excluded. In fact, in Gautam et al. (2015), mortality of test fish was observed. Therefore, the studies are considered unreliable.

In the following table information from the most relevant studies has been summarised. Further information on the studies and on additional studies not considered relevant or reliable for the B assessment can be found in the Annex 2.

Table 12-5. Overview of the most relevant fish bioaccumulation studies

Test substance/exposure concentration	Test species	Result	Remarks	Reference
Aqueous exposure				
4-NP (likely branched) 4.9 ug/L	Three-spine stickleback (*Gasterosteus aculeatus*)	Whole body BCFss of 1200 and 1300 L/kg ww (based on total 14C) BCFss5%-L 833 to 896	Reported BCF values are based on total 14C and on drawn curves fitted by eye Extraction efficiency of NP from fish was 47%	Ekelund et al. (1990)

			→ High uncertainty	
Branched 4-NP 3.6 ug/L	Medaka (*Oryzias latipes*)	Whole body BCFss: 167 L/kg ww BCFss5%-L : 380 L/kg ww Depuration rate 0.07 h^{-1} and half-life 9.9 h	7 day uptake phase, 1 day depuration phase Not indicated whether steady state reached	Tsuda et al. (2001)
4-NP (not indicated whether branched or linear) 0.33, 0.93, and 2.36 ug/L	Fathead minnow (*Pimephelas promelas*)	Whole body BCFss: 434 L/kg ww	Uptake phase 42 d, no depuration phase	Snayder et al. (2001)
4-NP (chemical name for branched isomer, but CAS for a linear isomer) 2.5 ug/L (nom.)	Common carp (*Cyprinus carpio*)	Whole body BCFk: 576 L/kg ww, lipid normalised, but not indicated what the fish lipid content was	Uptake phase 7 d, depuration phase7 d No information on measured water concentration	Cantu (2013)
NP (not indicated whether branched or linear) 5.6, 12.4, 27.6, 59.5 and 126 ug/L 9.3, 19.2, 38.1, 77.5 and 193 ug/L	*Lepomis macrochirus*	Whole body BCFss 231 (test conc. 5.6 ug/L)	Uptake phase 28 d, no dep.phase Lipid content not measured Some test concentrations above NOEC mortality and sublethal effects possible at some of the lower concentrations NOEC growth for Pimephales promelas 38 ug/L	Brooke et al. (1993) ASTM 1993 E1022-84
	Pimephales promelas	Whole body BCFss 769 and 984 (test conc. 9.3 and 19.2 ug/L) (steady-state not reached) Whole body BCFss 876 (test conc. 38.1 ug/L)		
Dietary exposure				
4-NP (not indicated whether branched or linear)	*Pleuronected yokohamae*	Steady-state BMFs: 0.018, 0.006 and 0.0014 for low, medium	Uptake phase 14 d, no depuration phase Extraction efficiency of 4-NP in fish was 60%	Nurulnadia et al. (2016)

13.6 µg/g (low), 49.7 µg/g (medium) and 259µg/g (high		and high exposures	NP detected also in control diet and fish at low concentrations	

In the study by Ekelund et al. (1990), BCFs of 1200 and 1300 for 4-NP (based on total 14C) in *Gasterosteus aculeatus* based on whole body tissue wet weight are reported. The results have been 5% lipid normalised based on the lipid values provided in the report, resulting in calculated BCFs of 833 to 896. The recovery of 14C was low (47%) in the fish tissue, which causes uncertainty to the results.

Tsuda et al. reported a mean steady state BCF based on whole body tissue wet weight of 167 for O. latipes (average lipid content 2.2% and water content 78% in the test fish). Steady state was reached at 48 h. The reported elimination rate K_2 is 0.07 h^{-1} and half-life 9.9h.

Snayder et al. (2001) determined a maximum BCF of 434 (whole body tissue wet weight) for Pimephelas promelas. In Brooke et al. (1993) a BCF of 808 is calculated for the same species. However, the some of the test concentrations in Brooke et al. (1993) were above the NOEC values for P. promelas, and hence the study is not fully reliable.

Cantu (2013) reports a BCF of 576 (whole body ww, lipid normalised, but not indicated what the fish lipid content was) for *Cyprinus carpio.*

Furthermore, Nurunaldia et al. (2016) and Carnevalia et al. (2017) performed dietary exposure tests with *Pleuronected yokohamae* and *Sparus aurata*, respectively. No depuration phases were included in the study. The BMFs were calculated as the ratio of the concentration in the organism to the diet at steady state. Low BMFs values are reported in both studies, 0.002-0.02 in Nurunaldia et al. (2016) and 0.001-0.028 in Carnevalia et al. (2017). However, the study of Carnevalia et al. (2017) is not considered reliable for the assessment as the fish were exposed to 4-NP in a mixture together with 4-OP or BPA and there seemed to be cross contamination between the different treatments. Furthermore, it is not clear whether steady state was reached in the study.

Considering all the BCFs reported in the available studies, it seems that 4-NP has low bioaccumulation potential in fish. This could be due to rapid metabolisation and excretion of the metabolites.

Bioaccumulation in invertebrates studies

No standard guideline BCF studies with aquatic invertebrates are available for the Substance. Several studies from open literature are available and a weight-of evidence approach is performed. None of the studies is fully reliable and some of them cannot be used in the assessment.

In the following tables information from the most relevant studies (Table 12-6) is summarised. Further information on the studies and on other studies not considered reliable can be found in the Annex 2.

Table 12-6 Overview of the most relevant bioaccumulation studies in invertebrates

Test substance/exposure concentration	Test species	Result	Remarks	Reference

Aqueous exposure				
14C-labelled 4-NP (likely branched) 5.9 and 6.2 ug/L 6.4 and 7.4 ug/L	*Mytilus edulis*	BCFss 2700 ww (Lipid content 1.6-1.9%.) (based on total 14C but corrected for content of radioactive metabolites)	Steady state not reached → concentrations in steady state extrapolated	Ekelund et al. (1990)
	shrimps *(Crangon crangon)*	BCFss 110 and 90 L/kg ww (lipid content 1.4 and 1.7 %) (based on total 14C)	Steady state reached	
4-n-NP 305 ng /L	*Mytillus galloprovincialis*	BCFk 1700 L/kg (dw) Depuration rate constant (k2) 0.04 d^{-1} and half-life 17 d BCFss 1954 L/kg (dw)	Steady state not reached BCF in dw higher than BCF in ww Uncertainty in the calculated k1	Gatidou et al. (2010)
technical 4-NP 3 µg/L	shrimp *Gammarus pulex*	BCFk 3838 ww (no information on lipid content) (water exposure only)	Steady state not reached NP desorbed to water from leaf disks placed in the test vessels (disks not available for shrimps)	Gross-Sorokin et al. (2003)
Sediment exposure				
4-NP (not indicated whether branched or linear)	amphipods: *Eohaustoriu estuarius*, *Grandidierella japonica* and *Corophium salmonis*	BAF (dw): 895 (E.estuarius), 1074 (G.japonica), 253 (male C.salmonis), 743 (female C.salmonis) BSAF (dw): 33.9 (E.estuarius), 17.3 (G.japonica), 7.1 (male C.salmonis), 16.0 (female C.salmonis) Lipid contents:4.65-5.96 %, 7.23-11.23% and 6.57-11.81	Results for the treatments without TOC amendment	Hecht et al. 2004

Technical 4-NP 2 µg/g dw	*Lumbriculus variegatus*	BSAF 24 g carbon dw/g lipid ww Lipid content 0.9-1.1 % TOC of sediment 9.29%	US EPA (EPA/600/R-99/064) No depuration phase Steady state not reached Worm concentration in ww, sed. concentration in dw Ammonia conc. in water varied by well more than 50% (validity criterion not met, but well below the 10d LC50 of 21.4mgN/L)	Croce et al. (2005)
14C-labelled 4-NP 2.86, 1.98 and 2.20 µg/kg sed.	*Lumbriculus variegatus*	BAFss 1.5 and 5.9 BAFk 1.8, 6.5 and 33.6 BSAF: 55.4, 14.1 and 34.8 lipid content 1.23% TOC of sediments: 24.28, 3.20 and 1.64 %, for S1, S2 and S3 (based on total 14C)	Steady state not reached Worm concentration in ww, sed. concentration in dw Lipid content not measured in the test, but in the same culture earlier The oligochaetes were not purged for gut content	Mäenpää and Kukkonen (2006)

BCF values above the B criterion were observed in the available studies. In Ekelund et al. (1990) BCFss of 2700 (ww) determined for mussels, and in Gatidou et al. (2010) BCFss close to 2000 were determined for mussel (in dw, BCF in ww lower) but steady state was not reached in the studies. A BCFk of 3838 (ww) is reported for shrimp Gammarus pulex in Gross-Sorokin et al. (2003).

BSAF values in the range of 14.1-55.4 for Lumbriculus variegatus in two studies (Croce et al., 2005, Mäenpää and Kukkonen, 2006) and in the range of 7.1-33.9 for three amphipods in another study (Hecht et al., 2004). According to ECHA Guidance R.11 (Appendix R11-3) lipid and organic carbon normalized BSAF values of 0.5 and higher are an indication of high bioaccumulation. The ECHA Guidance further indicates that the relationship between BSAF and BCF can be expressed as BSAF= BCF(lipid)/ Koc. The Koc of the Substance is reported to be 11060. Applying the above mentioned equation using as input the Koc value and the lowest and highest BSAF values results in estimated BCF(lipid) of 78526 and 374934, respectively. Converting these values to 5% lipid normalized BCF values results in BCF of 3926 and 18747, respectively. As indicated in the above table Table 12-6, there is some uncertainty in the available BSAF values, and hence, the estimated BCF values should be interpreted only as supporting information.

In most of the available studies with invertebrates steady state was not reached during the exposure phase. This suggests that 4-NP has slow elimination in most invertebrates.

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

No experimental information on soil dwelling organisms is available for the Substance.

Some toxicokinetic studies with mammals are included in the registration dossier.

The toxicokinetic of branched 4-NP (CAS RN 84852-15-3) was assessed in two non-guideline GLP studies in SD rats by Unnamed (2001) and a toxicokinetic study in CD rats conducted by Green (2003).

In a pilot pharmacokinetic study in male and female SD rats (Unnamed 2001) single i.v. and gavage application of branched NP was compared. The elimination half lives in plasma after i.v administration of 5 mg ring-14C labelled NP was 9.6 h (males) and 9.3 h (females); after oral administration it was 12.4 h (males) and 8.5 h (females). Cmax in blood was 10 fold lower administered by gavage than i.v. administration in female rats and 22 fold lower in male rats. Four radioactive moieties were detected in blood after i.v. application: protein-bound NP, NP glucuronide, NP itself and an unidentified glucuronidated metabolite. Protein-bound radioactivity decreased with time and was detected at all time points during the 24 h experiment, whereas free nonylphenol was below the limit of detection within 4 h of dosing (i.v.) in female rats. The plasma protein-bound fraction was not detected after oral administration.

Liver, lung, testis, epididymis, subcutaneous fat, abdominal fat and spleen were collected from male rats at 2, 8, and 24 h following i.v. dosing. Liver contained 11.3% of the dose 2 h after application; subcutaneous fat and abdominal fat contained 0.31 and 0.2% of the dose per gram of tissue, respectively. Estimating the amount of dose contained in total body fat (~ 9%) indicates that fat is a significant reservoir for NP when administered intravenous. When administered by gavage, the peak levels in the liver were 1.8% of the dose 2 h after dosing and 2.2% after 8 h, declining to 0.8% by 24 h. Fat tissue contained approximately 0.01% of the dose per g tissue at all time points, indicating that fat is a non-significant reservoir for NP when administered orally, in contrast to i.v. application. On gavage administration to male rats, less than 0.02% of the dose was recovered in lung, spleen, testis and epididymis after 24 h. The levels of radioactivity in plasma and tissue were highly variable following gavage. This variability was particular pronounced in animals at the 50 and 100 mg/kg group, implying individual differences in uptake from the gut and/or metabolism.

An associated study conducted by Unnamed (2001) focussed on distribution and metabolic profile in SD rats after single gavage of 0, 5 and 200 mg/kg bw. The majority of the administered dose was excreted within seven days in faeces (81-85%) and to lesser extend in urine (12-17%). The total dose recovered in excreta amounted to approximately 90-97%, except for the high dose group (200 mg/kg bw/day), in which approximately 75% of the dose was recovered. A small amount of radioactivity was exhaled as CO2in male rats in the low dose group (amounting to 0.14% of the applied dose), suggesting a breakdown of NP into volatile metabolites. The rate of excretion of radioactivity in the urine was faster in female rats compared with males. In addition qualitative differences were observed in urinary metabolites between male and female rats. No radioactivity was detectable in the reproductive organs (testes, ovaries, epididymis, and uterus) examined at day 7 after administration. The majority of the radioactivity recovered after 7 days was in the liver (0.14%), the intestinal tissue (small: 0.08%; large: 0.1%) and the contents of the small (0.5%) and large (0.8%) intestines. There was no accumulation in abdominal and subcutaneous fat tissue in accordance with the findings of the pharmacokinetic study (Unnamed 2001).

Green (2003) published a toxicokinetic study in SD rats. Branched 4-NP was administered i.v. (10 mg/kg bw) and oral (10 and 100 mg/kg bw) for up to 14 days. 75% of the radioactivity applied i.v. was being eliminated within 24 h, mainly in the faeces. After 7 days 13% of the dose applied i.v. was found in the carcass. Concentration of radioactivity in fat increased 4-5 fold over the duration of the study. The absolute amounts in fat, however, were less than 0.06% of the amount found in excreta on day 14 of the study. Up to 64% of the dose was eliminated in bile following 10 mg/kg oral dose and up to 49% at the higher dose. Similar amounts were excreted in bile after i.v. application. From the proportion of the dose eliminated in bile and urine, an absorption rate of 65% and 80% can be concluded at 10 mg/kg, respectively. Absorption rate was calculated to be ~50% at the 100 mg/kg dose. Following absorption, 4-NP was metabolised in the liver, with the majority of the metabolites excreted in bile, mainly as glucuronide conjugates.

Sex related difference was seen in the blood and plasma, with the maximum concentration in males being 2-3 fold higher than that in females. The ability to clear 4-NP and its metabolites from blood was also different. In males, the half live in plasma was not affected by the increase in dose from 10 to 100 mg/kg (7 vs. 9 h), whereas in females the half-life increased approximately 4-fold with the increase in dose (3 vs. 13 h). The capacity of the female rat to metabolize and excrete 4-NP is lower than that of males at high doses. The sex-related difference was also seen in the metabolic profiles in urine, bile and faeces. NP-glucoronide (NPG) represents the only significant metabolite in the bile at the 10 mg/kg dose; following 100 mg/kg significant amounts of 4-NP itself were present in female but not in male bile. Similar, 4-NP was a major component in female urine following a 100 mg/kg, but not a 10 mg/kg dose. Both findings suggest that the capacity of the liver to form glucoronide is saturated at the higher dose in females.

4-NP was more extensively metabolized in male rats, with a number of metabolites present in urine, bile and faecal extracts that were not seen in female rats. NPG, the major metabolite in female rats was not present in male urine, although it was present in bile.

Following repeated dosing, steady state was reached within 7 days. There was no evidence of significant accumulation into tissue compartments or of a significant change in clearance or metabolite profiles in urine. Extraction of faecal samples revealed that faeces contained mainly 4-NP itself.

There are also some studies on linear 4-NP. These studies are not considered relevant for most of the isomers of branched 4-NP or for branched 4-NP as a whole substance although they could be used as supporting information for B assessment of 4-NP isomers with very low level of branching.

12.4.3. Monitoring and information from field studies

Several field studies are available, measuring concentrations of 4-NP in animals and in their surrounding environmental media. 4-NP is widely detected in sediments and water and in organisms (up to hundreds of ng/g ww) from different trophic levels, including in top predators such as sharks (Ademollo et al., 2016) and in human hair (Nehring et al., 2017). Overview of the studies reporting bioaccumulation parameters (BAF, BSAF, BMF and TMF) is included in the below tables. Further information on the studies can be found in the Annex 2. Most of the results do not indicate high accumulation potential of 4-NP but for some organisms BAF values above 2000/5000 or BMF values above 1 are reported. The available trophic magnification factors (TMFs) are all below 1 indicating trophic dilution.

Uncertainties were identified for the studies, e.g. low number of individuals sampled, results reported in dry weight. The eMSCA has not done an in-depth assessment of the field studies.

Furthermore, field BCF/BMF/BAFs/BSAFs determined for 4-NP have uncertainties as the 4-NP measured in the organisms may partly be due to metabolism of precursors of 4-NP being taken up by the organisms (as seen e.g. in Cailleaud et al. (2011) laboratory study).

Table 12-7 Overview of the available BAF (C_{biota}/C_{water}) reported in field studies

BAF (L/kg) (water)	Species	Comments	Reference
In the range of 4423 (detritivores) - 28883 (brackish carnivores)	Fish with different feeding habits: brackish carnivores planktivores	the Pearl River, China Based on dry weights	Fan et al. (2019)

	molluscivores freshwater carnivores detritivores		
log BAF values of 4-NP reported for the different fish tissues are in the range of approx. 2-4.5 (read from a graph included in the publication) and are in the following decreasing order: bile>plasma>liver>muscle.	tilapia (*Tilapia aurea*), snakehead (*Ophiocephalus argus*), mud carp (*Cirrhinus molitorella*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idellus*), crucian carp (*Carassius auratus*), bream (*Parabramis pekinensis*), and chub (*Hypophthalmichthys molitrix*)	the Pearl River, China	Lv et al. (2019)
1029 245 5667 1143 290 564-8762	Zoo and phytoplankton+detritus Benthic invertebrates Ghost shrimp Oyster Mussel Fish, marine mammals and birds (livers)	Coastal zone at several locations on the North American Pacific of California and Canada Low n for most species	Diehl et al. 2012
12400 (mean), 5900 (median)	Mussel (*Mytilus trossulus*)	the Gulf of Gdansk (southern Baltic Sea) Based on dry weights	Staniszewska et al. 2017
1800 2600 1300 200-600	Phytoplankton Zooplankton Mussel Fish	Gulf of Gdansk (Baltic Sea) Based on dry weights	Staniszewska et al. 2014

2788-3649	Clam (*Corbicula fluminea*)	Miño River estuary (NW Iberian Peninsula) Based on dry weights	Salgueiro-Gonzalez et al. 2015
284-825	Mussel (*Mytilus galloprovincialis*)	Thermaikos Gulf, Northern Aegean, Greece Based on dry weights	Arditsoglou and Voutsa 2012
11-258 41-338 26-250	Shrimp Mussel Fish	the Pearl River estuary system, China	Diao et al. 2017
2900 2200	Sea snail Oyster	Coastal water and coastal sediments in southern Taiwan Based on dry weights	Cheng et al. 2006
10000	Clam (*Rangia cuneata*)	Vistula Lagoon (southern Baltic Sea) Based on dry weights	Graca et al. (2021)
3200-5800	Mussel *(M. galloprovincialis)*	Venice Lagoon, Italy	Pojana et al. 2007
4000-15000	carp, crucian carp and silvery minnows	Panlong river, Yunnan-Guizhou plateau, China	Wang et al. (2016)

278 325	Grass carp Japanese seabream	Luomo Lake region, China Based on dry weights	Liu et al. (2017)

Table 12-8 Overview of the available BAF ($C_{biota}/C_{sediment}$) and BSAF values reported from field studies

BAF (sediment) /BSAF (normalised to lipid and OC content)	Species	Comments	Reference
BAF: 93 10 152 63 12 25-319	Zoo and phytoplankton+detritus Benthic invertebrates Ghost shrimp Oyster Mussel Fish, marine mammals and birds (livers)	Low n for most species	Diehl et al. 2012
BAF 2.6	Clam (*Corbicula fluminea*)	Miño River estuary (NW Iberian Peninsula) Based on dry weights	Salgueiro-Gonzlez et al. 2015
BAF : 1.75-7.96 6.6-3.19 1.63-7.72	Shrimp Mussel Fish	the Pearl River estuary system, China	Diao et al. 2017
BAF 251	Clam (*Rangia cuneata*)		Graca et al. (2021)

BAF 40	Mussels (*Mytilus trossulus*)	the Gulf of Gdansk (southern Baltic Sea).	Filipkowska & Lubecki (2016)
BSAF 0.1-4.5	Clam (*Ruditapes philippinarum*)	Venice Lagoon, Italy Variation between different months Clam concentration in ww, sed. concentration in dw	Ademollo et al. 2017
BSAF 0.11-0.48	Clam (*R. philippinarum*)	Sacca di Goro lagoon, Adriatic Sea, Italy	Casatta et al. 2015

Table 12-9 Overview of the available BMF values from field studies

BMF	Species	Comments	Reference
All BMFs below 1 except Otter liver/oyster 2.2 Otter liver/mussel 10.9 Sculpin liver/goby liver 2.7	Zoo and phytoplankton+detritus Benthic invertebrates Ghost shrimp Oyster Mussel Fish, marine mammals and birds (livers)	Low n for most species	Diehl et al. 2012
Mean 2, median 0.7	Mussel/phytoplankton	Based on dry weights	Staniszewska et al. 2017
All BMFs below 1 except : Cod/herring 1 Herring gulls/herring 1-4	Phytoplankton Zooplankton Mussel Fish	Based on dry weights	Staniszewska et al. 2014

Herring gulls/flounder 1 Herring gulls/cod 1	Herring gulls		
0.7-2.4	Sea snail/Oyster	Based on dry weights BMFs higher (>1) in summer than in winter (<1)	Cheng et al. 2006
0.57-1.51	herring gull/different fish species	Bohai Bay, North China	Hu et al. 2005

Table 12-10 Overview of the available TMF values from field studies

TMF	Species	Comments	Reference
0.72	18 fish species, 2 prawn species, 3 mollusc species	Yangtze River Delta , East China Sea	Gu et al. 2016
0.83	Phytoplankton, zooplankton, 5 invertebrate species, 6 fish species and herring gull	Bohai Bay, North China	Hu et al. 2005

12.4.4. Conclusion on bioaccumulation

The eMSCA applied a weight of evidence approach for the determination of the potential for the Substance to bioaccumulate in aquatic and air-breathing organisms. No standard guideline studies are available for fish or aquatic invertebrates but several published studies from open literature are included in the registration dossier and some further studies were found by the eMSCA. Several field and monitoring studies are also available. As in the case of persistence, also the bioaccumulation potential of the different isomers is expected to differ as the degree and type of branching may affect metabolism and elimination in organisms. However, in the available tests the results are given for the whole substance and hence no conclusion can be drawn on individual isomers.

Considering all the BCFs (all below 2000) reported in the available laboratory studies with fish, it seems that 4-NP does not bioaccumulate in fish to the extent to fulfil the B criterion in Annex XIII. This could be due to rapid metabolisation and excretion of the metabolites.

BCF values above the B criterion were observed in some of the laboratory studies available for aquatic invertebrates. In Ekelund et al. (1990) BCFss of 2700 (ww) determined for mussels, and in Gatidou et al. (2010) BCFss close to 2000 were determined for mussel (in dw, BCF in ww lower) but steady state was not reached in the studies. A BCFk of 3838 (ww) is reported for shrimp *Gammarus pulex* in Gross-Sorokin et al (2003).

BSAF values in the range of 14.1-55.4 for *Lumbriculus variegatus* in two studies (Croce et al. 2005, Mäenpää and Kukkonen 2006) and in the range of 7.1-33.9 for three amphipods in another study (Hecht et al 2004). According to ECHA Guidance R.11 (Appendix R11-4),

lipid and organic carbon normalized BSAF values of 0.5 and higher are an indication of high bioaccumulation.

In most of the available studies with invertebrates steady-state was not reached during the exposure phase. This suggests that 4-NP has slow elimination in most invertebrates.

Based on the available toxicokinetic studies with mammals, 4-NP may be quickly metabolised and eliminated by mammals.

Several field studies measuring concentrations of 4-NP in animals and in their surrounding environmental media are also available. No clear conclusion can be drawn based on these studies as the results are varying and sometimes contradicting and there are a several uncertainties. However, in some of the studies bioaccumulation of 4-NP was observed.

In conclusion, based on the weight-of-evidence assessment, the Substance is not expected to be B/vB in fish, but it could fulfil the criteria for B at least in aquatic invertebrates based on the available information. Further assessment on the differences of bioaccumulation potential of different isomers of 4-NP would be needed to firmly conclude. However, as no firm conclusion on the persistence can be drawn, the bioaccumulation potential was not further assessed by the eMSCA.

13. Environmental hazard assessment

Many aquatic toxicity studies with 4-NP, branched are available in the registration dossier and in the open literature. Most of the data available have been recently evaluated and summarised by EU regulatory authorities both for the SVHC dossier (ECHA, 2012) and the restriction proposal for NPEOs (KEMI 2013). Summary tables are included in this document and further information and discussion on the studies can be found in the SVHC Support document (ECHA 2012), the RAC opinion and its background document for the NPEOs restriction (ECHA 2014a and 2014b) The eMSCA has therefore only evaluated the studies included in the registration dossier but not taken into account in the RAC opinion and SVHC dossier, hence studies performed from 2014 onwards. Only a few of such studies are included in the registration dossier. Three of the new studies with invertebrates were conducted following the requests in the Substance Evaluation decision of the Substance.

13.1. Aquatic compartment (including sediment)

Table 13-1 Summary of the lowest relevant and reliable acute and chronic toxicity values of nonylphenol for aquatic species considered in the RAC opinion (ECHA 2014a) (in white) and amended by two new studies performed post-2014 and leading to lower values (in yellow)

Trophic level	Species	Endpoint	Concentration	Reference
Freshwater fish	Fathead minnow (*Pimephales promelas*)	Mortality (96 h LC50)	128 µg NP/L	Brooke (1993a)
	Rainbow trout (*Oncorhynchus mykiss*)	Growth (91d-NOEC)	6 µg NP/L	Brooke (1993b)
	Japanese medaka (*Oryzias latipes*)	Reproduction in F1 generation (140d-NOEC)	1.27 µg NP/L	Watanabe et al. (2017)

Marine water fish	Winter flounder (*Pleuronectes americanus*)	Mortality (96 h LC50)	17 µg NP/L	Lussier et al. (2000)
	No marine fish long-term toxicity is available			
Freshwater invertebrates	*Hyalella azteca*	Loss of mobility (96 h EC50)	20.7 µg NP/L	Brooke (1993b)
	Daphnia magna	Surviving offspring (21-d NOEC)	24 µg NP/L	Comber et al. (1993)
	Potamopyrgus antipodarum	Reproduction (28-d EC10)	4.09 µg NP/L	Unpublished (2020)
Marine water invertebrates	*Mysidopsis bahia*	Mortality (96 h LC50)	43 µg NP/L	Ward and Boeri (1991)
	Mysidopsis bahia	Growth – length (21-d NOEC)	3.9 µg NP/L	Ward and Boeri (1991)
Freshwater algae	*Scenedesmus subspicatus*	Growth rate (72 h EC50)	323 µg NP/L	Kopf (1997)
		Growth rate (72 h NOEC)	25.1 µg NP/	
Marine water algae -		No marine algae toxicity data is available		
Freshwater aquatic plants	*Lemna minor*	Frond production (96 h NOEC)	901 µg NP/L	Brooke (1993b)
Freshwater sediment species	*Chironomus riparius*	Emergence rate (28-d EC10)	231 mg NP/kg dw	Bettinetti and Provini (2002)
Marine water sediment species	*Leptocheirus plumulosus*	Mortality, reproduction (28-d NOEC)	61.5 mg NP/kg dw	Zulkosky et al. (2002)

Table 13-2 Summary table on endocrine disrupting effects of 4-nonylphenols in different taxonomic groups (Taken from the RAC opinion (ECHA 2014a))

Taxonomic group	No. of species	Indication of hormonal activity?	Apical adverse effects observed?	Indication that apical endpoints fit to mode of action
Fish	9	Yes, In all species (increased vitellogenin level	Yes, Effects in all species with tested apical	Yes, based on studies with NP clear link

		in males and females, changes in female gonadal staging, changes in sperm stages in males, testis-ova, secondary sex characteristics, elevated estradiol levels)	endpoints (6 species). Most sensitive adverse endpoints: Sex-ratio (*Oryzias latipes, Danio rerio, Poecilia reticulata, Gambusia holbrooki*); growth (*Onchorhynchus mykiss, Pimephales promelas*) Most sensitive fully reliable LOEC = 10 µg/L (growth *O. mykiss* and sex-ratio *D. rerio*) with some indication that effects may start at 0.75 µg/L (semen volume On. mykiss)	for four fish species Effects observed in all species substantiate the endocrine mode of action and are known to be estrogen sensitive
Amphibians	7	Yes In vitro receptor binding for one species. Some hints that effects might be endocrine mediated in another species but not conclusive.	Yes, in 3 species (change in sex ratio, occurrence of intersex gonads, changes in development) Most sensitive LOEC≤ 10 µg/L (sex ratio in *Rana sylvatica* and *Rana pipiens*, Klimisch 2)	Effects observed on sex-ratio in *Xenopus laevis* in low quality study and changes in sex ratio in *R. sylvatica* and *R. pipiens* in a Klimisch 2-study point to an estrogen mediated mode of action
Invertebrates	2 crustacean species	Yes, effects on androgen metabolism in *D. magna* Depression of 20-hydroxyecdysone production during a moult cycle	Yes (reproduction, development, moulting) Most sensitive fully reliable EC50 = 8 µg/L (reproduction in *Cerodaphnia dubia*)	Some indication but no clear conclusion possible due to lack of knowledge
	2 echinoderm	Effects observed are similar to	Yes (larval malformations)	Some indication but

	species	those observed for a known anti-estrogen and thyroid active substance (pentachlorphenol)	Most sensitive reliable LOEC = 0.9 µg/L (larval malformation in *Arbacia lixula*)	no conclusion possible due to lack of knowledge
	4 mussel species	Induced hermaphrodism effects fit to those observed for 17ß-estradiol and knowledge about the influence of estrogens on female sexual maturation	Yes (sex ratio skewed to females in one study, survival of offspring) Most sensitive reliable LOEC ≤ 1 µg/L (survival, sex-ratio in Crassostrea gigas). Less conclusive end points like sperm motility were also affected below 1 µg/L.	Some indication but no clear conclusion possible
	1 snail species		Yes (fecundity, hatching success F1 generation, growth) Most sensitive reliable LOEC 1 µg/L (embryonic toxicity in *Haliotis diversicolor*)	No conclusion possible

13.1.1. Fish

A published study by Watanabe et al. 2017 according to OECD TG 240 (Medaka extended one generation reproduction test (MEOGRT)) study with 4-NP, branched is included in the registration dossier. Three generations of Japanese medaka (*Oryzias latipes*) were exposed to 4-NP over a 20-week period: 3-week parental generation (F0), 15-week in the 1st generation (F1) and 2-weeks in the 2nd generation (F2). The study was run under flow-through conditions with a control and mean measured test concentrations of 1.27, 2.95, 9.81, 27.8 and 89.4 µg/L.

No effects on the survival, total length and weight of the F0 generation was observed. The NOEC based on survival of the adult F1 generation was 27.8 µg/L for male fish and 2.95 µg/L for female fish giving an overall NOEC for male and female F1 adult fish of 2.95 µg/L. The NOEC based on total length and weight for the F1 adult fish were 9.81 and 27.8 µg/L. For the subadults of F1, effects were seen in the length and weight even at the lowest concentration of 1.27 µg/L. However, it is indicated by Watanabe et al. (2017) that this might be due to the enhanced statistical power based on the use of individual fish rather than tank replicate, and therefore, it should be treated as a reference value.

In the F0 generation, the replicate mean numbers of total eggs, fertilized eggs, and fertility rate (%)/pair/day during the 21-day exposure period remained stable in all treatment groups, with no significant difference among NP treatment groups over time.

In the F1 generation the NOEC values for total egg production and fertility rate were 2.95 µg/L, and the NOEC for fertilised eggs was 1.27 µg/L.

In the F1 generation, the mean hatching rate, and survival 14 and 21 days post fertilisation were significantly reduced ($p<0.05$) at the mean measured concentration of 89.4 µg/L, and therefore the NOEC values for these endpoints were 27.8 µg/L.

In the F2 generation, there were no fertilised eggs at the highest tested concentration of 89.4 µg/L. There was no significant difference in mean hatching rate or embryo survival at the mean measured concentration of 27.8 µg/L and below.

In F1 generation, an increased effect was also observed in anal fin papillae in males (NOEC = 9.81 µg/L) and VTG induction in female adult fish (NOEC = 27.8 µg/L), while the effect on VTG induction in male adult fish was slightly decreased in F1 compared to F0; the NOECs were 2.95 µg/L in F0 and 9.81 µg/L in F1. Internal sex change and sex reversal were observed at 27.8 and 89.4 µg/L, respectively, in F1 sub-adults. Furthermore, all genotypic males in breeding pairs at 89.4 µg/L NP showed female phenotypes and some males even demonstrated spawning. Therefore, F2 generation could not be initiated at 89.4 µg/L NP.

The mean and range of total eggs in the control were 32 and 27-34 eggs/pair/day, respectively, and mean fertility during 21 days in the control was 92-100%, which met the test validity criteria. Mean water temperature in each treatment tank over the entire test duration ranged from 26.9–27.2 °C, which was slightly above the range of the test validity criteria (=24-26 °C) but excursions from the mean remained within ± 2 °C. The mean DO achieved test validity criteria (≥ 60% of air saturation) except for a short period (overnight) in the juvenile stage during the analysis of the genotypic sex (described in F1 generation, F1 sub-adult stage). The pH ranged from 7.3 to 8.7, varying by more than ± 0.5 unit in each treatment.

The Watanabe et al. (2017) study is considered reliable for the assessment and is used as a key study by the registrants. The lowest NOEC value of 1.27 µg/L for fertilised eggs in the F1 generation is lower than the NOEC of 6 µg/L determined for rainbow trout in Brooke (1993a) and used as the lowest value for long-term fish toxicity in the RAC opinion (ECHA 2014a).

There are three further supporting studies performed after the RAC opinion (ECHA 2014c) and included in the registration dossier.

In Shirdel and Kalbassi (2016) non guideline study juvenile *Salmo trutta* caspius were exposed to 4-NP at nominal concentrations of 1, 10 and 100 µg/L for 21 days. Ethanol was used as solvent. Control and solvent control were also included in the test. The total volume of water and exposure solution in the tanks was renewed twice per day Concentration of the test substance was only measured immediately after the preparation of exposure solutions and the measured concentrations were 0.95 ± 0.34, 8.72 ± 2.03 and 91.67 ± 11.72 µg/L, respectively for 1, 10 and 100 µg/L nominal concentrations. No effects in length and weight were observed at the highest test concentration of 100 µg/L after 21 days of exposure. Effects of 4-NP on thyroid hormones (TSH, T3 and T4) and the growth hormone GH as well as on histopathology were also studied. The results showed that the HIS (liver weight / whole body weight × 100) and plasma total calcium of male and female fishes exposed to 100 µg/l nonylphenol were significantly increased compared with the control groups. The male plasma T3 level was significantly decreased in 10 and 100 µg/L treatments. The female T3 level increased in 1 µg/L concentration. The plasma T4 of males showed significant elevation in fish exposed to 100 µg/L, but no change for females in any of treatment groups relative to controls. No significant effect of nonylphenol exposure was observed on male plasma TSH levels, whereas, in females, nonylphenol at all concentrations significantly reduced TSH levels. A bell-shaped response was observed in

male and female plasma GH levels. Moreover, various histopathological lesions were observed in gill and intestine tissues of fish exposed to different nonylphenol concentrations.

Maltais and Roy (2014) performed a 21day chronic toxicity of nonylphenol to immature copper redhorse (*Moxostoma hubbsi*) under flow through conditions. Fish were exposed to control, solvent control, and test chemical nominal concentrations of 1, 10 and 50 µg/L. The 21 day NOEC value, based on mortality was >50 µg/L.

Puy-Azurmendi et al (2014) study was conducted exposing embryos of *Danio rerio* to concentrations from 0 to 500 µg/L of technical NP for 42 days. The study was not conducted according to guidelines or the principals of GLP. The NOEC value based on mortality was 50 µg/L as in the 2 highest concentrations tested, 250 and 500 µg/L, embryos died after 1 day. The concentrations were based on nominal concentrations, however limited analytical determination of exposure concentrations was carried out which demonstrated a reduction in concentrations.

13.1.2. Aquatic invertebrates

Following the Substance Evaluation decision, a chronic toxicity test with the freshwater mudsnail *Potamopyrgus antipodarum* following the OECD TG 242 (*Potamopyrgus antipodarum* Reproduction Test) and GLP principles was performed. The study was performed using five test concentrations of the Substance ranging from 6.25 to 100 µg/L nominal concentration. For each test concentration and the blank control, six *Potamopyrgus antipodarum* were exposed to the test item for 28 days in a semi static test system. After 28 days mortality, shell length and reproduction of test organisms (mean number of embryos per female) were measured. The concentration of the test substance at the start of the test, and at every 48-hour medium renewal period (plus one 72 hour medium renewal period), was analysed using HPLC. The measured concentrations were between 79 % and 102 % of the nominal concentration in fresh media, and between <LOQ % and 67 % of nominal in aged media. Therefore, the determination of the results was based on the time weighted geometric mean of the measured concentrations. Endpoints were significantly different to the control at the highest concentration for mortality; the two highest for shell length; and the three highest for reproduction. 28-d NOEC values of 24.7, 14.7 and 6.01 µg a.i./L (based on TWA measured concentrations) are reported for mortality, shell length and reproduction, respectively. The 28-d EC10 values for the same endpoints are 44.4, > 66.7 and 4.09 µg a.i./L. The validity criteria of the test guideline were fulfilled. The EC10 for reproduction is lower than the lowest chronic values for freshwater invertebrates considered in the RAC opinion (ECHA 2014c).

A toxicity test to investigate the effect of the Substance in the fertilisation success of the sea urchin *Lytechinus pictus* was also performed following the SEv Decision. The test was performed following the Environment and Climate Change: Canada's Biological Test Method: Fertilization Assay using Echinoids (sea urchins and sand dollars). Report EPS 1/RM/27. The study was carried out according to GLP (apart from the analysis of exposure concentrations that was carried out at an accredited laboratory (ISO 9001:2008 Quality Standard, compliant with ISO 17025, and holds a Health Canada Establishment Accreditation Cooperation (ILAC)). According to the test guideline, sperm are exposed to the test substance. Eggs are then added, and the success of fertilization under continued exposure to the same concentration of test substance is measured. The endpoint is decreased success of fertilization, described in terms of the concentration estimated to cause a specified percent inhibition (ICp).

Artificial seawater was used as test medium. 20 mL glass scintillation vessels containing 10 mL of test solution and 10 ml of headspace were used as test vessels. A single stock of 100mg/L (nominal) was prepared with the test substance, using a water accommodated fraction method. 1.0012 g of test item was added to 10L of seawater in a 20L glass aspirator bottle, the solution was stirred for 24 hours using a stir bar and stir plate after which the solution was settled for approximately one hour. A portion of this stock was used to prepare the lower test concentrations. In the study gametes (sperm and eggs < 4 hours

old) collected from at least 3 sexually mature adults from an in-house stock were used as test organisms. A sperm: egg ratio of 20,000:1 was used, and the sperm density per test vessel was 40000000. First the sperm were included in the test solution and exposed for 10 minutes. Then the eggs were added, and the exposure was continued for another 10 minutes. After the total study duration of 20 minutes, the eggs were fixed with buffered formalin and the counts for the success of fertilisation was done approximately 24 hours after the study termination. The counts of fertilised and unfertilised eggs were done based on assessment of the first 100 eggs. Counts were made under a microscope at 100x magnification using a counting cell. Approximately 1000ml of all test solutions (including controls) were collected in 1L glass pre-cleaned jars with Teflon lined caps, immediately prior to start of the test (0 hours before the addition of gametes) for analysis. Samples were not taken at the termination of the test due to the short duration of the test. Gas chromatography-mass selective detector (GC-MSD) was used in the analysis.

A range finding study was carried out initially with nominal exposure concentrations of 1, 10 and 100mg/L and measured concentrations of 0.42, 0.67 and 2.69 mg/L. An IC25 of 0.42 to 0.67mg/L and NOEC and LOEC of <0.42 and 0.42 mg/l, respectively, were determined in the range finding study. A definitive study was then carried out with lower test concentrations. The nominal exposure concentrations were 0.7813, 1.5625, 3.125, 6.25, 12.5, 25.0, 50.0, 100 mg/L, with measured concentrations of 0.03, 0.11, 0.22, 0.51, 0.85, 1.51, 3.39, 6.47 mg/L. All concentrations other than the highest concentration were below the estimated limit of solubility. In addition, procedural WAF controls were prepared and treated in the same manner as the test solutions except that no test item was added to the glass jars. A negative control (consisting of 100% artificial seawater without undergoing WAF procedure) and a blank (containing eggs but no sperm) were also included. Four replicate vessels for each test concentration and treatment were included. Copper in the form of sulphate was used as a reference substance and the results were in the range of the historical mean value. The validity criteria regarding the average success of fertilisation in the negative control was fulfilled (89-91 % which is in the range of >60% and <98%). From the results of the definitive study, the IC25 and IC50 for 4-NP with 95% confidence intervals were calculated to be 1.01 (0.97 to 1.03) and 1.20 (1.16 to 1.22) mg/L respectively. The NOEC and LOEC of 4-NP were estimated to be 0.85 and 1.51 mg/L respectively. Some uncertainty arises as the results of the range finding study are lower than those of the definitive study.

The third long-term toxicity test performed following the Substance Evaluation decision is a study on long-term and transgenerational effects of sublethal exposure to the Substance at a key developmental window of Eastern oysters (Crassostrea virginica). This full life-cycle method involved a 48-hour exposure of oyster larvae (filial generation F0), which were grown out to sexual maturity and then the effects on their gametes and offspring (second filial generation F1) viability were assessed. This study sought to repeat and improve upon previous work by Nice et al. (2003) where an increased incidence of hermaphroditism (17%) and sex ratio skewed towards females resulting from an exposure of C. gigas to nonylphenol was described. In the study by Nice et al (2003) a transgenerational effect was also observed as 4-NP had an influence on the quality of the developed gametes so that they are of poor quality resulting in a reduced survival rate of the offspring from parents where at least one had been exposed to 4-NP during the larval development. In the study by Nice et al (2003) rapid decrease of test concentrations was observed and the number of individuals sampled to assess the incidence rate of hermaphrodites was low which add uncertainty in the results. The new study utilized passive dosing, or partition-controlled delivery, to maintain exposure concentrations for the full duration of the intended exposure period, and higher number of test concentrations, replicates and individuals sampled.

The test design followed the method described by Nice et al. (2003) and augmented by the methods described in the OECD No. 121 Detailed Review Paper on Mollusc Life Cycle Toxicity Testing Protocol (OECD 2010) were adopted where methodological information was lacking.

As the study has not been officially submitted in an updated registration dossier and thus is not disseminated at the ECHA website[7], no detailed information on the study can be given in the public part of this document. Further information on the study is included in the confidential annex. No clear effects, following a clear dose-response, were observed in the studied endpoints. The determined NOEC values are higher than in some other studies with aquatic invertebrates.

13.1.3. Algae and aquatic plants

Zaytzeva et al 2015 performed a study to assess the effect of nonylphenols (no further information given on the test material, e.g. whether 4-NP, branched or linear) on the growth rate of the microalgae *Anabaena variabilis Kutz*. (CALU 458), *Aphanizomenon flosaquae* (CALU 1033), *Microcystis aeruginosa Kütz*. (CALU 972), *Microcystis aeruginosa Kütz*. (CALU 973), *Nodularia spumigena Mert (*CALU 795), *Oocystis parva* (CALU 391), *Oscil latoria agardhii Gom* (CALU 1113), and *Scenedesmus quadricauda* (CALU 1248) over a 14-day period. No test guideline was followed. Analytical measurements of the test material were not performed and the results are based on nominal concentrations. Therefore, the study is not considered relevant and reliable.

Medvedeva et al (2017) performed a study to assess the effect of 4-NP on the growth rate of the cyanobacterial strain *Planktothrix agardhii* 1113 and the degradation of 4-NP over a 14-day period. No test guideline is available for this species. Analytical measurement data of test material in solution revealed that after 7 days of exposure 5% of NP were sorbed and 10% biodegraded by the algal cells. Abiotic biodegradation due to mainly photodegradation was instead estimated around 60 %. Growth of the algae was followed by measurement of dry weight. Exposure of the cyanobacteria to NP concentrations from 0.0001 to 4 mg/L did not affect their growth rate up to a concentration of 0.4 mg/L, indicated as NOEC. The study is not considered relevant and reliable for the assessment.

Zhou et al. (2013) study was conducted using the green microalgae, *Scenedesmus obliquus*, exposed to concentrations from 0.25, 0.5, 1.0, 2.0 and 4.0 mg/L of NP for 5 days. Measured concentrations of NP revealed that the NP concentrations decreased rapidly, being only approximately 10 % of the nominal concentrations after 24 hours. The results are only indicated for the exposure period of 5 days and based on nominal concentrations. Therefore, the study is not considered relevant and reliable for the assessment.

13.1.4. Sediment organisms

In the registration dossier, one study presenting long-term sediment toxicity data for two freshwater organisms *Chironomus riparius* and *Tubifex tubifex* (Bettinetti and Provini 2002) and one study presenting long-term sediment toxicity data for saltwater organisms *Leptocheirus plumulosus* (Zulkosky et al. 2002) are included. These studies were considered in the RAC opinion (ECHA 2014c and 2014d).

13.1.5. Other aquatic organisms

No information in the registration dossier. In the SVHC Support document of the Substance (ECHA 2012) information from toxicity tests assessing endocrine disrupting properties of 4-NP e.g. on amphibians can be found.

[7] Last checked on 10 April 2024.

13.2. Terrestrial compartment

13.2.1. Soil macroorganisms, arthropods, plants and microorganisms

Regarding the toxicity of the Substance to terrestrial organisms, the RAC opinion and its background document (ECHA 2014a and 2014b) refer to the EU Risk Assessment for nonylphenol (ECB 2002) and the UK revised draft version of June 2008 (EA 2008) which is an addendum report to the EU risk assessment (ECB 2002) produced by the UK in 2008 and has not been published. There are no additional tests that were not considered in the RAC opinion that are included in the registration dossier. The below summary table, taken and amended from the RAC opinion (ECHA 2014a), lists the most relevant and lowest toxicity values for plants, terrestrial invertebrates and microorganisms. The toxicity values have, when possible, been normalised to the standard TGD soil (2% organic carbon or 3.4% organic matter). Using Equation R.10-4 NOEC or L(E)C50(standard) = NOEC or L(E)C50(exp) x Fomsoil(standard)/(Fomsoil(exp)) in the ECHA guidance Chapter R.10. Further information on all available studies can be found in the RAC opinion and its background document (ECHA 2014a and 2014b). No new information on toxicity to soil organisms was requested in the Substance Evaluation decision and hence this endpoint was not further evaluated by the eMSCA.

Table 13-3 Summary of the most relevant and lowest toxicity values for plants, terrestrial invertebrates and microorganisms (taken and amended from the RAC opinion (ECHA 2014a))

Species	Endpoint NOEC/EC10	Results	OC/OM	Normalised* NOEC/EC10 (mg NP/kg dw)	Reference
Terrestrial invertebrates					
Collembolan *Folsomia candida*	64 d NOEC (survival)	32 mg/kg	3% OMb	36	Widarto et al. (2007)
Collembolan *Folsomia fimetaria*	21 d EC10 (reproduction)	23 mg/kg	3% OMb	26	Scott-Fordsmand and Krogh (2004)
Enchytraeid *Enchytraeus crypticus*	28 d EC10 (reproduction)	24 mg/kg	6.9% OM	12	Domene et al. (2009)
Earthworm *Eisenia andrei*	28 d EC10 (reproduction)	56 mg/kg	6.9% OM	28	Domene et al. (2009)
Earthworm *Eisenia fetida*	28 d NOEC	≥38 mg/kg	8.3% OMa	16	Teixeria (2002)
Collembolan *Folsomia fimetaria*	21 d EC10 (reproduction)	24 mg/kg	0.88% OC	54	Krogh et al. (1996)
Plants					
Plants *Sorghum bicolor Helianthrus*	21 d NOEC (growth)	100 mg/kg	-	100	Windeatt and Tapp (1987

rodeo Gycine max					
Plants *Brassica rapa*	15 d EC10 (germination)	575 mg/kg	6.9% OM /3.45 OC	283/333	Domene et al. (2009)
Plants *Lolium perenne*	15 d EC10 (germination)	739 mg/kg	6.9% OM 3.45 OC	364/428	Domene et al. (2009)
Microorganisms					
Soil micro-organisms	40 d NOEC (CO2 production)	100 mg/kg	11% OC	18.2	Trocmé et al. (1988)

13.2.2. Birds

Three studies on avian toxicity are included in the registration dossier.

Cheng et al (2019) carried out a study to assess the sub-lethal and reproductive effects of 4-NP on Japanese quails (*Corurnix japonica*) over a 21 week period. The study was carried out according to the OECD guideline 206, but not according to the principals of GLP. The study included several deviations from the guideline, including that the concentration in the feed was not verified. Several standard endpoints from the original guideline were not included, however many of the suggested reproductive endpoints from the update 2018 guideline were included. The experiment was separated into 5 phases, the acclimation phase, the initial phase, the second phase, the final phase and the withdraw phase. The quail were 14 days old at the start of the exposure period (initial 14 day acclimation phase). Of the 21 weeks, the quail were exposed to NP for 17 weeks via their feed (the initial phase, the second phase and the final phase), at three different exposure concentrations, 10, 20 and 50mg/kg diet, plus a control. A large number of parameters were assessed over the course of the experiment and at the end of the experiment. NOECs determined for male body weight, fertilisation rate, survival of newborn quails after 14 days, histopathology of male gonads were <10mg/kg diet; the NOEC for female body weight was 10mg/kg diet; the NOEC for number of eggs produced, eggshell thickness, number of broken eggs, sex ratios and female ovarian tissue were >50mg/kg diet; and the NOEC for hatching rate was 20 mg/kg diet. Overall, the study indicated potential effects of 4-NP on quail reproduction at less than the lowest treatment concentration of 10mg/kg diet.

An additional study (Cheng et al 2017) was included which also assessed the effect of the test substance on the Japanese quail, *Coturnix japonica*, for 18 weeks period via drinking water to NP concentration of 0.1, 1, 10, 100µg/L. The study followed the OECD 206 "avian reproduction test". However, no analytical monitoring of the concentration of NP in the drinking water was performed, and hence the results are not considered reliable. A NOEC of 0.001 mg/L was determined for changes in the body weight of males, fertilization rate, hatchability and average of 14 d survival rate. A NOEC of 0.01mg/L was found for changes in weight of females during exposure and no effect was found up to the maximum concentration tested for reproductive parameters (8 weeks) and changing in the eggshell thickness, determining a NOEC >0.1 mg/L. The feed consumption was also tested for 21 weeks, starting from the 18th week of exposure to NP. No detrimental effect was found.

The registration dossier includes also a study on the effects of the read across substance 4-tOP on a non-standard bird species (Millam et al., 2001). However, in the Substance Evaluation decision, ECHA indicated that this study has several shortcomings and is not adequate for regulatory purposes.

13.3. Microbiological activity in sewage treatment systems

In a 3-hr acute toxicity study, domestic activated sludge was exposed to NP at nominal concentrations of 125, 251.6, 500, 1000, and 200.2 mg NP/L under static conditions in accordance with OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test). The EC50 of 950 mg NP/L was a calculated respiration rate based on percent inhibition of respiration of 22.1, 39.9, 43.7, 47.7, and 44.0 for each of the increasing test concentrations, respectively. Based on the results of this study, NP would be classified as toxic to domestic activated sludge. The authors concluded total inhibition of activated sludge respiration cannot be expected due to the limited water solubility of NP.

13.4. PNEC derivation and other hazard conclusions

The PNEC values have been calculated based on the toxicity data on the "traditional" apical endpoints. This was also done by RAC in the RAC opinion (ECHA 2014a). However, as also noted in the RAC opinion, these PNEC values may not cover the endocrine disrupting effect in all organisms, as there is information from non-standard studies that endocrine disrupting effects may occur at lower concentrations. In general, currently it is considered that it is difficult to define a threshold for endocrine disrupting effects. Therefore, minimisation of emissions and exposure must be applied to ED substances. In conclusion, the PNEC values presented in the below table should not be considered as threshold values for safe use of the Substance as they are likely not protective enough for the endocrine disrupting effects.

The lowest relevant chronic value for freshwater organisms is the 140-d NOEC of 1.27 µg/L determined for Japanese medaka in Watanabe et al. (2017). As chronic data is available for all three trophic levels, an assessment factor of 10 could be used for PNEC freshwater.

Regarding the marine water PNEC, in the SEV decision it was indicated that Winter Flounder (*Pleuronectes americanus*) is a sensitive fish species in acute tests, with a reported 96-h LC50 of 17 µg/L (Lussier et al., 2006). No long-term toxicity data on the Substance are available for this or other marine fish species. As described in the RAC opinion (ECHA, 2014a), the long-term NOEC for Winter Flounder might be around 0.4 or 0.8 µg/L, based on the acute:chronic ratio for Rainbow Trout or Fathead Minnow, respectively. The SEV decision included a request for an estimated chronic NOEC for Winter Flounder based on the reported 96-h LC50 of 17 µg/L from Lussier et al. (2006) and the worst case acute:chronic ratio from other fish species in the aquatic toxicity data set. The eMSCA has used as a worst case the acute:chronic ratio calculated for rainbow trout based on LC50 220 µg/L and NOEC 6 µg/L. This resulted in an estimated NOEC of 0.46 µg/L for Winter flounder. This is the lowest relevant value for marine organisms.

According to ECHA Guidance R.10, it is proposed that data on freshwater or marine fish, crustacea and algae be used interchangeably for evaluation of the risks to either compartment when there is no reason to believe that a systematic bias to freshwater or marine species would exist. The difference between the lowest determined chronic value for freshwater and marine organisms for the Substance (fish in this case) is a factor of 2.8. This is similar to the average differences typically observed in sensitivity for freshwater and marine species comparisons (within a factor of ~2) indicated in the ECHA Guidance R.10. Long-term studies with additional marine invertebrates are available for the Substance and they did not show significantly higher sensitivity when compared to freshwater invertebrates. Therefore, the eMSCA considers that no significant differences in the sensitivity of freshwater and marine organisms to the Substance are expected. Hence, the eMSCA derived only one aquatic surface water PNEC that covers both freshwater and marine compartments. The PNEC is derived based on the estimated NOEC of 0.46 µg/L for Winter flounder.

Table 13-4 PNEC derivation and other hazard conclusions

Compartment	Hazard conclusion	Remarks/Justification

Freshwater and marine water	$PNEC_{aqua}$ (freshwater and marine): 0.046 µg /L	Assessment factor: 10 Extrapolation method: The lowest relevant chronic toxicity value is the NOEC of 0.46 µg/L estimated for Winter flounder based on the LC50 of 17 µg/L determined in Lussier et al (2006). As chronic data is available for all three trophic levels (fish, invertebrates and algae) and for additional marine taxonomical groups (e.g. echinoderms and molluscs), an assessment factor of 10 is used.
Sediments (freshwater)	$PNEC_{sed}$ (freshwater): 4.62 mg/kg dw	Assessment factor: 50 Extrapolation method: The lowest relevant chronic toxicity value in freshwater sediment organisms is the 28-d NOEC of 231 mg/kg determined for *Chironomus riparius* in Bettinetti and Provini (2002). As two long-term tests (NOEC or EC10) with species representing different living and feeding conditions are available, an assessment factor of 50 is used.
Sediments (marine water)	$PNEC_{sed}$ (freshwater): 1.23 mg/kg dw	Assessment factor: 50 Extrapolation method: The lowest relevant chronic toxicity value in marine sediment organisms is the 28-d NOEC of 61.5 mg/kg determined for *Leptocheirus plumulosus* in Zulkosky et al. (2002). As three long-term tests with species representing different living and feeding conditions are available, an assessment factor of 50 is used.
Sewage treatment plant (STP)	$PNEC_{WWTP}$ >: 9.5 mg/L	Assessment factor: 100 Extrapolation method: The lowest relevant toxicity value in STP microorganisms is the EC50 of 950 mg/L determined in Hüls-Diefenbach (1999).
Soil	$PNEC_{oil}$: 1.2 mg/kg dw	Assessment factor: 10 Extrapolation method: The lowest relevant chronic toxicity value in soil organisms, when normalised to the standard TGD soil (2% organic carbon or 3.4% organic matter), is the 28-d EC10 of 12 mg/kg dw determined for *Enchytraeus crypticus* in Domene et al. (2009). As long-term toxicity tests for three species of three trophic levels are available, an assessment factor of 10 is used.
Secondary poisoning	$PNECoral_{bird}$: 0.33 mg/kg food	Assessment factor: 30 Extrapolation method:

		The lowest relevant chronic toxicity value in birds is NOEC of < 10 mg/kg food determined for Japanese quails in Cheng et al (2019).

13.5. Conclusions of the environmental hazard assessment and related classification and labelling

The Substance has a harmonized classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The registrants have applied M-factors of 10 for both acute and chronic classifications. The eMSCA supports the registrants' conclusion for the acute M-factor. However, the eMSCA has considered as the lowest relevant pelagic toxicity value the NOEC of 0.46 µg/L estimated for Winter flounder using the acute:chronic ratio and based on the LC50 of 17 µg/L determined in Lussier et al (2006). Based on this value and considering the Substance as non-rapidly degradable, a chronic M-factor of 100 should be used.

The Substance is identified as SVHC due to endocrine disrupting properties in the Environment. Therefore, it fulfils the classification criteria as ED ENV Cat.1 (EUH430) under CLP.

14. Human health hazard assessment

Not evaluated by the ES eMSCA.

15. Endocrine disrupting (ED) properties assessment

Not assessed as the Substance has already been identified as a SVHC due to its endocrine disrupting properties in the environment.

16. PBT/vPvB and PMT/vPvM assessment

16.1. Persistence

Weight of evidence approach was used by the eMSCA to assess the persistence of different potential isomers of 4-NP. Based on the available information, the degradation of the different isomers of 4-NP may significantly differ, depending on the position and degree of branching as well as the length of the side chains of the alkyl chain. Therefore, the results for the linear 4-n-NP isomer or for a mixture of branched 4-NP as a whole cannot be used to conclude on the persistence of all isomers of 4-NP. Due to the high number of different possible isomers and limited number of studies where the degradation of different isomers has been studied, it is not possible to firmly conclude on the degradation of different isomers with the available information.

Based on the lag-phase observed in several studies, and the faster degradation observed in pre-exposed than in not pre-exposed environmental matrices, it seems that adapted micro-organisms can degrade rapidly at least most of the 4-NP isomers. However, the similar substance 4-t-OP showed no degradation in an OECD TG 302C study, which suggests persistence of isomers of 4-NP with similar branching. Considering all the available information including the OECD TG 302C study and BODIS test with 4-t-OP, the non-standard degradation studies with 4-t-OP and with the mixture of different branched isomers of 4-NP and the QSAR predictions for example isomers of 4-NP, it appears that at least some of the isomers of branched 4-NP may degrade slowly, especially by non-adapted micro-organisms. Therefore, it cannot be excluded that some of the isomers of 4-NP fulfil the criteria for P and possibly also for vP in freshwater and seawater according to Annex

XIII. Hence, the eMSCA concludes that based on the available information it is not possible to conclude that all the isomers are not-P.

16.2. Bioaccumulation

The eMSCA applied a weight of evidence approach for the determination of the potential for the Substance to bioaccumulate in aquatic and air-breathing organisms. No standard guideline studies are available for fish or aquatic invertebrates but several published studies from open literature are included in the registration dossier and some further studies were found by the eMSCA. Several field and monitoring studies are also available. As in the case of persistence, also the bioaccumulation potential of the different isomers is expected to differ as the degree and type of branching may affect metabolism and elimination in organisms. However, in the available tests the results are given for the whole substance and hence no conclusion can be drawn on individual isomers.

Considering all the BCFs (all below 2000) reported in the available laboratory studies with fish, it seems that 4-NP does not bioaccumulate in fish to the extent to fulfil the B criterion in Annex XIII. This could be due to rapid metabolisation and excretion of the metabolites.

BCF values above the B criterion were observed in some of the laboratory studies available for aquatic invertebrates. In Ekelund et al (1990) BCFss of 2700 (ww) determined for mussels, and in Gatidou et al. (2010) BCFss close to 2000 were determined for mussel (in dw, BCF in ww lower) but steady state was not reached in the studies. A BCFk of 3838 (ww) is reported for shrimp *Gammarus pulex* in Gross-Sorokin et al (2003).

BSAF values in the range of 14.1-55.4 for *Lumbriculus variegatus* in two studies (Croce et al. 2005, Mäenpää and Kukkonen 2006) and in the range of 7.1-33.9 for three amphipods in another study (Hecht et al 2004). According to ECHA Guidance R.11 (Appendix R11-4), lipid and organic carbon normalized BSAF values of 0.5 and higher are an indication of high bioaccumulation.

In most of the available studies with invertebrates steady-state was not reached during the exposure phase. This suggests that 4-NP has slow elimination in most invertebrates.

Based on the available toxicokinetic studies with mammals, 4-NP may be quickly metabolised and eliminated by mammals.

Several field studies measuring concentrations of 4-NP in animals and in their surrounding environmental media are also available. No clear conclusion can be drawn based on these studies as the results are varying and sometimes contradicting and there are a several uncertainties. However, in some of the studies bioaccumulation of 4-NP was observed.

In conclusion, based on the weight-of-evidence assessment, the Substance is not expected to be B/vB in fish, but it could fulfil the criteria for B at least in aquatic invertebrates based on the available information. Further assessment on the differences of bioaccumulation potential of different isomers of 4-NP would be needed to firmly conclude. However, as no firm conclusion on the persistence can be drawn, the bioaccumulation potential was not further assessed by the eMSCA.

16.3. Mobility

Not evaluated by the eMSCA. However, a log Koc of 4 is reported by the registrants based on the results of an OECD TG 106 study from the open literature (Milinovic et al. 2015). The registration dossier includes several other studies that also support the conclusion that the log Koc is not below 3, and hence, the Substance is not expected to be mobile according to the CLP criteria.

16.4. Toxicity

The Substance is an SVHC due to its endocrine disrupting properties in the environment. The lowest chronic toxicity values in the available aquatic toxicity tests are well below 0.01 mg/L. Therefore, the Substance is T according to the Annex XIII criteria. However, it is noted that there can be differences in the toxicities and endocrine disrupting properties of the different isomers of 4-NP (ECHA 2012a).

16.5. Conclusions of the PBT/vPvB/PMT/vPvM assessment and related classification and labelling

The Substance is a UVCB consisting of several isomers of 4-NP with different branching. The PBT properties of the different isomers differ.

It is not possible to conclude on the persistence of all isomers. Concern remains that some of them could potentially be P/vP. At least some of the isomers are likely to be B (or even vB) in aquatic invertebrates. The Substance as a whole is T.

In conclusion, no definitive conclusion on the PBT/vPvB properties of the isomers of the Substance can be drawn.

Impurities:

In the SEV decision of the Substance, it was indicated that *dinonylphenol was an impurity of NP identified previously under the ESR (EC, 2002) and is mentioned in some (but not all) registration dossiers. 2,4-Dinonylphenol (EC number 284- 323-4) was identified as having potential vPvB properties (PBT list no. 103) by the PBT Working Group of the Technical Committee for New and Existing Substances because it was not considered to be readily biodegradable and has a predicted log KOW value above 5. No measured data were available. It is likely that all dinonylphenol isomers will have similar vPvB properties.* No experimental data on degradation, bioaccumulation or toxicity of dinonylphenol was found by the eMSCA during the Substance Evaluation. Therefore, no definitive conclusion on its potential PBT/vPvB properties can be drawn.

17. Exposure assessment

The ES eMSCA has not performed an in-depth exposure assessment for the Substance in the follow-up phase of the Substance Evaluation process. The UK eMSCA evaluated information on exposure during the initial phase of the process. The Substance is identified as SVHC due to endocrine disrupting properties in the environment. The eMSCA considers that based on the available information it is not possible to define a safe use threshold for the endocrine disrupting properties and the PNEC values derived based on apical effects are not sufficiently protective for the endocrine disrupting effects of the Substance. Therefore, it must be ensured that the emissions and exposure of the Substance are minimised.

There are several emissions pathways leading to exposure of the environment to the Substance. These include manufacture and uses of the Substance as such at industrial sites (e.g. as intermediate in the manufacture of other substances and polymers) as well as uses of substances and polymers that contain the Substance as constituent or impurity or that can degrade to the Substance in the environment. Although most of the Substance is used at industrial sites, based on the available registration information, uses of the Substance as such in mixtures by consumers and professional workers cannot be excluded either. Furthermore, polymers and other derivatives of the Substance may have wide dispersive uses. Information on exposure was requested in the SEV Decision of the Substance. The registration dossier of the Substance includes some exposure information on some of the polymers, but not on other derivative substances that have their own registration dossiers under REACH. Therefore, the eMSCA considers that further

assessment on the exposure of the environment to the Substance could be done in the context of the proposed restriction covering a wider group of relevant substances.

Based on the most recent available monitoring data found in the Norman database[8] (from year 2020), the concentrations of the Substance measured in surface waters in different locations in the EU were mostly below LoQ of the studies, but in several locations (e.g. in Germany, France, the Netherlands) concentrations ranging from 0.01 to up to approx. 2 µg/L are reported. Hence, the concentration of the Substance may exceed the PNEC value of 0.046 µg/L (which does not consider ED properties) determined by the eMSCA for surface water in this dossier, as well as the current EQS for annual average concentration (0.3 µg/L) set in the WFD. It is noted that new lower EQS values of 0.037 and 0.0018 µg/L for freshwater and marine water, respectively, have been proposed for the Substance (SCHEER 2022).

18. Risk characterisation

Not applicable as no exposure assessment was performed in this evaluation.

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20. Abbreviations

4-NP	4-nonylphenol
4-n-NP	linear 4-nonylphenol
4-t-OP	4-(1,1,3,3-tetramethylbutyl)phenol
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BCFk	Kinetic BCF
BCFss	Steady-state BCF
BMF	Biomagnification factor
BPR	Biocidal products regulation (EU) 528/2012
BSAF	Biota-soil or Biota-sediment accumulation factor
CAS RN	CAS registry number
CCH	Compliance check
CLP	Classification, labelling and packaging
CoRAP	Community rolling action plan
DMEL	Derived minimal effect level
DNEL	Derived no-effect level
EC	European community
ECHA	European chemicals agency
ED	Endocrine disruption
EQS	Environmental Quality Standard
ESR	the Existing Susbtances Regulation
EU	European union
EUSES	European union system for the evaluation of substances
GC	Gas chromatography
GLP	Good laboratory practice
HPLC	High performance liquid chromatography
MSCA	Member state competent authority
NER	Non-extractable residues
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NONs	Notification of new substances
OECD	Organisation for economic co-operation and development
PBT	Persistent, bioaccumulative and toxic
PMT	Persistent, mobile, and toxic
PNEC	Predicted no-effect concentration
POP	Persistent organic pollutants
PPP	Plant protection products regulation EC 1107/2009
QSAR	Quantitative structure-activity relationship
RAR	Risk assessment report
REACH	Regulation No 1907/2006 concerning registration, evaluation, authorisation, and restriction of chemicals
STOT RE	Specific target organ toxicity – repeated exposure
STOT SE	Specific target organ toxicity – single exposure
SVHC	Substances of very high concern
TG	Test guideline
TGD	Technical guidance document
TMF	Trophic magnification factor
tNP	technical 4-nonylphenol

TOC	Total organic carbon content
TPE	Testing proposal examination
UNEP	United nations environment program
UVCB	Unknown or variable composition, complex reaction products or of biological materials.
vPvB	Very persistent and very bioaccumulative
vPvM	Very persistent and very mobile
WAF	Water accommodated fraction
WFD	Water Framework Directive
WoE	Weight of evidence
WWTP	Wastewater treatment plant

Annex 1 Persistence weight-of-evidence assessment

1 Introduction

Problem Formulation

Is there sufficient evidence on the persistence properties of the isomers of branched 4-NP to fulfil the Annex XIII of REACH and CLP criteria for P/vP?

Collection and documentation of all information

Information/evidence used in the approach include:

- Experimental studies from Endpoint study records from corresponding IUCLID Registration Dossiers
- Studies found from open literature (searched e.g. in PubMed using key words nonylphenol/octylphenol + degradation, nonylphenol + isomer)
- QSAR Results
- EU RAR for nonylphenol (CE, 2002)

2 Assessment of quality of individual evidence

Abiotic degradation

Hydrolysis

No tests on hydrolysis are available. Hydrolysis is not expected to be a significant degradation process due to lack of functional groups susceptible to hydrolytic attack in 4-NP.

HYDROWIN (v2.00) cannot be used for 4-NP as it does not contain any of the structural fragments for which the model estimates hydrolysis rate.

Phototransformation in air

AOP Program (v1.92) QSAR model predicts for the example isomers of 4-NP for the reaction with OH-radicals in air an overall rate constant of approx. 48 x 10^{-12} cm^3 $molec.^{-1}$ s^{-1} and a half-life of 0.2 days (assuming 12-hr day and a concentration of 1.5 x 10^6 OH radicals/cm^3 in the atmosphere). In view of the short half-life, photo-oxidation may be an important degradation process for the removal of nonylphenol and further alkylphenols and possibly for lower alkylphenol ethoxylates in the atmosphere.

This half-life is < 2 days, which is the cut off for potential long-range transport[9]

The fraction sorbed to airborne particulates predicted by AOP Program is low (0.00496-0.0617), and therefore, significant hindrance of photo-oxidation due to adsorption is not expected. As the substance does not contain halogens and does not have the potential to reach the stratosphere it is not expected to have ozone depletion potential.

9 http://www.unece.org/fileadmin/DAM/env/documents/2000/ece/eb/ece%20eb%20air.60.e.pdf

Phototransformation in water

Information from three non-guideline photolysis studies is available, one with branched 4-NP and two with linear 4-n-NP. Significant deviations from the standard guideline are observed, and hence the studies are not considered fully reliable. Furthermore, photodegradation of linear and branched 4-NP isomers may differ. So, these results are considered as supporting information suggesting that 4-NP could be photochemically degraded in the surface layers of natural water. However, they are not used for assessing photodegradation rate.

Ahel et al (1994) assessed the rates of photochemical transformation of 4-NP (a mixture of differently branched isomers). Sunlight phototransformation of 4-NP was performed in 50 mL quartz tubes which were suspended in a shallow flat-bottomed container filled with tap water or in Chriesbach creek at a depth of 20-25 cm. The solutions of 4-NP were prepared in filtered (0.45 ~tm) lake water (Greifensee, DOC=4 mg/L) with an initial concentration of 0.39-1 mol/L. During the experiment the creek water was clear and the temperature, varied between 14.5-17 °C, depending on the time of day. The temperature in the shallow vessel was adjusted (addition of ice) to be similar to that of the creek (17±3 °C). The total sunlight irradiation was determined by integrating the values which were recorded in time intervals of 10 min. The experiments were performed twice on 11th September, 1985 (from 11:20 to 17:20 h; total duration 351 min) and on 18th September, 1985 (from 11:05 to 16:05 h; total duration 288 min). The average sun irradiation intensities during the experiments were 0.705 kW/m^2 and 0.760 kW/m^2 respectively. The first experiment was performed at the original pH value of lake water (8.4), while in the second it was adjusted to 9.4 by the addition of NaOH but during the experiment the pH decreased to 8.7. Additionally, laboratory experiments were performed for 4-NP (1.13 µmol/L) in distilled water or filtered lake water (4 mg/L DOC) using a merry-go-round reactor (MGRR) supplied with medium pressure mercury lamp. Moreover, to examine the photo-oxidation via singlet oxygen mechanism and the dependence of the photolysis rate of 4-NP on pH of the solution were examined in MGRR using a tungsten-halogen lamp. Rose bengal dye (5 mg/L) was added as sensitiser to the 4-NP solutions in ordinary water (H_2O) or heavy water (D_2O) with a phosphate buffer to produce singlet oxygen in the pH range from 7.0 to 10.7. Quantitative determinations of the analytes were performed by normal-phase HPLC after a simple extraction of the water samples with n-hexane.

The photolysis rate constants (kp) were calculated by presuming first-order kinetics. The photolysis was much faster (kp=0.09 h-1) in tubes suspended in a water-filled flat-bottomed container than in the tubes suspended in a creek (kp=0.06 and 0.05 h-l). This could be attributed to differences in the radiation intensity which was estimated to be about three times higher in the flat-bottomed container than in the creek at a depth of 20-25 cm based on measurements by actinometry with p-nitroanisol. It is indicated that the half-life of the photochemical degradation was estimated from the irradiation dose needed for 50 % degradation. The authors report estimated half-life values in the range of 15-20 hours corresponding to a continuous sunlight intensity of 0.700 kW/m2 which is a typical value for late summer at noon in Diibendorf. Furthermore, the authors speculate that under continuous clear sky, noon, summer sunlight in the surface layer of natural waters the half-life values are expected to be considerably lower (approximately 10-15 hours).

The rate constants for photolysis of 4-NP in distilled water (direct photolysis) and filtered lake water (indirect photolysis) using the MGRR were 0.20 and 0.92 h^{-1}, respectively. Hence, the photolysis in the presence of natural organic matter seems to be much faster than the direct photolysis. It is stated that the mercury lamp (700 W) and Solidex glass filter (λ>280 nm) employed in the apparatus provided approximately 10 times more light intensity than sunlight during a sunny summer day. Moreover, the photolysis of 4-NP in the pH range between 7.0 and 9.0 was virtually constant (kp=0.45 and 0.50 h^{-1}), while the photolysis at pH 10.7 is only slightly faster (kp=0.92 h^{-1}). The experiments with D_20 revealed that singlet oxygen was not an important photooxidant of NP at pH values usually found in natural waters.

The second study by Martinez-Zapata et al (2013) considered photo degradation of 4n-nonylphenol, rather than 4-nonyphenol, and investigated both direct and indirect

photolysis. Direct photolysis was assessed in ultra pure water at pH 5 and 9. Indirect photolysis was assessed using ferric ion (Fe(III)) at two different concentrations and with or without the presence of humic acids as the sensitisers, in addition to the effects of pH on transformation rates. Photodegradation was also assessed using two natural reservoir waters with different physico-chemical conditions. The test was performed using a Suntest CPS/CPS+ photosimulator from Atlas (Ref. 55007017, Chicago, USA) equipped with a xenon lamp filtered by a UV filter that delivered a light emission spectrum similar to that of the sun (250±10 W m^{-2}). Pyrex flasks containing 200ml of 4n-NP at a concentration of 2 mg/L were used as test vessels. Each experiment was performed in duplicate. In addition, darkness assays were performed at room temperature and at the two selected pH values to determine whether 4n-NP hydrolysed. The concentration of 4n-NP was measured using GC/MS for up to 5 hours in most experiments (up to 25 hours on occasion).

In the direct photolysis experiments, 4n-NP was degraded 98% after 19 h at pH 5 and 97% after this time at pH 9. It should be noted that in the test vessels that were kept in darkness, it is indicated that 25% of the test substance disappeared at pH 9 while at pH 5 it seems that < 10% disappeared. This could indicate that hydrolysis took place and was higher at pH 9. For the indirect photolysis experiments, a multivariate analysis of variance for the degradation of 4n-NP was carried out which demonstrated that pH was the most important factor in the process. There was a positive synergistic effect between the concentration of Fe(III) and humic acids at pH9. Humic acids without the influence of other variables had a negative effect by inhibiting photolysis in all other conditions. Up to approximately 70% disappearance occurred in the natural waters after 5 hours and was thought to be dependent upon the physico-chemical conditions of the test waters. The photodegradation kinetics of 4n-NP in ultrapure water were investigated under the most favourable conditions according to the study, i.e., at pH 9, Fe(III) concentration of 2 mg/L and a humic acid concentration of 1 mg/L. A first-order reaction kinetics was adjusted to describe the photodegradation of this compound with a half-life of 2.3 h.

The third study by Dulov et al (2013), also considered direct and indirect phototransformation of 4-n-nonylphenol with hydrogen peroxide (H_20_2) and Fe^{2+}as sensitizers (Fenton/photo-Fenton processes) and the influence of pH on these transformation rates. A mercury low-pressure OSRAM lamp with an energy input of 10 W located in a quartz tube inside the reactor was used as an UVC (thereinafter UV) source. UV radiation photon flux at 254 nm measured by potassium ferrioxalate actinometry (Gordon and Ford, 1972) was 5.52±0.38 µEinstein/sec. A constant temperature of 22 ± 1°C was maintained in the reactor. NP solutions were treated in a 1-L cylindrical glass reactor with permanent agitation for a period of 30–180 min. The pH values of the different experiments ranged from 3 to 11. The removal of 4n-NP from the aqueous solution was assessed for up to 3 h by measurement of NP concentration using HPLC. Nonylphenol removal by the direct and hydrogen peroxide photolysis was shown to be pH dependent, being faster in alkaline medium. The 90% conversion times ($T_{90\%}$) for the direct UV photolysis at pH 7 and pH 11 were 97 and 29 min, respectively. The pseudo-first order rate constant for the experiment at pH 11 was (13.2 ± 0.33) × 10−4 sec−1 , which was three times higher than the rate constant for trial at pH 3. The addition of 50, 100 and 500 µmol/L of H_2O_2 resulted in faster removal. The application of the hydrogen peroxide photolysis at pH 11 and at H_20_2dose of 250 µmol/L demonstrated the highest nonylphenol removal rate ((31.7 ± 0.55) $\times 10^{-4}sec^{-1}$, T90% 12 min). The time taken for 90% conversion of 4n-NP in all the experiments ranged from 12 minutes to > 180 minutes, but was generally at the lower end of the time scale. The results of 4n-NP degradation by the photo-Fenton process revealed similarity in terms of performance with the hydrogen peroxide photolysis.

Biodegradation

Three screening studies for biodegradation in water for branched 4-NP are available in the registration dossier. In addition, one study for 4-n-NP and four studies for the read across substance 4-t-OP are also available.

In the first study (Unnamed, 1999a at ECHA dissemination website), the biodegradability of 4-NP (EC 284-325-5) was determined according to OECD TG 301B and GLP. 4-NP was

tested at a concentration of 13 mg/l (equivalent to 10 mg DOC/l) for 35 days in duplicate vessels. Actual concentrations added to each flask were calculated from the measured weights added to the test vessels dispensed on cleaned glass microfiber filters. The inoculum came from a New Bedford Wastewater Treatment Plant, which is indicated to have a high nonylphenol ethoxylates concentration, and hence, it is considered to be pre-adapted to 4-NP. In the registration dossier it is stated that most of the time the test temperature was in the range of 22 +/- 2 °C indicated in the guideline but on several occasions of the study, the minimum/maximum temperatures (19 - 29 °C) were outside this range but these deviations were temporary (< 48 h). Sodium benzoate and diisotridecyl adipate procedural controls, two blank controls and a sterile control were also included. Sampling was done on days 1, 2, 4, 6, 9, 13, 18, 22, and 28. On day 28 the first $Ba(OH)_2$ CO_2 trap was removed from each vessel and analysed. On day 35 the test was finalised and all CO_2 traps were removed and analysed. Ready biodegradability tests may be prolonged beyond 28 days when the curve shows that biodegradation has started but that the plateau has not been reached by day 28, but in such cases the chemical would not be classed as readily biodegradable. Degradation of nonylphenol reached a mean of 47.5% after 28 days and 48.2% after 35 days using an adapted activated sludge. The sodium benzoate and diisotridecyl adipate procedural controls reached 94.1 and 66.5% degradation, respectively, after 28 days and 95.4 and 78.3 % degradation, respectively after 35 days. The study is considered reliable with restrictions. Due to the use of inoculum pre-exposed to the substance, the study is not adequate to be used to assess persistence. However, it can be used as part of weight of evidence assessment.

In a study by Staples et al (2001), the biodegradability of 4-NP (CAS RN 84852-15-3) was determined according to OECD TG 301B and GLP. 4-NP was tested at an initial concentration of 13 mg/l (equivalent to 10 mg DOC/l) for 35 days in duplicate vessels. The inoculum came from a New Bedford Wastewater Treatment Plant, which is indicated to receive wastewater containing measurable levels of polyethoxylated alkylphenols, similar to nearly all municipal wastewater treatment plants in North America according to Staples et al. (2001). Hence, the inoculum is considered to be pre-adapted to 4-NP. CO_2 traps were removed from all vessels on days 1, 2, 4, 6, 9, 13, 22, 28 and 35 for analysis. On days 15 and 35 samples were collected from each vessel for analysis of 4-NP. Total samples were analysed, including all dissolved and suspended solids material. Samples for DOC measurements were also collected on days 15 and 35. Temperature was within 22±2 °C, and pH remained within 7.4 to 7.6 during the test. Sodium benzoate positive controls reached 60% mineralisation on day 6 and 94.5% by day 35. 4-NP reached 48.2 % degradation after 35 days based on evolved CO_2. Based on the results of HPLC and GC/MS analyses from samples collected on days 0, 15 and 35, only low concentrations of the test substance (18 µg/L) remained by day 15, and by day 35, 4-NP was no longer extractable or identifiable as parent compound in the test media that included both 4-NP dissolved in the test solution and sorbed into particulate matter. This seems to be the same study as Unnamed (1999) but published later. The study is considered reliable with restrictions. As indicated above, due to the use of inoculum pre-exposed to the substance, the study is not adequate to be used to assess persistence. However, it can be used as part of weight of evidence assessment.

In another study Staples et al. (1999) examined the biodegradability of 4-NP (CAS RN 84852-15-3) (at an initial concentration of 31 mg/L) in a study following OECD TG 301 F. Activated sludge from a municipal sewage treatment plant (Somerset-Raitan Valley Authority, Bridgewater, NJ, USA) was used as inoculum. It is not specified whether the inoculum was pre-adapted or not. The test substance was tested in triplicate. Blank controls containing only inoculum and mineral medium were prepared in triplicate and the positive control, sodium benzoate (SB), was tested in duplicate. Based on oxygen consumption, approximately 62 % of 4-NP was biodegraded in 28 days. The degradation reached 10% by day 8 and reached 60% by day 25, and hence the 10-day window criteria were not met. The positive control, sodium benzoate degraded to approximately 94% by day 28.

But uncertainties due to acclimation rise since compounds show the same degradation kinetic than under the adapted industrial activated sludge. The source of the inoculum,

Somerset Raritan Valley Sewerage Authority, established in 1958, is a regional wastewater treatment plant located in Somerset County, New Jersey. It is a local governmental agency serving seven communities; Branchburg, Bridgewater, Hillsborough, Manville, Raritan, Somerville and Warren, and operates a 23.0 millions of gallons per day secondary advanced wastewater treatment system. According to information available from a survey conducted by the New Jersey Department of Environmental Protection in 2021 requiring all industrial dischargers and significant indirect users to provide information on their discharges of PFAS[10], it appears that there are discharges of industrial wastewater to the Somerset Raritan Valley Sewerage Authority. Therefore, pre-exposure of the inoculum to 4-NP used in the OECD TG 301F study cannot be excluded and the study may not be adequate to be used to assess persistence. However, it can be used as part of weight of evidence assessment.

Stasinakis et al. (2008) studied the degradation of 4-n-NP (CAS RN 104-40-5; linear isomer) in OECD TG 301F. Activated sludge from a municipal wastewater treatment plant (Mytilene, Lesvos), indicated to be non-adapted to the test substance, was used as inoculum. The initial concentration of 4n-NP was 30 mg/L (89.5 mg ThOD/L). Solution of 4-n-NP in methanol (1–2 ml) was poured into AQUALYTIC(R) flasks and it was allowed to stand at room temperature for complete methanol evaporation. Three replicates were included. Blank control as well as a positive control containing sodium acetate trihydrate (217 mg/l or 102 mg/l as ThOD and a toxicity control containing a mixture (0.88:1 as ThOD) of 4-n- NP and sodium acetate trihydrate were included in the test. As a deviation from the guideline, allylthiourea (CAS RN 109-57-9) was added in all flasks at a concentration of 10 mg/l to prevent nitrification; according to the author, it has been proved to be an effective inhibitor of nitrification and has often been used in biodegradation tests. Allylthiourea was also included in the blank control, and when calculating the results, the amount of oxygen consumed in the test vessel with 4-NP was corrected for the uptake in the blank control. After an initial lag phase of 7.9 ± 0.6 days the degradation of 4n-NP reached 61.5% by day 28 (O2 consumption). Degradation reached 10% by day 8 and exceeded 60% by day 14, in approximately 6 days, therefore fulfilling the 10d-window. The study is reliable with restrictions. Information from linear 4-n-NP is not adequate to assess the degradation of branched isomers of 4-NP. However, it can be used as part of weight of evidence assessment, especially for isomers of 4-NP with only low level of branching.

Further information from screening studies is available for the read across substance 4-t-OP at the ECHA dissemination site. This information is considered relevant for the isomers of 4-NP with similar branching as 4-t-OP. In the study by Staples et al. (2001), degradation of 4-t-OP (purity 99.64%) was also investigated using the same conditions and study procedures as indicated above for 4-NP. 69.9 % degradation of 4-t-OP was observed after 35 days based on CO2 evolution. No 4-t-OP was extractable from the test medium after 15 and 35 days. However, as noted above for the study by Staples et al. (2001), the inoculum used in the study came from a STP with high concentration of polyethoxylated alkylphenols and hence it is considered to be pre-exposed to the test substance. In the dissemination site another OECD 301B test (Unnamed 1999b) is also reported for 4-t-OP. As in the case of 4-NP, this study seems to be the same as the one reported in Staples et al. (2001) based on the same conditions, procedure and results reported. The degradation of 4-t-OP reached 62.1% degradation after 28 days and 69.9 % after 35 days. As indicated for the study by Staples et al (2001), the inoculum was pre-exposed to alkylphenols, and hence, this study is not considered adequate for the persistence assessment. However, it can be used as part of weight of evidence assessment.

In a GLP compliant test (Unnamed 1991a) following the ISO Draft (BOD Test for Insoluble Substances) guideline (BODIS test), the read across substance 4-t-OP (purity 95.6%) at an initial concentration of 27.5 mg/L was incubated for 28 days in a mineral medium containing activated sludge from a predominantly domestic sewage plant (sewage plant Marl-Ost, Germany) as an inoculum. The inoculum is stated to be non-adapted. Narrow

[10] https://www.nj.gov/dep/dwq/pfas.htm

neck bottles with glass stoppers, 2/3 filled with mineral solution were used as test vessels. 3 replicates with inoculum and test substance, 4 test vessels with inoculum without test substance (1 of them for determination of the cell concentration, discarded afterwards) and 3 test vessels with inoculum and reference substance (diethylene glycol). Sampling (O2 measurement) was done on days 7, 14, 21 and 28. Aeration of each flask through a glass frit prior to the addition of test or control substance and after every O2 measurement. 4-t-OP reached a mean degradation of 20% after 28 days based on O2 consumption. There was high variation between the test vessels (14-26% degradation observed). According to OECD TG 301 guideline, the test is considered valid if the difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-d window, as appropriate, is less than 20%. In this test the difference between the replicates was much higher, 46 %. However, as all results were well below the pass level, the test can be used as supporting information indicating slow degradation in a ready biodegradation test. On day 14 the mean degradation was only 2% and, on day 21, 6 %, although there was high variation between the three test vessels on day 21 (0, 4 and 15 % degradation observed). Therefore, it seems that there was a lag phase of at least 14 days in all vessels. The reference substance did not reach a biodegradation level of 60% after 14 days, but the tested reference substance diethylene glycol is considered to biodegrade slower than the recommended reference substances sodium acetate and sodium benzoate. Therefore, with a degradation of 93% of the reference substance after 28 days the inoculum is considered to have been viable. Due to the high variation in the results of different replicates and the failure to meet the validity criteria of the test guideline, the test is not considered fully reliable. However, it is adequate to be used in WoE approach to assess degradation of 4-NP isomers with similar branching as 4-t-OP

An OECD TG 302C test (Unnamed 1991b), non-GLP compliant, is also available for the read across substance 4-t-OP in the ECHA dissemination site. The inoculum was a mixed population of activated sewage sludge microorganisms. Activated sewage was prepared by sampling 10 different sites around the UK in accordance with OECD Guideline No. 302C. The mixed sludge was fed daily with 0.1% synthetic sewage and maintained on a constant aeration at 25 ± 1°C. Concentration of inoculum in the test vessels was 100 mg dry weight/L. The initial concentration of 4-t-OP (purity 98.97%) was 30 mg/L. Incubation was done at 25 ± 1°C in darkness with agitation by magnetic stirrers. Oxygen consumption was measured daily by direct manometer reading. Aniline was used as reference substance. Aniline attained 74% biodegradation after 14 days thereby confirming the suitability of the inoculum and culture conditions. Aniline attained 4% degradation within 7 days. However, this is not considered to affect the overall integrity of the study given that aniline attained 74% degradation within 14 days. Total organic carbon analysis of the test media at day 0 and day 28 showed that aniline attained 87% degradation within 28 days. 0 % degradation was observed for 4-t-OP after 28 days. There is no further information available on the test in the registration dossier of 4-t-OP, and hence, its reliability cannot be assessed. The registrants of 4-t-OP have given the study a Klimisch score of 1. The test can be used as part of WoE assessment to assess degradation of 4-NP isomers with similar branching as 4-t-OP.

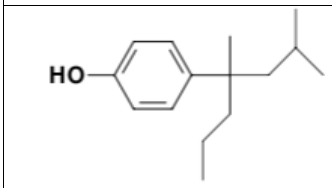
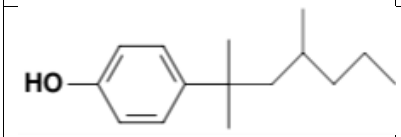
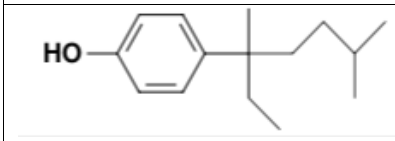
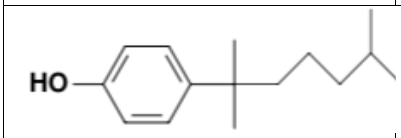
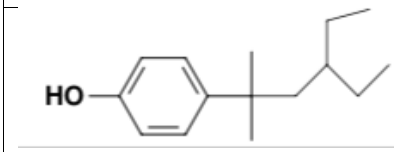
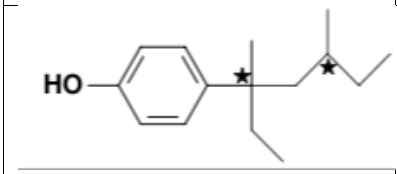
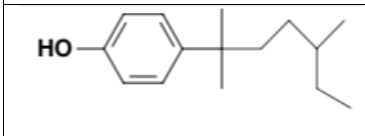
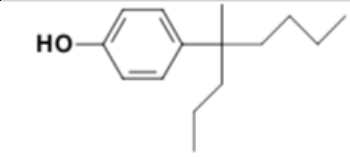
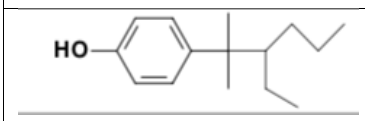
BIOWIN v4.10 model was performed for example constituents of branched 4-NP. For most of the example constituents, Biowin 2 and 6 indicate that they do not biodegrade fast (probability < 0.5) and Biowin 3 gives an ultimate biodegradation timeframe prediction: weeks-months (value in the range of >2.25 - < 2.75). Hence, according to the screening criteria included in the ECHA Guidance R.11, most of the example isomers of 4-NP are borderline cases for screening potentially P/vP based on the BIOWIN predictions.

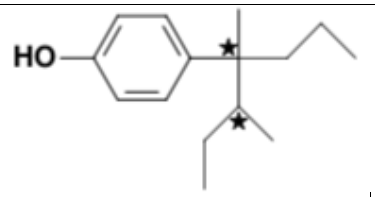
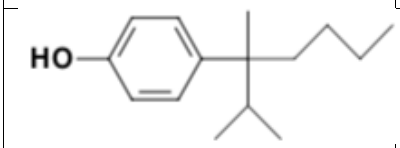
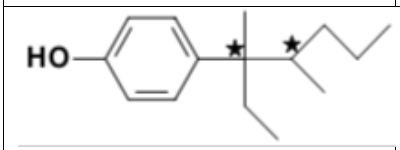
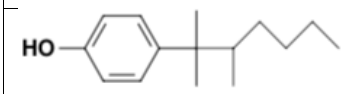
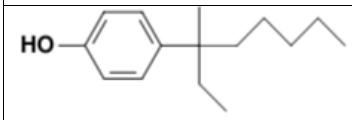
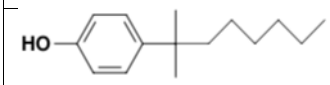
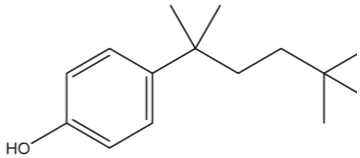
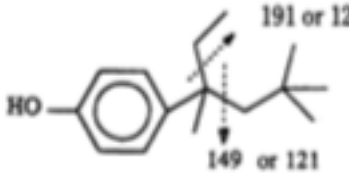
Some of the example isomers (4-NP152, 143, 35, 65, 9) have BIOWIN 3 results above 2.75 and therefore do not screen for potential P/vP. All these isomers have a linear C4 fragment in the alkyl group.

BIOWIN models were also performed for the linear 4-n-NP isomer and the read across substance 4-t-OP to compare the predictions with 4-NP isomers. The BIOWIN 2, 3 and 6 results were 0.9612, 2.9921 and 0.5086, respectively, for the linear 4-n-NP, and 0.0788, 2.3753 and 0.2428, respectively, for 4-t-OP. Hence, based on BIOWIN predictions, the

linear 4-n-NP isomer is expected to degrade faster than most of the branched isomers, and the results for 4-t-OP are very similar to those of the two 4-NP isomers with similar branching (the last two isomers in the below table).

Table Annex 1 - 3 Biowin results for the examples of constituents of branched 4-NP. See smiles codes in Table 8-2 **and** Error! Reference source not found..

Structure	Short name	BIOWIN 2	Biowin 3	Biowin 6
	4-NP194	0.2819	2.5565	0.2312
	4-NP36	0.2819	2.5565	0.2312
	4-NP112	0.2819	2.5565	0.2312
	4-NP38	0.2819	2.5565	0.2312
	4-NP128	0.2819	2.5565	0.2312
	4-NP111a,b	0.2819	2.5565	0.2312
	4-NP37	0.2819	2.5565	0.2312
	4-NP152	0.7127	2.8548	0.4349
	4-NP119	0.2819	2.5565	0.2312

	4-NP193a,b	0.2819	2.5565	0.2312
	4-NP143	0.7127	2.8548	0.2312
	4-NP110a,b	0.2819	2.5565	0.2312
	4-NP35	0.7127	2.8548	0.2312
	4-NP65	0.7127	2.8548	0.4349
	4-NP9	0.7127	2.8548	0.4349
	4-NP96	0.0655	2.3443	0.2474
		0.0655	2.3443	0.2474

Surface water and water-sediment simulation tests

No standard guideline simulation studies are available for branched 4-NP or for the similar substance 4-t-OP or linear 4-NP. Several studies from open literature are available for the substances.

Johnson et al (2000) studied the degradation of the read across substance 4-t-octylphenol (purity 97%) in river water. The study included several experiments with samples of river water and sediments taken from the Aire and Calder rivers in the United Kingdom, running through urban/industrial areas, as well as the Thames River running through a more rural area. The authors indicate that a long reach of the Aire River in 1994 was very estrogenic to fish, an effect believed to be related to chronic alkylphenol contamination, and the sampling sites at Aire river were 15 and 40 km downstream of the sewage treatment works outlet with high nonylphenol and estrogenic potency. The water samples were taken 2-5 cm below the surface at a distance about 2 m from the river Banks. The authors indicate that this sampling method may collect variable quantities of suspended sediment and

hence bacteria. In the laboratory the river samples were filtered (0.45 µm) and their pH, dissolved organic carbon, and the number of viable heterotrophic aerobic bacteria were determined by viable count. In most of the experiment octylphenol dissolved in methanol was added to 30 or 50 ml of river water to obtain an initial concentration of 100 µg/L, and the vessels were incubated at 20 °C without shaking. 125-ml polytetrafluoroethylene (PTFE) conical flasks were used as test vessels. Three replicate vessels per treatment were used. The methanol concentration in the final solution was 0.04 %. In additional experiments the impact of methanol (at concentrations of 0.04 and 0,43 %), initial concentration of octylphenol (0.5, 20, 50, 75 and 100 µg/L) and shaking (at 108 rpm) versus static conditions were examined. In one experiment, 25 µg/L of radiolabelled octylphenol and 75 µg/L of unlabelled octylphenol was used as test material and the evolution of $^{14}CO2$ was measured. Sterile controls were included in the experiments. Sampling was done weekly in most experiments by withdrawing a 1 ml sample of the test solution and analysing the octylphenol concentration using high performance liquid chromatography (HPLC).

Furthermore, in one experiment, water-sediment slurry (10 g dry weight) and river water (20 ml) were incubated with octylphenol at an initial concentration of 600 µg/kg (200 µg/L) under anaerobic conditions. At the start of the test, before placing the test vessels in the anaerobic jars, the test vessels were shaken for one hour to mix the test substance evenly into the sediment. Three replicate vessels were sacrificed for sampling on days 0, 28, 50 and 83.

Several half-lives (at 20°C) ranging from 8 to 71 d are reported in the study for the water samples obtained from the three rivers at different times, with most curves fitting a zero-order reaction. However, half-lives calculated with zero-order kinetics depend on the initial concentration (FOCUS 2014) and hence they are not considered relevant for P assessment. The initial concentration of 100 µg/L is quite high for obtaining degradation half-lives to compare with the P/vP criteria as in OECD TG 309 studies usually concentrations in the range of <1-10 µg/L are used in order to ensure that the degradation follows first order kinetics. Five half-lives obtained with first-order kinetics are also reported in the study and they range between 8 and 51 days. These are considered more relevant for the P assessment. Converting these half-lives measured at 20°C to values at 12 °C using the Arrhenius equation results in half-lives of 17.4 days for Calder River, 23.7, 38.8 and 43.1 days for Thames river and 109.1 days for Aire river. In the sterile samples approximately 10 % of the test substance disappeared after 35 days. The authors speculate that it could be related to sorption of the test substance to suspended sediment.

The Calder River was sampled at four separate points along a 45-km length, encompassing rural to increasingly urban/ industrial reaches. Little degradation was observed in the sample from the upland/rural reach during the 26-day incubation period (a half-life of 36 days reported based on zero order kinetics), while half-lives of 8 to 13 d (based on zero order kinetics except one that is based on first order kinetics) were seen in the more urban/industrial reaches, which have been highly exposed to alkylphenols. The degradation rate in Thames River water did not vary significantly between test vessels without methanol or methanol at 0.04 or 0.4 %, or between shaken and static test vessels. However, a lag phase of about 6 days with increasing concentration was observed in the test vessel without methanol. Mineralization of the phenyl ring, detected by evolution of $^{14}CO2$ from ring-labelled octylphenol, was only observed in water from the Calder River sample (25 % mineralisation after 56 days). Degradation rate was similar for a range of concentrations from 20 to 100 µg/L when tested with river water from the Thames River, with half-lives of 12 to 18 d calculated by using the first-order reaction. For the lowest test concentration, no half-life was determined as the data did not follow zero or first order kinetics. However, based on the figure included in the study, the degradation was not slower than at the higher concentrations. No degradation was observed over 83 d when bed sediments were spiked with octylphenol and incubated under anaerobic conditions.

It is noted that the half-lives reported in the Johnson et al (2000) study are from vessels incubated without shaking. According to OECD TG 309 continuous shaking must be used in order to maintain particles and microorganisms in suspension as well as to facilitate

oxygen transfer from the headspace to the liquid so that aerobic conditions can be adequately maintained. Hence, the static conditions could lead to lower degradation rates compared to standard OECD TG 309 conditions. Johnson et al (2000) studied the effect of shaking vs. static conditions in water samples from the Thames River. It is stated that no significant difference in the degradation rate was observed between the test vessels with static and shaken incubation over the 21-day period, and therefore shaking was not used in the other experiments of the study. It is also indicated that the original water sample had 2.1 x 10^5 cfu/ml (cfu, colony forming units) culturable organisms (SD 2 x 10^4), but after static incubation over 21 d with 100 µg/L OP, there were 4.2 x 10^6 (SD 2 x 10^6) cfu/ml, and with agitation, 5.9 x 10^6 (SD 4 x 105) cfu/ml. However, the authors note that based on visual observations the colony diversity had decreased over this period. It is noted that in the ISO ring test of the OECD TG 309 method, the waters examined were reported to have a bacterial biomass corresponding to 10^3 to 10^4 cfu/ml. Hence, it seems that the bacterial biomass was in line with the OECD TG 309 conditions. However, there was another deviation from the OECD TG 309 conditions as according to the guideline the appropriate volume of test water is about 100 ml, as small sample volumes may influence the length of the lag phase. Therefore, the test water volume of 30-50 ml used in the Johnson et al (2000) adds some uncertainty to the results. Furthermore, there is not very detailed information available on the study and its results, and hence, their reliability cannot be fully assessed. However, it can be used as part of the weight of evidence approach indicating that in some freshwaters, especially in those that have not been pre-exposed to alkylphenols, slow degradation of 4-NP isomers with similar branching as 4-t-OP may occur.

In a study by Ekelund et al (1993), 11 µg of branched 4-nonyl-[^{14}C]phenol isomers was incubated in 2-litre Erlenmeyer flasks either with 1 litre of seawater or with 1 litre seawater and 50 ml of sediment (1 mm). It is stated that the seawater was collected below the halocline from "a less polluted coastal area" in Sweden. Half of the flasks containing seawater and sediment were bubbled with nitrogen gas for 15 minutes. Four flasks with seawater were used as sterile controls (supplied with formalin). The flasks were sealed and shaken vigorously after addition of the test substance, after which they were incubated at 11±2 °C in darkness, with shaking twice a week for one minute (except the flasks with nitrogen gas). Formed ^{14}CO2 was collected with KOH traps and analysed after 1, 2, 4 and 8 weeks of incubation, and in the flasks without sediment also after 16 weeks, four replicates of each kind. After the collection of ^{14}CO2 from the flasks incubated for 8 weeks, 15 ml of n-hexane:diethyl ether (1:1) were added to each flask, which was shaken for 30 min. The organic phase was saved and the water was shaken with another portion of hexane:diethyl ether (10 ml) and the combined organic phases were evaporated. Scintillation cocktail was added and the radioactivity was measured. The degradation was expressed as percent radioactivity in the CO2 fraction per radioactivity of the added 4-NP. However, it is indicated that all the radioactivity trapped in the CO2 traps did not necessarily represent CO2, other volatile metabolites may also have been present but in every case all the radioactivity should correspond to transformation or degradation products of 4-NP.

The degradation in the absence of sediment was very slow during the first four weeks (lag phase about 0.06% of NP degraded day-l) but increased after 28 days to about 1% day^{-1}. Approx. 50 % degradation was reached after 8 weeks. The degradation rate in the presence of sediment and oxygen was high from the beginning (about 1.2% of NP degraded day-1) reaching approx. 40 % degradation after 8 weeks. The degradation was decreased by half in the flasks with nitrogen gas (i.e. with low concentrations of oxygen). In the sterile flasks, no 14CO2 was formed and 84% of the added radioactivity was extracted by hexane:diethyl ether. From the flasks with seawater, 64% of the activity was recovered, 44% in the CO2 fraction and 20% in the organic solvent. The remaining 36% of the radioactivity not recovered, may have existed partially as 4-NP metabolites with high water solubility. From the flasks with sediment, 49% of the added activity was regained, 46% in the CO2 fraction and 3% in the organic solvent. The remaining radioactivity may consist of 4-NP sorbed to the sediment phase or water soluble metabolites remaining in the aqueous phase. Due to the low recovery of radioactivity, the results of the study are considered to have high uncertainty. It should be taken into account that c.a. 20% longer DegT50s would be

expected at 9ºC, which is the relevant temperature for the marine compartment (50 % degradation after 8 weeks would result in a DT50 of 71d at 9ºC for the marine water). The study is not fully reliable but can be used as part of WoE assessment.

Ying and Kookana (2003) studied the degradation of linear 4-n-NP together with other endocrine disrupting chemicals (bisphenol A, 17β-estradiol, 17α-ethynylestradiol, 4-tert-octyl phenol) in sea water and sediment collected in South Australia. In the experiment with seawater only, two 2-L samples of fresh seawater were collected in two 2.5-L Winchester bottles. One of the bottles was sterilized by autoclaving and used as a sterile control. Both bottles (sterilized and unsterilized seawater) were spiked with an initial concentration of 5 µg/L of each of the five chemicals. The bottles, covered with cotton wool bungs, were incubated at 20±3 °C and aerated by bubbling air through them at a rate of approx. 1 L/h. All the bottles were shaken before sampling. The concentrations of the five compounds were monitored on days 0, 1, 2, 4, 7, 13, 21, 28, 35, 42, 49, and 56. A 100-mL aliquot was sampled each time from the two bottles and each sample was analysed by online solid-phase extraction HPLC. Following the above experiment, the dissipation of 4-t- OP and 4-n-NP in seawater was further investigated without air bubbling. Seawater samples of 800 mL each were measured into four 1-L Schott bottles, and two of the bottles were sterilized. Then all the bottles were spiked with the two compounds at the concentration of 5 µg/L. The lid of each bottle was tightened after spiking to minimize any loss due to volatilization. Other conditions used were the same as above.

In the experiments with sediment, 5 ml of seawater and 5 g of marine sediment were mixed to make a slurry. The concentration applied for each of the five chemical was 1 µg/g in the sediment. After spiking, the bottles or tubes were mixed with a vortex-mixer. The incubation temperature used in the experiments was 20 °C. The experiments were done both under aerobic and anaerobic conditions. The concentrations of the five compounds were monitored on days 0, 1, 3, and 7, and then weekly until 70 d. Two bottles were sacrificed from every treatment and from control group at each sampling time. Each of the sediment-seawater slurry samples was extracted twice with 20 mL of ethyl acetate by shaking 2 h each time. The extracts were dried under a gentle nitrogen stream and redissolved in methanol. The recovery for 4-*n*-NP was 88±4.6%. Water samples were directly analysed by online solid-phase extraction and HPLC. A Varian high-performance liquid chromatographic system was used to analyse the five substances in the study.

In the experiment with seawater and air bubbling, 4-n-NP concentration decreased rapidly during the first days of incubation both in the sterile and biotic vessels, to approx. 2 µg/L and less than 0.5 µg/L, respectively. In the biotic vessel the decrease continued and after one week of incubation the concentration was 0.06 µg/L. In the sterile control after the initial drop, the concentration remained quite stable. The authors speculate that the initial drop was probably caused by volatilisation and adsorption to test vessel. This is supported by the results of the experiments without shaking or air bubbling which showed much smaller loss of 4-n-NP in sterile seawater water. The half-life of 4-*n*-NP in nonsterile seawater without air-bubbling was 5 d. In contrast, 4-t-OP in seawater underwent much slower biodegradation. In the experiment with air bubbling, it took 42 days for 4-t-OP to reach a concentration of 0.03 µg/L, and in the experiment without air bubbling the half-life of the substance was 60 days. Hence, it seems that the branched alkyl chain of 4-t-OP significantly reduces the degradation rate compared to that of the linear chain 4-NP. Therefore, the degradation rate observed for 4-t-OP in this study is considered more relevant for branched 4-NP than that of the linear 4-NP. In the aerobic sediment slurry experiment, 4-n-NP concentration decreased rapidly with a half-life of 5.8 d based on first-order reaction kinetics. For 4-t-OP, there was an acclimation period with a concentration of 0.84 µg/g at 21 days after test start, followed by quick degradation with its concentration decreasing to only 0.09 µg/g within a week. Under the anaerobic conditions no degradation was observed.

It is not clear how the DT50 of 60 d was calculated for 4-t-OP at 20°C in the Ying and Kookana (2003) study since based on a figure included in the article, after 60 days almost complete disappearance of 4-t-OP was observed. However, based on a visual determination from the figure, a DT50 of 21-28 d can be considered at 20°C which results

in DT50 of 60 d at 9°C. -The study is not fully reliable but can be used as part of the WoE approach to assess degradation of 4-NP isomers with similar branching as 4-t-OP

Writer et al (2011) studied the degradation of radiolabelled linear 4-n-NP (together with other endocrine disrupting chemicals) in the water, sediment and biofilm formed in the surface of the sediment sampled in Boulder Creek from sites upstream or downstream the WWTP. Both sites are influenced by urban stormwater discharge and the downstream site is also influenced by effluent discharge from the City of Boulder WWTP. Previous studies on the distribution, fate, and biological impact of EDs, including 4-n-NP, have been performed on Boulder Creek and show that WWTP effluent discharge influences stream chemistry and corresponding biological function effluent discharge influences stream chemistry. Similar concentrations of 4-NP (1600-1700 ng/g) were measured in the biofilm on the surface of the sediment in upstream and downstream sites of the WWTP at the Boulder Creek. Therefore the microorganisms were pre-exposed to 4-NP in both sampling sites. The mineralization experiments were set up within 48 h of sample collection in microcosms test vessels (11 mL volume) by dispensing approximately 5 mL of the biofilm matrix solution (about 2.5 mg dry mass/ mL), approximately 5 g dry streambed sediment and 3 mL water, or for the water only vessels approximately 8 mL of Boulder Creek water. The sediments consisted of sand and gravel (size range 1-10 mm) and associated detritus. The initial test substance concentration is not indicated. Triplicate experimental controls, duplicate autoclaved controls, and a single sediment-free control were prepared for each matrix and location. Treatments were incubated concurrently in the dark at 23°C. The production of 14CO2 and 14CH4 over time was monitored by gas chromatographic separation with radiometric detection at time intervals of approximately 0, 7, 70, and 185 d. Mineralisation of 4-n-NP was slow in the test vessels with only water, reaching only approximately 10 and 40 % mineralisation in the upstream and downstream sites, respectively, after 180 days. Hence, mineralisation was significantly higher in water collected downstream from the WWTP than from upstream, likely due to microbial enrichment from the wastewater effluent and potential adaptation of microorganisms. In the test vessels with sediment the mineralisation was faster, reaching approximately 50% already after 70 days. The process was a bit slower in the sediments collected downstream from the WWTP than the sediments collected upstream from the WWTP, and this could be attributed to the biological oxygen demand (BOD) and oxygen limitations in sediments downstream from the WWTP. Mineralization was not observed in autoclaved matrices, and therefore it was attributed to biodegradation. Following deviations from the OECD 308 standard guideline are observed: sediment samples are associated to detritus and the microorganisms were pre-exposed to 4-NP. The system test vessels were setup with 5 g of sediment versus a minimum of 50 g of sediment (dry weight basis) recommended in the OECD TG 308, 3 ml of water was added but no information on the system geometry can be inferred, no information on method for aeration or ventilation is provided, nor potential disturbance of sediment layer. No information on mass balance and potential dissipation of the 4-n-NP out of the system is provided. Therefore, this experiment with the linear 4-n-NP cannot be compared to the Annex XIII criteria for P. However, the information from the water only experiment can be used as supporting information indicating slow mineralisation of linear 4-n-NP in the water phase, even in the presence of pre-exposed microorganisms.

Yuan et al (2004) studied the aerobic degradation of 4-NP (not indicated whether branched or linear) in water–sediment system. Surface sediment samples were taken in July 2000 from four sites in the Erren River, which is one of the most heavily contaminated rivers in southern Taiwan (Putri et al 2018). One of the sampling sites was located upriver (E1), and three sites downriver (E4, E5, and E6). Sediment organics were 1.25% and 0.93% for E1, and E4 sediment samples (for E5 and E6 samples organic material content is not indicated). Part of the sediment samples were pre-exposed to the test substance by adding 4-NP (1 μg/g concentration) to 500 g of sediment at 14-day intervals under static incubation at 30 °C without light for 1 year. Experiments were performed using 125 ml serum bottles containing 45 ml of mineral medium and 1 g of river sediment, to which 2 μg/g of 4-NP was added. First aerobic degradation of 4-NP in the E1, E4, E5, and E6 sediment samples not pre-exposed in the laboratory was measured. After that, comparisons were made between the results of the test vessels with or without the pre-

exposed sediment samples. Furthermore, the following factors were manipulated to investigate their effects on NP anaerobic degradation in the pre-exposed sediment: pH (5.0, 6.0, 7.0, 8.0, or 9.0); temperature (20, 30, 40, or 50 °C); shaking/static conditions, and the addition of various nutrient sources (yeast extract, ammonium, sulfate, and phosphate) and pollutants (heavy metals and phthalic acid esters [PAEs]). Sterile control (three separate 1 h autoclaving treatments at 121 °C) were also included in the study. It is stated that the test vessels were shaken prior to incubation at 30 °C and pH 7.0 in darkness, unless indicated otherwise. In the experiments with sediment pre-acclimated in the laboratory shaking was used also during the incubation, except the test vessels used to study the effect of static conditions. However, it is not clear whether shaking was used during incubation also in the experiments with sediment not pre-acclimated in the laboratory. All experiments were performed in triplicate. Aqueous samples were periodically collected in order to measure residual 4-NP concentrations. Gas chromatograph connected to an ion-trap mass spectrometer and equipped with a DB-5 MS capillary column was used in the analysis. 4-NP recovery percentage was 88.2 ± 1.8% and detection limit was 50 µg/l.

The aerobic degradation of 4-NP varied in the test vessels with sediments (without pre-exposure in the laboratory) from the four sites and showed the following descending order: E1(fastest degradation) > E6 > E4 > E5 (slowest degradation). Being the fastest degradation rate corresponding to E1 collected at the upper part of the river and expected to be less polluted. In the same order, respective 4-NP biodegradation rate constants (first-order kinetics) were determined at 0.051, 0.010, 0.009, and 0.007 day^{-1}, with half- lives of 13.6, 69.3, 77.0 and 99.0 days. Converting these half-lives (determined at 30°C) to 12°C using the Arrhenius equation results in half-lives of 71, 362, 401 and 517 days. The sites E4, E5 and E6 came from downstream sites of the heavily contaminated river, and hence they are assumed to be exposed to alkylphenols already before the pre-exposure performed in the study. The E1 site was in the upper stream and may be less exposed to alkylphenols than the downstream sites, but pre-exposure cannot be excluded for it either as in Taiwan industrial and high population areas are located also in upstream areas of rivers and water pollution has been observed to be spread along the rivers (Putri et al. 2018).

The results for the other treatments are given only for the sediment E1. In the sterile control, 93.1 % of the initial concentration of 4-NP remained after the 84-d incubation period. 4-NP was completely degraded by day 70 without pre-exposure of the sediment in the laboratory. In comparison, the substance was completely degraded by day 28 when sediment pre-exposed in the laboratory was used. The degradation of 4-NP was positively correlated with temperature, and pH 7 was the most optimal pH value for degradation. Shaking of the test bottles also significantly increased the degradation rate (half-lives with and without shaking were 5.1 and 20.4 days, respectively). Biodegradation was enhanced by the addition of yeast extract whereas a delay in 4-NP biodegradation was observed when concentrations of available ammonium, phosphate, or sulphate were reduced by half. Degradation was decreased when heavy metals or PAEs were included. Additionally, pure microbial strains were isolated from the river sediment samples in the study. Sixteen bacterial strains were capable of aerobically degrading 4-NP, the isolate showing the greatest degrading power was identified as *Pseudomonas* sp.

Based on the results of the study by Yuan et al (2004), micro-organisms present in sediments previously exposed to alkylphenols can degrade 4-NP under aerobic conditions, but the degradation is expected to be slower under environmentally relevant lower temperatures. It is also noted that in the study it appears that 4-NP concentrations were determined only for aqueous samples taken from the test vessels, and hence the reported half-lives seem to be for water phase. The disappearance of 4-NP from the water phase may partly be due to sorption to sediment and non-extractable Residues (NER) formation. However, as in the sterile controls only approx. 7 % decrease was observed, it seems that degradation occurred in the water phase. It should also be noted that when 4-NP is degraded in the water phase, desorption from the sediment to water phase may occur to account for the decreased concentration in water, as was observed in de Weert et al (2010b). It is also noted that at least in most of the experiments shaking was used, and

hence, the study conditions resemble more of OECD TG 309 suspended sediments test than OECD TG 308 sediment test, in which static conditions are used. However, the concentration of sediment is higher (1 g of sediment in 45 ml of medium used) than what is indicated in the OECD TG 309 for the suspended sediment test (0.01-1 g/L dry weight). Sediments used in this study were collected from the Erren river, one of the most heavily contaminated rivers in southern Taiwan and therefore pre-exposure to 4-NP cannot be excluded. Furthermore, in some of the experiments the sediment was pre-exposed in the laboratory by adding NP at 14-day intervals for 1-year. Moreover, the water phase used in the study was not natural water, but mineral medium was used instead. The water phase may have contained microorganisms from the sediment phase, but the microorganism community (e.g. diversity and density) may have differed from that of a natural freshwater. In conclusion, the half-lives reported in the Yuan et al (2004) cannot be directly compared with any of the P/vP criteria of Annex XIII. The study is considered not relevant for the P assessment of 4-NP.

Bradley et al. (2008) studied degradation of linear 4n-NP in stream sediments under aerobic and anaerobic conditions. Sediments were collected from three streams in the USA, (Fourmile Creek, Boulder Creek and South Platte River), in each one both upstream and downstream of wastewater treatment plants. The sediments in the downstream sites are exposed to 4-NP , its precursors and other alkylphenols contained in the WWTP effluent. However, it is indicated in the study that also the upstream sites may be impacted by anthropogenic activity as all three streams are located in or near urban centres. It is noted that in a study by Writer et al (2011) similar concentrations of 4-NP were measured in the biofilm on the surface of the sediment in upstream and downstream sites of the WWTP at the Boulder Creek. Hence, pre-exposure of the upstream sediments to 4-NP was also likely, at least in Boulder Creek. A series of microcosm studies was conducted with a single isomer, [U-ring-14C]-radiolabelled 4-*n*-nonylphenol (4-*n*-NP). The test vessels consisted of 10-ml serum vials with 5 ml of saturated sediment and an atmosphere of air (oxic treatments) or nitrogen (anoxic treatments). Four replicates for each sediment and treatment were included. Triplicate control microcosms were prepared for each sediment by autoclaving three times for 1 h each time. All microcosms were amended with approximately 0.027 µCi of [U-ring-14C]4-n-NP to yield initial dissolved concentrations of approximately 0.25 µM (55 µg/L). Microcosms were incubated under static conditions in the dark at 23°C for up to 154 d. Static conditions were applied to minimize the advective oxygen supply and maximize the potential impact of the sediment BOD. The bed sediments in all three systems consist of coarse-grained deposits that facilitate the infiltration and recirculation of oxygenated surface water. Headspace concentrations of CH4 and CO2 as well as of ^{14}CH4 and ^{14}CO2 were monitored by analysing 0.5 ml of head-space using gas chromatography with thermal conductivity detection and radiometric detection, respectively. Furthermore, the sediment biochemical oxygen demand (BOD) was estimated from the difference in the microcosm oxygen decline between experimental and autoclaved control microcosms over the initial 20 day of incubation.

In the upstream sediments of the three streams, 4n-NP had a fast linear initial 14CO formation for the first 5-6 days followed by slightly slower phase under aerobic conditions. In all cases, recovery of ^{14}CO2 was greater than 90% of the theoretical CO2 evolution within 32 days of incubation. In the downstream sediments slower degradation was observed. Complete recovery of ^{14}CO2 was observed in both Fourmile Creek sediment treatments within 154 days of incubation; however, the mean initial linear rate of ^{14}CO2 production was 70% lower in downstream sediment microcosms (2.3% ± 0.1%/d) than in upstream sediment microcosms (8% ± 2%/d). In contrast, the final recoveries of ^{14}CO2 in downstream sediment from Boulder Creek and the South Platte River were only approximately 7.5-12.5 % of the theoretical CO2 evolution (estimated visually from a graph included in the published study). Furthermore, the mean initial linear rates of 14CO2 production in Boulder Creek and South Platte River treatments were two orders of magnitude lower in downstream sediment microcosms (approx. 0.07%/d) than in upstream sediment microcosms (approx. 9%/d). No significant formation of 14CO2 was observed in the sterile controls. No accumulation of ^{14}CO2 was detected (minimum detection limit, 2% of theoretical) in any of the collected sediments (both upstream and downstream of the WWTP) when incubated under anoxic conditions. The decreasing

efficiency of 4-n-NP mineralization observed in downstream sediments corresponded to significant increases in sediment BOD downstream of the WWTP outfall in each stream system. These results are in line with the study of Yuan et al (2004) where highest aerobic degradation rates were observed in the upper stream river site (E1). The study is reliable with restrictions but it is not adequate to assess the biodegradation of branched isomers of 4-NP nor to compare the half-lives with Annex XIII criteria. However, it can be used as part of the WoE approach.

De Weert et al. (2010a) sampled sediments polluted with branched 4-NP (at a concentration of 14±1.5 mg/kg dry weight) in Huerva River, Spain, and followed the degradation of the substance in laboratory experiments. In the experiment 2 g of the sediment (dw) and 50 ml of aerobic medium were incubated in 250 ml vessels. This resulted in an initial concentration of 2.5 nmol 4-NP per millilitre slurry added with the sediment. The bottles were closed with viton stopper with crimp cap and incubated horizontally shaken (110 rpm) in the dark at 30°C. In the study further experiments were done by enriching the bacterial cultures and by adding technical 4-NP mixture or linear 4-NP in the enriched cultures to further study the degradation and formation of metabolites. Sterile controls were included in the experiments. It is indicated that in the original polluted sediment with no additional 4-NP added, the 4-NP isomers present in the sediment started degrading after 2 days of lag phase and in 8 days 95 % of the initial amount had degraded. The observed DT50 value was 1.5±0.4 days. In the further experiments with enriched bacterial cultures, the DT50 values of technical 4-NP ranged from 4 to 5 days. In these latter experiments, significant decrease (over 50% in approx. 17 days) of the test substance was also observed in the sterile controls. Therefore, it can be assumed that other dissipation processed than degradation also occurred in the biotic test vessels. There is no information in the publication on the results of the sterile control with the original sediment with no additional technical 4-NP. However, it can be expected that also in this experiment other dissipation processes may have affected the observed DT50 value. Furthermore, due to continuous shaking at 110 rmp, a sediment slurry was formed, which is not comparable to the static conditions and stratified sediment column used in OECD TG 308 studies. Agitation by means of continuous shaking at 100 rpm is indicated in the OECD TG 309 to facilitate oxygen transfer and resuspension of particles and microorganisms which would increase biodegradation rate of sediments. Moreover, as the sediment was environmentally polluted with branched 4-NP, sediment microorganisms were adapted to the substance. Therefore, the results of the study are not considered relevant for the P assessment. However, the study can be used as part of the WoE approach. In the experiments with linear 4-NP, nitro-nonylphenol metabolites were formed, which were further degraded to unknown compounds. The attached nitro-group originated from the ammonium in the medium.

Wang et al. (2014) assessed the degradation of linear 4-n-NP in a laboratory sediment-water system study. The sediment was collected from Wenyu River, Beijing, which is indicated to be impacted mainly by urban run-off and domestic sewage. The total NP concentration of the sediment at the time of sampling is stated to be below 1 µg/g dw with no linear NP detected. The test systems consisted of 100 g river sediment (dry weight) and 70 ml artificial test medium in 250-ml vessels. Linear 4-NP was added at a concentration of 150 µg/g. Blank and sterile controls were included. All treatments were done in triplicate. The test vessels were incubated in horizontal shakers (120 rpm) at 25 °C during 9 days. Sampling was done in 3-day intervals. After 6 and 9 days of incubation, an average of 54.7 and 93.2 % reduction, respectively, occurred in the non-sterilised sediment. In the sterile control only an average of 14 % reduction was observed after 9 days. Due to the continuous shaking, the conditions of the study are not considered comparable to an OECD TG 308 study. Not adequate to assess the biodegradation of branched isomers of 4-NP nor to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

De Weert et al. (2010b) studied the combined processes of branched NP desorption and biodegradation in river sediment systems. Sediment polluted with NP was collected from Huerva River, Spain. After sampling the sediment was stored at 4°C for two years after study initiation. No 4-NP was added to the test sediment. The method and principals of the

study were loosely based on the OPPTS 835.3180 guideline, with some deviations. The method was a flow-through system with variable stirring conditions, including large periods of complete settling (35 and 9 days) and a resuspended sediment period of 14 days, where 5 minutes of stirring was done every 8 hours. The total duration of the study was 59 days. A reactor setup was used as a test system containing two biotic and two sterile control replicates. Each reactor contained 100 g sediment (ww) and 650 ml mineral medium. The influent was pumped into the reactor (approx. 1 L/day) just above the surface of the sediment, and the effluent was pumped from the liquid surface through a glass tube, using the same pumping rate as the influent. Tenax was placed in the glass tube to sorb the NP present in the effluent. The concentration in the water phase was calculated with the amount sorbed to Tenax divided by the volume of the medium passed through the Tenax. Sediment samples were collected at the start and end of the experiment. The study was conducted at 20°C. In addition, a separate experiment for degradation was performed, where 5 g of the sediment and 50 ml of mineral medium or 50 ml of river water (collected at the same site and time as the sediment) were added to 250 ml bottles which were closed with a viton stopper. The bottles were incubated at 20°C in darkness and horizontally shaken. The NP concentrations were measured in various timepoints by taking samples of the slurry.

In the desorption-degradation experiment, under settling sediment conditions, a continuous amount of NP was transferred from the sediment bed to the bulk water (desorption). The authors of the study assumed that the desorbed NP in the non-sterile reactors was biodegraded in the first 20 days of the experiment as the concentrations in the bulk water were lower in the non-sterile than in the sterile reactors. At the end of the settled sediment conditions, the biodegradation became very limited, and the mass transfer was comparable to the mass transfer under sterile conditions. When the sediment condition was resuspended, the mass transfer of NP to the bulk water initially increased sharply under sterile and non-sterile conditions due to the release of NP from pore water and enlargement of the sediment desorption area. This initial mass transfer was larger than the amount of NP that was biodegraded and lead to an initial increased concentration. After 2 days of resuspending the sediment, more NP was released under non-sterile conditions than under sterile conditions, thought likely due to biological processes most affecting the adsorption properties of the sediment. During a final period of non stirring, the NP absorbed back on to the sediment and the concentrations in the aqueous solution decreased. There was a difference between the mass balance of NP in the sediment at the end of the study in the sterile and non sterile reactors, with 64 to 72% measured in sediment in the sterile reactor and 47 to 56% measured in the sediment in the non sterile reactor. This was considered due to biodegradation. It is not possible to determine whether the degradation occurred in the water or sediment matrices due to a combination of mixing and non mixing events. In the additional degradation experiment with slurry, during the first 2 days of incubation, an initial decrease (approx 50%) in NP concentrations were observed both in the sterile and in biotic vessels, and hence this was not caused by degradation. After that, the concentration in the sterile control remained quite stable for the 6 days of test duration while in the biotic vessels almost 100% decrease in the concentrations was reached after four days. The mixed slurry conditions of the test are not comparable to OECD 308 test conditions. Furthermore, as indicated above for the other studies of De Weert et al. (2010a, 2011), the sediment used in these studies was polluted with NP and hence was adapted to the substance. Therefore, the results are not considered relevant for the P assessment. However, the study can be used as part of the WoE approach.

Toyama et al (2011) investigated degradation of technical nonylphenol (tNP) in *Phragmites australis* rhizosphere sediment by conducting degradation experiments using sediments spiked with tNP. *Phragmites australis* plant, sediment, and surface water samples were obtained from a pond at Osaka University, Japan. Young *P. australis* plants were collected from within the *P. australis* vegetation zone, and a rhizosphere sediment sample (pH 6.9; organic carbon content [ignition loss], 2.2%; sediment density, 2.07 g dry-sediment/cm^3; NP content undetectable [<0.001 mg kg 1]) was collected at 0 to 20 cm depth from around *P. australis* roots. Another sediment sample was collected from an unvegetated area (unvegetated sediment sample) (pH 7.3; organic carbon content, 1.4%; sediment density,

1.98 g dry-sediment/cm^3; NP content undetectable) from the same depth, but about 3 m from the nearest *P. Australis* plants. The unvegetated sediment sample did not contain any root materials. A water sample was collected from the surface waters of the pond near the plants. The 150-ml test vessels contained 100 g dry-weight sediment (either rhizosphere sediment or unvegetated sediment), 10 ml of surface water and tNP at 25 mg/kg dry sediment. In the treatment with rizosphere sediment *P.australis* plant was replanted while in the unvegetated sediment treatment no plant was included. A sterile control including autoclaved rizosphere sediment and surface water and a 2-month- old sterile *P. australis* plant was included in the study. The test vessels (12 per treatment) were incubated under static conditions at 28 °C and 16:8 h light/dark cycle, and water was added to each test vessel every few days. Three test vessels from each treatment were sampled at the start of the 42-day experimental period and at 7, 21, and 42 days for analysis of the tNP level. Accelerated tNP removal was observed in *P. australis* rhizosphere sediment (52.7 % after 42 days), whereas tNP persisted in unvegetated sediment without plants (only 9.2% of tNP disappeared after 42 days) and in autoclaved sediment with sterile plants, suggesting that the accelerated tNP removal resulted largely from tNP biodegradation by rhizosphere bacteria. However, if a DT50 of approx. 40 days (at 28°C) is assumed for the vegetated sediment, this results in a DT50 of 173 days when converted to 12 °C using the Arrhenius equation. In an additional aerobic control experiment using the unvegetated sediment spiked with tNP (25 mg/kg dry sediment) and mineral medium incubated at 28°C and under continuous shaking at 120 rpm, only 10.2% of the tNP was removed from the sediment over 42 days. Based on the GC-MS results for days 0 and 42 for the rhizosphere and unvegetated sediments, several peaks decreased during the study, but other peaks remained almost unchanged. This suggests that some of the isomers were not degraded significantly during the 42 days study period. The study is not adequate to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

Lu and Gan (2014b) studied degradation of different 4-NP isomers in river sediment systems. Two surface sediments (0−5 cm) were sampled from Santa Ana River in Southern California. Sediment A was from a location in the upper river located in the San Bernardino National Forest. Sediment B was from a middle segment of the river located about 200 m below the outlet channel of Prado Dam in Corona, California. There was no detachable NP residue in both sediments. For aerobic incubation, 10 g (dry weight) sediment and 5 mL of 0.01 mol/L CaCl2 solution were added into 125-mL amber glass jars. These samples were acclimated for 3 days and then 50 μL of 1000 mg/L technical NP in acetone was added. The spiked systems were gently vortexed for 1 min, and loosely covered with aluminium foil. Water loss was checked every other day by weighing and deionized water was added when significant water loss occurred. The test vessels were gently vortexed periodically to ensure adequate aeration during the incubation. For anaerobic incubation, amber glass vials (40 mL) were filled with 10 g sediment (dry weight) and 5 mL of 0.01 mol/L CaCl2 solution, and then the uncovered vials were placed in a plastic glove chamber and flushed with nitrogen. After equilibration in the inflated glove chamber for 1 day, vials were tightly closed with screw caps with Teflon-lined butyl rubber septa in the glove chamber and these samples were further acclimated for 12 days to ensure anoxic conditions. The sample vials were taken out of the glove chamber and then 50 μL of 1000 mg/L tNP in acetone was spiked using a microsyringe. The spiked samples were gently vortexed for 1 min, and then returned to the nitrogen-filled glove chamber. The glove chamber was checked daily and filled with more nitrogen if noticeable deflation occurred. Sterile controls were prepared by steam autoclaving at 121 °C for 45 min on 3 days. The sterilised sediment samples were also amended with 200 mg/L sodium azide, and then included in both the aerobic and anaerobic incubation experiments using sediment A. All of the samples were incubated at 21 ± 0.8 °C. 3 replicate samples from each treatment were randomly removed on days 0, 1, 3, 7, 14, 21, 35, 56, and 84. Additional samples of sediments A and B under both oxic and anoxic conditions were sacrificed for measurement of redox potential and pH at the same time intervals. The sediment samples were extracted using a Dionex accelerated solvent extraction system. The separation and identification of 4-NP isomers (only quaternary α-C isomers quantified) were carried out on a GC-MS.

The DT50 values of different 4-NP isomers studied in the sediment A under oxic conditions were in the range of approx. 1-13 days. Isomers with short side chain and bulky α-

substituents generally were more persistent. In the sediment B, a lag phase or slower degradation phase was observed during the first 7−14 days followed by a faster degradation phase with half-lives in the range of 15-20 days. It is stated in the publication that sediment B was likely well acclimated to NP contamination, as the river receives effluents discharged from multiple WWTPs. The slower degradation in sediment B compared to sediment A may be caused by the reducing conditions of sediment B for which the actual redox potential was measured to be about 255 mV as compared to 413 mV in sediment A under the same conditions. The redox potential in sediment B was reflective of nitrate reducing conditions. This was likely due to its properties, such as its higher organic carbon content and lower sand content. In the sterile controls no significant reduction in the 4-NP concentration was observed. Biodegradation of 4-NP isomers was extremely slow in both sediments exposed to nitrogen. The redox potentials of sediment A and B incubated under nitrogen were −92 and −122 mV, respectively, indicating sulphate reducing conditions. Under anaerobic conditions, in sediment A, the estimated first-order half-lives of all 4-NP isomers were greater than 200 days, while in sediment B, no detectable degradation occurred for any of the isomers after 84 d. The study is not adequate to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

The registrants have also included studies on degradation of 4-NP (not specified in all studies whether branched or linear) by isolated bacterial (Gabriel et al 2005 and 2008), yeast (Corti et al. 1995), fungi (Junghanns et al. 2005, Rozalska et al. 2010) or algae (Gao et al. 2011, Liu et al. 2010) cultures. Varying levels of degradation were observed in these studies. However, these studies are not considered relevant for comparison with the Annex XIII criteria and are not further assessed here.

The registrants provided (January 2023) additional information regarding an article of Klecka et al. (2008) in which studies on degradation of alkylphenol ethoxylates, nonylphenol and octylphenol were reviewed. Most of the studies have been included in the registration dossier and had already been assessed by the eMSCA. Only one additional study by Dutka et al (1998) investigating degradation of branched 4-NP in sediments was included in the review of Klecka et al. (2008). In this study, sediment was collected from heavily polluted Hamilton Harbour, in Lake Ontario, Canada. The sediment was divided into two portions, one that was not spiked with aniline and nonylphenol (sediment A) and the other that was spiked with 100 ppm branched nonylphenol and 1500 ppm aniline (sediment B). 2.5 kg of each sediment was placed in large wide mouth Erlenmeyer flasks, lightly covered with cotton batten, and placed on a reciprocating shaker (40 cycles/min) at 21°C. Two further Erlenmyer flasks were prepared with A and B sediments for incubation under anaerobic conditions. The flasks were purged with a mixture of N_2 and CO_2 gases (30:70 v/v) and then sealed with parafilm. Subsamples of all test flasks were collected after 1, 3, and 6 months for aniline and nonylphenol level testing. In the aerobic test vessel with sediment A (without spiking, initial concentration of NP 390 ppm), 4-NP levels decreased approximately 80-85% with no real difference between 4 weeks and 6 months. In the aerobic test vessel with sediment B (spiked) nonylphenol levels initially increased 15 and 30% at 4 weeks and 3 months, then decreased to approximately 50% (176 ppm) of the original spiked level. In the anaerobic test vessels no decrease of NP was observed. Aniline spiked in the sediment B at initial concentration of 1220 ppm decreased by more than 90% in one month and was not detected after 6 months under aerobic conditions. Under anaerobic conditions no change occurred in aniline levels during the first month, but after 3 months over 90% had disappeared and by 6 months it was not detected anymore. No sterile controls were included in the study, and hence, it cannot be assessed to what extent the disappearance of 4-NP was due to degradation and to what extent due to other dissipation processes. Furthermore, as the sediment contained 4-NP it was pre-exposed to the substance.

The registrants have also included in their dossier two studies that used only anaerobic conditions. De Weert et al. (2011) studied the anaerobic degradation of 4-n-NP and branched 4-NP using as inoculum the same environmentally polluted sediment as in the aerobic study by De Weer et al. (2010). The other conditions of the experiment were similar in the two studies. No degradation of branched 4-NP was observed in the study under

anaerobic sediment conditions. However, degradation of 4-n-NP did occur under nitrate reducing conditions and was enhanced by pre-adaptation of the bacteria. In enriched microbial cultures, half-lives between 0.38 and 0.43 days (after a lag phase of 1-3 days) were calculated for 4-n-NP under nitrate reducing conditions. Without enrichment of the microbial community, complete degradation of 4-n-NP took up to 104 days under denitrifying conditions.

Chang et al (2004) studied the anaerobic degradation of 4-NP (not indicated whether branched or linear) in sediments sampled in the Erren river, at the same sites and in the same time as Yuan et al. (2004). Part of the sediment samples were pre-exposed to NP in the laboratory as explained above for Yuan et al (2004). Experiments were performed using 125 ml serum bottles containing 45 ml of mineral medium and 1 g of river sediment, to which 2 µg/g of 4-NP was added. Bottles were filled with N2 gas for 10 min, capped with butyl rubber stoppers and wrapped in aluminium foil to prevent photolysis. All experiments were conducted in an anaerobic glove box filled with N2 (85%), H2 (10%), and CO2 (5%) gases. First anaerobic degradation of NP in the E1, E4, E5, and E6 sediment samples not pre-exposed in the laboratory was measured. After that, comparisons were made between the results of the test vessels with or without the pre-exposed sediment samples. Furthermore, the following factors were manipulated to investigate their effects on NP anaerobic degradation in the pre-exposed sediment: pH (5.0, 6.0, 7.0, 8.0, or 9.0); temperature (20, 30, 40, or 50 °C); and the addition of yeast extract (auto- lysed yeast cells), electron donors, electron acceptors, the phthalic acid esters (PAEs), or microbial inhibitors. Sterile controls (three separate 1 h autoclaving treatments at 121 °C) were included in the study. The test vessels were incubated without shaking at 30 °C and pH 7.0 in darkness, unless indicated otherwise. All experiments were performed in triplicate.

Bottle contents were periodically sampled in order to measure residual 4-NP concentrations, oxidation–reduction potential (ORP), and pH values. Methane was sampled from the headspace of serum bottles. 4-NP was extracted from acidified water samples and the 4-NP concentrations of the extracts were measured using gas chromatograph connected to an ion-trap mass spectrometer and equipped with a DB-5 MS capillary column. 4-NP recovery percentage was 88.2 ± 1.8% and detection limit was 50 µg/l.

The degradation of 4-NP varied in the four sites and showed the following descending order: E6 (fastest degradation) → E5 → E4 → E1 (slowest degradation). The anaerobic degradation followed first order kinetics, and the degradation rate constants of 4-NP determined for the sites were 0.015, 0.013, 0.012, and 0.010 1/day, with half-lives of 46.2, 53.3, 57.8, and 69.3 days, respectively. Converting these half-lives (determined at 30°C) to 12°C using the Arrhenius equation results in half-lives of 241, 278, 302 and 362 days. The remaining percentage of 4-NP in the sterile control after an 84-day incubation period was 89.6%. 4-NP was completely degraded by day 84 without pre-exposed sediment. In contrast, the substance was completely degraded by day 63 with pre-exposed sediment. However, the Erren river has been referred as one of the most heavily contaminated rivers in southern Taiwan and therefore pre-exposure cannot be excluded. This is in line with the fact that E1 river sediments, which refers to the upper part of the river, present the lowest anaerobic degradation rate. E1 was referred by Yuan et al (2004) as the site with the highest aerobic degradation rate.

The results for the other treatments in Chang et al. (2004) are given only for the sediment E6. The results showed that between 20 and 50 °C the degradation rate of 4-NP positively correlated with temperature and the optimal pH value for degradation was pH 7. There is limited information on the study available, and hence, its reliability cannot be fully assessed. However, it can be used as part of the weight of evidence approach. Based on the results of the study, micro-organisms present in sediments exposed to alkylphenols can degrade 4-NP under anaerobic conditions, but the degradation is expected to be slow under environmentally relevant temperatures, i.e. lower temperatures. Therefore, the half-lives determined based on concentrations in water are not reliable for P assessment. No half-lives for the sediment phase or total system are reported in the study. However, as in the sterile controls only approx. 10% decrease of 4-NP was observed, it seems that degradation occurred in the biotic systems under anaerobic conditions. Although results

would indicate slow degradation even under favourable conditions (pre-adapted microorganisms).

Table Annex 1 - 4 Overview of degradation studies in surface water and water-sediment systems considered less relevant and not included in Table 12-3 **in the main part of the document.**

Type of Evidence	Test substance	Test medium	Results	Consistency & Specificity	Likelihood/ Biological Plausibility	Confidence / Strength of Evidence	Remaining Uncertainty
Read across from linear 4-n-NP, freshwater and freshwater-sediment-biofilm microcosms study, **IUCLID dossier, Writer et al. (2011)**	Linear 4-n-NP	Freshwater Freshwater + sediment+ biofilm (aerobic)	Water: **10 and 40% mineralisation after 180 d** in water from upstream and downstream a WWTP (at 23°C) Water+sed+biofilm: approx. 50% mineralisation after 70 days (at 23°C)	Not consistent with other studies indicating rapid degradation of linear 4-NP in water. Consistent with other studies indicating rapid degradation of linear 4-NP in sediments.	Plausible	Low	High Linear 4-NP may degrade faster than branched isomers Water and sediment sampled from sites pre-exposed to 4-NP Test conditions not comparable to OECD 308 or 309 Low test medium volume (8ml) No info on initial test concentration or mass balance
Water-sediment microcosms study, IUCLID dossier, Yuan et al. (2004)	4-NP (not indicated whether branched or linear)	Mineral medium and sediment (aerobic)	DT50s: 13.6 (upstream site), 69.3, 77.0 and 99.0 d (at 30°C) → 71, 362, 401 and 517 d (at 12°C) Only aqueous samples collected →the DT50s	No conclusions can be drawn as sediment phase not sampled in the study.	Plausible	Low	High Downstream sites more polluted than the upstream site, but it may also be exposed to 4-NP as in Taiwan industrial/urban areas are found also in upstream areas.

			are not for the sediment phase, but the DT50s cannot be used to conclude on degradation on water either because desorption from sediment to water may have occurred (as observed in de Weert et al. 2010b)				Not clear whether shaking or static conditions used → Not relevant for P assessment
Read across from linear 4-n-NP, Sediment microcosm study, IUCLID dossier, Bradley et al. (2008)	Linear 4-n-NP	Freshwater and sediment (aerobic)	Upstream sediments: > 90 % mineralisation in 32 d (based on 14CO2) (at 23°C) Downstream sediments: ca. 7.5, 12.5 and 100% mineralisation in 154 d (based on 14CO2) (at 23°C)	Consistent with other studies indicating that linear 4-NP may degrade rapidly in (pre-exposed) sediments	Plausible	Low	High Linear 4-NP may degrade faster than branched isomers. Sediment collected from sites upstream and downstream WWTP, but upstream sites also likely pre-exposed to 4-NP 5 ml of saturated sediment used in test vessels
Read across from linear 4-n-NP, Freshwater sediment slurry microcosm study, IUCLID dossier, Wang et al. (2014)	Linear 4-n-NP	Freshwater+ sediment (aerobic)	4-n-NP concentration decreased 54.7 and 93.2% after 6 and 9 d (at 25°C) In the sterile control 14 % reduction after 9 d.	Consistent with other studies indicating that linear 4-NP may degrade rapidly in sediments	Plausible	Low	High Linear 4-n-NP may degrade faster than branched isomers Shaking at 120 rpm, not comparable with OECD 308 conditions
Water-sediment microcosms study, IUCLID	4-NP (not indicated whether	Mineral medium and sediment (aerobic)	half-lives of 46.2, 53.3, 57.8, and 69.3 days (at 30°C)	No conclusions can be drawn as sediment phase not sampled in	Plausible	Low	High Downstream sites more polluted than the

dossier, Chang et al. (2004)	branched or linear)		→ half-lives of 241, 278, 302 and 362 days when converted to 12°C using the Arrhenius equation Only aqueous samples collected →the DT50s are not for the sediment phase, but the DT50s cannot be used to conclude on degradation on water either because desorption from sediment to water may have occurred (as observed in de Weert et al. 2010b)	the study.			upstream site, but it may also be exposed to 4-NP as in Taiwan industrial/urban areas are found also in upstream areas. Not clear whether shaking or static conditions used → Not relevant for P assessment

Soil simulation studies

No standard guideline simulation tests for biodegradation of branched 4-Nonylphenol are available for soil. However, the registrants have included several non-standard studies.

Trocmé et al. (1988) studied the fate of technical NP (85% branched 4-NP and a lesser proportion of 2-nonylphenol and decylphenol) in a simplified soil system and its effect on microbial activity. The soil system was made up of sewage sludge compost (1/3 dry matter) and sandstone (2/3 dry matter) and had the following characteristics: pH 6.8, total nitrogen 0.5%, organic carbon 11%, carbon:nitrogen ratio 20, total phosphorus 1%, cation exchange capacity 22.1 meq/100g, water holding capacity 51%. Nonylphenol was dissolved in ethanol (0.4 ml/g spiked compost) and mixed with part of the compost, the ethanol was then left to evaporate. The spiked compost was mixed with the remaining compost to give a 60 g sample. Two concentrations of nonylphenol were applied (100 mg/kg and 1,000 mg/kg) plus a control sample spiked with ethanol. The cells were incubated at 60% field moisture capacity at 25°C in the dark for 40 days. Carbon dioxide was removed periodically by flushing the cells with carbon dioxide free air, the adsorbed carbon dioxide was determined by a conductivity method and volatilisation of nonylphenol measured by phenol traps. Residual 4-NP, adenosine triphosphate (ATP), pH and humidity were measured after 0, 5, 10, 20 and 40 days of incubation. Nonylphenol persistence was also studied under aseptic conditions. Samples were sterilised by gamma irradiation, spiked with 100 mg/kg nonylphenol then incubated for 24 days under the above conditions. The authors found that carbon dioxide evolution was significantly depressed by the 4th day in the 1,000 mg/kg vessel, and a decrease was noted in the ATP content in the 1,000 mg/kg vessel after 5 days. No significant changes in carbon dioxide evolution or ATP content were observed in the control and 100 mg/kg vessels. After 40 days incubation 11% nonylphenol remained in the 100 mg/kg treatment and 38% remained in the 1,000 mg/kg treatment. Volatilisation was insignificant, with 0.22% volatilisation over 40 days in the 1,000 mg/kg treatment. In both treatments NP concentrations started decreasing after 5 days incubation; loss was rapid at first then either slowed down (100 ppm) or stopped (1000 ppm). NP was more persistent under the semi-sterile conditions (the sterilisation was incomplete) with 76% NP recoverable after 24 days. The authors suggested that NP underwent microbial degradation after a period of induction of the micro-organisms. They also noted that part of the decrease of NP observed in the test may have been caused by formation of NERs. The chromatographic profile for NP taken at various times during the test indicated that certain isomers of NP degraded more easily than others. This may also explain the two different phases of faster and slower disappearance of 4-NP in the test as the more easily degraded isomers may disappear first and after that the disappearance becomes slower due to the slower degradation of the remaining isomers. As the soil was amended with sewage sludge at a relatively high concentrations (1/3 of the dry weight), previous exposure of the mircoorganisms with NP cannot be excluded. The study is not adequate to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

In the experiment conducted by Dettenmaier and Doucette (2007) column microcosms (diameter, 7.5 cm, length, 36 cm), containing a mixture of soil/biosolids planted with crested wheatgrass (*Agropyron cristatum*), were used to evaluate the fate of NP with emphasis on plant uptake and soil mineralization. The biosolids, containing approximately 1,000 mg/kg of NP, were obtained from a municipal WWTP receiving industrial, commercial, and domestic wastewater. These biosolids were spiked with uniformly ring-labelled [14C]NP (not indicated whether branched or linear) dissolved in ethanol. The ethanol was evaporated and after that the jars with the spiked biosolids were purged with N2, sealed, and placed in the dark at 4°C for 14 d. During the 14-d aging process, jars were removed from refrigeration and rotated daily for 4 h at 60 rpm. After completion of the aging process, samples of the spiked biosolids were collected from each jar for 14C analysis. No loss of 14C was observed during the aging period. The biosolids were then mixed with a recently collected, loamy sand soil (soil to biosolids ratio of 99.5:0.5 by dry wt) to yield three initial nominal soil/biosolids concentrations of 6, 24, and 47 mg/kg dry weight. These concentrations include the amount of compound originally in the biosolids and the amount of spiked 14C-labelled compound. Triplicate planted, unplanted, and

unplanted column poisoned with HgCl2 microcosms were used for each of the three concentrations. The microcosms were incubated for 150 days in a greenhouse with a 16:8-h light:dark photoperiod and a day/night temperature of 20/16 ± 1°C. Soil moisture was maintained at 60 to 80% of field capacity by weighing the columns and adding tap water as needed. The observed mineralization (to 14CO2) of NP was 7% and was independent of the initial exposure concentration of 6 to 47 mg/kg. The presence of crested wheatgrass did not enhance the percentage mineralization. Degradation of NP in soil based on test material analyses, performed at the end of the study for the triplicate 47 mg/kg initial concentration microcosms, was 90% at the end of the study, to 4.5±3.3 mg/kg (mean ±95% CI of triplicate column microcosms) for the planted, 1.5±1.0 mg/kg for the unplanted, and 5.26±3.84 for the unplanted poisoned. Calculated half-lives for NP averaged from 31 to 51 d in the various planted, unplanted, and unplanted aseptic (incomplete sterilisation based on CO2 measurements) systems. Based on the similar decrease in NP concentration observed in the soil poisoned with HgCl2 and in the unpoisoned soils, either the sterilisation was incomplete or other processes than biodegradation caused the decrease. The authors speculated that biotransformation and formation of NER would be the most significant processes. The reference compound phenol was mineralized 53±1.9% (average±95% CI) after 75 days. The study is not adequate to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

Topp and Starratt (2000) studied degradation of 14C labelled 4-NP and technical NP (tNP) in six different soils incubated statically at 30°C. The soils tested included three cultivated soils, a noncultivated temperate soil, and two soils from the Canadian Far North, which were presumably pristine with respect to exposure to 4-NP through agricultural or other sources. Hexane solutions (20 µL) of [ring-U-14C]4-NP and non-radiolabelled technical NP were added to fine sand, and the solvent was allowed to evaporate. Samples of moist soil (100 or 10 g dry wt) added to 150-ml baby food jars were supplemented with NP by thoroughly incorporating 100 mg of sand containing the amount of chemical required to achieve the desired final concentration. The baby food jars were placed in sealable 1-L Mason jars containing a scintillation vial with water to provide moisture and a scintillation vial with 7 ml of 1 M NaOH as a carbon dioxide trap. The traps were replaced periodically during the incubation and the recovered radioactivity measured by liquid scintillation counting. At the end of the incubation, the relative amounts of extractable and non-extractable 14C residues were established by extracting soil with ethyl acetate (see below) and determining the radioactivity in the extract by liquid scintillation counting. Mineralisation of the 14C labelled 4-NP (with an initial concentration of 1 µg/g soil and 80 Beq/g soil) was biphasic in all six soils, with a rapid initial phase lasting about 10 days, during which about 30% of the initial radioactivity was converted to carbon dioxide, followed by a much slower second phase with the final amount of carbon dioxide accumulated representing about 40% of that initially applied. At the end of the incubation of the sandy soil, only 10% of the initially applied radioactivity was extractable; 50% remained bound. Half-lives estimated from the initial (rapid) mineralisation rates of 4-NP ranged between 4.5 and 16.3 d. The 4-NP mineralisation did not occur in the autoclaved soil. In further experiments of the study, the relative biodegradability of the various GC-detectable isomers of technical 4-NP was investigated in a sandy agricultural soil. Following a 10-d incubation, all peaks in extracts prepared from the unsterilized sandy soil amended with 50 mg/kg tNP were reduced to low levels. In contrast, all isomers were stable in soil that had been autoclaved. In additional experiments incubated at different temperatures in the range of 4-30°C or at different moisture contents of soil, it was observed that mineralisation decreased with decreasing temperature and that mineralisation was maximal at intermediate moisture contents and was inhibited by either an overabundance of water or its near absence. Mineralisation of 14C-4-NP was rapid in the concentration range of 1 to 250 mg/kg soil dw. Furthermore, in experiments where the soils were amended with STP sludge solids at low concentration (100 mg), the mineralisation of 4-NP was comparable to that in soils with no sludge amended. However, sewage sludge amended at high concentration (50% v/v) was inhibitory, apparently because of high BOD. In summary, the results of the study indicated that microorganisms that are able to metabolise 4-NP can be found in a variety of soils, including two originating from the Canadian Far North, which presumably have not been exposed anthropogenically to this

chemical. The study is not adequate to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

In Mortensen and Kure (2003) the degradation and possible uptake of NP in agricultural plants was studied in greenhouse pot experiments. Different waste products including anaerobic and aerobic sludge, compost, and pig manure were incorporated in sandy soil. The soil came from a Danish experimental station and had not received any pesticides, sludge, or other domestic waste products. Sludge was received from sewage treatment plants, which receive wastewater from households and small industries. Compost was from a Danish plant receiving waste from households and smaller industries. The waste products (except the pig manure) contained branched NP leading to concentrations in the range of 13-246 ppb dry weight in the final soil mixture. In addition, linear 4-NP was used to spike the test soils resulting in concentrations in the range of 320-534 ppb dry weight. Rape (Brassica napus L. ev Hyola 401) was sown in the pots. There were 3.5 kg soil in each pot. Pots were not drained, and moisture in them was kept at 60% of the soil water-holding capacity. After 30 d, the rape plants were cut 1 cm above the soil surface. Visible roots were removed, and the remaining soil in the pots was thoroughly mixed and sampled for analysis. In order to investigate the influence of plant growth in the degradation, plant-free pots were also established. When NP was added to soil with waste application and homogenous mixtures were established, plant growth stimulated the degradation process of NP. In experiments with anaerobic and aerobic sludge, respectively, 13 and 8.3% of NP remained in the soil from pots planted with rape compared with 26 and 18% in soil without plant growth. When linear 4-NP was added as spike to soil, the degradation was more complete and plant growth did not influence the degradation. 2.2 and 1.8 % of the initial spiked concentration were still in the soil after 30 days for planted and plant-free pots, respectively. More complete degradation of NP was observed in soil amended with sludge compared with compost-treated soil. As the sewage sludge and compost mixed with the soil contained NP, they were adapted to the substance. Linear 4-NP can degrade faster than branched isomers of 4-NP, especially those with higher degree of branching, and hence, the results for the treatments spiked with linear 4-NP are not relevant for branched 4-NP. The study is not adequate to compare the half-lives with Annex XIII criteria but can be used as part of the WoE approach.

Jacobsen et al. (2004) studied degradation and mobility of NP in a lysimeter study using a sandy loam soil and 45-cm soil columns. Anaerobically digested sewage sludge was incorporated in the top-15-cm soil layer to an initial content of 0.56 mg NP/kg soil dry wt. Spring barley (*Hordeum vulgare* L.) was sown onto the columns. The lysimeters were placed outdoors and therefore received natural precipitation but were also irrigated to a total amount of water equivalent to 700 mm of precipitation. Leachate and soil samples from three soil layers were collected on days 0, 10, 20, 30, 50, 70, and 110. Leachate samples and soil extracts were concentrated by solid-phase extraction (SPE) and analysed using high performance liquid chromatography (HPLC) with fluorescence detection. The concentrations in the top-15-cm soil layer declined fast within the first 10 day of the study, to 45% of the initial content of NP. After that the decline was slower, and at the end of the study, the NP content was below the detection limit. Assuming first-order degradation kinetics, a half-life of 37 days was estimated for NP based on the data from samplings on days 20-110. NP was not measured in leachate samples in concentrations above the analytical detection limit of 0.5 µg/L for NP. In addition, NP was not measured in concentrations above the detection limit of 50 µg/kg dry wt in soil layers below the 15 cm of sludge incorporation, indicating negligible downward transport of the substance in the lysimeters. It is not possible to assess whether part of the NP present initially in the test system was lost through other processes than biodegradation, e.g. volatilisation or formation of NER. Furthermore, as NP was present in the sewage sludge introduced to the soil, the microorganisms were already adapted to the substance. Therefore, the results of the study cannot be used to compare with the Annex XIII criteria, but they can be used as part of the WoE approach.

The registrants have also included a study by Montgomery-Brown et al. (2008) where the degradation pathway of nonylphenol ethoxyacetic acid (NP1EC) was studied in oxic microcosms constructed with organic carbon-poor soil from the Mesa soil aquifer treatment

(SAT) facility (Arizona). The facility has been in operation since 1990 and discharges tertiary treated wastewater effluent into rapid infiltration basins for groundwater recharge and subsequent non potable reuse. 15 g of soil was added to the microcosms (125 ml glass bottles) and spiked with 10 µl of a contaminant containing stock solution. The microcosms were lightly capped, covered with aluminium foil, placed on a rotary shaker (~100 RPM), and incubated for up to 146 days. Samples were analysed for NP1EC and its metabolites using GC-MS on a regular basis. Para-NP1EC was transformed to 4-NP before being rapidly transformed to nonyl alcohols (NOHs). Between days 7 and 14, almost all NP1EC was converted to NP and between days 14 and 16, the NP was rapidly converted to NOHs. The soil used in the study is expected to be adapted to NP and its precursors due to the discharge of wastewater. The results are not considered relevant for the P assessment of NP.

The registrants have also included a study by Soares et al (2005), which was conducted to examine the potential of four white-rot fungi (*Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolor* and *Bjerkandera sp.* BOL13) for their ability to degrade nonylphenol, amongst others, in soil. Degradation of nonylphenol was followed by test material analyses (HPLC-UV). The fungi were observed to degrade NP. However, the results are not considered relevant for the P assessment of the substance.

Degradation of different isomers

As seen above (Lu and Gan 2014b, Trocmé et al. 1988, Topp et al. 2000, Toyama et al. 2011) and below, some of the studies included in the registration dossier have compared degradation of different isomers or isomer groups of 4-NP. Further studies were searched for in the open literature.

Gabriel et al. (2005 and (2008) studied degradation of different 4-NP isomers using preculture of *Sphingobium xenophagum* Bayram isolated from activated sludge of sewage treatment plant. These isolates do not metabolize NP by oxidative attack on the alkyl side chain, but rather release the alkyl moiety as a volatile alcohol derivative with unchanged carbon connectivity and most likely utilize only the aromatic ring as a carbon and energy source. In Gabriel et al. (2008) Incubation was done in Erlenmeyer culture flasks each containing 50 mL of minimal medium and 1 ml of the *Sphingobium xenophagum* Bayram and 1 mg/mL tNP and took place on a rotatory shaker (250 rpm) at 25 °C. One or two test vessels were sacrified for sampling after 9, 18 and 27 days of incubation. Sterile control was sampled at the end of the experiment. They observed a significant shift in the isomers composition of the mixture from the start of the incubation to day 9 of incubation. Chromatograms of extracts from cultures sacrificed at 9 (duplicate), 18, and 27 days were nearly identical, indicating that no further degradation occurred after 9 days. The authors speculate that the cease of degradation might have been related to the accumulation of chemically reactive 2-nonyl-p-quinones derived from corresponding alkyl-hydroquinone metabolites, a phenomenon that have been described for other bacterial strains in other studies. The isomer pattern in the sterile control at the end of the study was identical to that of technical NP at the start of the study. By means of GC-MS, a strong correlation between transformation of individual isomers and their α-substitution pattern, as expressed by their assignment to one of six mass spectrometric groups, was observed. As a rule, isomers with less bulkiness at the α-carbon and those with an optimally sized main alkyl chain (4-6 carbon atoms) were degraded more efficiently. By mass spectrometric analysis, the authors identified the two most recalcitrant main isomers of the technical mixture (Group4) as 4-(1,2-dimethyl-1-propylbutyl)phenols (NP193a and NP193b), which are diastereomers with a bulky α-CH3, α-CH(CH3)C2H5 substitution.

In Gabriel et al. (2005), degradation of five isomers of 4-NP in *Sphingobium xenophagum* Bayram (in the article referred as *Sphingomonas xenophaga* Bayram) cultures were compared. Differential degradation was clearly evident, as isomers with more highly branched alkyl side chains were degraded much faster than the others. *Sphingobium xenophagum* was able to utilise 4-(1-ethyl-1,4-dimethylpentyl)phenol (4- NP112), one of the main isomers of technical nonylphenol, as the sole carbon and energy source. Isomers

with quaternary α-carbon served as growth substrates with the subsequent formation of C9 alcohols, while the isomers with one or two hydrogen atoms in the α-C did not.

The review article by Lu and Gan (2014a) cites several further studies where information on the presence in the environment or degradation of 4-NP at isomer level is provided. Based on some of the cited studies, the relative proportion of some isomers is higher in some environmental matrices than in the tNP mixture (e.g. Horii et al. 2004, Kim et al. 2005), which may suggest differential degradation of isomers in the environment. However, it is noted that in other studies the relative proportions of the isomers or chromatogram peaks are similar in the sampled environmental matrices and tNP (e.g. Isobe et al. 2001). The degradation of different isomers has been compared in microorganisms cultures, soils, sediments, wastewater and biosolids, in some studies differences, up to ten-fold, have been observed between the degradation rates and half-lives of the studied isomers or isomer groups (Das and Xia, 2008; Eganhouse et al., 2009; Gabriel et al., 2005, 2008; Hao et al., 2009; Lu and Gan, 2014b; Shan et al., 2011; Toyama et al., 2011), while in other studies no significant differences have been observed (e.g. Topp et al. 2000). Although in many studies relatively rapid degradation has been observed for several isomers, it is noted that other isomers (or isomer groups) have been observed to degrade slowly or persist in some of the studies (e.g. Tomaya et al. 2011). However, it should be noted that the co-elution of isomers in chromatograms makess the interpretation of results at isomer level difficult, especially for the ones present at low concentrations. Based on the studies using isolated bacterial cultures (e.g. Gabriel et al. 2005 and 2008), there may be differences also between the degradation capacities of different microorganisms, some being able to degrade faster some isomers and other microorganisms other isomers.

Das and Xia (2008) studied the disappearance of 4-NP isomer groups with structures relative to α- and β-carbons of the alkyl chain detected as peaks in GC-MS analysis from biosolids during composting process in a bioreactor. Isomers with α -methyl- α -propyl structure disappeared significantly slower than those with less branched quaternary α -carbon and those with tertiary α -carbon, suggesting isomer specific degradation of 4-NP during composting process. The half-lives reported for the different isomer groups ranged from less than one day to 28.5 days. However, they are not considered relevant for the P-assessment as the conditions of the biosolid composting in bioreactor are not considered environmentally relevant.

Shan et al. (2011) studied degradation of five 4-NP isomers including four branched (4-NP38, 4-NP65, 4-NP111, and 4-NP112) and one linear (4-NP1) isomers in a rice paddy soil under oxic conditions. One of the isomers (4-NP111) was 14C –labelled to study its metabolites and bound residues in the soil. Degradation followed an availability-adjusted first-order kinetics with the decreasing order of half-life 4-NP111 (10.3 days) > 4-NP112 (8.4 days) > 4-NP65 (5.8 days) > 4-NP38 (2.1 days) > 4-NP1 (1.4 days). The mineralization of 14C-4-NP111 in the rice paddy soil was low (about 5% of the initially applied 14C within 58 days) and did not have a lag phase, indicating that microorganisms in the soil did not need an apparent adaptation time for mineralizing 4-NP111. In the sterilized soil less than 0.5% of 14C-4-NP111 was mineralized. the bound radioactivity increased rapidly to 31.1% within the beginning 5 days and to 54.4% at the end of the incubation in the active soils while in the sterile soil less than 20% of AR was in the bound fraction at the end of the experiment.

3 Integration & Weighing of evidence (WoE analysis)

See section 12.1 of the main part of the document.

4. Conclusions

See section 12.1 of the main part of the document.

Annex 2 Bioaccumulation weight-of-evidence assessment

1. Introduction

Problem formulation

Is there sufficient evidence on the bioaccumulation properties of the isomers of branched 4-NP to fulfil the Annex XIII of REACH and CLP criteria for B/vB?

Collection and documentation of all information

Information/evidence used in the approach include:

- Experimental studies from Endpoint study records from corresponding IUCLID Registration Dossiers
- Studies found from open literature (searched e.g. in PubMed using key words nonylphenol + bioaccumulation)
- QSAR Results

2. Assessment of quality of individual evidence

Bioaccumulation in fish studies

Ekelund et al. (1990) performed an aqueous OECD TG 305 study with the 14C labelled 4-NP (no EC or CAS RN mentioned). The marine fish three-spined stickleback (*Gasteroteus aculeatus)* was exposed to the substance during 16 days followed by a depuration phase of 32 days. Two test assays were performed using a flow-through system: Test1 using fish with 6.7% lipid content and a Tª of 10-13ºC and Test 2 using fish with 7.8% lipid content and a Tª of 8-13ºC. The tests were conducted in tanks containing 10 litre of water and 110 animals (85g of sticklebacks) under flow-through conditions. For the determination of total NP concentration in the exposure tanks, water samples were taken daily. Dosing of NP lasted for 16 days followed by an elimination period of 32 days. Four samples containing three animals each were taken 2, 4, 8, 16, 18, 20, 24, 32 and 48 days, respectively, from the start and were stored in glass jars at - 20 ° C until analysed. The fish were fed with mussels once or twice a week but never less than one day before sampling. Average measured exposure concentrations were 4.8 ug/L in Test 1 and 4.9 ug/L in Test 2, using acetone (at a concentration of 20 mg/L) as carrier. After few days steady-state was reached. Authors provided mean BCFs of 1200 and 1300 for 4-NP in *Gasterosteus aculeatus* for Test 1 and Test 2, respectively, based on whole body tissue wet weight. The results have been lipid normalised based on the lipid values provided in the report, with calculated BCFs of 833 to 896. The reported BCF values are based on total 14C and on drawn curves fitted by eye. It is indicated in the Ekelund et al. (1990) that if the BCF value is based on the mean tissue concentration after 16 days, the value should, with 95% probability, lie above 1000 in test 1 and above 840 in test 2. The determined extraction efficiency of NP from the sticklebacks was 47 ±12%(±95%CI). The authors of Ekelund et al (1990) speculate that the low extraction efficiency from sticklebacks probably resulted from NP being retained in skeletal parts remaining after the enzymatic treatment done in the sample treatment. Due to the low extraction efficiency, the results are considered to have high uncertainty.

Spehar et al (2010) studied the bioconcentration of 4-NP, a technical grade mixture of ring and chain isomers (90% pure), in whole fish *Lepomis macrochirus* (11–15-week-old) according to a method equivalent to guideline ASTM E1022-94, under flow-through aqueous exposure. Exposure concentrations (21.6, 43.9, 86.5, 211 and 444 ug/L), some of these are well above the 96h-EC50 (growth) of 0.096 mg/L and the 28d-NOEC (growth) of 0.038 mg reported for P. promelas in the ECHA dissemination site and all are above the lowest NOEC of 8.2 µg/L mortality at embryo and larval stages observed for Oryzias latipes in a two-generation test. Therefore, adverse effects cannot be excluded even in a short

exposure. The tests were conducted with 30 organisms per concentration and control (15 per duplicate tank) Duration of the exposure phase was 96 h, after which fish from the highest two no-effect exposure concentrations (43.9 and 86.5 ug/L) were put in clean water for 8 h and subsequently analysed to evaluate NP depuration in tissues. Two fish from each treatment and control chamber were collected at the start of the test (0 h before the toxicant flow was initiated) and at 1, 2, 4, 8, 12, 24, and 48 h. Four to six fish were collected from each treatment at the end of the 96-h test. At least two fish from each of the highest two no-effect exposure concentrations were also collected at 1, 2, 4, and 8 h after the toxicant flow was terminated to determine elimination kinetics. Mortality during the 96-h test was monitored every 2 h during the first 8 h, every 4 h for the next 24 h and then every 8 h until the end of the test. Bioconcentration was directly proportional to exposure concentration until steady state seemed to be reached although only two measures, between 24 to 48h exposure, were done. Then concentrations of NP decreased rapidly when placed in clean water (8h) after exposure (96h). Tissue concentrations decreased approximately to 60%, when placed in clean water for 8 h after exposure. Mean, 1% lipid normalised BCF of 53, associated with no-effect concentration, is reported. This can be converted to a 5% lipid normalised BCF of 265. However, uncertainties are raised due to lack of information on test conditions and confirmation of no effects on organisms. Furthermore, it is noted that according to OECD TG 305, a steady-state is reached in the plot of test substance in fish (Cf) against time when the curve becomes parallel to the time axis and three successive analyses of Cf made on samples taken at intervals of at least two days are within ± 20% of each other, and there is no significant increase of Cf in time between the first and last successive analysis. As the duration of the exposure phase in the study was only 96h, it is not possible to assess whether the steady-state was actually reached according to the criteria of OECD TG 305, or whether the concentrations could have slowly increased in a longer exposure period.

Spehar et al (2010) also studied the bioconcentration of nonylphenol, technical grade mixture of ring and chain isomers (90% pure) in *Pimephales promelas* (four-week-old) according to a method equivalent to guideline ASTM E1022-94. Same test procedure as in the above study with Lepomis macrochirus were applied. Measured exposure concentrations were 18.4, 41.9, 83.1, 230 and 544 ug/L, some of those are well above the 96h-EC50 (growth) of 0.096 mg/L and the 28d-NOEC (growth) of 0.038 mg/L reported for P.promelas in the ECHA dissemination site and all are above the lowest NOEC of 8.2 µg/L for mortality at embryo and larval stages observed for Oryzias latipes in a two generation test. Duration of the exposure phase was 96 h, after which fish from the highest two no-effect exposure concentrations (41.9 and 83.1 ug/L) were put in clean water for 8 h and subsequently analysed to evaluate NP depuration in tissues. Bioconcentration was directly proportional to exposure concentration until steady state was reached between 24 to 48h exposure and concentrations of NP decreased rapidly when placed in clean water (8h) after exposure (96h). The same uncertainties as mentioned above for *Lepomis macrochirus* regarding the reaching of steady-state apply. Tissue concentrations decreased approximately to 40%, when placed in clean water for 8 h after exposure. The mean, 1% lipid normalised BCF of 176, associated with no-effect concentration, is reported. This can be converted to a 5% lipid normalised BCF of 880.

Spehar et al. (2010) also studied the bioconcentration of 4-NP technical grade mixture of ring and chain isomers (90% pure) in whole fish blue gill (Lepomis macrochirus) and fathead minnow (*Pimephales promelas*) according to a method equivalent to guideline ASTM E1022-94 in tests with exposure phase of 28 d and no depuration phase included. Under flow-through aqueous exposure. Exposure concentrations were 5.6, 12.4, 27.6, 59.5 and 126 ug/L in the blue gill test, and 9.3, 19.2, 38.1, 77.5 and 193 ug/L in the fathead minnow test. Most of these are near or above the lowest 28d-NOEC of 0.038 mg NP/L for growth reported in the ECHA dissemination website for Pimephales promelas. Even lower NOECs are reported for other species, e.g. a NOEC of 8.2 µg/L for mortality at embryo and larval stages observed for Oryzias latipes in a two generation test. The 28-d tests were conducted with 40 organisms per concentration and control (20 per duplicate tank). Two fish were collected from each treatment and control chamber at the start of the test (0 h before toxicant flow was initiated). During the exposure, four fish from each treatment

were collected from each treatment at day 14 and six fish at day 28. Mortality was monitored every 4 h for the first 24 h, every 8 h for the next 72 h, and twice daily thereafter (more often if mortality rates were high) until the end of the test. Mean 1% lipid normalised BCFs of 49 and 186, associated with no-effect concentration, are reported for blue gill and fathead minnow, respectively. These can be converted to 5% lipid normalised BCFs of 245 and 930, respectively. Authors calculated these data associated with no effect concentrations on mortality, which was thoroughly monitored. However, Giesi et al (2000) published increase in mean plasma concentration of E2 with died organisms during 42 day long-term exposures at 0.05 µg/L. In addition, a current NOEC of 8.2 µg/L is available at the ECHA dissemination website for 4-NP. Therefore, potential sublethal adverse effects, which could have affected the results of the bioconcentration test, cannot be excluded.

Tsuda et al. (2001) studied a 7-day bioconcentration of 4-NP, mixtures of compounds with branched sidechain, to *Oryzias latipes* under flow-through conditions in a laboratory following principles similar to OECD TG 305. Juvenile fish were exposed to an average measured concentration of 3.6 ug NP/L for 7 days. Ethanol was used as vehicle. Glass tanks holding ca. 10 and 5 l were used for the accumulation and excretion tests, respectively. In the tests, all drainage was passed through activated charcoal. For the accumulation tests (168 h), the aqueous stock solutions of NP (400 mg/L) was diluted 50 times continuously with dechlorinated city water and supplied to aquaria containing 250 fish. The aqueous stock solutions of NP (400 mg/L) was prepared by diluting ethanol solutions of NP (1000 mg /mL, 4 ml) with dechlorinated city water to 10 L, respectively. For the excretion tests (24 h), about 100 fish remaining in the aquarium were separately transferred into another aquarium supplied with dechlorinated city water. Twenty fish were taken at 3, 6, 12 and 24 h of the depuration phase. During the tests, the flow rate (6.0 L/h) and temperature (19 °C) of water were maintained. Solvent control was included in the test. The pH and hardness of the water in the exposure phase and depuration phase were 6.9–7.0, and 44–45 mg/L as CaCO3, respectively. The dissolved oxygen concentrations were 7.3–7.8 and 8.2–8.4 in the exposure and depuration phases, respectively. The temperature (19±1ºC) is below the range recommended in the OCDE 305 (20-25ºC) which could slightly decrease the metabolism during the uptake phase. Fish whole body tissue (20 fish per sampling) and water column concentrations were measured at 0, 6, 12, 24, 48, 72, 120, and 168 h for mean BCF determination. Mean steady state BCF based on whole body tissue wet weight was 167 (average lipid content 2.2% and water content 78% in the test fish) (BCF 758 dw). 5% lipid normalised BCF of 380 is calculated. Steady state was reached at 48 h. The reported elimination rate K_2 is 0.07 h^{-1} and half-life 9.9h.

Giesi et al. (2000) studied the 42-day aqueous bioconcentration of NP, a mixture composed of several isomers with a purity of greater than 98% to *Pimephales promelas* under flow-through conditions in a laboratory test. Adult fish (12 -18 months old) were exposed to a control and solvent control and chemical test measured concentrations of 0.05, 0.16, 0.4, 1.6, and 3.4 µg NP/L for 42 days. All treatments were done in triplicate (each containing two males and two females placed into 19-L aquaria) expect solvent control which had only two replicates. NP was measured in 5-7 fish from each exposure treatment. It is not known when steady state was reached. No depuration phase was performed. BCF values of 203, 252 and 268 are reported for exposure concentrations of 0.4, 1.6 and 3.4 µg/L, respectively, based on whole body tissue wet weight. However, in the published article, the concentrations of NP in fish tissue are given in ng NP/L instead of ng/kg. Therefore, it is not clear based on which values the BCF values, which should have a unit of L/kg, were calculated.

Study authors conclude that NP affects adult fathead minnows not as a result from direct-acting estrogenic agonist mechanism, but rather from changes in the endogenous concentrations of E2 through an indirect activation mechanism of action. In females, exposure to 0.05 µg NP/L resulted in the greatest mean plasma concentration of E2 (24 ng/ml), which was approximately 10-fold greater than in the controls. Thus, in both males and females, an inverted U–type response was found. Additionally, deaths were observed in all treatments, except in controls. This would indicate that the test was carried out under

stressing concentrations. Additionally, concentrations proving lethal should not be used for BCF determination. This study is not considered for B assessment.

Brooke et al. (1993) studied the 28-day chronic toxicity and bioconcentration of NP (EC 246-672-0) to *Lepomis macrochirus*, *Pimephales promelas* under flow-through conditions in a laboratory test. The test was performed following the principles of the ASTM 1993 E1022-84 (reapproved 1988) for fish and saltwater bivalves. Juvenile *Lepomis macrochirus* were exposed to measured test concentrations of 5.6, 12.4, 27.6, 59.5 and 126 ug/L. Juvenile *Pimephales promelas* fish were exposed to measured test concentrations of 9.3, 19.2, 38.1, 77.5 and 193 ug/L of NP/L. The uptake phase duration was 28 days. No depuration phase was performed. Fish whole body tissue (8 fish per sampling event) concentrations were measured at day 0, 14 and 28 for mean BCF determination. Water concentrations were measured at 4h from test start and three times per week after that. The fish were held in 2.5 L test vessels, 20 fish in each vessel. Two replicate per test concentration and two controls. Lipid contents of the fish were not measured.

For *Lepomis macrochirus* it is indicated that steady state was reached and the BCFss ranged from 173 to 253. A chronic NOEC of 59.5 ug/L and LOEC of 126 ug/L based on mortality are reported in the same study by Brooke et al 1993 and sublethal chronic effects could occur even at lower concentrations. Therefore, the BCF of 231 reported for the lowest test concentration (5.6 ug/L) could be considered the most reliable.

For *Pimephales promelas* steady state was reached for the three highest concentrations between days 14 and 28. NOEC for mortality of *Pimephales promelas* was 81.3 µg nonylphenol/L and LOEC was 230 µg/L. However, it is noted that a NOEC of 0.038 mg/L for growth of P. promelas is reported in the ECHA dissemination site and hence adverse effects cannot be excluded in the two highest test concentrations, and these are not considered reliable. The adequacy of the test concentration of 38.1 µg/L is also questionable as it is equal to the NOEC determined for growth. The BCFs of the two lowest test concentrations were 769 and 984, but steady state was not reached. For the 38.1 µg/ concentration a BCF of 876 is determined.

Snayder et al (2001) studied the 42-day bioconcentration of nonylphenol (not indicated whether branched or linear) to *Pimephales promelas* under flow-through conditions in a laboratory. Adult fish (12 -18 months old) were exposed to a control and solvent control (0.0001% ethanol) and measured concentrations test of 0.33, 0.93, and 2.36 ug NP/L for 42 days. Fish were randomly assigned to 20 groups, each containing two males and two females, and placed into 19 L aquaria. Treatments were in triplicate with the exception of the solvent control group, which contained only two aquaria. NP was measured in 5-7 fish from each exposure treatment. Fish whole body tissue and water column concentrations were measured periodically during the test for mean BCF determination. It is not known when steady state was reached. No depuration phase was performed. Test temperature 26ºC. A maximum BCF of 434 (whole body tissue wet weight) was determined.

Cantu (2013) (MSc Thesis,) carried out a BCF test trying to demonstrate a shortened testing design for the OECD TG 305. The study includes exposure and depuration periods reduced to a total of 14 days (7d exposure, 7d depuration). The study was performed with *Cyprinus carpio* that were exposed to a nominal concentration of 2.5 ug/L of 4-NP (4-(2,4-dimethylheptan-3-yl)phenole (CAS RN 104-40-5). It is not clear what isomer has been used since the indicated name correspond to a branched isomer but the provided CAS RN correspond to a linear one. No measured concentrations were provided. Study well documented. A BCF of 576 whole body ww is stated (lipid normalised, but not indicated what the fish lipid content was). However, BCFs for muscle and liver are also estimated. In the case of muscle steady state is confirmed although only by two measures but in the case of liver steady-state is confirmed by the author not to have been reached, with the concentration of 4NP still clearly increasing after 7 days exposure. Anyway, it is recognised that it would have increased the proposed BCF, but it is not a crucial deviation from the guideline that would modify the proposed BCF.

Gautam et al (2015) carried out a 60-day study investigating the aqueous bioaccumulation of 4-NP (99% purity) (no CAS RN or EC number indicated, nor whether branched or linear) in the catfish *Heteropneustes fossilis* (30-40g). Two semi-static tests were conducted at different times of year to encompass different periods in the reproductive cycle, The first experiment was started in the resting phase and terminated in the preparatory phase (January 15 to March 16). The second experiment was started in the preparatory phase and terminated in the prespawning phase (March 15 to May 16). The test fish were mature female catfish *Heteropneustes fossilis* (30–40 g), purchased from a local fish market near Varunariver, India, during resting (January) and preparatory (March) phases of the annual reproductive cycle. Two nominal test concentrations were included (64 and 160 µg/L) in addition to a control group, each treatment group containing 40-50 fish. The fish were fed goat liver twice daily ad libitum. Water was changed twice daily after feeding and the desired concentration of 4-NP was added. After 15, 30, 45 and 60 days of exposure, blood samples were collected, and the fish were dissected and the concentration of 4-NP was analysed in the liver, gill, plasma, brain, muscle, ovary and kidneys using HPLC with a UV-detector. Variation in the bioaccumulation was found across the different exposure concentrations, the different experimental runs and the different tissue types. No BCFs are calculated in the study but based on the tissue concentrations estimated from the graphs included in the publication, BCFs for the catfish, *Heteropneustes fossilis*, after 60 days exposure are estimated to be between 19 to 113 L/kg. The greatest BCF was noted in the brain, followed by the gill, kidney, liver, ovary, plasma and muscle. In the chromatograms, a prominent peak after the 4-NP peak and also some more minor peaks in brain, liver, kidney and ovary were observed indicating metabolism of 4-NP. However, these metabolites were not identified due to non-availability of standards. The study did not follow any standard guideline and several deviations from the OECD TG 305 are observed: BCFs for the whole body not calculated, water sample analysis not carried out/reported, species not recommended by the guideline, no depuration phase included, no lipid content reported, no information on real exposure concentrations, no steady-state reached at 60 days and exposure concentrations well above the lowest NOEC of 8.2 µg/L observed for fish (O.latipes). Therefore, the study is not considered reliable nor assignable to be used in B assessment.

Nurulnadia et al (2016) conducted a dietary bioaccumulation study with several endocrine disrupting chemicals (EDCs) including 4-NP (not indicated whether branched or linear) following largely the principals of the OECD 305 guideline. Deviations included the lack of depuration phase and the use of a non standard test species, *Pleuronected yokohamae,* which is a benthic marine fish. The test fish were purchased from the Regional Government Aquaculture Center in Yamaguchi, Japan.

NP was spiked to food in two experiments assessing the assimilation efficiency and the biomagnification. For the biomagnification experiment food stuff was prepared individually at three different concentrations: 13.6 µg/g (low), 49.7 µg/g (medium) and 259 µg/g (high). Diethyl ether was used as solvent in the spiking, but there is no indication whether a solvent control diet was prepared. However, the spiked diet was left overnight in a draft chamber, so it can be expected that most of the solvent evaporated during that time. Aerated flow-through tanks (35x48x18 cm, one per test concentration and 1 control) were used for the exposure test. Twenty-four of P. yokohamae were placed in each tank. The average body weight of individual *P.yokohamae* was 2.95 ± 0.38 g and 3.51 ± 0.49 g, for the control and exposure groups, respectively, at the beginning of the experiment. The test water (30 L) was completely replaced 300 L/10 times/ d with natural sand-filtered seawater. On day 0, three *P.yokohamae* individuals were sampled as a baseline. *P.yokohamae* in each tank were then administered the spiked diet at a daily feeding rate of 2% of their body weight during the 14 day uptake phase. Non-consumed food residue was removed daily. Five fish in each tank were sampled on days 2, 4, 7 and 14. No depuration phase was included 4-NP concentrations in fish and diet were measured using LC-MS/MS. Based on the recovery test, the extraction efficiency for 4-NP was 60±7.4%.

BMFs were calculated as the ratio of the concentration in the organism to the diet at steady state as follows: BMF =(EDC concentration in *P.yokohamae* at steady state)/(EDC concentration in spiked diet) on a wet weight basis. Mean water temperature, dissolve

oxygen, pH, and salinity were 26.7 ± 0.9 _C, 6.67 ± 0.4 mg/L, 8.28 ± 0.1, and 28.0 ± 0.2 ppt, respectively, during the biomagnification experiment.

Steady state was determined as reached after 7 days of exposure. The maximum concentrations of NP in the low, medium, and high exposure groups were 248 ± 56, 299 ± 85, and 380 ± 25 ng/g w.w., respectively. Whole body BMFs of 0.02, 0.007 and 0.002 are reported for low, medium and high test concentration of 4-NP, respectively. In the Materials and method section of the publication it is indicated that the BMFs were calculated on wet weight basis. However, in the table where the BMFs are presented, concentrations in diet and fish are included in dry weight basis. However, based on the available information on the wet weight concentrations of 4-NP in the fish, BMFs of 0.018, 0.006 and 0.0014 can be calculated for low, medium and high test concentration. No information on lipid content is included and, hence the BMFs, cannot be lipid-normalised. NP was detected also in the control diet, at concentration of 28.3 ± 1.8 ng/g. NP level in the test medium was below detection limit (0.15 ng/mL) suggesting that it was not released from the diet to the test medium. On day 0, NP was detected in *P.yokohamae* at a concentration of 53.6 ± 21 ng/g wet weight. NP was also detected in control fish as *P.yokohamae* accumulated very low concentrations of these compounds from the control diet during the acclimation period.

Carnevalia et al (2017) investigated the uptake of tNP in Gilthead seabream (*Sparus aurata*) via dietary exposure in a laboratory experiment. The test was not conducted according to standard guideline procedures, although it follows similar principals to the OECD 305. The study was based on assessment of bioaccumulation as part of a mixed exposure to 2 or 3 chemicals: NP, octylphenol (OP) and BPA. The different diets containing a nominal concentration of 5 mg/kg bw of each substance, were formulated as follows: NP+tOP, BPA+NP, BPA+tOP and NP+BPA+tOP (NBO) The test fish were divided into groups of 20 individuals, in duplicates. The food stuff was dosed with the mixtures and fed to the fish equal to 2.5% of their body weight once a day for an uptake phase of 21 days. No depuration phase included. Concentrations in the foodstuff and the fish were determined, however the fish were only sampled on day 21. The BMF was calculated based on the foodstuff concentration and the tissue (muscle) concentrations, assuming steady state. Concerning the diets spiked with NP, it is indicated that t-NP was the main form detected and low levels of 4-NP (± 1.6 mg/kg feed) were also measured in these diets. It is not clear based on the information available in the publication whether this refers to branched or linear 4-NP. Steady state BMFs in muscle of 0.028, 0.0084 and 0.001 are reported for NP when exposed together with Bisphenol A or tert-octylphenol or the three substances together, respectively. Several deviations from the OECD guideline are observed, e.g. BMF derived for a particular tissue. It was not possible to determine whether the chemicals had reached steady state as no samples were taken prior to day 21, underestimation in the uptake of nonylphenol cannot be excluded due to potential competence of the exposure compounds during the uptake phase.

Additionally, based on the information on BPA, NP and 4 -t-OP concentrations in the diets of each experimental group, it would seem that contamination of exposure has happened since BPA is measured in the test feed with NP+OP and t-NP appears in higher concentrations than expected based on nominal concentrations. Therefore, this test is considered not assignable for B assessment.

Invertebrates

Table Annex 2 - 3 Overview of the invertebrate studies not considered relevant/reliable for the assessment.

Test substance/exposure concentration	Test species	Result	Remarks	Reference

Aqueous exposure				
14C radiolabelled 4-(3,5 dimethyl-3 heptyl)Phenol 5 -150 ug/L	*Daphnia magna (*adult and neonates)	BCFk 760 and 4271 L/kg ww (total 14C) BCFk 31 and 302 L/kg ww (based on 4-NP) (not indicated at which exposure concentrations)	Uptake phase 24-96h Depuration phase 96-168h Some concentrations above NOEC for D.magna → Not reliable	Preuss et al (2008)
4-NP (CAS RN 104-40-5) 75 µg/L (nom.)	Mytilus galloprovincialis	BCFk 6850 L/kg (dw)	Daily spiking with the 4-NP stock solution to maintain test concentrations High concentration above NOEC for aq. invertebrates → not reliable	Vidal-Linan et al. (2015)
4-NP 100 µg/L (nom.) 34 µg/L (mean meas.)	Mytilus edulis	Whole body BCFss 196 Whole body depuration rate 58.9 d	4-NP water concentration decreased rapidly, but not in control without mussels → likely rapid adsorption/uptake by mussels High concentration above NOEC of aq.invertebrates → Not reliable	Ricciardi et al. (2015)
technical 4–NP and nonylphenol acetic acid (NP1EC), mixed exposure 480 ng/L (4-NP) + 520 ng/L (NP1EC)	copepod *Eurytemora affinis* (collected from the Seine Estuary)	4-NP: BCFss 324 L/kg (dw)	Similar concentrations of 4-NP in exposed and control copepods after 86h uptake phase. 4-NP conc. increased in copepods during depuration phase due to metabolism of NP1EC to 4 –NP. → Not reliable	Cailleaud et al. (2011)

Ekelund et al (1990) carried out a study to assess the bioconcentration of NP to shrimps (*Crangon crangon)* under flow-through conditions in a laboratory. Shrimps were exposed to average measured concentrations of 6.4 and 7.4 µg 14C-labelled NP/L for 16 days (Test1 and Test2, respectively). A depuration phase of 32 days was included in the study. Acetone was used as vehicle with 20 mg/L. Each test tank contained 10 litres of water and 10

animals (45g of shrimps). Shrimp whole body tissue and water column concentrations were measured on days 2, 4, 8, 16, 18, 20, 24, 32, and 48 (from the start of the exposure, including depuration) for mean BCF determination. Four samples containing three animals each were collected at each sampling event. The shrimps were fed with mussels once or twice a week, but never less than one day before sampling. Temperature varied between 10 and 13°C in test 1, and between 8 and 13°C in test 2. Steady state was reached after a few days. Mean BCF of 110 based on whole body tissue wet weight for Test 1 and 90 for Test 2. The lipid content of the shrimps was 1.4 and 1.7 % in tests 1 and 2, respectively. Rapid elimination was observed in the depuration phase. The study does not follow any guideline. There is no information on survival or potential adverse effects on the organism.

Ekelund et al.(1990) also studied bioconcentration of NP in *Mytilus edulis* under flow-through conditions in a laboratory. Each test tank contained 60g soft tissue fw of mussels. The average measured concentration were 5.9 and 6.2 ug 14C-labeled nonylphenol/L for Test1 and Test2, respectively. Otherwise, the same study conditions and protocol as in the shrimp study were used. Steady state was not reached over the duration of the exposure and therefore results were extrapolated to determine BCF value. The increase of the tissue concentration for the mussels was adapted to a mathematical expression using a least square regression (Box et al., 1978):

$Y= C(1 - e^{-t})$

where

C = limit value of NP concentration in tissue as mg kg^{-1}fw for the steady state condition

Y= concentration in mg kg^{-1}fw for each sample

t = exposure time in days.

A TLC separation of the tissue extracts of Mytilus showed that the major part (> 80%) of the radioactivity in the extract co-chromatographed with 14C-NP. BCF's of 2740 for Test 1 and 4120 for Test 2, based on the extrapolated total 14C whole body concentration in wet weight are reported. When the BCFs are corrected for the content of the radioactive metabolites, a mean BCF of about 2700 is reported. Lipid content measured 1.6-1.9%. Elimination of NP from Mytilus was a biphasic process, and a significant residue remained even after 30 days in clean water which is an indication of slow elimination in mussels.

Gatidou et al. (2010) carried out a study that largely followed the principles of the EPA OPPTS 850.1710 (oyster bioconcentration test). The report did not state compliance with this guideline and departed from the guideline based on the choice of test species, depuration duration, and particular methodological variations associated with the choice of test species. Mediterranean mussels (*Mytillus galloprovincialis*) (shell length 40–55 mm, wet tissue mass 1.22 ± 0.58 g) were collected from the wild (different clean sites along the Thermaikos Gulf and Lesvos Island, Greece) and, after an acclimation period of 7 days in the laboratory, exposed to a nominal concentration of 300 ng/L concentration of 4-n-NP (EC/CAS, not specified) (meas. Concentration 305 ng/g) in a semi-static system. Glass tanks that contained 10 L of unfiltered seawater and 25 individuals were used as test vessel. One replicate was included for 4-n-NP and one for control group. The duration of the exposure phase was 28 days. During this period, both water and target compounds were renewed every 24 h and the water was gently and continuously aerated. Two mussels and 100 mL of seawater were regularly sampled from each tank (0, 7, 14, 21 and 28 days). These samples were analyzed for the presence of the target compounds. The uptake phase was followed by a depuration phase of 28 days. Two individuals from each tank were sampled every seven days during the depuration phase. Analysis of samples was performed using a Hewlett Packard Gas Chromatograph 5890 Series II that was connected to a Hewlett Packard Mass Spectrometer HP5971 MSD (USA). The analysis of tissue samples was performed using the wet weight of each sample; however, for the purpose of data comparison and consistency, the final results were expressed as dry weight, with corrections for the sample humidity. To evaluate the trueness of the method, recovery

experiments were performed for tissue and seawater samples. Low recoveries were calculated for 4-n-NP in both sample types (59±3.8% and 34±3.2%, respectively). Therefore, recovery correction was made for 4-n-NP by dividing the observed concentrations by the recovery rates. However, this adds uncertainty to the results of the study.

The average, initial concentrations of 4-n-NP in the Mediterranean mussels that were used in the accumulation experiments were 88 ng/g.

Kinetic bioconcentration factors (BCFs) were calculated in the study. However, the determination of the uptake rate constant (K_u) differs from the calculations presented in the OECD TG 305 for determining k1, because the depuration rate constant (Kd in Gatidou et al. (2010)) is not taken into account in the calculation of Ku. According to Gatidou et al. (2010), for the accumulation procedure Kd can be neglected, and, assuming a constant concentration in water (Cw), the below equation can be used for concentration in tissue (Ct) during the uptake phase. The value of Ku was obtained by linear regression of Ct versus time.

$C_t = K_u C_w t + C_{t0}$

where C_t = the concentration of the substance in the tissue (ng g^{-1} d.w.), C_w = the concentration of the substance in the water (ng L^{-1}), K_u = the rate constant for uptake (L g^{-1} day^{-1}), K_d = the rate constant for depuration (day^{-1}) and t = time (days)

A kinetic BCF of 1700 L/Kg dry weight was determined in the mussel, *Mytillus galloprovincialis*, in the study by Gatidou et al. (2010). Uptake rate constant (k1) is estimated as 0.067 (L g^{-1} day^{-1} dw). Based on the depuration phase, it is indicated a half life of 17 days and a depuration rate (k2) of 0.040 (day^{-1} dw). The eMSCA has calculated a steady state BCF based on the concentration in mussel on the last day of the depuration phase and the measured water concentration of 305 ng/L. Use of dry weights for expression of the BCF is not considered a standard unit and will lead to an over-estimation of the BCF. However, Georgina el al (2022) has published a weight-to-weight conversion factors. In the case of Bivalvia a conversion factor of 0.481 has been calculated which will result in a BCF of 817 ww.

Hecht et al. (2004) studied the 16-day bioaccumulation of nonylphenol (not indicated whether branched or linear) in three estuarine amphipods under static renewal conditions in a laboratory. No specific guideline for bioaccumulation testing with these organisms. The sediments were spiked with a mixture of 14C radiolabelled and non-labelled 4-NP in a 1 to 99 ratio in methanol (v/v) using acetone as a vehicle. The nominal concentration was 400 disintegrations per minute (dpm) per gram dry weight of sediment, that is, NP concentration of 0.256 µg/g dw. Measured concentration averaged 65% of nominal, 0.167µg/g dw. Methanol and acetone were used as vehicle but left to evaporate from the sediment after spiking. The sediment was collected from a high, sandy, intertidal site in the Yaquina Bay estuary (Newport, OR, USA). The sediment was divided in three portions, and two sediment portions were enriched to 1.5 times (to 0.25-0.27 % TOC/g dw) their initial TOC content, which was TOC at 0.17 ± 0.01%/g dry weight with either Ulva sp. (LA treatment) for labile organic matter or lignin from refractory organic matter (RE treatment). The sediment portion not amended with additional organic carbon was used for positive (with 4-NP) (C+) and negative (no 4-NP) (C-) controls.

The test species were *Eohaustoriu estuarius* (Haustoriidae), *Grandidierella japonica* (Corophiidae), and *Corophium salmonis* (Corophiidae). *E.estuarius* is a free-burrowing, infaunal species that typically remains below the sediment surface and is abundant in Yaquina Bay in high intertidal sandy habitats. *G.japonica* and *C. salmonis* are tubicolous species inhabiting mid to low intertidal sediments in Pacific Northwest estuaries, including Yaquina Bay. Adult *E.estuarius* were collected from the Yaquina Bay two days before the

test start, and the other two test species were collected immediately before the experiment from laboratory cultures at the U.S. Environmental Protection Agency laboratory.

1-L test beakers contained 290 g of wet sediment and 775 ml of seawater (28‰ salinity). 20 test organisms were added to each beaker. Organisms were exposed for 16 days and no depuration phase was included. Three replicate beakers from each treatment were sampled after 16 days of exposure to measure NP concentrations in whole body and sediment. In addition, uptake and elimination kinetics were determined in *E. estuarius* by sampling three replicate beakers of each treatment on days 0, 1, 3, 6, 10, and 16. Duplicate sediment samples for analysis of 14C-NP, TOC, TN, and NP chemistry were taken on days -28, -1, 0, 1, 3, 6, 10, and 16 from each beaker. Overlying water (5 ml) was sampled from each beaker on days 0, 1, 3, 6, 10, and 16, and immediately analyzed for 14C. At the start of the test, three samples of each species (20 organisms/sample, including separate samples for male and female *C. salmonis*), were taken immediately before animals were placed into experimental beakers to determine initial dry weight, lipid content, and background levels of 14C. Radioactivity of sediment, water, and tissue samples was determined by liquid scintillation counting. Radioactivity was expressed as dpm/g dry weight (for sediment and tissue) and dpm/ml (for water). Radioactivity was converted to NP µg/g dry weight tissue or µg/g dry weight sediment by using the relationship 1 dpm = 0.183 ng NP, based on the specific activity of 14C-NP in the spike solution. In addition, actual sediment NP concentration was determined by using a modified normal-phase HPLC method to determine the percentage of parent NP.

Measured sediment concentration of NP at day -28 averaged 65% of nominal target concentration. The concentrations in sediment (both 14C and HPLC measurements) declined with time from day -28 to the end of the experiment. A mean of 63% of 14C-NP in sediment was attributed to parent NP. This relationship was then used to adjust 14C-derived NP concentrations in pore water, overlying water, and tissue. Adjusted values were used for accumulation factor determinations. According to the authors this approach ensured a conservative estimate of NP concentration because they could not determine which analytical method was more accurate, the HPLC or liquid scintillation counting of 14C-NP.

Lipid contents on day 16 were in the range of 4.65-5.96 %, 7.23-11.23% and 6.57-11.81 for *E.estuaris, G.japonica and C.salmonis*, respectively. In *G.japonica*, the lipid content was significantly different in the organism of the TOC amended treatments than in the positive control. *C.salmonis* females had a higher lipid content than males. Weight and lipid content of *E. estuarius* did not change significantly from day 0 to day 16. In *G.japonica* weight decreased by 54% in the positive control and by 41% in the RE treatments during the experiment, but remained unchanged in the LA treatment. Tissue lipid content of *G. japonica* tended to decrease over time in all treatments, but these decreases were only significantly different from the positive control in the RE treatment, where a 47% reduction in tissue lipid was measured. Both male and female *C. salmonis* decreased in weight during the experiment. Female *C. salmonis* exhibited a 56% increase in lipid content.

The BCF, BAF and BSAF values calculated for the three species in the different treatments are shown in Table Annex 2 - 4**Error! Reference source not found.**. According to the equation indicated in the article, the BCFs were calculated by dividing NP tissue concentration in µg/g wet wt by NP overlying or pore-water concentration in µg/L. It is noted that this would lead to a BCF values in L/g units, which differs from the L/kg units calculated in the OECD TG 305. As the concentrations of NP in the organisms in wet weight (only mean concentrations in dry weight reported) or in the pore water and overlying water are not indicated in the article, it is not possible to verify the BCF calculations. Furthermore, there is some uncertainty on whether wet weight concentrations in tissue were actually determined as it is not indicated whether the organisms were weighed upon sampling, and it is only stated that the dry weight of the organisms were determined.

The BAF values were calculated based on µg/g dry weight in the organisms and sediment. The BSAF were calculated using the equation:

$BSAF = (C_t/L_t)/(C_s/TOC)$

where *C*t is NP tissue concentration (g/g tissue dry wt), *L*t is tissue lipid concentration (g/g tissue dry wt), *C*s is NP sediment concentration (µg/g sediment dry wt), and TOC is total organic carbon concentration (µg/g sediment dry wt). According to OECD TG 315 in the calculation of BSAF the lipid normalised concentration in tissue and organic carbon normalised concentration in sediment should be in the same units, g/Kg. In the BSAF calculations of the Hecht et al (2004) it is not clear whether this was the case as the tissue concentrations were indicated to be in g/g and the sediment concentrations in µg/g. However, when converting the BAF values reported in the below table to BSAF values using the formula

$BSAF = BAF \times f_{OC}/f_L$

indicated in the OECD TG 315, where f_{oc} is the fraction of sediment organic carbon, and f_L is the fraction of worm lipid, and using the mean lipid contents and TOC contents reported in the article, similar BSAF values are obtained as the ones reported by Hecht et al. (2004).

Table Annex 2 - 4 BCF, BAF and BSAF values reported in the Hecht et al (2004)

Species and treatment	Overlying water BCF	Porewater BCF	BAF	BSAF
Eohaustoriu estuarius				
C+	3744	770	895	33.9
RE	1542	448	200	8.7
LA	827	246	225	8.5
Grandidierella japonica				
C+	4505	926	1074	17.3
RE	1170	340	149	5.8
LA	791	235	208	4.7
Corophium salmonis				
Male C+	1129	232	253	7.1
Female C+	3226	663	743	16.0

C+= positive control; RE = refractory lignin treatment; LA= Ulva treatment.

Mean BSAF based on whole body tissue dry weight was in the range of 8.5-33.9, 4.7 -17.3 and 7.1-16.0 for *E.estuaris*, *G.japonica* and *C.salmonis,* respectively. Kinetic uptake data was obtained from sampling *E.estuarius* on days 0, 1, 3, 6, 10, and 16. 14C-NP body residues reached steady state during the experiment and followed first-order kinetics. The rate of 14C-NP uptake (in dpm g dry wt tissue/dpm g dry wt sediment/d) was significantly greater in the positive control (355.91) compared with the RE (48.96) and LA (76.06) treatments. Elimination rates were modelled from uptake curves and were in the range of 0.25-0.40 dpm g dry wt tissue/dpm g dry wt sediment/d. However, as no depuration phase was included in the study, these depuration rate constants are considered to have uncertainty. Dividing the uptake rates with the elimination rates results in kinetic BAFs of 889.8, 195.8 and 190.2 for *E.estuarius in* positive control, RE and LA treatments. These are similar to the steady state BAFs included in the above table.

It is noted that *E. estuarius* does not construct burrows, but remains beneath the sediment surface, essentially swimming in pore water. Thus, it was more likely exposed to porewater

concentrations. A BCF of 1,542 from the overlaying water is estimated by the authors based on whole tissue wet weight. However, as indicated above, there is uncertainty in the reported BCF values due to use of different units for concentrations in tissues and in water than in the OECD TG 305.

Study authors of Hecht et al. (2004) conclude that the marine amphipods accumulated significant NP, particularly in sediment not amended with additional OC, suggesting that pore water is the dominant mode of uptake. Bioaccumulation of NP was inversely proportional to TOC concentration of the sediments but was unaffected by TOC nutritional quality.

Preuss et al (2008) studied the 48h bioconcentration of 4-NP to *Daphnia magn*a under static renewal conditions in a laboratory. Test material used was 14C radiolabelled 4-(3,5 dimethyl-3 heptyl)Phenol (p353-NP) (chemical purity > 97%). P353-NP is referred as the most prominent isomer in technical NP mixtures, representing about 20% of the total mass (Russ et al., 2005). Adult, juvenile and neonate (<24h) Daphnids were exposed to average measured concentration 5 -150 ug/L radio-labelled NP/L for 24-96 h Test concentrations ranged from no effect concentrations to LC20 concentrations. Experiments with neonates (<24h) were conducted according to the Daphnia Immobilization Test (OECD 202), in which 5 neonates were incubated in 10 ml Elendt M4-media with radiolabelled p353-NP. For adults and juveniles 4 daphnids were tested in 80 ml media. Daphnids were not fed during the experiments. In excretion experiments daphnids were transferred after 24 h of exposure to fresh untreated M4-medium, which was renewed daily. The depuration phase seemed to last between 96 and 168 hours. In the experiments with uptake phase only the duration was 48-96 hours. In total 5, 6 and 7 independent bioconcentration experiments were conducted for neonates, juveniles and adults, respectively. All experiments were conducted at constant temperature (20 ± 1 °C) and a light-dark rhythm of 16:8 h in open borosilicate glass vessels. Measurements of radioactivity were conducted with liquid scintillation counting. Biotransformation of p353-NP was measured using radio-HPLC analysis of daphnid extracts at different time points to determine the amount of p353-NP, the conjugates, and metabolites in percentage of total radioactivity.

Daphnid whole body tissue and water concentrations were measured for mean BCF determination. Kinetic BCFs were calculated for total radioactivity and for p353-NP separately. In the HPLC analysis, in addition to p353-NP two conjugates and two metabolites could be detected. One metabolite was more and one was less hydrophilic than p353-NP. The proportion the internal p353-NP concentration from the total radioactivity decreased from 6 to 48h at constant exposure level, whereas the proportion of the concentration of conjugates and metabolites increased. p353-NP concentration in the daphnids was calculated taking the biotransformation into account. Biotransformation rate (kMET) was determined to be 0.427 h^{-1}. Since kMET was high, the authors claim that the elimination rates found in the excretion experiment were not due to elimination of p353-NP as such (they found no p353-NP in the daphnids after 24 h of depuration), but due to the conjugates. Therefore, only the metabolization rate was taken into account and excretion was ignored in the BCF calculation for p353-NP.

The elimination rates for adults and neonated are reported to be 0.0172 and 0.0302 h^{-1}, respectively, based on total radioactivity. Mean kinetic BCF based on total radioactivity of 760 and 4271 are reported for adults and neonates, respectively. The kinetic BCF determined for the p353NP are 31 based on wet weight for adults and 302 for neonates. Based on the graphical information provided in the publication, no steady-state was reached in any of the experiments with adults. However, as the BCFs determined in the study are kinetic, they are not affected by that. It is not indicated at which exposure concentrations these values were determined.

Gross-Sorokin et al (2003) studied the uptake and elimination of technical 4-NP (85% purity based on content of para isomers) in the detritus-feeding freshwater shrimp *Gammarus pulex*. Two experiments were performed: one with exposure from water only (Experiment 1) and another one with exposure from both water and diet (Experiment 2).

The uptake phase lasted 48 h followed by a 144 h depuration phase. The diet consisted of dried horse chestnut leaf disks (2 cm diameter) dosed with NP by exposing 4 g of disks to a 1-L volume of stream water with a concentration of 1 mg/L NP for 24 h on a rotary shaker to achieve a nominal leaf concentration of 100 µg/g. The leaf disks were drained and combined, and excess water was removed by centrifugation. Measured NP concentrations in leaf disks were determined by GC/MS. 40 gammarids, collected from the River Mimram, a tributary of the River Lea near London, U.K. and acclimatised in the laboratory for 48h, were placed into each of the four 500-mL glass Beatson jars containing 250 mL of filtered stream water. Each experiment was set up in triplicate. In both experiments, NP concentrations were measured in the gammarids, water, and leaf disks at 8, 12, 24, and 48 h, respectively. For Experiment 2, the amount of leaf material ingested relative to the total weight of the gammarids was measured in each replicate at each sampling time point.

Experiment 1. A total of 0.5 g of NP-dosed horse chestnut leaf disks with a nominal concentration of 100 µg/g was enclosed in mesh netting (mesh size: 0.25 mm) and immersed into each Beatson jar. Aeration lines were placed under the netting to ensure movement and circulation of water around the leaves as in the dietary exposures. This design precluded the gammarids from feeding on the leaf disks and ensured that NP uptake could only occur through desorption of NP from the leaf disks into the water.

Experiment 2. A total of 0.5 g of NP-dosed horse chestnut leaf disks with a nominal concentration of 100 µg/g was added to each jar with no partition between leaf disks and gammarids, thus allowing the gammarids to feed on the disks.

Depuration. Separate depurations were performed for both sets of exposure experiments and were initiated after 48 h of exposure by transferring gammarids to clean water. Uncontaminated leaf disks and water were replaced daily. Samples were then taken in triplicate as above for analysis at 24, 48, 72, and 144 h (72, 96, 120, and 192 h after initiation of uptake phase, respectively).

Mean measured exposure concentration in water was 3 µg/L in Experiment 1 and 2.9 µg/L in Experiment 2. The measured concentration in the leaf disks was 83.4 and 82.9 µg/g in the Experiments 1 and 2, respectively. The uptake rate of NP from aqueous sources was modelled using the one-compartment model as described by Spacie and Hamelink (1985). Based on the data of the depuration phase of Experiment 1, a depuration rate constant *k*2 of 0.01 was obtained by regression (*r*2 0.87) with a corresponding half-life (*t*1/2) of 62.4 h. Regression results using data from Experiment 2 gave a similar value of k2=0.01 (r 2 0.90) and a corresponding half-life (t1/2) of 60.3 h. An uptake rate constant *k*1 of 31.38 h-1 was determined by non-linear regression to the bioconcentration data obtained in exposure 1. The uptake rate for the Experiment 2 was also determined in the study, but it is not considered relevant for the B assessment as the exposure came from both the water and diet, and there is uncertainty in the actual feeding rate. A higher body burden of NP was achieved in the gammarids by dietary uptake than from aqueous uptake, which indicates that there was uptake from feed also.

The eMSCA calculated a kinetic BCF of 3838 for *Gammarus pulex* after 48h exposure period based on the k1 and k2 values determined in the Experiment 1. Based on the graphical information steady-state was not reached during the exposure phase, but considering the estimated kinetic BCF steady state does not seems to be relevant. This well documented study is considered for the B assessment of NP.

Vidal-Linan et al., (2015) investigated the bioaccumulation of 4-NP (CAS RN 104-40-5 indicated in the study publication) in mussels (*Mytilus galloprovincialis*) based on the guidelines OPPTS 850.1710 for the bioaccumulation in the oyster. Mussels between 40 and 45 mm shell length were collected from a pristine area from the outer part of Ria de Vigo (NW Iberian coast), cleaned from epibionts, and acclimated to incubation conditions in the laboratory for one week prior to experiments. Exposure was made in 30 L glass tanks with 20 mussels per tank, at constant temperature (15 °C), in darkness, using 1 mm filtered seawater with oceanic characteristics. The tanks were continuously aerated with filtered

air, and were allowed to equilibrate for 1 h, before introducing the mussels. Water was renewed three times per week after feeding mussels for 1 h with a mixed diet of Isochrysis galbana, Tetraselmis suecica and Chaetoceros gracilis. Exposure tanks were spiked using a stock solution of 4-NP (99.9% purity, CAS RN 104-40-5) dissolved in dimethylsulphoxide (DMSO) to obtain a nominal concentration of 75 µg/L of 4-NP (<0.1% (v/v) DMSO). This is well above the lowest NOEC value (0.0095 mg nonylphenol/L for reproduction of A. Bahia), determined for marine aquatic invertebrates, and hence adverse effects cannot be excluded. Seawater control and DMSO control were also included. Preliminary trials showed an exponential decay in actual 4-NP concentrations in the water; consequently, every day without water renewal, 4-NP was spiked to maintain nominal concentrations. Water samples were taken at 0 h to check the initial concentration and at 24, 96 and 120 h before water renewal to monitor the concentration of 4-NP in the exposure media. In total over 450 mussels were exposed for 30 days to seawater control and experimental solutions containing 75 µg/L of 4-NP, followed by a 10 days depuration period. Mussel samples were taken for biochemical and chemical analyses after 0, 2, 5, 9, 15, 20 and 30 days exposure, and after 10 days of depuration.

Additionally, to study the concentration-response relationship between 4-NP exposure and biomarker responses, over 240 mussels were exposed for 30 days to seawater control and experimental solutions containing 25, 50, 75, 100 µg/L of 4-NP. After 0, 7 and 30 d exposure 6 individuals from each treatment were sampled for chemical analyses.

The analysis of water samples was carried out by dispersive liquid-liquid extraction (DLLME) followed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Mussel tissues were extracted by selective pressurized liquid extraction (SPLE) and determined by HPLC-MS/MS.

Bioaccumulation was modelled assuming first-order kinetics and constant 4-NP concentration in water according to equation in Landrum et al. (1992).

The measured initial 4-NP concentrations were 88-112% of the nominal concentrations. A marked decrease in actual 4-NP concentrations was observed already after 24 h; however, daily spiking with the 4-NP stock solution generally restored nominal concentrations. 4-NP concentrations in mussel tissues measured before exposure were 0.295 µg/g dw. The uptake of waterborne 4-NP was very rapid and approximately linear with time up to 9 days of exposure. Saturation was later observed, and a maximum of 599 µg/g was reached at day 15, with decreasing 4-NP concentrations further on. Kinetics of bioaccumulation were fitted to the classical saturation model described by equation of Landrum et al. (1992) resulting a R2=0.84, and statistical significance $p < 0.005$. According to this model, the BCF was 6850 ± 1850 L/kg (based on dry weight body concentrations), with an uptake rate coefficient (Ku) of 1702.5 ± 1549.6 L/Kg d^{-1} and an excretion rate of 0.248 ± 0.263 d^{-1}. The dose response experiments showed that uptake rate of 4-NP was dose-dependent during the first few days; however, saturation took place disregarding exposure concentrations, and at the end of the 30-day exposure 4-NP concentrations were 246, 282, 380 and 382 µg/g dw, in mussels exposed to 25, 50, 75 and 100 µg/L, respectively.

Use of dry weights for expression of the BCF is not considered a standard unit and will lead to an overestimation of the BCF. Additionally, due to the semi-static conditions and the additional daily dosing of 4-NP, there is uncertainty on the real exposure concentration, and the exposure concentrations were above the NOEC values determined for marine invertebrates. Furthermore, the CAS RN indicated for the test material refers to the linear 4-NP. The accumulation of linear and branched isomers of 4-NP may differ. Considered as not reliable for B assessment.

Cailleaud et al. (2011) investigated the bioaccumulation of technical 4 -NP (purity > 98%) and nonylphenol acetic acid (NP1EC), in a mixed exposure, to the invertebrate copepod, *Eurytemora affinis*. The study was run as an 86 h uptake phase, via a flow through continuous dosing design, with a 1 week depuration phase. The study was not conducted according to test guidelines. The copepods were collected from the Seine Estuary, France, in autumn 2004, and acclimatised in the laboratory for 3 days prior the test start. 25 L glass tanks were used as exposure tank and control tank. Freshly filtered sea water

sampled in the English Channel, mixed with ultra-pure water in order to reach the selected salinity of 15 PSU was used as test medium. Approx. 250,000 copepods were transferred into the experimental continuous flow-through system (1,000,000 individuals/m3). The concentrations of 4 NP and NP1EC were 480 and 520 ng/L, respectively. Acetone was used as solvent in the preparation of test solutions and was present in the final test medium at a concentration <15 µL/L of water. During exposure experiments, food was not provided to the copepods. Some copepod aliquots (n=3 pools) were sampled and immediately frozen in liquid nitrogen in situ at sampling time, after 3 days in clean water and at the end of exposure experiments for chemical analyses.

The accumulation of the test substances was determined by comparing concentrations of the substances in the exposed group and in the non-exposed (control) group at the end of the exposure. A BCF of 324 L/kg was determined for 4-NP based on whole body concentration in dry weight. During the depuration phase, increasing 4 NP levels were observed. 4-NP levels are around six times higher after 3 days of depuration and nine times higher after 1 week of depuration in comparison with background levels measured in the copepods at the in situ sampling time. However, similar concentrations of 4 NP are observed both in exposed (610±29 ng g−1 dry weight) and non-exposed copepods (674±62 ng g−1 dry weight) after 86 h of exposure. Furthermore, total amounts of NP1EC eliminated by the copepods placed in clean water (547± 165 ng/g dry weight) are equivalent to those of 4-NP that are accumulated by these copepods during the same depuration period (596±58 ng/g dry weight), with a strong correlation indicated. This indicated the apparent metabolism of NP1EC to 4 -NP which was then accumulated by the copepod to some extent. A BCF of 3020 is determined for NP1EC. Use of dry weights for expression of the BCF is not considered a standard unit and will lead to an overestimation of the BCF. REACH criteria for assessing bioaccumulation are based on wet weights and are therefore not applicable. On the other side, potential competence in the bioaccumulation process could happen between both compounds present in the mixture, resulting in lower BCFs for both substances.

Ricciardi et al. (2015) carried out a bioaccumulation study to determine the bioaccumulation of 4-NP (EC 203-199-4) in the Mussel, *Mytilus edulis*. The method used is similar to the principals of the EPA OPPTS 850.1710 (oyster bioconcentration test), although it is not stated in the publication. The method was amended to suit the requirements of different test organisms and a particular set up of the experiment was used i.e. different species, different uptake and depuration periods, different modelling approach to kinetics than those specified in the test guideline. Mussels (45.6–51.8mm length), collected from wooden breakwater on Spectacle Island (Boston Harbor, MA, USA) and acclimated in the laboratory for 12 days prior the test start, were exposed to 14C-4-NP (not indicated whether branched or linear) dissolved in seawater. The uptake phase was carried out for 38 days and the depuration phase for 35 days. The test was run using a semi-static method, with water changes every 3 -4 days. Nominal exposure concentration was 100.138 µg/L (454 nM). The concentrations is high compared with most levels measured environmentally because of the specific activity of the commercially available radiolabelled material and the need to have an acceptable radioactive count in the seawater and tissue samples). The concentration is well above the lowest NOEC value (0.0095 mg NP/L for reproduction of A. Bahia), determined for marine aquatic invertebrates, and hence adverse effects cannot be excluded. The control aquarium was spiked with a comparable volume of the ethanol carrier. Water quality was periodically monitored in the control (nonradioactive) tank. Parameters remained well within optimal range throughout the present study (temperature, 11.2 ± 0.7 °C; salinity, 33 ± 1 PSU; dissolved oxygen, 9.07 ± 0.47 mg/L or approximately 100% oxygen saturation). Mussels were fed 2 h prior to the water change with *Isochrysis galbana* and *Thalassiosira weissflogii* (approximately 2.7 10 7 cells/mussel). Four mussels were removed from each aquaria (one for 4-NP and one for control) on days 0, 3, 7, 10, 17, 24, 31 and 38 (exposure period) and on days 40, 43, 50, 57 and 73 (35-d depuration period). Each mussel was dissected into 6 tissues: cell-free blood plasma, posterior adductor muscle, gill, digestive gland, gonad, and remaining viscera. Water samples were taken before and after each water change done at 3-4 days intervals, and before and after feeding to monitor possible changes in the 4-NP concentration as a result of the algal food. Additional water samples were taken periodically

at shorter intervals to better determine the rate of loss of the radiolabelled compounds over the interval between water changes.

Initial concentration 4-NP decreased exponentially in the seawater aquaria minutes after being spiked. Minimum values were reached by 18 h to 24 h post spike, after which time seawater concentration rose slightly over the 3-d to 4-d period between water changes. In additional experiments, it was observed that 4-NP concentrations remained more stable in the seawater when no mussels were present, suggesting that most of the fast initial drop in the concentrations could be caused by e.g. rapid uptake of the substance, binding to the shell or to mucus sheets on mussel epithelia. A time-integrated average concentration of seawater was modelled, and the average value of 153 nM (33.75 µg/L) was used to represent the time-integrated concentration of 4-NP over the course of the exposure period.

A whole body BCF of 196 is reported in the study, calculated by dividing the 38-d wet weight tissue concentration by the integrated water concentration. The whole body concentrations were "reassembled" by summing the individual 4-NP tissue bioburdens for each mussel and dividing by the summed total wet weight of the tissues. Radiolabelled NP was used in the study, which did not allow for differentiation between metabolites and NP and therefore the concentrations of NP are likely overestimated. Additionally, it is known that under high exposure concentrations of toxicants mussels protect themselves by isolating from the environment by closing the valves. So, it is uncertain that NP was uptaken at maximum by mussels. Based on the graph presented in the registration dossier, the steady state does not seem to have been reached since the concentration seems to increase after the 38d exposure period. Therefore, this BCF has not been considered in the B assessment.

Uptake and depuration kinetics were also studied. However, traditional kinetic modelling was not used for the uptake data as according to the authors the data suggested that uptake was not exponential within any of the components of the mussel in the early stages of exposure. An alternative model was therefore developed to predict the uptake of 4-NP in the various tissues within the mussel during exposure. The uptake of the EDCs in the components of the mussel during exposure is modelled using a coupled system of ordinary differential equations. In this system, the rate of uptake for each component is modelled as a function of the concentration of EDC in the upstream component times a rate-limiting term that is based on the concentration of EDC within the component. Depuration was modelled using the traditional kinetic model. Uptake of 4-NP was curvilinear throughout exposure and the uptake in different tissues showed the following descending order: digestive gland > gill ≥ remaining viscera > gonad > adductor > plasma. Half-lives in the tissues based on total 14C: gill (57 days), plasma (15 days), digestive gland (30 days), gonad (33 days), adductor (32 days), viscera (48 days). Reassembled whole tissue: reassembled whole tissue half-life 58.9 days. These data would indicate low elimination rate in mussels.

Mäenpää and Kukkonen (2006) studied the 11-day bioaccumulation of NP (EC 203-199-4) to the sediment worm, *Lumbriculus variegatus*, under static conditions in a laboratory. Worms were exposed to three sediment (S1-S3) average measured concentrations of 0.013 (S1), 0.009 (S2), and 0.01 (S3) mmol/Kg of 14C-NP in spiked sediments for 11 days. That means, S1: 2.86 µg/Kg sed, S2: 1.98 µg/Kg sed and S3 2.20 µg/kg sed. Ethanol was used as solvent and control was also included. The organic carbon content of the sediments were 24.28, 3.20 and 1.64 %, for S1, S2 and S3, respectively.

The sediments (S1-S3) used in the bioaccumulation and toxicity experiments originated from freshwater lakes situated in North Carelia, Finland. In the laboratory, sediments were sieved through 2-mm sieve and homogenized by stirring. The sediments were characterized including dry weight, particle size, organic matter and carbon and nitrogen content. Tests were performed by using an aerated artificial freshwater as an overlying water, pH adjusted to 6.5. Artificial fresh water was prepared to deionized water yielding 0.5 mM inorganic salt concentrations. Following inorganic salts were added: MgSO4, KCl,

Na- HCO3 and CaCl2. The water was buffered against pH alteration by adding phosphate buffer (Na2HPO4) yielding a concentration of 0.5 mM.

Sediment was spiked with the test compound in one large (4 L) beaker. The 14C-labelled chemical was added to give a final concentration of approximately 10,000 dpm per gram of dry sediment. The stock solutions of 14C-labelled chemicals were added dropwise in sediment while mixing. The sediments were mixed with rotating metal blade for 2 hours at a room temperature to achieve a homogenous distribution of radioactivity in the sediment. Control sediment was treated similarly with solvent only added.

An experimental unit was 200 mL glass jar (height 10 cm, Ø 6 cm) containing 50 g (wet weight) of sediment and 100 mL of artificial fresh water. Water was cautiously added on the top of the sediment to avoid sediment disturbance. Test chambers were stored in a cabin incubator at +4°C for 2 days after spiking to let the sediment-chemical interactions to take place. After two days, ten test organisms were placed into each beaker. The experiments were conducted at a room temperature (+20 ± 0.5°C) with 16/8 hour light/dark period using yellow light source (>500 nm), that averts photo degradation of chemicals. Evaporated water was replaced daily by adding aerated deionized water (DI-water).

For each sampling time (0, 8, 24, 48, 72, 169, and 240/264 h) three replicate test chambers were destructed and sampled for pH, oxygen content, and chemical concentration of overlying water, sediment and animals. After sieving from the sediment, worms from a test unit were carefully blotted dry, weighed, but no purging of their gut content was done prior the sample treatment. The samples were analyzed by Liquid Scintillation Counter (LSC). The pore water pH was determined for triplicate control test chambers at the beginning (0 h) and at the end (240 h) of the experiment.

The pH measured from water, sediment and pore water varied among the sediments but did not differ among the experiments with the same sediment. Furthermore, the pHs did not significantly change during the experiments. Water oxygen concentration decreased during the experiments from 7.05 (0.27) at 0 h to 5.30 (0.13) at 240/264 h. Water temperature remained at 20°C (±0.5). The concentration of 4-NP in the sediment decreased from 12.69 to 13.94% during the 240 hours.

Bioaccumulation data were fitted for least squares non-linear regression method to de-scribe the bioavailability of sediment associated 4-NP (SigmaPlot 5.0, SPSS corpo- ration, Chicago, IL). The data of each accumulation experiment was fitted to a two- compartment, first-order kinetic model (Landrum et al. 1992):

$$C_a = \frac{k_s \times C_s}{k_e} \times (1 - e^{-k_e t})$$

where Ca is the concentration of a chemical in the animal (µmol/kg wet weight), ks is the uptake clearance coefficient (kg dry sediment/kg organism wet weight/h), ke is the conditional elimination rate coefficient (1/h), Cs is the concentration of a chemical in sediment (µmol/kg dry weight), t is the time (h). The bioaccumulation of 4-NP was estimated by calculating bioaccumulation factors (BAF): BAF = $C^{\text{steady state}}_a / C^0_s$. BAFs were estimated also from kinetic factors (k_s / k_e) according to Bailer et al. (2000). Biota-to-sediment accumulation factors (BSAF) were estimated similarly by fitting the kinetic data after normalizing the chemical concentrations for sediment organic carbon content and the organisms' lipid content. Worm lipid content (1.23% ww) used in BSAF calculations was examined earlier for the same L. variegatus culture as was employed in the current experiments (Leppänen and Kukkonen 2000).

The accumulation of 4-NP was higher in one of the sediments than in the two others. No steady state was reached in any of the three sediments during the 11 days study. The BSAF values determined for the three sediments ranged from 14.1 to 55.4. The BSAF was calculated based on *L. variegatus* tissue residue normalised to lipid content in the organism (µmol/kg lipid ww), and sediment chemical concentration normalised to organic carbon

content in the sediment (µmol/kg OC dw). It is noted that the BAF and BSAF were calculated based on worm wet weight concentrations and sediment dry weight concentrations. However, the calculations should be done for both either on dry weight or wet weight basis.

Depuration rate constant (k2) was estimated by authors to be very low 0.006 (S1), 0.008 (S2) and 0.002 (S3) 1/h.

Uptake rate constants (k1) were 0.011 (S1), 0.053 (S2) and 0.074 (S3) kg dw / kg ww/h. Mean BAF of 1.5 to 5.9 whole body tissue wet weight (for lipid content 1.23%). No mortality occurred during test. Number of test organisms increased from initial 10 per replicate to 12-15, due to reproduction, while the mass of individual worms decreased. But it can be a normal process after reproduction and not interpreted as an adverse effect. Dilution of contaminant due to organism growth was not taken into account.

The study is considered as supporting information indicating a clear magnification tendency in Lumbriculus and potential lack of depuration capacity.

Croce et al. (2005) performed a study following US EPA Guideline for Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates (EPA/600/R-99/064). In the study oligochaetes *Lumbriculus variegatus* were exposed for 56 d to lake sediment spiked with technical 4-NP. Sediment spiking was done by first preparing a concentrated spiked sediment by adding, drop by drop, a 1-g/L methanolic technical 4-NP solution to 1 L of sediment while mixing. The sediment was then mixed for 24 h with a rotating metal blade. A known amount of concentrated sediment was then added slowly to 50 L of Monate sediment, which was mixed for two weeks in the daytime until the beginning of the test to achieve a 4-NP nominal concentration of 2 µg/g dw. TOC of sediment was 9.29 %. The test was conducted in 3-L glass vessels containing 1.5 L (1.3 kg) of sediment and 1.5 L of reconstituted freshwater (hardness 106±4 mg/L as CaCO3, alkalinity 1.2±0.1 meq/L, conductivity 318±34 µmS/cm, and pH 8.07±0.42). Overlying water in the test vessels was aerated continuously with a Pasteur pipette and received two automatic volume additions per day. The vessels were held in a temperature-controlled water bath at 23± 1°C on a 16:8-h light:dark photoperiod at about 150 lux. Before worm addition the sediment water system was conditioned in the test vessels for 10 days. At the test start, oligochaetes (originating from a laboratory culture of Department of Biology of the University of Joensuu, Finland, and acclimated to the test laboratory conditions before the test start) corresponding to about 1.5 to 3 g fresh weight (1.1 ±0.1% fresh wt of lipid) were introduced into the test vessels. Three pools of oligochaetes were also collected at the start of the test for chemical analyses. The oligochaetes were not fed during the test. Three replicate vessels were included, which deviates from the EPA/600/R-99/064 guideline where minimum of 5 replicates are recommended for the *L.variegatus* bioaccumulation study. In addition, three kinds of control vessels were prepared: Control 1 vessels, in which worms were exposed to non-spiked sediment; control 2 vessels containing only non-spiked sediment; and control 3 vessels with spiked sediment and without added worms.

Three samples of sediment without spiking and six samples of sediment spiked with 4-NP were collected for chemical analyses on the same day homogenized sediments were placed in the exposure unit (10 d before the start of the exposure). One sample was collected for particle size and total organic carbon analysis. Operation of the exposure system, dissolved oxygen, temperature, and behaviour of oligochaetes were checked daily. Conductivity, pH, total hardness, alkalinity, and total ammonia of the overlying water were measured on the day the exposures started (before adding the organisms), and weekly in the control units and in six randomly selected experimental units with 4-NP spiked sediment.

A bioaccumulation screening assay was performed before the definitive test. In the screening test, an exposure unit with 4-NP spiked sediment was sampled to collect oligochaetes for 4-NP determination after 5 h of exposure, every day during the first exposure week, every 2 d during the second exposure week, and finally on days 21, 28, and 35 of exposure. The control units were sampled at the end of the exposure time (day

35). In the definitive test, for each sampling day (days 7, 14, 28, 35, 44, and 56), concentration of 4-NP was measured in three oligochaete samples collected from three different exposure units; a fourth sample was collected to determine worm lipid content. The control units were sampled only at the end of the experiment (day 56).

Upon sampling, oligochaetes were isolated from the sediment by passing the sediment through a 250-µm mesh sieve and then collecting the contents of each sieve in a glass pan. The oligochaetes were separated from detritus and kept for 6 h in a 1-L beaker containing aerated reconstituted freshwater for purging out gut content according to a standardized procedure. Afterward, the worms were rinsed to remove any remaining debris, weighed after the removal of excess water by a Pasteur pipette, and frozen at -20°C for analysis.

Sediment samples were Soxhlet extracted with methanol and the methanol extracts were purified to alumina columns. Worm samples were mixed with anhydrous sodium sulphate and Soxhlet extracted with hexane: acetone (1:1 v/v). Extracts were dried, recovered with 1 to 2 ml of methanol, and purified onto an activated Florisil column. Both alumina and Florisil columns then were eluted with 15 ml of 10% acetic acid in methanol. The purified extracts were concentrated to 0.5 ml under a gentle stream of nitrogen and filtered through a 0.45-µm Teflon filter before analysis. Overlying and pore water samples were filtered on a glass microfiber filter (0.7 µm nominal pore size), then subjected to solid phase extraction on C-18 (500 mg) cartridges, and recovered with 10 ml of acetone. The acetone was concentrated to 0.5 ml under a gentle stream of nitrogen and filtered through a 0.45-µm Teflon filter before analysis. The 4-NP analytical determinations were performed by high-performance liquid chromatography with fluorescence detection. The mean recovery and the detection limit of the sediment samples analysis were 79 ±24% and 0.095 µg/g dry weight, respectively; the mean recovery and the detection limit of the worm samples determination were 85±15% and 0.03 µg/g fresh weight, respectively; the mean recovery and the detection limit of the aqueous samples determination were 82±9% and 0.33 µg/L.

The total organic carbon content and the total nitrogen content in the lake sediment used in the two tests were 9.29 ± 0.02% and 1.05±0.01%, respectively. The main sediment fraction (59% dry wt) was the silty one (<0.063 mm), whereas 34% (dry wt) of the sediment particles had a size between >0.063 mm and <0.25 mm, and only 7% (dry wt) of the sediment particles had a size >0.25 mm. Mean 4-NP concentrations of the spiked sediment were 1.4 ±0.5 and 1.8±0.3 µg/g dry weight for screening test and definitive test, respectively. No detectable concentration of 4-NP was found in the unspiked sediment. The 4-NP content was also measured in the overlying water during the exposure period and in pore water at the end of the test, but the concentrations were always below the detection limit (<0.33 µg/L).

The dissolved oxygen was always above 2.5 mg/L. All the other variables measured in the overlying water, except ammonia, did not vary significantly during the tests. Total ammonia was not present in the reconstituted freshwater used to renew the overlying water of the experimental units, but it diffused from sediment into the overlying water, attaining concentration for the definitive test, from 4 to 264 µgN/L. It is noted that one of the validity criteria of the US EPA Guideline EPA/600/R-99/064 states that the ammonia concentration should typically not vary by more than 50% during the test. In the study publication it is pointed out that this concentration is well below the 10-d LC50 of 21.4 mgN/L at pH 7.8 of ammonia determined for *L. variegatus* in Schubauer-Berigan et al. (1995). US EPA Guideline EPA/600/R-99/064 refers to the same study stating that 4-day LC50 values for *L. variegatus* in water-column (no sediment) exposures ranged from 6.6 to 390 mg/L total ammonia as pH was increased from 6.3 to 8.6 (Schubauer-Berigan et al., 1995). Hence, even though the ammonia concentration varied significantly more than 50 % during the study, the concentrations remained well below the LC50 values.

No dead organisms were recovered from either the test or the control units. *L. variegatus* exposed to control and test sediments did not appear to avoid the sediments, but oligo-chaetes were observed processing only the top 2 to 3 cm of the sediment. Wet weight of worms recovered from the sediment (both spiked and non-spiked sediments) at the end

of the test was slightly lower than the starting weight of the worms added to the sediment, being 102 ± 5% at the beginning of the exposure and 89±4% at the end of the definitive test. According to the authors, an explanation is that this test species loses weight when transferred from the culture, where it is fed with high organic carbon–content food, to sediment with lower organic carbon content. Lipid concentration of oligochaetes remained quite constant during the whole exposure time, ranging from 0.9 to 1.1% fresh weight for the definitive test.

The 4-NP concentrations in *L. variegatus* before starting the test and in organisms recovered from the control units (control 1) were always below the detection limit. *Lumbriculus variegatus* body concentration increased over the course of the tests (from 0.50 (day 7) to 4.40 (day 56) µg/g fresh wt). A plateau was not reached by day 56 of the exposure to the 4-NP–spiked sediment. Based on the increasing concentrations of 4-NP in the worms during the whole study duration, the elimination of the substance in *L. variegatus* seems to be slow. Biota sediment accumulation factors (BSAF) were calculated as the ratio of the mean concentration of 4- NP associated with the tissue of the oligochaetes recovered from the sediment (normalized to lipid contents) to the mean concentration of 4-NP in sediment (normalized to the total organic carbon content at the start of the exposure). The BSAF values increased during the whole study period (both in the screening and definitive study) reaching a value of 24 g carbon /g lipid at the end of the 56 days definitive study. The increase of BSAF was constant and did not reach a plateau. It is noted that in the study all concentrations of 4-NP in the worms as well as the lipid content of the worms are expressed for fresh weight. However, the concentration of 4-NP in sediment is for dry weight. When calculating BSAFs both concentrations should be either in wet weight or in dry weight. Therefore, the BSAFs calculated in the study are not fully reliable. However, the concentration in the worms based on dry weight would be higher than that based on wet weight, and, hence the BSAF would be higher. Also, if converting the sediment concentration to wet weight, would result in a higher BSAF. In the ring test of OECD TG 315 (Egeler et al. 2006), the worm dry weight ranged from 12.3% to 28% of wet weight for *L. variegatus*.

The study followed quite well the EPA/600/R-99/064 guideline, but there were some deviations. Only three replicates were included while the guideline recommends a minimum of five replicates. Furthermore, the ammonia concentration varied by more than 50% as indicated above.

In additional experiments of Croce et al (2005), laboratory-cultured *L. variegatus* were exposed for 14 d to sediment collected from the Po River (Northern Italy) downstream of its most polluted tributary. At the end of the exposure time, the organisms were collected for 4-NP quantification. Sediment treatment, experimental conditions, and chemical analysis were the same as reported for the main test procedure. Natural sediment collected from the Po River used for *L. variegatus* exposition had a 4-NP concentration of 0.31±0.07 µg/g dry weight and a total organic carbon content of 1.9±0.4%. After 14 d of exposure to this sediment, the level of 4- NP in *L. variegatus* tissue was 1.0 ±0.1 µg/g fresh weight. A BSAF value of 6 g carbon sediment/g lipid worm is reported.

Furthermore, in another experiment included in Croce et al (2005), native oligochaetes and sediment were collected in the same site (Parco Lambro, Milan, Italy) from a highly polluted Northern Italian river, Lambro River, in July 2002. Native oligochaetes and sediments also were collected simultaneously from four sites of the Tevere River in Rome (Central Italy), in January 2003. The Tevere River sediments from the different sites were analyzed separately, and the oligochaetes were pooled together because the number of animals collected in each site was not sufficient for chemical analysis. Organisms and sediment were analyzed with the same procedures reported for the test samples. The concentration of 4-NP in sediment and in field oligochaetes recovered from the Lambro River sediment in the year 2002 were 6.9±0.3 µg/g dry weight and 123.9 µg/g fresh weight, respectively; total organic carbon content of the sediment was 2.3±0.3%. The concentration of 4-NP and the total organic carbon content of the sediment collected from the Tevere River ranged from 0.05 to 0.3 µg/g dry weight and from 0.4 to 2.7%, respectively. The concentration of 4-NP in the pooled oligochaetes recovered from these

sediments was 6.0 µg/g fresh weight. The BSAF values calculated in the study assuming for the field collected individuals the same lipid content as the laboratory oligochaetes ranged from 39 to 55 g carbon/g lipid.

Field studies

Cheng et al. (2006) investigated the occurrence and seasonal variation of 4-NP and 4-t-OP in oysters (*Crassostrea gigas*), snails (*Thais clavigera*), coastal water and coastal sediments of the western coast of southern Taiwan. The organisms were collected from oyster cultural sites of Tai-shi and Chi-ku in August (average water temperature 32°C) and December (average water temperature 22 °C) 2003. The number of oysters collected at each site and each sampling event ranged from 7 to 44 and the number of snails ranged from 39 to 84. Coastal water (from depth of 1 m) and sediment (10 cm surface layer) samples were collected from the same sites in November 2003.

Chemical analyses were performed on gas chromatograph directly connected to ion-trap mass spectrometry. The biota tissue and sediment samples were extracted according to steam distillation extraction technique (Cheng et al., 2005). Briefly, ground tissue sample 0.5 g with 100 ml deionized water (pH 7.0) was added to a 250-ml flask with 0.05 g of NaCl and a TeflonTM coated stir bar. The flask was then placed onto a heating mantle with a magnetic stir plate below the mantle. *n*-Hexane (4 ml) was added to the column as extraction solvent. The extraction was performed for 1 h and the water layer in the distillation column was discarded. The hexane layer was collected and dried by Na_2SO_4. The extract of 4-t-OP and 4-NP residues was then completely evaporated to dryness using a gentle stream of purified nitrogen. For recovery experiment, spiked oyster tissue samples were followed the extraction and GC-MS measurement procedures. Average recoveries were 91 and 92% for 4-t-OP and 4-NP, respectively, with RSD ranging from 7 to 14% (n = 6), indicating good recovery and repeatability of the method. The quantitation limit of this method was less than 20 ng/g in 0.5 g of the sample (dry weight). The water samples were extracted by Oasis HLB solid-phase extraction. The recoveries and reproducibility for the SPE method were determined by spiked coastal water (final concentration 100 ng/l for each standard). Average recoveries ranged from 91 to 97% with RSD ranging from 7 to 10% (n = 6). The limits of quantification (S/N > 10) of this method was 2 and 5 ng/l for 4-t-OP and 4-NP, respectively. It is stated that no 4-t-OP and 4-NP were detected in the blank samples. However, it is not indicated whether these were field blanks or laboratory blanks.

Both alkylphenols (AP), 4-NP and 4-t-OP, were detected in all oyster and snail samples indicating that APs are ubiquitous contaminations in coastal Taiwan. There is no exact information on the concentrations of 4-NP in the organism but approximate values can be obtained from the figures included in the publication. In August, the geometrical mean concentrations of 4-NP in oysters were approx. 1000 ng/g (dry weight) and there was no significant difference between the sampling times. The geometrical mean concentrations of 4-NP in snails differed between the sampling times being above 1000 (up to 1500) ng/g (dry weight) in August and approx. 750 ng/g (dry weight) in December. The mean concentration of 4-NP in the water were 370 and 290 ng/L and in sediment 190 and 130 ng/g dw in the two sites. Based on the average concentrations in oysters, snails, and coastal water during the winter, the bioconcentration factors (BCF) for oyster were estimated to be 4100 and 2900 for 4-t-OP and 4-NP, respectively. For snails, the BCF values for 4-t-OP and 4-NP were estimated to be 2500 and 2200, respectively. BMF values for snails/oysters are also calculated in the study. It is indicated that the snails (*T. clavigera*) are common in the west coast of Taiwan, especially in oyster cultural sites where they feed mainly on oysters. The BMF values calculated for 4-NP on dry weight basis were 1.4 and 2.4 in August for the two sites and 0.7 and 0.8 in December. It is noted that the BCF and BMF values are normally calculated on wet weight basis.

Graca et al. (2021) investigated the sequestration of 4-NP and other phenolic endocrine disrupting compounds in the shells of the mature clam *Rangia cuneata* from the Vistula Lagoon (southern Baltic Sea) and aimed to determine the influence of sex and shell length on bioaccumulation of these contaminants. Individual *R. cuneata* were collected in August

2017, in the area where water depths are ~1.5 m and the bottom is covered with silty sands. The samples were obtained with the use of a bottom dredge and kept for 24 h in aerated water in a lab- oratory to depurate prior to biological analysis. In addition, within a radius of about 50 m of the site from which the clams were collected, 3 sediment cores (surface layer of 0–3 cm) and near-bottom water samples were taken. A total of 96 adult individuals was subjected to chemical analyses. Prior to chemical determinations (EDCs and lipids in soft tissues), the individuals of *R. cuneata* were pooled into groups according to tissue type (soft tissue or shell), sex and size class, then weighed (±0.001 g) and frozen (− 20 °C). The frozen soft tissues and shells were freeze-dried and homogenised. The weight of soft tissues and shells was determined after lyophilisation. The concentration of 4-NP in the extract of sediments, water, soft tissue, and shell samples were analysed using HPLC with fluorescence detection. Validation of the method was conducted based on recommendations of Council Directive 96/23/EC. Phenol derivative standards (10–100 ng/cm^3) were prepared from high purity reagents (4-NP > 97%; SIGMA-ALDRICH®). The linear correlation coefficient was >0.999. The average recovery was: >85% and the precision (coefficient of variation) was <8.0%. The limits of quantification (LOQ) were: 4.0 ng/dm^3 for water and 0.5–1.0 ng/g d.w. for solid samples (soft tissue, shell, sediments). The background values of blank samples were below the limit of quantification. Field-derived bioconcentration factors (BCF) (L/kg d.w.) and the biota-sediment accumulation factors (BSAF) of phenol derivatives were calculated as the decimal logarithm of the ratio between the concentration of the chemical in the soft tissue (ng/kg d.w.) and the mean concentration of the chemical in the water (ng/L) and sediment (ng/kg d.w.) respectively. It is noted that the biota-sediment value calculated in the study is actually a non-normalised BAF and not a BSAF which is usually used to refer to values determined by normalising BAF for the lipid content of the organisms and the sediment total organic carbon content.

The mean concentration of 4-NP in the water samples (n=3) was 25 ±1.3 ng/L and in the sediment samples (n=3) 1.2 ±0.2 ng/g dw. In the soft tissue of *R. cuneata,* the mean concentration of 4-NP was 290±109 ng/g dw (range 172-528 ng/g dw). In the shells the mean concentration of 4-NP was 24±5.4 ng/g dw (range 15-32 ng/g dw). With respect to total body burden (ng/individual), 79±5% of 4-NP was deposited in the shell. Both in the soft tissues and in the shells, the females of *R. cuneata* exhibited 2.4 times higher concentrations of 4-NP than males, corresponding with higher concentrations of lipids in the soft tissue of females. No significant difference in the 4-NP concentrations in the soft tissue were observed between individuals of different shell lengths, while the 4-NP concentration in shells increased approximately linearly with the increasing shell length. Based on concentrations in the soft tissue (on dry weight basis) and water, field-derived bioconcentration factors (BCF) of 4-NP in *R. cuneata* from the Vistula Lagoon were calculated in the study. It is noted that the concentration in soft tissue was in dry weight basis, while the OECD TG 305 recommends calculating the BCF values in fish in wet weight basis. The calculated mean value of log BFC was 4.0 ± 0.1 for 4-NP. In the turbid water of Vistula Lagoon, filter feeding organisms are strongly exposed to resuspended sediment. For this reason, biota-sediment accumulation factors were also calculated in the study and an average log BAF of 2.4_± 0.1 is reported for 4-NP.

Fan et al. (2019) determined the presence of 4-NP in abiotic compartments (water, sediment, suspended particulate matter (SPM)) and fish with different feeding habits along 14 sampling sites along the Pearl River, China, which is known to be polluted by 4-NP and other toxic organic compounds. At each sampling site, water, sediment, SPM, and fish samples (for each, n=6) were collected every month during the rainy (June and July) and dry (November and December) seasons of 2016. The water, sediment, SPM, and fish samples were stored at 4 °C, transported to the laboratory, and processed within 24 h. Sediment samples were lyophilized and homogenized. Fish samples were dissected upon arrival at the laboratory. Fillets of fish were harvested with an acetone-rinsed bistoury. The diet composition of individual fish species and trophic links of food webs were analysed by Wang et al. (2018a). All processed samples were packed in solvent-rinsed glass bottles with Teflon-lined caps and stored at -20 °C before pretreatment and analysis. The concentrations were based on dry weight (dw) and corrected by the results of procedural blanks and the recoveries of isotope-labelled internal standards. BAFs were calculated by

dividing the concentration of 4-NP in the fish muscle, C_{fish}, (ng/g dw) by the concentration of 4-NP measured in water, C_{water} (ng/L).

In the abiotic compartments, 4-NP had concentrations of 61-2996 ng/L in water, 53-12042ng/g in sediment, and 1.3-217 mg/g in SPM. In fish muscle, 4-NP concentrations showed the following trend: brackish carnivores (9097 ng/g) > planktivores (6152 ng/g) > molluscivores (5764 ng/g) > freshwater carnivores (5052 ng/g) > detritivores (2498 ng/g). The average BAFs calculated for 4-NP in the study were in the range of 4423 L/kg for detritivores and 28883 L/ kg for brackish carnivores.

Lv et al. (2019) studied the concentrations of 4-NP and other phenolic EDCs in the river water and different fish tissues (bile, liver, plasma, and muscle) of Pearl River system. Sampling was carried out during July 2012 (wet season) and December 2012 (dry season). A total of 60 water samples (three replicates per site every season) and 74 fish specimens were collected from ten sites in the Pearl River Delta region, including the mainstream of the Dongjiang River and its three tributaries, Shima River, Danshui River, and Xizhijiang River. Fish samples were captured using fishing nets, and included 24 tilapia (*Tilapia aurea*), 6 snakehead (*Ophiocephalus argus*), 7 mud carp (*Cirrhinus molitorella*), 8 common carp (*Cyprinus carpio*), 7 grass carp (*Ctenopharyngodon idellus*), 8 crucian carp (*Carassius auratus*), 7 bream (*Parabramis pekinensis*), and 7 chub (*Hypophthalmichthys molitrix*) samples. The bile, liver, and plasma samples could not be collected for each fish, owing to the complexity of fish structure and difficulty of dissection. Consequently, a total of 73 bile, 64 liver, 44 plasma, and 74 muscle tissue samples could be obtained. A strict QA/QC system was set up to ensure credible results. For every fifteen samples, blank samples and an independent check standard (standard solution, 100mg/L) were used to monitor carryover, background pollution, as well as precision and accuracy of the instrument. Only BPA and 4-NP were detected in trace amounts in the procedural blanks. Furthermore, recoveries were assessed by spiking standards and internal standards into blank samples. Method detection limits (MDLs) and quantification limits (MQLs) were calculated as three and ten times of the signal-to- noise ratio (SNR), respectively. Calculation of the bioaccumulation factor (BAF, L/kg) was based on the 4-NP concentration in biological samples [Cb, ng/g wet weight (ww)] and corresponding water samples (Cw, ng/L), expressed as BAF=1000 * Cb/Cw.

4-NP was found in every water sample, at average concentrations of 52.0-8643 ng/L. 4-NP was also detected in almost all the fish tissue samples. The mean concentrations of 4-NP (ng/g ww) for the different tissues in wet/dry seasons were: 4750/ 5499 in bile, 1417/418 in liver, 3702/3749 in plasma and 46.0/48.8 in muscle. The log BAF values of 4-NP reported for the different tissues are in the range of approx. 2-4.5 (read from a graph included in the publication) and are in the following decreasing order: bile>plasma>liver>muscle.

Salgueiro-Gonzales et al (2015) examined the occurrence, distribution and bioaccumulation of 4-n-NP and NP technical mixture in water, sediment and biota (clam *Corbicula fluminea*) collected from the Miño River estuary (NW Iberian Peninsula). BAF estimated field values of between 2788 and 3649 (L/kg dw) for NP for May and November 2012, respectively. It was considered that seasonal differences could be explained by environmental parameters such as temperature, pH and total organic carbon (TOC) as well as organism's characteristics such as lipid content, health status and gender/life stage, which could affect metabolism of compounds. Biota-sediment accumulation factor (BSAF) were estimated to be 2.9 (dw) for NP in this study.

Georgina el al (2022) has published a weight-to-weight conversion factors. In the case of Bivalvia an average conversion factor of 0.481 has been calculated which will result in BAFs of 1341 to 1755 ww and BSAF of 1.4 ww.

Dielh et al., (2012) collected samples of water, sediment and 3 marine species from the coastal zone at several locations on the North American Pacific of California and Canada. Fish liver analyses of 4-NP were conducted on three species: the amphipod, *Corophium salmonis* Arrow gobies (Clevelandia ios), Staghorn sculpins (*Leptocottus armatus*).

Bioaccumulation from seawater to test organisms was calculated by comparing weight 4-NP concentrations with seawater concentrations. Accumulation from sediment to test organisms was calculated for each species using dry weight 4-NP concentrations. Analysis showed 4-NP was present in biota, water and sediment samples at all stations sampled for this study. The BCFs (BAF) for the Goby, Goby liver and Sanddab/sclupin livers were 564, 4929 and 5104 respectively. The BMF_{lipid} for the Goby and Sculpin liver based on their respective diets were 0.3 and 2.7. Similarly, to determine bio-magnification factor (BMF) between predator and prey species lipid weight 4-NP concentrations were used. However, these tissue BCFs cannot be compared with the Annex XIII criteria. The invertebrate-based BCF (BAF) values were 245, 5667, 1143 and 290 for benthic invertebrates as a group for ghost shrimps, oysters and mussel. BAF values relative to sediments were all <152. Lipid-corrected BMF values for the invertebrates using in this study were 0.1, 0, and 0.2 for the oyster, mussel and ghost shrimp.

Additionally, the average levels of 4-NP in arrow gobies from all sampling sites ranged from 9,000 to 40,100 ng/g lw in liver. Average 4-NP levels were lowest at the least populated site (Bamfield Inlet, Canada), but were not statistically different from gobies from the most 'urbanised' sampling station in San Francisco Bay, California. A factor contributing to this may have been that gobies from California were younger and had a smaller mean size than those from other sampling sites.

Liver analyses of marine seabirds and mammals was conducted as 4-NP may accumulate in lipid-rich tissue. Seabird and marine mammal livers concentrations ranged from 14,000 to 138,000 ng/g ww. Average 4-NP levels were lowest at the least populated site (Bamfield Inlet, Canada), but were not statistically different from gobies from the most 'urbanised' sampling station in San Francisco Bay, California.

The authors used the same calculation method for BCF and BAF, the concentration of NP in the organism/ concentration NP in the seawater. The eMSCA prefers the consideration of BAF as it is likely that the exposure comes mainly from diet more than from aqueous exposure. The BAF or values for seabird liver, Otter liver, Porpoise liver and Sea lion liver were, 621, 8762, 1921 and 1795 respectively. None of the BAF values relative to sediment were greater than 319.

Lipid-corrected BMF values for the seabirds and marine mammals examined in this study were all less than 1, other than for the Otter liver based on a prey item of the oysters or mussels which were 2.2 (otter liver/oyster) and 10.9 (otter liver/mussel), respectively. However, it is observed that these two higher ratios correspond to those relationships in which invertebrates (bivalves) are involved as prey and predators´liver.

Even though there is no direct trophic link between sea otter and bivalves, authors indicate that sea otter diet consists of a variety of invertebrates including sea urchins, bivalves and gastropods, placing them in a different trophic pathway to the other species investigated in this study, which consume mainly fish and cephalopods. Therefore, this study would confirm that consumption of invertebrates as prey would increase the levels of 4-NP in the predator, due to the low metabolic capacity of 4-NP by invertebrates. In addition, the reduced levels of 4-NP in some marine mammals (in comparison to fish) may be due to the higher clearance rate for 4-NP by mammals compared to fish hepatocytes.

Average levels of 4-NP in the invertebrates used in this study from all sampling sites ranged from 103 to 2380 ng/g ww. The invertebrate-based BCF (BAF) values were 245 (for benthic invertebrates as a group), 5667 (for ghost shrimps), 1143 (for oysters) and 290 (for mussels). BAF values relative to sediments were all <152. Lipid-corrected BMF values for the invertebrates using in this study were 0.1, 0, and 0.2 for the oyster, mussel and ghost shrimp.

Nehring et al., 2017 determined the concentration of 4-tert-octylphenol (OP), and 4- NP) in in human hair, the fur of Baltic grey seals and the feathers of herring gulls. The feathers from the 25 dead herring gulls (*Larus argentatus*) were collected from inhabiting Gdynia

harbour, the Vistula estuary, and the fishing port in Władysławo over the period 2010–2012. Fur samples were taken from the 17 Baltic grey fur seal (*Halichoerus grypus*) living at the Marine Station of the Institute of Oceanography, University of Gdansk (Poland). Samples included juveniles and adults. Assays of phenol derivatives were conducted using HPLC with fluorescence detection technique. NP was measured in the fur of the Baltic grey fur seal (*Halichoerus grypus*) (aged 10–29 years) and the feathers of the herring gull (*Larus argentatus*) at mean concentrations (min and max) of 39.1 (5.5-91.3) and 37.7 (4.9 to 151.3) ng/g dry weight respectively. In human hair, the mean OP 131.2 ng g^{-1} dw, NP 4478.4 ng g^{-1} dw, in seal fur OP 62.8 ng g^{-1} dw, NP 39.1 ng g^{-1} dw, and in feathers, OP 162.0 ng g^{-1} dw, NP 37.7 ng g^{-1} dw.

Gatidou et al., 2010 collected mussels from field for analysis of environmental concentration from different sites along the Thermaikos Gulf and Lesvos Island (Greece). Results of mussels collected from the wild showed that 4-n-NP was detected in 39% (7/18) of the samples that were collected. The concentrations ranged from <LOD to 913.7 ng g-1 for 4-n-NP d.w.

Staniszewska et al., 2017 collected *Mytilus trossulus* in 2011 and 2012 to assess factors determining the accumulation of bisphenol A, 4-tert-octylphenol (OP) and 4- NP at a low trophic level in the coastal zone of the Gulf of Gdansk (southern Baltic Sea). BCF and BAF were determined and the relationship between concentrations and age, weight and condition of specimens examined. Mussels (*Mytilus trossulus*) were calculated to have mean BCFs of 12,400, ranging from 100 to 116700, L/Kg based on dry weight tissues. BAFs of 2 was also presented based on consumption of phytoplankton, with a range from 0 to 10.1. These BAFs are considered more representative of BMFs since they are based on food consumption. Seasonality, sex and proximity to sources of NP were shown to influence the concentration of NP found in mussels.

Filipkowska and Lubecki (2016) carried out a monitoring study of the concentration of 4-NP (names not indicated) in sediments and mussels (*Mytilus trossulus*) in the Gulf of Gdansk, in the Southern Baltic. Potential habitats of blue mussels *Mytilus trossulus*, are characterized by organic-poor sandy sediments, good oxygen conditions in the near-bottom water, and low salinity. Samples were collected over three sampling years (2008, 2012 and 2013), the tissues and sediments extracted and analysed for concentrations of NP. 4-NP concentrations in whole mussels ranged from 30 to 111 ng g−1 d.w. (mean 74 ng g−1 d.w.) and the concentration of 4-NPs in sediments were distinctly lower in comparison with mussels and varied from 0.8 to 2.7 ng g^{-1} d.w. (mean 1.9 ng g^{-1} d.w). Being filter feeders, this information would represent a mean BAF of c.a. 40.

Ademollo et al (2016) investigated the concentration 4-NP, its mono- (NP1EO) and di-ethoxylate (NP2EO) precursors in the muscle and liver tissue of the Greenland shark, Somniosus microcephalus. The sharks were caught from different regions in Greenland waters. 4-NP and NP1-2EO were found to be present in the tissues and livers of Greenland shark in 75 and 87.5% of the 23 sharks analysed. The 4-NP and NP1-2EO mean content in liver was 43.5 ng/g and 288.5 ng/g wet wt respectively, while in muscle mean concentrations was 20.3 ng/g for 4-NP, 171.1 ng/g wet wt for NP1-2EO. The authors suggest that the large variation in results is likely due to a complex array of factors such as different feed stuff (opportunistic species), growth rate and the reproduction process which in reproducing females requires use of fat stores and can effectively work as an elimination source.

Casatta et al., (2015) determined NP and octylphenol (OP) in sediment and biota samples; with higher concentrations observed at sites more directly exposed to sources of NP. The biota-sediment accumulation factors (BSAF) were calculated as the ratio of a pollutant lipid-normalised concentration in aquatic organisms to its organic carbon-normalised concentration in sediments. BSAF values for NP were estimated ranging from 0.11 to 0.48 from the different sites and 0.14 for OP (only measured in site A). This was considered to reflect the low trophic levels of bivalves. Manila clams (*Ruditapes philippinarus*) were collected from Sacca di Goro lagoon, Adriatic Sea, Italy. Clams were wild specimens (i.e. from outside areas of clam farming) with commercial size (>25 mm). Age was not

determined, however, shell lengths suggested they were between 1 and 2 years. NP was detected at all 3 sites, at relatively high concentrations (326 - 424 ng/g dw) while OP was detected at lower concentrations 0.75 – 3.8 ng/g dw. Whole sediment concentrations of NP were in the range 4.2 -26.3 ng/g ww.

Ademollo et al., (2017) collected samples of sediment and biota (*Ruditapes philippinarum*, Manila clam) from 3 sites within Venice Lagoon. Clams (3.4 - 3.6 cm shell length) and surface sediments were collected in October 2003, and January, April and July 2004 from 3 sites within Venice Lagoon, Italy chosen for their different pressures. The sites were selected for their different characteristics; mainly domestic, industrial or reference conditions. After extraction, the sediment and clam whole tissue samples were analysed for 4-NP, 4-Nonylphenol mono- (NPEO1) and di- (NPEO2) ethoxylates among other compounds. Lipid content of the clam whole tissue was also determined. Surface sediments were characterised by particle size analysis. BSAF appeared inversely related to site contamination with highest BSAFs found at the 'reference' site (Poveglia). The BSAFs for NPs as a group were estimated from the graph provided in the report. They were between 0.2 and 4.5.

Wang et al., (2016) studied the occurrence and bioaccumulation of a mixture of phenolic EDCs including nonylphenol-di- ethoxylate (NP2EO), nonylphenol-mono-ethoxylate (NP1EO), 4-NP, in water, sediment and fish samples collected in 2014 from Panlong river, Yunnan-Guizhou plateau, China. The average BCF of 4-NP and 4-t-OP (BCF=Cf/Cw) for three species of freshwater fish (carp, crucian carp and silvery minnows) collected from Songhuaba reservoir to Lake Dianchi along Panlong River were calculated at 4-15 and 4-11, respectively. The units of the BCF values are not indicated in the publication. However, the units for the concentrations of the substances in fish and in water are in ng/g and ng/L. If the BCF was calculated using these units, the reported BCFs would be in L/g. When converted to L/kg the BCFs would be 4000-15000 L/kg and 4000-11000 L/kg for 4-NP and 4-t-OP, respectively.

Gu et al., (2016) collected marine biota samples from 8 coastal cities in the Yangtze River Delta area between June and December 2013. Wild samples, including 18 fish species (64 samples), 2 prawn species (23 samples), 3 mollusc species (8 samples) were randomly caught in the East China Sea. Four APs were analyzed (4-t-octylphenol, 4-t-OP (CAS: 140-66-9, branched compound; 4-octylphenol, 4-OP (CAS: 1806-26-4, liner compound;4-nonylphenol, 4-NP (CAS: 84,852-15-3, branched mixed isomers); 4-n-nonylphenol, 4-n-NP (CAS: 104-40-5, liner compound),). 4-NP was the predominant compound with the highest detected concentration of 19,891 ng/g ww. Higher residual concentrations of 4-t-OP), 4-OP, 4-n-NP and BPA were observed in fish species that consumed benthic organisms or demersal fish species, whereas 4-NP showed different results due to trophic dilution. The trophic magnification factors (TMFs) of the linear APs (4-OP and 4-n-NP) (1.22-2.93) were calculated higher than those of the branched ones (4-t-OP and 4-NP) (0.72-0.90), indicating the relative metabolism stability of linear APs. 4-NP has the lowest TMF value of 0.72, and its trophic dilution might be observed because the branched carbon chain exhibits the lowest dispersion force compared to that of the other APs. Concentration of 4-NP from Wild fish samples ranged from 14.5 to 1470 ng/g ww, prawn from 14.6 to 260 ng/g ww and molluscs from 76.1 to 1320 ng/g/ww (whole body measurements).

Lee et al., (2015) collected water and sediment samples at the same time and from the same locations as wild freshwater fish samples. Wild freshwater fish samples were collected downstream from 16 major Taiwanese rivers between 2009 and 2010. Only fish muscle was analysed. Lipid was extracted, but there is no indication of lipid normalization. Concentrations of NP (nonylphenol ethoxylates isomers, not specified) ranged from 1.01 to 277 µg/kg ww in wild freshwater fish muscle. BCFs of 74 to 2.6 x 10^4L/kg were calculated. BSAFs of NP in fish ranged from 0.003 to 18.3. Bioaccumulation of NP in fish was indicated to be very species specific, with considerable variation observed. BCFs of 74 to 26,000 were calculated. However, as only muscle samples were analysed these data are not considered in the B assessment.

Staniszewska et al., (2014a) collected samples of water, phytoplankton, zooplankton and mussels from the coastal zone of the Gulf of Gdansk (Baltic Sea) between 18 to 20 March 2011. Concentrations of 4-tert-octylphenol (OP), and 4- NP were determined in water and fish. For NP, BCFs for herring, flounder and cod at average (maximum) of 600 (900), 200 (400), 200 (400), respectively. Similarly for OP, BCFs for herring, flounder and cod at average (maximum) of 500 (1300), 1100 (2100), 100 (400), respectively.

A BAF was also provided for the fish, based on consumption of prey items. It is not clear how this was calculated, however it appears more equivalent to a BMF. These were on average less than 1 other than for the Cod preying on herring, with a maximum of 2. Use of dry weights for expression of the BCF is not considered a standard unit and will lead to an overestimation of the BCF.

Additionally, concentrations of NP were determined in water and mussel (*Mytilus trossulus*). The BCF for mussels based on 10 samples was determined at an average as 1300 L/Kg, with a maximum of 5,550 L/Kg, for NP and 1800 L/Kg, with a maximum of 6,400 L/Kg based on dry weight tissues. Use of dry weights for expression of the BCF is not considered a standard unit and will lead to an overestimation of the BCF. Criteria for assessing bioaccumulation (REACH) are based on wet weights and are therefore not applicable.

BAFs was also provided for mussels for OP and NP, based on consumption of phytoplankton. It is not clear how this was calculated, however it appears more equivalent to a BMF. These were determined at <1 on average. No numerical figure is provided.

These authors also considered, the role of guano in the removal of NP. NP concentrations in gull guano were 3.1 times greater than in herring suggesting that gulls are able to eliminate NP via this route. However, only the parent compound is mentioned and measured, and therefore uncertainties on the potential metabolization is raised. NP was detected in all gull specimens. In muscles and livers of gulls NP was detected in the range 5.0-76.6 ng/g dw. Concentrations of NP in gull guano were in the range 7.3-299 ng/g dw. Consumption of food containing NP was considered to account for the statistically significant relationship between NP concentrations in gull livers and their age ($r = 0.6$, $p<0.05$). BMF tissue values based on a range of herring gull diets were determined, with the averages for each diet ranging from 1 to 4. Maximum BMF of 15 was determined for the herring gull consuming a diet of herring liver. This study is considered not assignable for B assessment of NP.

Hu et al (2005) conducted the food web characterisation, trophic level calculation, trophic magnification and biomagnification factors of an aquatic food web. Aquatic food web components were collected in May, June and September 2002 from the Bohai Bay, North China. Phytoplankton, zooplankton, 5 invertebrate species, 6 fish species and *Larus argentatus* (herring gull). After preparation and extraction samples were analysed for NPEOs and 4-NP, (4 <s < 16), in 14 marine species including plankton, benthic invertebrates, fish, and marine birds.

4-NP was detected in all samples, and the concentration ranged from 142 to 678 ng g-1 lipid. The 4-NP concentration in fish was lowest in catfish (151 ng/g lipid) and highest in mullet and wolffish (>300 ng g-1 lipid). The 4-NP concentration in herring gull was 240 ng g-1 lipid. The total NPEOs concentration ranged from 183.9 ng/g lipid in short-necked clam to 678.2 ng/g lipid in catfish, which is similar with the 4-NP concentration in the same biota samples. Based on herring gull/fish (single prey diets) BMFs for 4 -NP ranged from 0.57 to 1.51 and 0.20 to 2.20 for NPEOs. Lipid equivalent concentrations of 4-NP and of all NPEOs did not exhibit a statistically significant correlation with trophic levels in the food web, and the TMF of NP was 0.83, which was similar to those of all NPEOs, mean of 0.80 (0.45-1.22).

Gatidou et al., 2010 analysed mussels (*Mytillus galloprovincialis*) (shell length 40–55 mm, wet tissue mass 1.22 ± 0.58 g) collected from different clean sites along the Thermaikos

Gulf and Lesvos Island, Greece. 4-n-NP) was detected in 39% (7/18) of the samples that were collected. The concentrations ranged from <LOD to 913.7 ng g-1 for 4-n-NP d.w. The geometric means of the concentrations that were found for all of the bivalves were 158 ng g-1 for 4-n-NP dw.

An accumulation study performed under exposure of 28 days at 300 ng/L. Mediterranean mussels were collected from clean sites and the levels of 4-n-NP determined before starting the experiments. The mussels were placed into an aerated 75 L tank, that contained unfiltered seawater, and they were acclimated for 7 days under laboratory conditions (20 ± 1 _C and 34 ± 2% salinity). After the acclimation process. Tank A was used as a control operating in the absence of EDCs, whereas tanks B, C and D contained 300 ng/L of 4-n-NP. The duration of the experiment was 28 days. During this period, both water and compound were renewed every 24 h and the water was gently and continuously aerated. Two mussels and 100 mL of seawater were regularly sampled from each tank (0, 7, 14, 21 and 28 days). After finishing the accumulation tests, the remaining organisms (n = 15) from the four tanks were caged and placed in clean seawater during other 28 days to investigate the depuration of the compound. During the uptake phase the tissue concentrations was constantly increased. Steady state was not observed up to the end of the experiment. Kinetic bioconcentration factors of 1.7 was calculated for 4-n-NP. The half-life of 17 days was estimated, which would reflect very low depuration capacity.

Diao et al (2017) collected water, sediment and biota samples from 4 locations within the Pearl River estuary system in China. Biota samples: *Mugil cephalus* (flathead grey mullet), *Parabramis pekinensis* (white amur bream), *Penaeus chinensis* (Chinese white shrimp) and *Corbicula fluminea* (Asian clam) were collected from the same stations. BCF and BSAF of 4-NP (a mixture of different isomers) and 4-tert-octylphenol (OP) are calculated based on tissue concentrations and environmental field measured concentrations.

Calculated BCFs for NP ranged from 25.7-250 for mugil, 33.6-124 for bream, 10.9-258 for shrimp and 40.9-338 for clams. Calculated BSAFs ranged from 1.85-7.72 for mugil, 1.63-7.31 for bream, 1.75-7.96 for shrimp and 6.60- 30.2 for clams. In the case of mugil and bream only edible fraction is considered and therefore only data from shrimps and clams (whole body) are used for the assessment.

The 4-NP and 4-tert-octylphenol concentrations in water ranged from 233 - 3352.86 ng/L and 1.20 – 3.99 ng/L, respectively and in sediments ranged from 7.55–20.80 ng/g dw and <0.17 (LOQ) ng/g dw. The concentrations of 4-NP were higher than those of 4-tert-octylphenol and bisphenol A in the water, sediment and organisms. The BCFs of 4-NP ranged from 10.88 to 257.99 in P. chinensis; 40.93 to 338.31 in *C. fluminea*; 25.71 to 250.23 in *M. cephalus*; and 33.63 to 123.96 in *P. pekinensis*. As these organisms are filtrate feedings these BCFs could be assimilated to BAFs.

Liu et al., (2017) collected water, sediment and biota samples from Luomo Lake region (China). BAFs of NP (and OP) have been estimated. Grass carp and *Lateolabrax japonicus* (Japanese seabream) were obtained directly from local fishermen. NP was found in all water, sediment and biota samples analysed during this study. Bioaccumulation factor (BAF) in both fish species were calculated 278 and 325 for two different species of fish, the Grass carp and Silvery minnow, based on dry weight tissues. Similarly, BAFs for OP were calculated 100 and 179 for the same fish species. No lipid content is provided. Use of dry weights for expression of the BCF is not considered a standard unit and will lead to an overestimation of the BCF. Criteria for assessing bioaccumulation (REACH) are based on wet weights and are therefore not applicable.

Casatta et al., (2016) calculated biota-sediment accumulation factors (BSAFs for clams to be between 0.11 to 0.49. Experimental sites were located in 6 lagoons of the Po River Delta (Italy), chosen to represent potentially different transitional ecosystems, receiving direct freshwater inflows from the Po River. In each lagoon juvenile clams (*Ruditapes philippinarum*) from a common batch were seeded on 12 and 13 March 2014. After 3 months of environmental exposure aprox. 100 clams were sampled at each site. After extraction, samples were analysed for alkylphenols (OP octylphenol, NP1EO nonylphenol

mono-ethoxylate, NP NP). Sediment samples were collected from the same sites. The following Biota-sediment accumulation factors (BSAFs) for clams were calculated to be between 0.11 to 0.49 for NP, 0.15 to 0.51 for OP and 0.11 to 0.65 for NP 1EO. Lipid content 5 - 7.6 %.

Arditsoglou and Voutsa (2012) collected water, sediment and biota samples (mussels of the genus *Mytilus galloprovincialis*, 4.5 - 5.5 cm long.) from a number of marine sites along the coast of Thermaikos Gulf, Northern Aegean, Greece. After extraction, samples were analysed by GC-MS for nonylphenol, octylphenol, their mono- and diethoxylate oligomers). Due to mussels are filtering organisms, BCF values for nonylphenol in this study could be assimilated to BAF. The BCF values for NP ranged from 284 to 825 l/g dw. For tOP, values up to 1250 l/g dw were calculated.

Additionally, low or moderate bioconcentration factors for NP in mussels, which ranged from 14 to 3400 l/g, have been previously reported (US EPA, 1996). Pojana et al. (2007) also reported BCF values for NP from 3200 up to 5800 (mean 4400) l/g based on fresh weight.

Granek et al (2016) studied the uptake of nonylphenol among a large number of other chemicals into the tissue of the Olympia oyster (*Ostrea lurida*) on the coast of Oregon, at two locations, Netarts Bay and Coos Bay. Oysters were sampled and the tissues extracted and analysed for four alkylphenols (4-nonylphenol, 4-n-octylphenol, 4-nonylphenol monoethoxylate, and 4-nonylphenol diethoxylate). Alkylphenols were detected in the tissue of the Olympia oyster (*Ostrea lurida*) collected from two locations on the Oregon coast, Netarts Bay and Coos Bay. The Olympia oysters had shell lengths between 30–46 mm and a total wet weight of 4–14 g (shell + tissue). The concentrations of NP were 20-31.6 µg/kg wet weight, 4-n-Octylphenol were 1.38-2.35 µg/kg wet weight, Nonylphenolmonoethoxylate were 2.22-2.50 and Nonylphenoldiethoxylate were 0.5 – 0.9 µg/kg wet weight. Concentrations of APs were not determined in the sediment. The concentrations were similar from both locations. No BFCs were estimated to be considered in this B assessment.

Weng-Lin et al (2014) found a positive correlation between APs (4-tert-octylphenol (OP), technical mixture of nonylphenol (NP), Nonylphenol monoethoxylate (NP1EO, mixture of branched isomers), nonylphenol diethoxylate (NP2EO, mixture of branched isomers), nonylphenoxy acetic acid (NP1EC, mixture of ring/chain isomers), 4-n-NP-13C6, 4-n-nonylphenoxyethoxy acetic acid, 4-n-NP1EO-13C6, and 4-n-NP2EO-13C6) concentration in fish (*Oreochromis niloticus*, Nile tilapia) tissue and water and sediment samples collected from 8 sites Kee-Lung River (a tributary of Dan-Shui River) and from the main Dan-Shui River, northern Taiwan. The mean BAFs were measured in eggs of tilapia at 20,971±25,175 for NP, 2,873±2,062 for NP1EO and 2,167±3,293 for NP2EO.

NIVA (2012) investigated the concentrations of a large number of contaminants in the tissues of Crab, Blue mussel, Cod liver and trout from Western Norway and Polar cod, Capelin, Blood plasma, Seal blubber from Arctic locations. The study included the analysis of NP (nNP, straight chained isomer and tNP, branched chain mixture), nonylphenol monoethoxylate (NP1EO), octylphenol (4nOP straight chained isomer and tOP, branched chain mixture), octylphenol monoethoxylate (OP1EO) and a range of other contaminants in the marine and freshwater aquatic environments collected in Norway and in a range of Arctic biota. Marine samples, Blue mussel (3-5 cm), Cod (41 to 81 cm and 720 to 4750 grams) and Brown crab (c.a. 20g soft tissue) were collected from different fjords. Fresh water samples, surface sediment samples and trout (Salmo trutta) (c.a. 70 cm) were collected from Lake Mjøsa and Lake Spjeldsjøen (reference lake). Arctic sampling included samples of glaucous gull, kittiwake, bearded seal, ringed seal, eider duck eggs and common guilemot eggs.

tNP was detected at much higher concentrations with a median concentration of 74 ng/g, and a maximum concentration of 383 ng/g in one sample. NP1EO was also detected in the majority of samples with a median concentration of 21.3 ng/g (<7-121 ng/g). OP oligomers

were detected less frequently and at lower concentrations. No compounds were detected in blue mussels.

4-n-NP was detected most frequently of the nonylphenol substances, with the highest concentrations in the cod liver samples, ranging from 1.5 to 114ng/g wet weight. Lower concentrations were detected in crab samples, <2 to 6.6 ng/g wet weight. NP was not detected in Blue mussel tissue from similar areas to the crab and cod samples. The samples of whole polar cod and capelin, seal blubber, kittiwake and glaucous gull blood/plasma, and eider and common guillemot eggs collected from the Arctic contained levels of nNP, tNP and NP1EO below the limits of detection for each analyte. The most commonly detected chemical of the nonylphenols was tNP in both the water and sediments, with low concentrations of 4 -n-NP and NP1EO.

3 Integration & Weighing of evidence (WoE analysis)

See section 12.4 of the main part of the document.

4. Conclusions

See section 12.4.4 of the main part of the document.