SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Nonylphenol, branched, ethoxylated

EC No 500-209-1

CAS No 68412-54-4

During the evaluation ECHA reviewed the identifiers and proposed the following were more appropriate however, at the time of writing these changes have not been made on the ECHA systems.

4-Nonylphenol, branched, ethoxylated
EC No 500-315-8
CAS No 127087-87-0

Evaluating Member State(s): United Kingdom

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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2016

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

Further information on registered substances here:

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

DISCLAIMER

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

Foreword

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

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

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

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

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

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Nonylphenol, branched, ethoxylated (NPEO) was originally selected for substance evaluation in order to clarify concerns about:

- environmental endocrine disruption.

During the evaluation another concern was identified. The additional concern was:

- invertebrate ecotoxicity.

The purpose of this Substance Evaluation (SEv) was to check the reliability of the toxic equivalence factor (TEF) approach used in the registration dossiers to derive a Predicted No Effect Concentration for surface waters ($PNEC_{water}$), including whether it takes account of endocrine effects and needs to be extended to other constituents of the registered substance and/or their breakdown products.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

NPEO is partially restricted under the REACH Regulation (Annex XVII entry 46) following pre-REACH regulatory activity which identified unacceptable environmental risks for some uses arising from a transformation product (4-nonylphenol, branched).

Under REACH, Risk Management Option Analyses were performed separately by Germany and Sweden, resulting in the identification of NPEO as a Substance of Very High Concern (SVHC) because it can degrade to 4-nonylphenol, branched (which had already been identified as an SVHC due to environmental endocrine disruption). NPEO was subsequently added to Annex XIV of the REACH Regulation with a sunset date of 4 January 2021 and latest date for application for authorisation of 4 July 2019. In parallel, a proposal to restrict NPEO in textiles has been adopted by the European Commission (updating Annex XVII entry 46, with practical effect from 3 February 2021).

3. CONCLUSION OF SUBSTANCE EVALUATION

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

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	✓
Harmonised Classification and Labelling	✓
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

NPEO does not currently have a harmonized classification in Annex VI of the CLP Regulation (EC) 1272/2008.

Whilst the self-classification for aquatic hazard proposed by the Registrants is appropriate for the registered substance, a range of polymeric substances may be supplied using the same CAS number, with various compositions. Toxicity data are available for both the whole substance and in some cases for one or both of the main constituent groups present in the registered substance. It might therefore be better to classify the main constituents of the registered substance (NP1EO and NP2EO) separately, and then classify supplied NPEO substances as mixtures. This would ensure that all classifications are based on the same basic data set.

The evaluating Member State Competent Authority (eMSCA) understands that another EU Member State Competent Authority is currently preparing a CLH dossier considering aquatic toxicity for submission to ECHA (personal communication). No further action is therefore planned by the eMSCA.

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

As noted in section 2, NPEO is already listed on the Candidate List and Annex XIV because it can transform to relevant amounts of an endocrine disrputing substance in the environment.

The potential for constituents of the registered substance to cause endocrine disruption themselves in aquatic organisms has not been clarified. A Fish Sexual Development Test according to OECD TG 234 could be performed. The results could affect the SVHC entry and subsequently the derivation of a Predicted No Effect Concentration for the purposes of authorisation under the REACH Regulation (which currently only has to be based on data for 4-nonylphenol, branched). However, given the high cost in terms of resources and vertebrate animal lives, the eMSCA decided that it would be disproportionate to request such a test from the Registrants subject to this SEv: emissions are site-limited, and both Registrants have given written commitment to cease manufacture and use of this substance by 31 December 2020 and will therefore not apply for authorisation. If new uses or registrations occur in future, the substance may need to be put on the CoRAP again to clarify the endocrine disruption concern.

4.1.3. Restriction

Restriction is not appropriate for the use identified in the registration dossiers, which concerns a very small number of specific sites. Further restriction activity for polymeric NPEO (e.g. in imported articles or used as a chemical intermediate) may be relevant in future, but these substances were not subject to the assessment made under SEv. As NPEO is now listed on Annex XIV, the need for additional restrictions could wait until the outcome of authorisation is known.

4.1.4. Other EU-wide regulatory risk management measures

The transformation product 4-nonylphenol is a Priority Hazardous Substance under the Water Framework Directive, but NPEO is not listed. Environment Agency (2007)

concluded that derivation of an Environmental Quality Standard for NPEO was unlikely to provide any significant additional benefit.

It is possible that conditions of future authorisations might be set to involve a requirement for environmental monitoring in some cases, which could provide useful information about local releases from specific processes.

Short-chain nonylphenol ether carboxylates (NPEC) are additional transformation products of NPEO. These have only been considered briefly in this report, but given signs that they might also have endocrine disrupting properties, further evaluation could be considered for nonylphenol mono-ether carboxylate since it has been registered under REACH.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

5.2. Other actions

Emissions from the manufacturing site are very low and unlikely to be of concern.

The local regulatory authority for the user site may wish to review the conditions of any permit to ensure that best available techniques are being applied to minimise environmental emissions until such time as use ceases. They could also consider a survey to evaluate whether fish in the local receiving environment are showing signs of endocrine disruption (this could involve non-lethal sampling of the fish epidermal mucus with a swab followed by analysis using a vitellogenin enzyme-linked immunosorbent assay (ELISA)).

SEv does not apply to the polymeric forms of NPEO, since there is no registration requirement. A trade body representing the interests of the NPEO suppliers, or a national authority, could consider the voluntary performance of a Fish Sexual Development Test according to OECD TG 234 for NP1EO to clarify whether this type of substance has endocrine disrupting properties in its own right. This may be useful for jurisdictions outside the EU, and could also lead to an update of the Candidate List entry (which might affect authorisation applications). It might also trigger a reconsideration of the prioritisation of NP1-2EO under the Water Framework Directive. Additional *in vitro* studies according to modern standard protocols could also be conducted voluntarily to confirm or refute the published findings.

Several recommendations to improve the quality of the registration dossiers have been made in Part B of this report.

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

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

Table 3

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
CLP Annex VI dossier for harmonised classification	Unknown	To be confirmed

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Nonylphenol, branched, ethoxylated (NPEO) was originally selected for substance evaluation in order to clarify concerns about:

- environmental endocrine disruption.

During the evaluation another concern was identified. The additional concern was:

- invertebrate ecotoxicity.

Table 4

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Physico-chemical properties relevant for interpretation of ecotoxicity data	Micelle formation and data for individual constituents could be considered in more detail, but this is unlikely to affect these specific registration dossiers.
Environmental degradation	Substance can be considered inherently biodegradable, but there is some evidence to suggest that the sediment half-life could be above 180 days at environmentally relevant temperatures.
Bioaccumulation	Insufficient data available - the fish BCF may be above 500 L/kg but will be less than that for 4-nonylphenol, branched.
Ecotoxicity, including endocrine disruption	C&L process to be initiated. TEF unreliable. Further investigation of endocrine disrupting properties could take place voluntarily.
Risk characterisation ratios reported by the Registrants	Impact of emissions in terms of environmental endocrine disruption could be considered at site level.

7.2. Procedure

The eMSCA held an introductory meeting with the Registrants by teleconference in June 2016. A copy of the draft SEv Report was shared with them for discussions about the need for *in vivo* fish toxicity data at the ECHA Endocrine Disrupter Expert Group Meeting in September 2016. A follow-up teleconference was held in October 2016, when the possibility of a request for mysid shrimp data was raised by the eMSCA for the first time. A further teleconference was held on 9 February 2017 to provide a progress update prior to submission of the draft decision to ECHA in March 2017. The Registrants responded to the draft decision in July 2017, and the eMSCA terminated the process in February 2018.

The Community Rolling Action Plan (CoRAP) justification document identified the initial concern as the potential for environmental endocrine disruption. Although NPEO has already been identified as a Substance of Very High Concern (SVHC) on the basis of environmental endocrine disruption, this is related to its transformation to a degradant that has these properties (4-nonylphenol, branched). The purpose of this Substance Evaluation was to check the reliability of the toxic equivalence factor (TEF) approach used in the registration dossiers to derive a Predicted No Effect Concentration for surface waters ($PNEC_{water}$), including whether it takes account of endocrine effects and needs to be extended to other constituents of the registered substance and/or their breakdown products. This could help in the evaluation of applications for authorisation of the registered substance, as well as potential restriction proposals for NPEO in future (e.g. due to any parts of the product life cycle that are not subject to authorisation). In addition, if endocrine disrupting properties of equivalent concern were confirmed for NPEO itself, the Candidate List entry may need to be updated to ensure that these properties are taken into account by applicants for authorisation.

This evaluation was therefore targeted to a review of existing information on environmental fate, behaviour and ecotoxicity relevant for characterisation of aquatic hazards. Registration data were taken from the ECHA dissemination site (http://echa.europa.eu/) unless indicated otherwise. The SVHC Background Document agreed by the ECHA Member State Committee in 2013 (ECHA, 2013) is quoted extensively. The reliability markings of studies included in that report have not been checked for the purposes of this evaluation. The ECHA Risk Assessment Committee opinion about the proposed restriction of NPEO in textiles (ECHA, 2014) is also cited in places. The evaluation also takes account of information in older regulatory reports from Canada (Servos, 1999; Servos et al., 2000), the UK (Whitehouse, 2002) and the Netherlands (Vlaardingen et al., 2003).

Coady et al. (2010) performed a literature search for NP1-2EO ecotoxicity data from 1997 up to mid-2009. ECHA (2013) does not include a literature search date, but the evaluating Member State Competent Authority (eMSCA) assumes that the German Competent Authority performed a search up until 2011 at least. On this basis, the eMSCA has performed a literature search in Scopus, EBSCO, Wiley Online Library and Science Direct for the period 2011 – July 2016, using the following search terms:

> (((nonylphenol OR alkylphenol) AND (ethoxylate OR ethoxylated)) OR Nonylphenoxydiglycol OR Nonylphenoxypolyethoxyethanol OR ("Poly oxy-1 2-ethanediyl alpha-nonylphenyl-omega-hydroxy") OR ("Polyethylene glycol mono branched nonylphenyl ether") OR "Polyoxyethylene branched-C9-alkylphenol" OR ("CAS registry number" AND ("68412-54-4" OR "85005-55-6" OR "155679-84-8" OR "158054-24-1" OR "501935-85-9" OR "158054-25-2" OR "155679-85-9" OR "26027-38-3" OR "127087-87-0"))) AND (aquatic OR ecotox* OR endocrine* OR reproduc* OR develop* OR vitellogen* OR estrogen* OR oestrogen* OR "sex ratio" OR chronic OR NOEC OR disorder OR disrupt* OR imposex OR intersex OR mimic OR modulat* OR ovotest* OR steroidogen* OR "testis-ova" OR xeno* OR "gonado-somatic" OR assay OR "ER-CALUX" OR "MCF-7" OR receptor OR binding OR reporter OR gene OR YAS OR Yeast OR Hepatocyte OR YES OR "Yeast estrogen screen") AND (Fish OR "Fathead minnow" OR "Pimephales promelas" OR Bluegill OR "Lepomis macrochirus" OR Guppy OR "Poecilia reticulata" OR swordtail OR "Xiphophorus helleri" OR Mosquitofish OR "Gambusia holbrooki" OR "Rainbow

trout" OR "Oncorhynchus mykiss" OR "Sheepshead minnow" OR "Cyprinidon variegatus" OR Zebrafish OR "Danio rerio" OR Medaka OR "Japanese ricefish" OR "Oryzias latipes" OR "Chinese rare minnow" OR "Gobiocypris rarus" OR goldfish OR carp OR "Carassius auratus" OR Cyprinus OR stickleback OR amphibian*) AND >2010

A search by the eMSCA of grey literature via the OECD eChemPortal (http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en) in July 2016 failed to identify data for relevant constituents of the registered substance.

The initial review was performed by the eMSCA during July 2016.

7.3. Identity of the substance

The following information was provided on the ECHA dissemination web site at the time the SEv began.

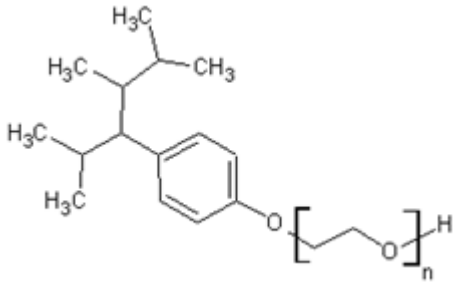
Table 5

SUBSTANCE IDENTITY	
Public name:	Nonylphenol, branched, ethoxylated
EC number:	500-209-1*
CAS number:	68412-54-4*
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	UVCB
Molecular weight range:	UVCB
Synonyms:	Trade names mentioned on the ECHA dissemination site include: (C9) Branched alkylphenol, ethoxylate Branched-nonylphenol, ethoxylate Nonylphenol, branched, ethoxylated Nonylphenoxydiglycol Nonylphenoxypolyethoxy-ethanol (branched ethoxylated nonylphenol) Poly(oxy-1,2-ethanediyl), alpha-(nonylphenyl)-omega-hydroxy-, branched Polyethylene glycol mono(branched nonylphenyl) ether Polyoxyethylene branched-C9-alkylphenol

*As noted below, during the initial evaluation year ECHA reviewed the identifiers used and concluded they should be changed to EC 500-315-8 (CAS 127087-87-0). The ECHA systems do not yet reflect these changes (February 2018).

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

Structural formula (example):



n = 1 - 2.5 (on average)

UVCB substance

The registered substance is one of Unknown or Variable composition, Complex reaction products or Biological materials (UVCB). It primarily comprises (4-)nonylphenol mono- and di-ethoxylates (NP1EO and NP2EO) and the alkyl chain has multiple branching patterns. Table 6 summarises information provided from the registrations on the ECHA dissemination site (accessed on 11 July 2016).

Table 6

COMPOSITION Constituents	Typical concentration	Concentration range	Abbreviation
2-(Isononylphenoxy)ethanol (CAS no. 85005-55-6)	Confidential	Confidential	NP1EO
2-{2-[4-(2,4,5-Trimethylhexan-3-yl)phenoxy]ethoxy}ethanol (CAS no. 155679-84-8)	Confidential	Confidential	NP2EO
2-(2-{2-[4-(2,4,5-Trimethylhexan-3-yl)phenoxy]ethoxy}ethoxy)ethanol (CAS no. 158054-24-1)	Confidential	Confidential	NP3EO
2-[2-(2-{2-[4-(2,4,5-Trimethylhexan-3-yl)phenoxy]ethoxy}ethoxy)ethoxy]ethanol (CAS no. 501935-85-9)	Confidential	Confidential	NP4EO
14-[4-(2,4,5-Trimethylhexan-3-yl)phenoxy]-3,6,9,12-tetraoxatetradecan-1-ol (CAS no. 158054-25-2)	Confidential	Confidential	NP5EO
17-[4-(2,4,5-Trimethylhexan-3-yl)phenoxy]-3,6,9,12,15-pentaoxaheptadecan-1-ol (CAS no. 155679-85-9)	Confidential	Confidential	NP6EO

Note: The order of the constituents is as presented on the ECHA dissemination site.

The amounts of longer chain NPEO decline as the ethoxylate chain length increases. As an illustration, the NPEO composition of a test substance for a study cited by Coady et al. (2010) was: 41.5 % NP1EO, 37.3 % NP2EO, 11.1 % NP3EO and 3.8 % NP4EO (i.e. NP1-2EO were present at around 80 % w/w). A variety of different test substances have been used for various end points in the registration dossier.

No ecotoxicologically relevant impurities (e.g. 4-nonylphenol or dinonylphenol) are present in the registered substance according to the ECHA dissemination site.

N.B. The information included in the registration dossiers indicates that despite the generic substance name, the alkyl group is at the 4- (para-) position of the phenol ring. A more appropriate identifier for this substance may therefore be:

- 4-Nonylphenol, branched, ethoxylated, EC no.: 500-315-8, CAS no.: 127087-87-0

This substance had not been registered at the time the SEv was started, but was included on the CLP Inventory. ECHA looked into this substance identity issue and is in the process of changing the identifiers. However, the eMSCA believes that no further action is required for the purposes of this SEv.

Additional potentially relevant registered substances included on the ECHA dissemination pages are:

- 4-Nonylphenol, ethoxylated, EC no.: 500-045-0, CAS no.: 26027-38-3.
- Nonylphenol, ethoxylated, EC no.: 500-024-6, CAS no.: 9016-45-9.

Based on the reported physico-chemical data, the eMSCA believes that they may have longer chain length constituents than the substance subject to this specific SEv.

Analogues

ECHA (2013) uses supporting information from 4-*tert*-octylphenol ethoxylates (OPEO) for some end points. The REACH Registrants use supporting information from 4-nonylphenol, branched (4-NP) for some ecotoxicity end points.

7.4. Physico-chemical properties

The following information is provided in the registration dossier on the ECHA dissemination web site (only data relevant for the environmental assessment are presented). The data are all considered to be reliable without restriction by the Registrants, and unless otherwise stated refer to the substance described in Section 7.3.

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Viscous liquid
Vapour pressure	0.043, 0.066 and 0.48 Pa at 20, 25 and 50 °C, respectively (OECD TG 104, vapour pressure balance, effusion method)
Surface tension	<55.92 mN/m at 0.34 mg/L and 20 °C (OECD TG 115)
Water solubility	ca. 4.55 mg/L at 20 °C (critical micelle concentration) – determined as part of the surface tension measurement NP2EO (purity not stated): 3.38 ± 0.12 mg/L (11.0 µmol/L) at 20.5 °C, with no significant differences in the range 2-25 °C (OECD TG 105, column elution method); the robust study summary (RSS) suggests that this may be an under-estimate since it does not match the calculated solubility value of 14.0 µmol/L (4.3 mg/L, calculation method unknown) The same RSS also mentions the water solubility of a commercial mixture containing NP1EO - NP3EO (Imbetin N/7A)[2] as follows: 10.8 µmol/L (slow stirring method) 10.9 µmol/L (generator column technique) 12.2 µmol/L (calculation, method not stated)
Partition coefficient n-octanol/water (log K_{OW})	5.39 estimated with KOWWIN v 1.67 using EPI Suite v4; the log K_{OW} was estimated for each of the main constituents, then a weighted average derived. Branched structures gave lower values than linear ones, so were not taken into account[3] [The eMSCA notes that the data are not provided in a QSAR Prediction Reporting Format (QPRF) and there is no discussion of model applicability or reliability for this type of structure]
Dissociation constant	Not measurable due to low water solubility and the UV/VIS absorption properties of the substance (OECD TG 112)

Discussion

A substance is considered surface active if aqueous solutions have a surface tension lower than 60 mN/m under the conditions of the OECD TG 115. Therefore the registered substance is surface active, which affects the measurement of its physico-chemical properties in water (as well as predictions of other properties based on octanol-water partitioning). For example, a reliable log K_{OW} can not be determined using the shake flask

[2] The registration dossier does not provide any detailed purity data for this substance. However, a study by Ahel et al. (1994b) (see Section 7.7.1.4) gives the composition of Imbetin N/7A as: 75% NP1EO, 20% NP2EO and 5% NP3EO.

[3] The registration dossier on the ECHA dissemination web site also includes a supporting study on "NPEO" (no further composition data provided), rated reliable with restrictions, which reported a log K_{OW} of 4.21 ± 0.18 at 20.5°C using the shake flask method. This is not an appropriate method for surface active substances, so this result is not reliable in the view of the eMSCA.

or high performance liquid chromatography (HPLC) methods. The eMSCA considers that the Registrants have taken this into account appropriately, and no further data are required for this end point given the focus of this evaluation.

Nevertheless, the substance is composed of several constituent groups, each of which may have different partitioning and other properties. The eMSCA therefore believes that it is not appropriate to derive "average" properties for the substance for use in hazard and risk assessment. For example, the critical micelle concentration (CMC) applies to the mixture, but does not provide any information about how solubility varies with ethoxylate chain length (i.e. some constituents may be more or less soluble than this value suggests). This is important when considering aquatic test data, since the exposure concentration should be kept below the CMC of the specific test substance (e.g. NP1EO). Measured properties for each of the main NPEO constituent groups would provide a more realistic input for exposure and other modelling. The alternative would be to use the average value but perform sensitivity analyses to reflect the range of possible property combinations.

For the purposes of this evaluation, physico-chemical properties have been predicted by the eMSCA for the main constituents of the registered substance using EPI-Suite v4.10, with ranges reflecting differences between a linear and highly branched nonyl- chain structure (the substances fall within the model domains):

Property (with method)		**Constituent**			
		NP1EO	**NP2EO**	**NP3EO**	**NP4EO**
Molecular weight (g/mole)		264.41	308.47	352.52	396.57
Water solubility at 25 °C (mg/L)	WSKOW v1.41	1.1 - 2.0	1.1 - 1.9	1.0 – 1.8	0.91 - 1.6
	Wat Sol (v1.01 est)	0.5 – 4.3	1.0 - 8.0	1.8 – 14.5	3.3 – 25.8
Vapour pressure at 25 °C (mmHg) (MPBPWIN v1.42, Modified Grain method)		2.8×10^{-6} - 1.8×10^{-7}	1.2×10^{-7} - 9.1×10^{-9}	5.1×10^{-9} - 3.9×10^{-10}	2.5×10^{-10} - 2.3×10^{-11}
Henry's Law constant (atm.m^3/mol) (HENRYWIN v3.20, Bond method)		1.6×10^{-7}	2.6×10^{-9}	4.0×10^{-11}	6.2×10^{-13}
Log K_{OW} (KOWWIN v1.67)		5.3 - 5.6	5.0 - 5.3	4.7 - 5.0	4.5 - 4.8

The predictions indicate that the degree of branching may affect some properties significantly (e.g. water solubility predicted using the Wat Sol model).

As expected, the vapour pressure and K_{OW} decrease with increasing number of ethylene oxide (EO) groups (reflecting molecular weight and increasing hydrophilicity). The Registrants state that the water solubility of (branched and linear) nonylphenol ethoxylate (NPnEO) oligomers increases with an increasing number of EO groups. One of the two models (WSKOW) does not suggest any major difference (i.e. all constituents may have a water solubility of around 1-2 mg/L). However, this model appears to under-predict water solubility for this type of substance: for example, the predicted water solubility of 4-NP using WSKOW is 1.5 mg/L, whereas the preferred measured value selected by the REACH registrants for that substance is 5.7 mg/L at 25 °C; the measured water solubility of NP2EO is around 3.4 mg/L, compared to a prediction of 1.1 – 1.9 mg/L. The Wat Sol model may therefore be more appropriate, but given the wide range of water solubility predictions for each constituent group, it is not very helpful in indicating the actual level of water solubility (especially as some of the predictions may significantly exceed critical micelle concentrations). Nevertheless, since measured data exist for NP2EO and the registered substance, there seems to be no need to generate any further measured water solubility data for other individual NPEO constituent groups for the purposes of this evaluation. The water solubility of NP1EO may be assumed to lie in the range 3.4 – 5.7 mg/L at 25 °C. This is consistent with a CMC of 4.55 mg/L.

The reliability of the predicted K_{OW} values as input for other predictions (e.g. of bioaccumulation behaviour or ecotoxicity) is highly uncertain, given the surface active nature of the substance. Measured data would therefore be preferable for these other end points.

By analogy with 4-NP, NPEO is likely to be un-ionised at environmentally relevant pH.

7.5. Manufacture and uses

7.5.1. Quantities

Table 8

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ **100 – 1000 t**	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

Table 8 refers to the collective registrations for the specific CAS no. 68412-54-4.

EC (2002) indicated that supply of NPEO to the EU for uses that have not since been restricted was in the region of 27 000 tonnes in 1997. Information provided in the publicly available background document developed in the context of ECHA's 6th recommendation for the inclusion of substances in Annex XIV (1 July 2015)[4] suggests that the overall amount of NPEO manufactured and/or imported into the EU is still likely to be in the range 10 000 – 50 000 tonnes/year. This includes NPEO that meets the REACH definition of a polymer so is exempt from registration requirements. It is therefore highly likely that the substance that is the focus of this Substance Evaluation is a relatively minor contributor to the overall volume of NPEO currently on the European market.

7.5.2. Overview of uses

The Registrants subject to this Substance Evaluation only supply/use NPEO for a specific application (flotation agent) in the mining industry.

There is little further information available on other uses due to the lack of registrations for the majority of NPEO substances. NPEO is a non-ionic surfactant, and has many potential applications that have not been restricted under Annex XVII of REACH. According to EC (2002), these may include:

- The manufacture of nonylphenol ether sulphates and nonylphenol ether phosphates, which are used as emulsifiers in the chemical industry and lubricant oil additives.
- Polymer manufacture.
- Additive packages for use in either fuel oil or lubricants.
- Paints and coatings.
- Photographic film developers (with the advent of digital photography this is presumably no longer a significant use, although there may be niche uses such as for medical imaging).
- The electrical engineering, civil and mechanical engineering industries, e.g. for wall construction materials; road surface materials; fluxes, dyes and etching baths used in the manufacture of printed circuit boards; and also some specialist cleaning products.
- Spermicide manufacture,
- Vehicle cleaning agents,
- Office products such as correction fluids.

[4] https://echa.europa.eu/documents/10162/13640/6th_axiv_rec_backgdoc_4-NPnEO_en.pdf

Most of these uses are still relevant according to the background document developed in the context of ECHA's 6th recommendation for the inclusion of substances in Annex XIV (1 July 2015), which mentions the following uses:

- formulation and use of paints;
- emulsion polymerisation;
- a processing aid in the manufacture of fine chemicals;
- (potentially) a reducing agent in surface treatment;
- admixtures for concrete;
- polyurethane systems;
- auxiliaries in the textile industry[5];
- emulsifying wax;
- auxiliaries for oil extraction; and
- formulation of products for scientific research and development.

These applications are thought to involve NPEO with a chain length longer than three EO groups (commonly in the ranges 7-15 and 30-70), which are not addressed by this Substance Evaluation and are not subject to registration if they are defined as polymers.

ECHA has requested more specific information on uses of NPEO under the Substance Evaluation of 4-NP, and use information would also be obtained if applications for authorisation are made in due course (although this would not apply to uses as a chemical intermediate (e.g. in the manufacture of nonylphenol ether sulphates/phosphates), or for any NPEO present in imported articles).

[5] The concentration of NPEO in textile articles is now restricted by Annex XVII of REACH; although it is thought that this will prevent intentional use, textiles containing NPEO can still legally be supplied as long as they contain it below the specified concentration limit.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

The substance does not have a harmonised classification in Annex VI of the CLP Regulation (EC) 1272/2008.

7.6.2. Self-classification

- In the registration(s): According to the ECHA dissemination web site, the Registrants do not classify the substance for physical or human health hazards (on the grounds that data are conclusive but not sufficient for classification, with the exception of reproductive toxicity and effects on or via lactation, for which data are lacking). They classify it for environmental hazards as follows:

  Aquatic Acute 1 (H400: Very toxic to aquatic life), M-factor 1

  Aquatic Chronic 1 (H410: Very toxic to aquatic life with long lasting effects), M-factor 10

- The following hazard classes are also notified among the thirty-seven aggregated self-classifications in the C&L Inventory (checked 4 July 2016) (http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database):

  Aquatic Chronic 2 (887 + 361 + 90 + 69 + 47 + 44 + 29 + 18 + 4 + 3 + 2 + 1 notifiers)

  Aquatic Chronic 3 (465 + 13 + 12 + 4 + 2 notifiers)

  Aquatic Chronic 4 (16 + 6 notifiers)

  No Aquatic Acute classification

  No environmental classification

  M-factors are not always proposed for Aquatic Acute/Chronic 1, or are different to those proposed by the Registrants (e.g. a chronic M-factor of 1).

  Notified human health hazards include Acute Tox. 4. STOT SE 3 (H335) and various classifications relating to eye damage/irritation and skin irritation/sensitisation.

7.7. Environmental fate properties

7.7.1. Degradation

The persistence or otherwise of a substance is an important consideration for its potential to cause long-term harm to populations of organisms. This is relevant for hazard classification under the CLP Regulation, and may be important for a decision about endocrine disruption under REACH.

ECHA (2013) provides a generic description of the biotic transformation of NPEO in the environment. In the case of long-chain nonylphenol n-ethoxylated (NPnEO) (n>4), ethylene oxide (EO) groups are rapidly removed as a first step yielding NPEO with less than four EO units, particularly the mono- and di-ethoxylates, i.e. NP1EO and NP2EO. The rate of removal of the EO chain increases with increasing chain length. Under aerobic conditions these short-chain NPEOs can be further oxidised to the corresponding carboxylic acid (e.g. nonylphenoxyacetic acid (NP1EC) or nonylphenoxyethoxyacetic acid (NP2EC)) as well as carboxylated alkylphenol ether carboxylates (CAmPEnC with m=5-9 and n=0 or 1). Under anaerobic conditions, NP1EO, NP2EO and NP1EC can be transformed to 4-nonylphenol (4-NP). Based on the evidence presented in EC (2002), the fate of a long-chain NPEO entering anaerobic wastewater treatment was assumed to be as follows (as a worst case, on a per cent weight basis):

Mineralised/highly degraded	*45 %*
Released as NPEC in effluent	*25 %*
Released as shorter chain NPEO in effluent	*8 %*
Released as 4-NP in effluent	*2.5 %*
4-NP in digested sludge	*19.5 %*

4-NP is only a minor transformation product from the aerobic degradation of short chain NPEO/NPECs and so it was assumed as a worst case that a further 2.5 % of any NPEOs released to the environment would eventually be transformed to 4-NP in water, with a half-life of around 100 days.

The following section discusses the available studies that provide information on the degradability of the shorter chain NPEO substances that are the main constituents of the registered substance (with a focus on NP1EO and NP2EO).[6]

7.7.1.1 Abiotic degradation

The ECHA dissemination website provides information on hydrolysis and phototransformation in water (as well as predicted degradation in the atmosphere, which is not the focus of this evaluation):

- A hydrolysis study performed according to OECD TG 111 was carried out in 2010 according to GLP. This study is rated reliable without restriction by the

[6] A large number of studies have examined the degradation of NPEO, and there are two major reviews (Melcer et al., 2007; Staples et al., 2008). Several studies focus on elucidating biodegradation pathways rather than rates under realistic environmental conditions. In addition, most studies have considered the degradation of long chain length NPEOs, so are not directly applicable to the registered substance. The Registrants have not performed a review of laboratory or pilot scale WWTP studies, or reviewed field studies that measured removal in WWTP (e.g. Lee and Peart, 1998; Nasu et al., 2001; Sheahan et al., 2002; González et al., 2007; Soares et al., 2008, etc.). Since many of these are confounded by the degradation of long chain length NPEO, and given the focus of this substance evaluation, the eMSCA has not performed a comprehensive review, but has previously summarised relevant information, which can be made available by the eMSCA on request.

Registrants. The actual test substance is not indicated, but is said to be the same as that covered in Section 1 of the registration dossier. The substance was found to be stable in the preliminary test at pH 4, 7 and 9 at 50 °C. Reaction rate constants and half-lives could not be calculated, and it assumed that the hydrolysis half-life is > 1 year for ambient temperature conditions.

- Ahel et al. (1994a) studied the rate of photochemical transformation of NP1EO (1 µmol/L [0.26 mg/L]) in filtered lake water (containing 4 mg/L of dissolved organic carbon, pH 8.4; a second experiment used an adjusted pH of 9.4) by exposing solutions to natural sunlight and measuring the analyte concentration intermittently by normal-phase HPLC after a simple extraction of the water samples with n-hexane. This study is rated reliable with restrictions by the Registrants.

  The test substance concentration remained essentially unchanged in the sunlight test, so the photochemical degradation of NP1EO was insignificant. Laboratory experiments using artificial light (ten times more intense than sunlight during a sunny summer day) showed that any photochemical degradation that did occur was due mainly to sensitized photolysis (rather than direct photolysis).

ECHA (2013) does not provide any additional useful information on abiotic degradation.

Discussion
The eMSCA agrees with the Registrants' assessment that abiotic degradation will only make a minor contribution to the overall fate of the registered substance in the aquatic environment.

7.7.1.2 Screening tests for biodegradation

Two relevant ready biodegradation studies are included in the registration information on the ECHA dissemination web site, both rated reliable with restrictions (a third RSS refers to 4-NP, so is not relevant in the view of the eMSCA):

- The biodegradation of NP1.5EO[7] was measured using OECD TG 301B [carbon dioxide evolution] (Gledhill, 1999). According to ECHA (2013) the test was run with adapted inoculum from a waste water treatment plant, but the RSS says that the adaptation of the inoculum was not specified.

  45.3 % biodegradation was observed after 28 days (58.7% after 35 days). The 10-day window was not met. Staples et al. (2001) calculated a first order primary degradation half-life of 18.9 days (with a lag time of 9 days).

  The Registrant considered that this study demonstrated inherent biodegradability.

- Another study was conducted to OECD TG 301D [closed bottle test] in 1998, although a full reference is not provided. The test substance was "isononyl ethoxylated" (CAS no. 37205-87-1). The Registrants assign a reliability rating of 4 (not assignable) since the study was not performed in accordance with GLP and there is uncertainty over the precise identity of the test substance (there was no analysis certificate). Standard closed bottles were inoculated from a semi-continuous activated sludge (SCAS) unit. Secondary activated sludge and primary settled sewage were collected from a Dutch waste water treatment plant (WWTP) (an activated sludge plant treating predominantly

[7] This is presumably an average level of ethoxylation, broadly equivalent to a mixture of NP1EO and NP2EO.

domestic sewage). 150 mL of secondary activated sludge containing approximately 2 g DW (presumed dry weight)/L of suspended solids was used as an inoculum for each unit. The test duration was 112 days and the initial test substance concentration was 3 mg/L. The sludge was pre-exposed to the substance in the SCAS test to acclimatize the microorganisms, although both unacclimated and acclimated sludge was used as inoculum in the ready biodegradability test. The pH of the media was 7.0 at the start of the test and 6.8 on day 28. The incubation temperatures ranged from 20 to 22 °C. No control was used, but the validity of the test was demonstrated by endogenous respirations of 2.1 and 1.3 mg/L at day 28. Furthermore, the differences of the replicate values at day 28 were less than 20%. Finally, the validity of the tests is shown by oxygen concentrations >0.5 mg/L in the bottles.

i. Using inoculum collected from the SCAS unit on day 0 (i.e. not intentionally acclimated), the start of degradation was delayed (length of time not indicated) and the substance achieved 21 % degradation by day 28 based on oxygen consumption. When the test was extended to 112 days, the substance achieved 112 % (*sic*) degradation. [eMSCA note: the same numerical figure is used for the number of days and percentage degradation – the latter may therefore be a transcription error. The viability of the inoculum over a three-month timescale is unknown.]

   Recommendation: The Registrants should clarify the level of degradation observed after 112 days in the OECD TG 301D closed bottle test, and assess the reliability of this result.

ii. When inoculum was introduced following 14 days in the SCAS unit (i.e. acclimated), degradation began immediately and the substance achieved 52 % degradation by day 28. The RSS also states that "almost 60% biodegradation was reached after 28 days in the Closed Bottle test inoculated with pre-exposed sludge", although it is not clear what part of the experiment this relates to.

The Registrant concludes that the substance is inherently biodegradable. The eMSCA cannot determine whether the test substance was a long or short-chain NPEO.

ECHA (2013) summarises two additional ready biodegradation studies for relevant substances, both rated reliable with restrictions[8]:

- Stasinakis et al. (2008) performed an OECD TG 301F [manometric respirometry (oxygen consumption)] study with NP1EO including an additional 10 mg/L allythiourea to prevent nitrification. After a lag phase of 17.3±0.7 days, NP1EO achieved 25.9±8.1 % biodegradation by day 28. No biodegradation was observed for NP2EO.
- Gledhill (1999) investigated the corresponding octylphenol ethoxylate (OP1.5EO), which achieved 61.6 % biodegradation after 28 days, failing the 10-day window. The calculated first order primary degradation half-life was 10.7 days (with a lag time of 4 days).

[8] In addition, the eMSCA notes that Di Gioia et al. (2009) and Frassinetti et al. (2011) performed studies using fixed-bed laboratory-scale bioreactors that demonstrated high levels of removal (around 70-98 per cent) for two commercial NPEO substances (Igepal CO-520 and Igepal CO-210, containing an average of 5 and 1.5 EO units, respectively) over 10-15 days up to two months. However, the inoculum was an aerobic bacterial consortium isolated from an oxidation pre-treatment plant receiving wastewater from a textile works. The consortium was therefore pre-adapted and intended to be a competent degrader of NPEO. These data are not considered further.

Discussion

NP1EO and NP2EO are not readily biodegradable using standard test methods (the pass level has to be 60 % by day 28 for respirometric methods, to be reached in a 10-day window following achievement of 10 % degradation within the test period; inocula must not be pre-adapted to the test substance).

The Registrants conclude that as NP1.5EO reached 59 % biodegradation after 35 days in a ready biodegradation test (OECD TG 301B), it can be considered to be inherently biodegradable. The eMSCA agrees with this conclusion, provided that the inoculum was not pre-adapted. Although a standard test for inherent biodegradability (OECD TG 302A-C) is not available, the REACH Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance (Version 3.0, February 2016) states that when results of ready biodegradability tests (which may include incubation beyond 28 days) indicate that the pass level criterion is almost fulfilled (i.e. theoretical oxygen demand (ThOD) or dissolved organic carbon (DOC) slightly below 60% or 70% respectively) such results can be used to prove inherent biodegradability. The conclusion for NP1.5EO is supported by data for the analogue OP1.5EO in the same test system.

Since the focus of this Substance Evaluation is on endocrine effects, the eMSCA does not consider it necessary to seek any additional confirmatory data for this end point.

7.7.1.3 Sewage treatment plant

No relevant studies are included in the registration dossier on the ECHA dissemination web site. Data on degradation in sewage works are relevant for risk assessment but less so for hazard identification.

ECHA (2013) summarises five studies (Rudling and Solyom, 1974; Ejlertsson et al., 1999; Lu et al., 2008a&b; and Ball et al., 1989). These studies clearly demonstrate that NP1EO and NP2EO can transform to 4-NP under anaerobic conditions in sewage sludge, although the rate depends on concentration, and in some circumstances NP1EO in particular may persist. Under aerobic conditions, NP2EO may also persist as temperature declines. The degree of mineralisation is low.

7.7.1.4 Surface water

The registration dossier on the ECHA dissemination web site includes data for four studies which investigated the degradation of longer chain NPnEO (n typically 9 or higher) in fresh and estuarine surface waters; these are also included in ECHA (2013) and they are rated reliable with restrictions:

- Jonkers et al. (2001) investigated the aerobic biodegradation of NP4EO (EO range 2-9) at a concentration of 10 mg/L in a closed loop laboratory-scale bioreactor filled with river water. Microorganisms ubiquitously present in the river water settled on the carrier material to form a biofilm, so this study is not equivalent to OECD TG 309. Small amounts of Octylphenol ethoxylates (OPnEO) and ethoxylated decylphenol were also present.

  Primary biodegradation reached 50% in 10 hours and was nearly complete (>99%) by 100 hours (4.1 days). The initiating step of the degradation was ω–carboxylation of the individual ethoxylate chains to form NPECs. NP2EO was the main lower molecular weight ethoxylate formed, peaking in concentration at 100 hours and disappearing by 300 hours (12.5 days). The eMSCA does not know whether this degradation rate would have been different at 12 °C since the temperature of the study is not stated.

The test water was obtained from the River Rhine, so may have been pre-adapted, as suggested by the fact that degradation started immediately with no observable lag phase.

- Potter et al. (1999) performed a static die-away test for NPnEO (n=7-24, average 18) using estuarine water from four sampling sites in Tampa Bay, Florida, USA. The sampling site temperature ranged from 27.5 to 31.0 °C, and tests were conducted at 28±1 °C. The concentration of NP2EO, NP1EO, NP2EC, NP1EC, NP and total surfactant were monitored at intervals of 4-8 days for 89 days and at 30-day interval thereafter until 183 days. Due to the different sampling locations the results are given as a range.

  NPnEO underwent relatively rapid primary degradation followed by accumulation and decay of NP2EO and steady accumulation of NP2EC that plateaued by the end of the study. Primary degradation was essentially complete by day 4-24, with an adaptation time between 0 and 12 days. Some of the sites were known to have microbial populations capable of degrading NPEO, and were likely to have been adapted due to continuous input. NP2EO reached a maximum concentration in 4-16 days, then disappeared later in the test (by day 56 in one sample; data for the other samples are not provided). The actual half-life of NP2EO cannot be established because it was being formed due to degradation of longer chain NPEOs.

  NP2EC increased until day 20-76 with little or no decrease until the end. It was present at the highest concentration of all metabolites by the end of the experiment, representing 63-93% of all metabolites detected (on a molar basis). NP1EC was detected in some samples, with a maximum concentration less than 20% of NP2EC. NP1EO was detected at intermediate time intervals, but only in trace amounts (<0.1 mg/L).

  Nutrients and microbial populations were not replenished during the course of the experiment, which could have influenced the limited removal of NP2EC. A further die-away experiment was therefore conducted after 296 days, when half the water in a sub-set of containers was replenished with freshly collected water. Three of five replicates for water collected from one site showed approximately 50 % degradation of NP2EC in 32 days, with a lag time of approximately 20 days. No change in the NP2EC concentration was observed in samples prepared with water collected at a different site. Presumably the primary degradation rate would have been slower at 12 °C.

- Naylor et al. (2006) studied the aerobic biodegradation of ^{14}C-NP9EO (uniformly labelled on the aromatic ring) in 5 L glass tanks in a river die-away experiment. The test substance was specially synthesised in a way that mimicked the industrial process. Whilst similar in alkyl group branching pattern overall, the test substance contained more of the highly branched isomers than a sample of a commercial substance. The number of EO groups ranged from 0 to 17, with a peak around 7 or 8 (depending on the detector used). River water was collected 7.5 miles (12 km) downstream from a wastewater treatment plant. After filtration, the water was amended (1 % by volume) with clarified secondary effluent from the wastewater treatment facility. Three tanks were prepared by the addition of 2.5 L of amended river water and test substance at a concentration of 100 µg/L. Carbon dioxide-free and water saturated air was passed over the head space of each tank to provide oxygen. The tanks were maintained at around 20 °C, and buffered to remain within the pH range 6.2 – 9.2. Changes in oligomer distribution and mineralisation to $^{14}CO_2$ were monitored at intervals over 128 days.

  By day 28, around 80 % of the radioactivity in the water extracts still remained as NPEO, but the EO oligomer distribution had shifted towards the lower mole ethoxylates, with an increased abundance of NP1-5EO. There was also evidence of metabolism to non-ethoxylate substances (at around 20 %). Carbon dioxide began to be evolved after 21 days.

Around 80 % of the initial radioactivity was recovered at the end of the experiment (the causes were not investigated, but were assumed to be due to experimental losses/error). After 128 days, over 40 % of the ^{14}C-NP9EO aromatic ring carbon was converted to $^{14}CO_2$ and another 21 % was incorporated in the biomass. Around 10 % of the initial radioactivity was recovered as NPEO oligomers (or co-eluting substances). The mineralisation half-life was determined to be 19 days from day 42 (when a constant rate was reached) to day 128.

A fourth tank was similarly prepared, but dosed with a mixture of non-labelled NP and NPEO oligomers at a concentration of 100 µg/L to simulate the fate of partially degraded NPEO in a sewage treatment outfall. The stock solution composition was 3 % nonylphenol, 2.5 % NP1EO, 24 % NP4EO (commercial product) and 70.5 % NP9EO (commercial product). The NPEO oligomer distribution was analysed on day 128 by HPLC, along with NPEC content by gas chromatography-mass spectrometry (GC-MS). By day 128, NPEO oligomers had been reduced to 2.5 % of initial levels, indicating a high degree of primary degradation. NPECs accounted for less than 2 % of the initial NPEO concentrations.

Overall, primary degradation (conversion to metabolites other than NP, NPEO and NPEC) was estimated to be 87-97 % over 128 days. 4-NP was a minor metabolite, accounting for less than 0.4 % of the initial NPEO. The study showed the opening, metabolisation and mineralisation of the phenolic ring of NPEO. The eMSCA notes that OECD TG 309 (aerobic simulation in surface water) is of shorter duration (typically 60 days), does not include the addition of secondary effluent to test samples and uses smaller vessels (0.5 or 1 L), although they are shaken.

- Kvestak and Ahel (1995) studied the aerobic biotransformation of NPnEO (n = 1-18, average 10) by estuarine mixed bacterial cultures under laboratory conditions using a static die-away method. The experiments were performed with autochtonous bacterial cultures from brackish and saline water layers from the Krka River estuary, Croatia. Experiments were conducted in 5 L glass vials incubated at temperatures corresponding to those found in the environment at sampling time (13 – 22.5 °C). The test substance concentration was 0.1 and 1 mg/L.

  Biotransformation kinetics were faster in the brackish water culture than saline water cultures at all temperatures examined and at both concentrations of NPnEO. This was thought to be due to better pre-adaptation of the brackish water bacteria to NPEO in their natural habitat. Under winter temperature conditions (13 °C), the estimated dissipation half-life was 23 – 69 days, while under summer temperature conditions (22.5 °C) it was 2.5 - 35 days. The main intermediate detected during the experiment was NP2EO. NPEC was not measured.

  Since the study used bacterial cultures, it is not equivalent to OECD TG 309, and so the half-lives cannot be considered to be environmentally relevant.

The eMSCA is also aware of a study by Ahel et al. (1994b), referenced in the risk assessment of 4-NP performed under the Existing Substances Regulation (EC) No. 793/93. The test substance (Imbetin N/7A) had the following composition: 75% NP1EO, 20% NP2EO and 5% NP3EO. Aerobic river water die-away tests were carried out at 20 °C using water spiked with the test substance at a concentration of 1.1 mg/L. The source of the water was the Sava River near Zagreb, which was described as a "polluted river". Die-away tests were also performed at 4 and 20 °C using secondary sewage effluent from a Swiss wastewater treatment plant (concentrations of NP1EO and NP2EO were 90

and 64 µg/L respectively).[9] In the river die-away test the first order rate constant for primary degradation of NP1EO and NP2EO was 0.35-0.37 d^{-1} (i.e. half-life of around 2 days) with continuous stirring and 0.23 d^{-1} (i.e. half-life of around 3 days) under static conditions. The rate of primary degradation of the two compounds was slightly lower in the sewage effluent, with rate constants of 0.09 d^{-1} (i.e. half-life of around 8 days) at 20 °C and 0.01 d^{-1} (i.e. half-life of around 69 days) at 4 °C. The eMSCA notes that since the test media was likely to have been pre-adapted due to prior exposure to NPEO, the experiment with river water may not provide reliable half-life data for comparison with degradation criteria.

Discussion

In general, the studies suggest that longer chain NPEO substances undergo fast primary degradation (complete within a matter of days) to form short chain NPEOs then NPECs under aerobic conditions, although this may require a period of adaptation (which was 28 days in one study). The NPECs subsequently transform to CAmPEnC (with m=5-9, n=0 or 1). Mineralisation was below 60% over a timescale of 120 and 180 days in two studies. Degradation might also be concentration dependant and the influence of micelle formation is unknown.

The eMSCA notes that most of the available information was collected at temperatures of 20 °C or higher. However, data at a temperature of 12 °C are preferred for hazard and risk assessment under REACH. The variety of conditions (including pre-adaptation), test substances and test temperatures mean it is not possible to estimate a reliable environmental degradation half-life at 12 °C for NP1EO, NP2EO or NP2EC from the data. The ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7b: Endpoint specific guidance (Version 3.0, February 2016) indicates that there can be no systematic or universal correction factor for temperature that should be applied to higher tier biodegradation studies, but it suggests that a correction based on the Arrhenius equation may be applied in some circumstances:

$$t_{½} \text{ at } 12\ °C = t_{½} \text{ at } T\ °C \times e^{(0.08 \times (T - 12))}$$

Applying this equation to the data measured by Ahel et al. (1994a) suggests that the primary degradation half-lives of NP1-2EO in "polluted" river water at 12 °C might be around 4 – 6 days. The validity of such an extrapolation is unknown and it may not be reliable since the micro-organisms involved were likely to have been pre-adapted to these substances.

7.7.1.5 Sediment

The registration dossier on the ECHA dissemination web site includes data for two studies that investigated the degradation of NPEO in sediments; both are considered to be reliable with restrictions by the Registrants:

- Yuan et al. (2004) studied the aerobic degradation of NP1EO in contaminated river sediments from Taiwan at a concentration of 2 µg/g sediment at pH 7 and 30 °C. It is not clear whether this temperature represents the conditions experienced by the micro-organisms at these sites.

  The primary degradation half-life ranged from 69.3 to 115.5 days at four of six sites (arithmetic mean: 87.1 days; geometric mean: 85.5 days), which is longer than the anaerobic half-life measured in the same sediments (see Chang et al., 2004). The paper goes on to say that based on the site with the fastest level of degradation, NP1EO was completely primarily degraded after 84 days when the sediment had not been intentionally acclimated to 4-NP, or 56 days when

[9] The study also reports the results of shake culture test measuring primary degradation using adapted bacterial cultures, but the eMSCA does not consider these to be useful in the context of this evaluation.

acclimation had been allowed to occur. It is not clear how this information relates to the half-life range presented earlier in the paper. In addition, the sediments were collected from a contaminated river, but an intentional acclimation step took a further 12 months, and the viability of the original microbial population over this time frame is unknown.

A test conducted at 20 °C gave a reported primary degradation half-life of 57.8 days at pH 7, which was 2.5 – 10 times longer than that at 30 °C at the same pH (5.7 days with shaking, or 23.1 days without shaking). Again, it is not clear why the half-lives in this part of the experiment differed from those reported earlier on in the paper for the tests performed at 30 °C.

- Ferguson and Brownawell (2003) studied the degradation of a specially synthesised ^{14}C-labelled NPnEO mixture (n=4 on average, but full composition not provided) for 116 days (aerobic conditions) or 129 days (anaerobic conditions) at a concentration of 14 µg/g dry weight (dw) in a high organic carbon content (ca. 8 %) and polluted estuarine sediment known to contain NPEO and 4-NP residues. The reported primary degradation half-life was 85 and 287 days under aerobic and anaerobic conditions, respectively. 4-NP was not detected under either aerobic or anaerobic conditions, and it is possible that insufficient time had passed for degradation to 4-NP to occur. NPECs were formed under both conditions. Only small amounts of the parent compound were mineralised to carbon dioxide under aerobic conditions (<2 % of applied radioactivity). The bioavailability of the substance may have been reduced and there may have been inhibitory effects of other organic contaminants and heavy metals known to be in the sediment.

ECHA (2013) summarises two additional studies:

- Teurneu (2004) studied the degradation of NP2EO under aerobic and anaerobic conditions at 27 °C and 10 °C. The batch experiments used sediment samples collected from the bottom of a sedimentation basin at an industrial site involved in the production of NPEO [eMSCA note: presumably pre-adapted]. The initial concentration of NP2EO was 500 mg/L [eMSCA note: well in excess of its measured and predicted water solubility values). Theoretical calculations indicated that after 44 days, the level of NP2EO degradation was 4 % at 27 °C and 0 % at 10 °C under aerobic conditions, and 5 % at 27 °C and 1 % at 10 °C under anaerobic conditions.
- Chang et al. (2004) investigated the anaerobic degradation of NP1EO in contaminated river sediments from Taiwan (using the same sites as Yuan et al., 2004) at a concentration of 2 µg/g sediment at pH 7 and 30 °C. The primary degradation half-life at 30 °C ranged from 49.5 to 77.0 days for sediment collected from four sites. After day 8, 4-NP was determined as an intermediate product, and the concentration increased until day 14.

  A test conducted at 20 °C gave a reported primary degradation half-life of 115.5 days at pH 7. The data reported at 30 °C for this part of the experiment indicate a half-life of 19.8 days. It is not clear why this differs from the data given earlier in the paper. Degradation rates for NP1EO were enhanced at higher temperature.

Four additional RSS for simulation studies in water/sediment are included in the registration dossier on the ECHA dissemination web site, but as they concern 4-NP they are not relevant for this evaluation.

Discussion

Applying the Arrhenius equation to the NP1EO half-life of 57.8 days at 20 °C obtained by Yuan et al. (2004) gives a primary aerobic degradation half-life of 110 days at 12 °C. This study reported *longer* half-lives for four sites at 30 °C (average of around 86 days) than that specifically reported at 20 °C, which is unexpected. The half-life at 12 °C would consequently be longer for these sites; the Arrhenius extrapolation would suggest an aerobic half-life of about one year based on these data. Since the sediments were intentionally pre-adapted, the half-life could well be longer in uncontaminated sediment.

Applying this equation to the NP1EO half-lives quoted at 20 °C by Chang et al. (2004) gives a primary anaerobic degradation half-life of 227 days at 12 °C. When the 30 °C data are considered, the half-life at 12 °C would be in the range of 208 – 325 days. Again, the half-life could well be longer in uncontaminated sediment.

The validity of such extrapolations is unknown and they may not be reliable. However, long sediment degradation half-lives (well in excess of 180 days) are also suggested by the study of Teurneu (2004) conducted at 10 °C for NP2EO under both aerobic and anaerobic conditions.

On the basis of this evidence, both NP1EO and NP2EO may be persistent in sediment under both aerobic and anaerobic conditions at 12 °C, but definitive data at environmentally relevant temperatures and concentrations are lacking.

Soil

Since the focus of this evaluation is on aquatic effects, soil degradation data have not been considered further.

Summary of aquatic degradation

The Registrants conclude that based on OECD screening studies and results from water/sediment simulation studies, the registered substance will be biodegraded under aerobic conditions in the aquatic environment, with a significant portion of the parent compound being mineralised. The eMSCA believes that this analysis is broadly correct based on data in the registration dossier and public sources. Abiotic degradation is unlikely to be relevant in the aquatic environment. Neither NP1EO nor NP2EO is readily biodegradable. Standard test guideline studies for inherent degradation are not available, but the extensive mineralisation observed for NP1.5EO (and the analogue OP1.5 EO) over 35 days in a ready test indicates that it can be considered inherently biodegradable.

Although primary degradation appears to be relatively fast in aerobic surface waters (e.g. forming nonylphenol ether carboxylates), it is not possible to estimate a reliable environmental degradation half-life at 12 °C for NP1EO or NP2EO from the available data. The degree of mineralisation is generally well below 60 % over a time period of one month in surface water. In addition, degradation might also be concentration dependant and the influence of micelle formation is unknown.

There is some evidence to suggest that the half-life for both NP1EO and NP2EO could be above 180 days in sediment at environmentally relevant temperatures.

Significant transformation products include 4-NP (under anaerobic conditions) and NP2EC (for NP2EO).

7.7.2. Environmental distribution

Not evaluated.

7.7.3. Bioaccumulation

The focus of this evaluation is on endocrine disruption in fish, so bioaccumulation is not wholly relevant (ECHA (2013) did not consider bioaccumulation potential). However, the available information has been briefly reviewed by the eMSCA since it could provide insights for fish exposure and metabolic potential.

The registration dossier on the ECHA dissemination web site includes five RSS, four of which provide data on short chain NPEO (the fifth is for 4-NP only so is not relevant). No standard test guideline studies are available, and instead the information relates to a quantitative structure-activity relationship prediction, and field data:

- Bioconcentration factor (BCF) values were calculated for the individual constituents of the registered substance using the BCFBAF v3.01 model (EPIWEB v 4.1, Arnot Gobas method). The eMSCA notes that the data are not provided in a QPRF and there is no discussion of model applicability or reliability for this type of structure. An "overall BCF" value of 648 L/kg was calculated on a weighted-average basis using the mole fractions of all the individual constituents.

  The eMSCA has not attempted to replicate this estimate, but points out that a single 'average' BCF value for a complex mixture is not appropriate for hazard or risk assessment purposes. Instead, the individual BCF data should be provided for each constituent. In addition, the Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7c: Endpoint specific guidance) says that the log K_{OW} might not be suitable for calculation of a BCF value for surface-active agents. Environment Agency (unpublished) also highlights some additional considerations about the choice of values from this model, which would need to be taken into account if the data were to be evaluated in more detail.

- Ahel et al. (1993) performed a field study to evaluate the concentrations of NP1EO and NP2EO in aquatic plants (three species), fish (three species) and birds (a duck species). The study is rated reliable with restriction by the Registrants. Samples were collected over two consecutive years from a creek and river which received secondary effluents from mechanical-biological domestic sewage treatment plants. The total sample numbers were very low. Water samples were also taken (number not specified) for NP1EO and NP2EO analysis.

  The highest concentrations were found in the alga *Chladophora glomerata*, i.e. 4.7 and 4.3 mg/kg dw for NP1EO and NP2EO, respectively. The concentrations in fish tissues were typically lower (NP1EO: 0.06 – 7.02 mg/kg dw; NP2EO: <0.03 – 3.07 mg/kg dw). In the duck, the values were <0.03 – 2.10 mg/kg dw for NP1EO and <0.03 – 0.35 mg/kg dw for NP2EO. The average water concentrations of NP1EO and NP2EO were 24 and 9.4 µg/L, respectively (arithmetic mean of three determinations).

  The authors calculated bioaccumulation factors (BAFs) comparing the measured data on a dry weight basis to concentrations in water. However, Staples et al. (1998) considered that this methodology was incorrect: when all data were expressed on a wet weight basis (assuming that fish muscle was 85 % water / 15 % dry matter and that algae were 95 % water / 5 % dry matter), the non-lipid-normalised BAFs were below 50 for all species. No recalculated values were presented for the duck, but the original measured values were in the same range as those for fish.

  The Registrants conclude that these BAF values suggest a relatively low bioaccumulation potential in the aquatic environment for NP1EO and NP2EO and no significant biomagnification in the food chain. However, the eMSCA considers that **the reported BAFs are unreliable** due to the uncertain

relationship between the biota and water concentrations, the very low sample numbers spread over two years, and the fact that whole fish concentrations were not reported (muscle will not necessarily contain the highest concentrations). The lack of lipid normalisation is also a confounding factor.

- Keith et al. (2001) evaluated the bioaccumulation potential of NP1EO, NP2EO and NP3EO using several freshwater fish species collected from Michigan (USA) on three occasions in 1999. The study is rated as reliable with restrictions by the Registrants. However, only the digestive/excretory system was chosen for analysis, so **no conclusion can be drawn about bioaccumulation potential** in the view of the eMSCA.
- Granmo et al. (1991)[10] evaluated the bioaccumulation of NP1EO and NP2EO in blue mussels (*Mytilus edulis*). The study does not appear to have been reported in the peer-reviewed literature so its overall reliability is unclear; the Registrant considers its reliability as not assignable in view of the poor documentation. Mussels were exposed for 50 days to 1, 10 and 100 % wastewater in tanks submersed in a relatively unpolluted area using a semi-static system where the water was changed every 4 hours. Mussels were measured, weighed and analyzed for NP1-2EO content at the end of the study. Nominal and measured concentrations were not reported, but the BCF was in the range 100 – 200 for NP1EO and 50 – 100 for NP2EO. No information is provided on lipid normalisation. The eMSCA considers that **this study should not be used for bioaccumulation assessment**, especially because the composition of the wastewater (which might have caused toxicity to the molluscs) is unknown. For comparison, the reported BCF for 4-NP was in the range 300 – 400 in this study, but the UK Substance Evaluation of that substance identified a BCF of up to 2 000 L/kg in mussels.

The eMSCA has identified two further scientific papers that report BAFs of NP1-2EO in fish, which are missing from the REACH registrations, and there may well be more since the literature search did not address this end point:

- Lozano et al. (2012) analysed whole samples of predatory Largemouth Bass (*Micropterus salmoides*) collected from a waste-water dominated stream in Chicago, USA. BAFs[11] were estimated using average concentrations from water samples collected around the same time as the fish and there is no information on fish lipid content. BAFs were 517, 360 and 49 for NP1EO, NP2EO and NP3EO, respectively, in spring 2007 (levels were lower in autumn). The highest NP1EO concentration in the fish was around 3.5 µg/g ww (value read from a graph); levels of NP2EO were lower.
- Lozano et al. (2012) mention that BAFs of 1 700 and 693 for NP1EO and NP2EO, respectively, were reported for Common Carp (*Cyprinus carpio*) by Mitchelmore and Rice (2006). The eMSCA has not evaluated this information further, although notes that as a benthic feeder, a significant amount of exposure may come from sediment.

In addition, the registration dossier includes a RSS for a study that examined fish toxicokinetics:

[10] A full study citation is not provided in the RSS, but some further information is provided in the CSR, and the eMSCA has previously seen this information so provides the full reference in Section 7.14.

[11] A BAF is not necessarily identical to a BCF since exposure will include a dietary contribution.

- Cravedi (2001) investigated the metabolic fate of NP2EO in mature Rainbow Trout (*Oncorhynchus mykiss*) over 72 hours[12].

  Recommendation: The Registrants should provide the full reference for this study in the RSS. The eMSCA believes that it is Cravedi et al. (2001).

  The Registrants consider the study to be well reported, and reliable with restrictions. The test substance was a specially synthesised radiolabelled straight chain NP2EO (the purity is not stated). The substance was administered to the fish as a single 10 mg/kg oral dose via a gelatine capsule by gavage. Urine was collected over 72 hours, and at the end of the test, the fish were sacrificed, the gall bladder excised and radioactivity was measured in tissues, viscera and carcasses. Metabolic profiles were analysed by radio-HPLC and metabolites were identified by LC/MS (where possible). For comparison, biotransformation was also investigated in freshly isolated hepatocytes incubated during 6 h with 50 µM of radiolabelled NP2EO.

  Liver, viscera, carcass, bile and urine accounted for 1.2, 12.5, 4.7, 14.7 and 1.8 % of the dose, respectively. This leaves around 65 % of the dose unaccounted for (faeces were not collected). NP2EO was found to be widely spread in the fish body including in brain tissue. Elimination occurred mainly via the bile, with urinary excretion a minor pathway. High levels of radioactivity were found in the liver (7.4 µg NP2EO equivalent/g), and viscera (8.8 µg/g) with lower residue concentrations in fat (3.4 µg/g), muscle (0.8 µg/g) and remaining carcass (0.4 µg/g). However, muscle represents around half of the body mass in trout. Since the fish weighed 200–350 g, around 150 – 260 µg NP2EO may have been present in this tissue.

  Due to the low amount of radioactivity present in urine samples, no further analysis of metabolites was performed on this excreta. Chromatographic analysis of biliary metabolites resulted in the separation of at least ten different minor peaks and a major peak accounting for 42 % of bile radioactivity (retention time TR=42 min). After enzymatic treatment with β-glucuronidase, this peak shifted to 48 min, a retention time corresponding to NP2EO, suggesting that the major metabolite excreted through the bile was the glucuronide conjugate of NP2EO.

  The radio-HPLC profile obtained with isolated hepatocytes exhibited fourteen separated peaks. Several of them (TR=25, 31, 36, 37 and 38 min, respectively) were modified after enzymatic treatment with β-glucuronidase indicating that these peaks correspond to glucuronides, but no trace of NP2EO glucuronide (the main *in vivo* metabolite) was found. The difference between *in vitro* and *in vivo* metabolic patterns might be related to the fact that NP2EO-glucuronide could undergo additional biotransformation steps in hepatocytes leading to more polar metabolites. This hypothesis was supported by the presence of several glucuronides in the incubation medium and by the capability of trout to convert the linear alkyl chain of nonylphenolic conjugates to various hydroxylated compounds and carboxylic acid metabolites.

  4-NP and/or 4-NP glucuronide were not detected in any bile or hepatocyte sample, suggesting that 4-NP is not a metabolite of linear NP2EO in this species during short exposures.

Discussion

The Registrant concludes that based on the available BAF data, the registered substance can be considered to have a low bioaccumulation potential in aquatic organisms, even

[12] In related papers, Leguen and Prunet (2001) and Sturm et al. (2001a&b) report the effects of a substance referred to as "NP2EO" on various biochemical/functional responses in trout liver and gill cells. These have not been considered further for this evaluation as they are not directly relevant.

though the predicted log K_{OW} value is above 3 (see Section 7.4). They say that this assumption is supported by the available mammalian toxicokinetic data as well as data on fish metabolism indicating efficient metabolism and rapid excretion of metabolites.

The eMSCA disagrees with this assessment. NP1EO to NP4EO (at least) are predicted to have log K_{OW} values above 5 (see Section 7.4), which implies potentially significant bioaccumulation potential. The Registrants' own prediction results in an "overall" BCF above 500 L/kg. However, the interpretation of predicted log K_{OW} and BCF values in the context of bioaccumulation assessment for surfactants is difficult in the absence of definitive fish BCF studies. On the basis of primary degradation in surface water and the study of Cravedi et al. (2001), it is likely that fish can metabolise these substances. However, the fish metabolism data included in the registration dossier relate to a *linear* NP2EO substance, and their relevance for branched structures is unclear. As well as being potentially less hydrophobic and/or sterically hindered, linear side chain alkylphenols may enter the β-oxidation pathway thereby producing shorter side-chain carboxylic acid metabolites. This pathway was established and extensively characterized *in vivo* for 4-n-nonylphenol by Cravedi and Zalko (2005). In addition, the data relate to a single oral dose with analysis only over a 72-h period, so the metabolic profile over longer/higher exposures is unknown. The Registrants' Chemical Safety Reports (CSRs) mention that simulation of metabolism has been assessed using the OECD Toolbox, but data are not presented in the dossier. The actual extent of transformation in fish is therefore unclear.
Field studies appear to show that NP1EO can have a fish BAF as high as 1 700 (e.g. Mitchelmore and Rice, 2006), although this might include a contribution from sediment exposure; another study by Lozano et al. (2012) indicated a non-lipid-normalised fish BAF slightly above 500 for the same substance.

The eMSCA considers that there are insufficient data to allow a definitive conclusion about bioaccumulation potential in aquatic organisms to be drawn. The available information suggests that the fish BCF may be above 500 L/kg. It will, however, be less than that for 4-NP which is more hydrophobic. EC (2002) used a worst case estimated BCF of 1,280 L/kg for that substance, although a more realistic value was thought to be 741 L/kg.

Since this evaluation is targeted on endocrine disruption, a further standard test guideline study is not specifically needed to address the aquatic bioaccumulation end point. However, it may be possible to include additional measurements in any further fish toxicity study, and this is considered in Section 7.10.3.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

The main focus of this evaluation is on the potential for endocrine (primarily estrogenic) effects in sensitive fish life stages during long-term tests. Short-term ecotoxicity data for fish, and both short- and long-term ecotoxicity data for invertebrates and algae, could therefore be considered irrelevant. However, brief details are presented in this section because they are still an important consideration for hazard classification and risk management decisions (particularly if a taxonomic group is significantly more sensitive than fish despite any endocrine interaction).

The Registrants consider that toxicity declines as the NPEO ethoxylate chain length increases (as the substances become more hydrophilic), and since NP1EO and NP2EO are the main constituents of the registered substance, the CSR focuses on these two substances, with "less emphasis" on NP3-6EO (though in fact these longer chain lengths are ignored due to the lack of relevant data). The eMSCA agrees that NP1EO and NP2EO should be the focus of the assessment.

Since NPEO can degrade to NP1EC and NP2EC under both aerobic and anaerobic conditions, it may be important to consider their environmental effects to ensure that hazard and risk assessment are sufficiently protective. This is provided in Appendix 1 of this report.

Table 9

Summary of relevant information on aquatic toxicity for the registered substance and relevant constituents					
NP1EO					
Species	**Endpoint**	**Results (mg/L)**	**Validity rating**	**Comment**	**Reference**
Short-term fish toxicity					
Fathead Minnow (*Pimephales promelas*)	Mortality	96-h LC_{50}: 0.22 (mm)	2	Guideline: US EPA OPP 72-1 (flow-through) *Not checked by eMSCA*	TenEyck and Markee, 2007
Japanese Medaka (*Ozyrias latipes*)	Mortality	48-h LC_{50}: 3 mg/L	?	*Not checked by eMSCA or Registrant*	Yoshimura, 1986
Short-term invertebrate toxicity					
Cladoceran (*Ceriodaphnia dubia*)	Mortality	48-h LC_{50}: 0.33 (im)	2	Guideline: US EPA 600/4-90/027F (semi-static) *Not checked by eMSCA*	TenEyck and Markee, 2007
Long-term fish toxicity					
Japanese Medaka (*Ozyrias latipes*)	Mixed secondary sexual characteristics	100-d LOEC: 0.10 (mm) 100-d NOEC: 0.035 (mm)	2	Guideline: similarities to OECD TG 234 (semi-static) 4-NP was detected in treatment groups	Balch and Metcalfe, 2006
Rainbow Trout (*Oncorhynchus mykiss*)	Plasma vitellogenin	21-d NOEC: 0.048 (mm)	2	Non-standard guideline (flow-through) NP1EO was detected in controls	Dussault et al., 2005

(cont.)

Table 9 (cont.)

NP2EO					
Species	**Endpoint**	**Results (mg/L)**	**Validity rating**	**Comment**	**Reference**
Short-term fish toxicity					
Fathead Minnow (*Pimephales promelas*)	Mortality	96-h LC_{50}: 0.32 (mm)	2	Guideline: US EPA OPP 72-1 (flow-through) *Not checked by eMSCA*	TenEyck and Markee, 2007
Short-term invertebrate toxicity					
Cladoceran (*Daphnia magna*)	Mortality	24-h LC_{50}: 0.56	2	Guideline: "Similar to OECD TG 202" (static) *Not checked by eMSCA*	Sun and Gu, 2005
	Mortality	48-h LC_{50}: 0.15	2	Guideline: ISO 6341 (1982) *Not checked by eMSCA*	Maki et al., 1998
Cladoceran (*Ceriodaphnia dubia*)	Mortality	48-h LC_{50}: 0.72 (im)	2	Guideline: US EPA 600/4-90/027F (semi-static) *Not checked by eMSCA*	TenEyck and Markee, 2007
Long-term fish toxicity					
Rainbow Trout (*Oncorhynchus mykiss*)	Plasma vitellogenin and decrease in testicular growth	21-d LOEC: ≤0.038 (mm)	2	Non-standard guideline (flow-through) Males only	Jobling et al., 1996
	Weight reduction	22-d LOEC: ≤0.001 (n)	3	Non-standard guideline (flow-through) Females only	Ashfield et al., 1998
NP1.5EO					
Species	**Endpoint**	**Results (mg/L)**	**Validity rating**	**Comment**	**Reference**
Short-term invertebrate toxicity					
Cladoceran *Ceriodaphnia dubia*	Immobilisa-tion	96-h EC_{50}: 0.63	?	*Not checked by eMSCA or Registrant*	England, 1995a
Mysid shrimp (*Americamysis* bahia)	Mortality	48-h LC_{50}: 0.11	2	Guideline: US EPA OPP 72-2 (semi-static) *Not checked by eMSCA*	Hall et al., 1989
Long-term invertebrate toxicity					
Cladoceran (*Daphnia magna*)	Reduction of reproductive output	21-d LOEC: 0.32 (n) 21-d NOEC: 0.1 (n) 21-d EC_{10}: 0.085 (n)	1	Guideline: OECD TG 211 (semi-static) *Not checked by eMSCA*	Unpublished (in dossier)
Cladoceran (*Ceriodaphnia dubia*)	Reproduction	7-d NOEC: 0.28	?	*Not checked by eMSCA or Registrant*	England, 1995a

(cont.)

Table 9 (cont.)

Species	Endpoint	Results (mg/L)	Validity rating	Comment	Reference
Mysid shrimp (*Americamysis* bahia)	Parental (F_0) survival, growth and reproduction and F_1 survival	28-d LOEC: 0.016 28-d NOEC: 0.0077	1	Guideline: EPA OTS 797.1950) (flow-through) *Not checked by eMSCA*	Sousa, 1999
Long-term fish toxicity					
Japanese Medaka (*Ozyrias latipes*)	Testis-ova	85 – 110-d LOEC: ≤ ca. 0.057 (mm)	2	Non-standard guideline (semi-static) 4-NP was detected in treatment groups at 48 h	Metcalfe et al., 2001
Rainbow Trout (*Oncorhynchus mykiss*)	Reduced testicular growth and development	21-d LOEC: ≤0.122 mg/L (n)	2	Non-standard guideline (semi-static) Males only. Plasma vitellogenin induced but LOEC hard to determine	Le Gac et al., 2001
Algae and aquatic plant toxicity					
Pseudo-kirchneriella subcapitata	Growth rate	72-h E_rC_{50}: >3.0 (n) 72-h E_rC_{10}: 1.2 (n)	1	Guideline: OECD TG 201 (static) *Not checked by eMSCA*	Unpublished (in dossier)

Notes: mm - mean measured concentration
n - nominal concentration
im - initial measured concentration

7.8.1.1. Fish

Short-term toxicity to fish

The registration dossier on the ECHA dissemination website indicates that acute toxicity of 4-NP, NP1EO and NP2EO was investigated in the Fathead Minnow *Pimephales promelas* under flow-through conditions (TenEyck and Markee, 2007). Test concentrations were verified analytically on samples collected at 0 and 96 h and from half of the tanks at 24, 48 and 72 h, and results appear to have been expressed in terms of measured concentrations (this is not clearly indicated in the RSS; the eMSCA has not checked the original reference on this issue). The procedures followed US EPA guideline OPP 72-1 and the studies are considered reliable with restriction by the Registrants (the study is an adaptation of the test guidance to accommodate the use of newly hatched fish (5 d); there are deviations from the guidance due to the age/size of the fish used including a reduced acclimation period of only 24 hours). The 96-h LC_{50} values were 0.136, 0.218 and 0.323 mg/L for 4-NP, NP1EO and NP2EO, respectively.

In addition, Coady et al. (2010) mention one additional study (not mentioned in either the REACH registrations or ECHA, 2013): Yoshimura (1986) reported a 48-h LC_{50} of 3 mg/L for Japanese Medaka (*Ozyrias latipes*) with NP1EO. The eMSCA has not reviewed this information.

Discussion

The Registrants conclude that the data show that the acute fish toxicity decreases with the degree of ethoxylation, and select the least toxic value (for NP2EO) to represent acute fish toxicity.

The eMSCA notes that this interpretation of the data suggests that NP1EO and NP2EO are around 1.6 and 2.5 times less acutely toxic than 4-NP, respectively. However, the reliability of this comparison is uncertain since the accuracy and representivity of the analytical measurements is unknown, and the results are all of the same order of magnitude (so may also reflect biological variation).

If the data are expressed on a molar basis, the 96-h LC_{50} values become 0.0006, 0.0008 and 0.001 moles/L, for 4-NP, NP1EO and NP2EO, respectively. Therefore whilst a trend is still apparent, the relative differences in toxicity are lower (NP1EO and NP2EO are actually 1.3 and 1.7 times less acutely toxic to fish than 4-NP, respectively).

The eMSCA also notes that other fish species are more acutely sensitive to 4-NP than *Pimephales promelas*. For example, a 96-h LC_{50} of 0.017 mg/L has been reported for Winter Flounder *Pleuronectes americanus* (Lussier et al., 2000). In drawing from the 4-NP data set, the Registrants should avoid selectivity and ensure that they take account of the whole data base. Assuming that the relative difference in toxicity observed for *P. promelas* may also apply to *Pl. americanus*, the 96-h LC_{50} for NP1EO and NP2EO could be around 0.03 and 0.04 mg/L, respectively.

Long-term toxicity to fish

The registration dossiers do not include a standard test guideline study for long-term fish toxicity (which is an Annex IX requirement for substances manufactured or imported in quantities of 100 tonnes or more). However, the registration dossier on the ECHA dissemination website includes RSS for two long-term fish studies for constituents of the registered substance, neither conducted to current standard test guidelines, but both considered reliable with restriction by the Registrants:

- Balch and Metcalfe (2006) investigated the effects of NP1EO on the Japanese Medaka (*Oryzias latipes*). The test substance was a "high purity experimental preparation" (actual purity not stated). The Registrants consider the study to be reliable with restrictions. It is a type of fish sexual development test (FSDT), although does not follow OECD TG 234 (for differences, see Table 10).

  Fish were exposed for 100 days post-hatch under semi-static conditions (test medium was completely renewed every 48 h, with the exception of the first two weeks when 15–20% of the test water was left so that the young fish did not need to be physically handled) to nominal concentrations of 0.010, 0.030, 0.100 and 0.300 mg/L, with acetone as a solvent. Average exposure concentrations measured during the 48-h period between the renewal of test solutions were 0.0035, 0.0105, 0.035 and 0.105 mg/L, representing a loss in nominal concentration of around 65 % in all treatments[13]. A nominal concentration of 1.0 mg/L was also tested but excluded because 100 % mortality occurred within the first week of exposure (which is consistent with the reported acute LC_{50} data for Fathead Minnow reported above). Two negative controls (acetone and clean control) and a positive control (1 µg/L of 17β-estradiol (E2)) were run in parallel for validation purposes. None of the

[13] Test solutions were sampled for chemical analysis in the first and last weeks of exposure, immediately following media renewal and again at 6, 24, and 48 h post-addition (with some further samples collected at random 24 h after renewal throughout the period between the first and last weeks of exposure). The mean exposure concentration (expressed as a percent of nominal) during the 48-h renewal period was determined using a linear best-fit relationship for a ln-normal plot, with the y-axis representing the natural log of the percent nominal concentration and the x-axis being the collection time (h).

The measured concentrations of the random samples collected at 24 h (post-addition) were not excessively variable, considering the changes in fish densities (resulting from growth) and associated changes in feeding rates, fish metabolism and excretory products.

treatments were replicated. The temperature was 27±1 ºC and pH was 7.4-7.8. The dissolved oxygen concentration was not reported but said to be at or near saturation.

Table 10

Comparison of OECD Test Guideline 234 requirements with the study by Balch and Metcalfe (2006)		
Validity criteria	**OECD TG 234**	**Balch and Metcalfe (2006)**
Abiotic parameters	Dissolved oxygen ≥ 60 % air saturation value	✓
	Water temperature differences ±1.5 °C	✓
Hatching success	> 80 %	Not stated
Post-hatch survival	≥ 70 %	✓
Solvent	No effects on survival or endocrine disruption	✓
	Max. final concentration 100 µL/L	Not stated
Test substance exposure start	Newly fertilized eggs (before cleavage of the blastodisc)	✗ (fry within 1 day of hatching)
Test substance exposure duration (days post hatch)	60	100
Flow-through: volume exchange (per day)	≥ 5	Not stated
Semi-static: volume exchange (per day)	≥ 66 %	✗ (50 %)
Photoperiod (light h / dark h)	12-16 / 8-12	✓
Light intensity (lux)	540 - 1 080	Not stated
No. of treatments	≥ 3	✓
No. of replicates per treatment	≥ 4	✗ (1)
No. of animals per treatment	≥ 120 eggs	✓
Validated test species	*Oryzias latipes, Danio rerio* or *Gasterosteus aculeatus*	✓
Endpoints	Sex ratio	✓ (gonadal sex)
	VTG level	✗
	Mortality	✓
	Standard length	✓
	Body weight	✓
	Time to start/end of hatching	Not stated
	Observed abnormalities (deformation, behaviour)	✗
	(Genetic sex)	✗
	(Histopathology)	✓

Exposure was initiated with 150 fry per concentration within 1 day of hatching to ensure at least 50 fish survived to the end of the 100-d exposure period. Survival and growth (total body length and weight of 20 euthanised fish at each sampling point) were monitored in each treatment after 30, 60 and 100 days. Fifty randomly chosen fish from each treatment were sacrificed at the end of the test for assessment purposes. Individual fish were viewed under a dissecting microscope to assess the expression of secondary sex characteristics (shape of the urogenital papilla, dorsal and anal fins and the presence or absence of papillary processes on the anal fin), with results recorded for the expression of either male or female characteristics. After this, fish were placed into Bouin's tissue fixative in preparation for histological examination. Two microscope slides were prepared for each fish with each slide containing between 4 and 6 sagittal sections. Gonadal tissues were examined to verify the gonadal sex of the fish and to monitor for evidence of gonadal intersex (i.e. presence of pre-vitellogenic oocytes within the testes of male fish (''testis–ova'')). The histological survey was performed by a single individual using a blind assessment protocol and the results for treatments exhibiting gonadal intersex were verified by a second person. Verification of the remaining groups was checked by the second scorer by examining randomly selected fish.

There were no statistically significant effects on survival and growth (length and weight) or testis-ova formation at any dose, although the mean length at the end of the study was lower than the controls in all NP1EO treatment groups (with lower weight for the top three). The 100-d NOEC for these endpoints was therefore ≥0.105 mg/L (mean measured concentration) or ≥0.3 mg/L (nominal).

Only one of the 29 histologically confirmed males in the nominal 0.3 mg/L (mean measured 0.105 mg/L) NP1EO treatment group had papillae on the anal fin, which was consistent with a weak estrogenic response (this is a dominant male secondary sex characteristic). Fish that exhibited both feminized and masculinized traits were identified as having ''mixed'' secondary sex characteristics (MSSC). The prevalence of fish exhibiting MSSC (22 %) was statistically significantly (chi square, $p < 0.05$) elevated above the incidence observed in the solvent and non-solvent control treatments (0 and 4 %, respectively; the study authors state that this latter finding reflected a "small number" of errors in assessing male and female traits). No intersex fish were observed in this or any of the other NP1EO treatments. In the nominal 0.1 mg/L (mean measured 0.035 mg/L) NP1EO treatment group, 10 % of fish had mixed secondary sexual characteristics, but this was not statistically significantly different from the controls. In comparison, only 1 of 49 fish was phenotypically male in the E2 treatment (indicating almost complete feminization).

The 100-d Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) for these end points are therefore 0.105 and 0.035 mg/L, respectively (measured concentration).

The registration dossier does not include all of these details.

4-NP was detected at a mean concentration of 1.5 µg/L in the 100 µg/L treatment group, and 3.8 µg/L in the 300 µg/L treatment, but there was no trend over time. For comparison, a full fish life cycle study with this species for 4-NP reported an EC_{10} (equivalent to a NOEC) of 8 µg/L – see Section 7.10.3. The eMSCA therefore considers that the fish were probably not responding to 4-NP, but there might have been additive effects.

- The same study (Balch and Metcalfe, 2006) also exposed *Oz. latipes* fry to "NP4EO" under comparable experimental conditions. The test substance was a

commercial product (Surfonic® N40) with a composition of 1 % NP1EO, 11 % NP2EO, 19 % NP3EO, 18 % NP4EO, 13.5 % NP5EO, 10.5 % NP6EO and 17 % NP>6EO (values read from a graph). Measured concentrations were around 38 % of nominal (based on peak area in a HPLC-mass spectroscopy chromatogram). There were no effects on survival, growth, secondary sexual characteristics or gonadal intersex at any dose, so the 100-d NOEC was ≥1 mg/L (nominal) (≥0.380 mg/L based on measured concentrations). 4-NP was not detected in the test solutions.

- The same study (Balch and Metcalfe, 2006) also includes data for an "NP9EO" test substance, but no effects were observed and these are not considered further for the purposes of this Substance Evaluation.

In addition, the registration dossier on the ECHA dissemination web site includes a fish early life stage test for 4-NP with a reported 91-d NOEC of 0.006 mg/L for growth in Rainbow Trout (*Oncorhynchus mykiss*) (Spehar et al., 2010). The Registrants assume that this is the most sensitive end point for long-term fish toxicity as a worst case.[14]

ECHA (2013) includes further relevant studies that are missing from the registration dossiers, all of which are rated reliable with restrictions (studies using NP>4EO are omitted here[15]):

- Metcalfe et al. (2001) exposed Japanese Medaka (*Oz. latipes*) from 1-day post-hatch to a commercial formulation known as "POE (1–2) nonylphenol" (54 % NP1EO, 44 % NP2EO and 2 % NP3EO) until they were approximately 1.5 cm in length, which occurred at 85 – 110 d after hatch. The study was performed under semi-static conditions with test medium renewal every 48 hours and acetone as a solvent. Three test concentrations were used initially (nominal 0.025, 0.05 and 0.1 mg/L) plus a solvent control; concentrations measured in two of the exposures at 0, 24 and 48 h indicated that NP1EO was present at an average concentration of 57 % of the nominals, and although NP2EO concentrations appeared close to nominal the analytical data were highly variable. 4-NP was detected at <10% of nominal concentrations in samples collected at 48 h, indicating some degradation over time. Each treatment included 60 fish at the start of the experiment. The number of replicates was not stated.

  Fish length, wet weight and condition factor (weight divided by length) were recorded at the end of the test, and gonads were examined histologically using blind scoring techniques to determine phenotypic sex as well as the

[14] The registration dossier on the ECHA dissemination web site also reports that the same study gave a 100-d NOEC for survival and growth of 0.029 mg/L. MSSC were observed, with a LOEC of 0.0087 mg/L (NOEC of 0.0029 mg/L), and phenotypic males had gonadal intersex (testis-ova) at a LOEC of 0.029 mg/L (NOEC of 0.0087 mg/L). These values are based on mean measured concentrations, which were around 30 % of nominal.

[15] For example, Nichols et al. (2001) exposed adult Fathead Minnows (*Pimephales promelas*) to a commercial NPEO called Surfonic® N-95 (with an average chain length of 9.5 EO units) in a continuous flow-through system at concentrations of 0, 0.21, 0.65, 2.1, or 7.9 µg/L (measured) for 42 days. There was no concentration-dependent response on survival, fecundity or plasma concentrations of VTG, 17β-estradiol and testosterone for males or females. The eMSCA notes that the test concentrations were much lower than those that appear to elicit VTG in *O. mykiss* for the shorter chain NPEO substances, and the study did not involve a sensitive life stage. An estrogenic response therefore cannot be excluded on the basis of this study.

presence of testis–ova. All fish with testis–ova were assumed to be genotypic males, but this could not be verified.

The experiment was run twice because on the first occasion the sex of a large proportion of fish (17 % at each concentration) could not be determined because the gonad was not sectioned properly during histologic preparation (i.e. unknown sex). The experiment was repeated using the highest concentration only plus a control. Of thirty fish identified as male, one testis-ova (a single oogonium observed in one section) was observed at the highest test concentration of 0.1 mg/L nominal (approximately 0.057 mg/L mean measured) and no effects on sex ratio were observed. No effects on growth were observed in either test series.

This study supports the findings of Balch and Metcalfe (2006).

- Jobling et al. (1996) performed an *in vivo* screening assay for NP2EO with groups of two-year-old male Rainbow Trout (*Oncorhynchus mykiss*) held in large glass tanks (500 L). The test substance was donated by ICI (Cleveland, UK) and was a mixture of different isomers and oligomers (the actual composition is not provided).

  The experiment involved a group of 15 fish and was conducted in May, when the testes were beginning to grow. Exposure involved a flow-through system for three weeks using a single nominal concentration of 30 µg/L (0.03 mg/L). The concentration in the tanks was monitored using HPLC analysis (weekly samples were taken from the inlet and outlet of each tank). The mean concentration was 0.038 mg/L (127 % of the nominal concentration). Blood plasma was sampled from all fish both initially and at the end of the exposure period and assayed for vitellogenin ("VTG") content using an established homologous radioimmunoassay. Gonads were removed, weighed to the nearest milligram, and their size expressed as a percentage of the total body weight (gonadosomatic index: GSI) in each case. Another group of fish was sacrificed at the onset of the experiment (initial control) to establish initial values for each of the variables measured. The middle portion of one of the testes of each fish was fixed and sectioned at three different positions. After staining in Mayer's hematoxylin, proportions of the different cell types in the sections were assessed. Five germ cell stages were identified: spermatogonia A, spermatogonia B, spermatocyte A, spermatocyte B, and spermatid. The area of the section occupied by cysts containing these cells was expressed as a percentage of the total weight of the testis.

  The plasma VTG concentration was in the range 10^3 – 10^4 ng/mL (values read from a graph) following the three-week exposure to NP2EO, compared to $<10^2$ ng/mL in controls. This was statistically significant ($p < 0.001$). Although all of the fish survived and grew (data not shown), there was a statistically significant decrease in the rate of testicular growth. At the beginning of the experiment, the GSI was 0.2 (i.e. the testes were 0.2 % of the body weight). After three weeks, the GSI of the control groups had risen to 0.9, reflecting the rapid growth rate of the testes during this period of gonadal development. Conversely, the NP2EO treatment had a GSI of around 0.73 (value read from a graph), which was significantly different from the final control value ($p < 0.05$). Histological examination of the testes revealed that most of the fish in the control group had actively developing testes with a predominance of cysts containing spermatocyte A (25–30% by weight), indicative of the beginning of the most active phase of spermatogenesis. Spermatogenesis was more advanced in the fish exposed to NP2EO, as shown by the fact that spermatogonia B was the most prevalent cell type. However, the distributions of the various cell types in the testes of all exposed groups of fish appeared to be skewed, whereas in control fish this distribution was normal (similar observations were made for NP).

Coady et al. (2010) point out that as the test substance composition was not confirmed, the results might possibly reflect contamination (although this is speculative). The eMSCA notes that the reported effects of NP2EO (in terms of VTG induction, GSI and germ cell stages) were very similar to those observed in the same study for 4-NP at the same nominal concentration of 0.03 mg/L (measured concentration: 0.036 mg/L).

- Ashfield et al. (1998) performed a test using newly hatched Rainbow Trout (*O. mykiss*) raised from eggs that were known to be all female. The NP2EO test substance was obtained from Aldrich Chemical, Gillingham, Dorset, UK, and was a mixture of different isomers and oligomers (the actual composition is not stated). Groups of 200 fish were exposed, in duplicate, via a flow-through system to three nominal concentrations of 0.001, 0.01 and 0.03 (Experiment 1) or 0.05 (Experiment 2) mg/L plus a solvent control. The study authors recognised that actual test concentrations may have been lower due to adsorption to glassware, etc., but this was not measured.

  Two experiments were run. In Experiment 1 exposure was terminated after 22 d, and the fish were monitored for a further 86 d. In Experiment 2, fish were exposed for 35 d and were monitored for a further 431 d. The eMSCA notes that this is a very long time for a study, and the effects of fish loading in the 80 L tanks are not discussed. No compensation was made for mortalities. Weight and length of the fish were measured at regular intervals during development, and relative ovary size was measured at study termination. The condition factor of each fish was calculated [(100 × weight)/length], and ovaries also weighed on day 466 of Experiment 2 to determine the ovosomatic index (OSI). No other end points were monitored.

  On day 108 of Experiment 1 all fish exposed to NP2EO displayed a statistically significant reduction in body weight relative to the control fish, giving a LOEC of 0.001 mg/L. A significant reduction in length and weight was also observed from day 55 of Experiment 2 in fish exposed to 0.001 and 0.01 mg/L. This reduction was sustained through to day 84, but the fish recovered and no reduction was observed after this period. The highest dose of NP2EO in Experiment 2 (0.03 mg/L) caused no effects on either weight or length at any time. No effect of NP2EO on condition factor or OSI was observed.

  Similar effects on growth were observed with 4-NP but these were sustained for longer in Experiment 2, and there was an effect on OSI at 0.03 mg/L.

  The lack of consistency between Experiments 1 and 2, and the lack of a clear dose-response in Experiment 2 make interpretation difficult. Whilst the effects may have been due to chemical exposure, it is possible that tank effects were also involved. The eMSCA does not consider the LOEC to be reliable, and notes that this study does not provide any information about effects if exposure had been continuous throughout the study period.

- Dussault et al. (2005) exposed immature (ca. 45 g) *O. mykiss* to NP1EO (purity >95 %) for 21 days in a flow-through system. Nominal concentrations were 0.001, 0.003, 0.01, 0.03 and 0.1 mg/L, and there was a solvent control (0.01 % ethanol) and a positive control of 0.1 µg/L E2 (NP and NP1EC were also tested separately as part of the same study). There were two 50 L tanks per treatment, each containing six fish. Pooled water samples were collected weekly for analysis, and the mean measured exposure concentrations were 0.0008 (n=1), 0.0039 (n=1), 0.0069 (n=3), 0.048 (n=3) and 0.281 (n=3) mg/L, respectively. The top two treatments had higher mean measured concentrations than the nominals, with considerable variation (standard deviations of 0.014 and 0.029 mg/L, respectively). NP1EO was

detected in the solvent control at 0.0008 mg/L.[16] After 21 days, fork length and weight were recorded, a sample of blood was taken for VTG analysis and sex determined for each fish (the method of sex determination is not stated). The paper only reports the VTG levels.

Plasma VTG was induced above the detection limit (not stated) in all twelve fish at the highest nominal NP1EO concentration of 0.1 mg/L (mean measured 0.281 mg/L). Although sex was determined, the paper only reports the mean level of VTG (with standard error) for all fish. The mean VTG level in this treatment group ($2.0\times10^6 \pm 4.3\times10^5$ ng/mL) was of the same order of magnitude as the positive E2 control ($5.8\times10^6 \pm 1.1\times10^6$ ng/mL). No VTG was induced above the detection limit at the next lowest nominal concentration of 0.03 mg/L (mean measured 0.048 mg/L).
NP induced plasma VTG at lower concentrations in an identical test system. The study authors report a relative potency of 0.22 for NP1EO based on their data, but the eMSCA cannot ascertain how this was calculated (since the levels of VTG and proportion of fish induced differed; for example, there was significant loss of fish due to mortality at nominal concentrations of 0.03 mg/L, and VTG was not induced in four fish at the next lowest treatment. One of 12 fish was induced at a nominal concentration of 0.003 mg/L, achieving a plasma VTG level of 5.5×10^2 ng/mL). In addition, the number of analytical measurements of exposure concentration was low, so there could have been more variation in exposure.

The eMSCA has also found an additional study in the academic literature that is not included in either the registration dossier or ECHA (2013)[17]:

- Le Gac et al. (2001) exposed 13-month old male Rainbow Trout (*O. mykiss*) for 3 weeks in February-March 1999 to a commercial product known as Igepal CO-210 (80 % NP1EO and 20 % NP2EO)[18]. The fish had a mean body weight of 306 ± 87 g and 95 % were expected to mature later in the same year. Exposure involved a semi-static renewal regime (half of the test water volume

[16] 4-NP was also detected in test solutions in the range 0.0008 – 0.0047 mg/L (it is not clear which treatments these values relate to); in the NP experiment, no plasma VTG was induced at a nominal concentration of 0.001 mg/L (mean measured concentration of 0.0062 mg/L). NP1EC concentrations in the water control were around 0.0025 mg/L.

[17] Another study by Crago et al. (2015) is potentially relevant. mRNA transcripts were used to measure relative VTG expression in livers of juvenile Rainbow Trout (*O. mykiss*) and adult male Fathead Minnow (*Pimephales promelas*) exposed for 7 days to two concentrations of a mixture of "analytical grade" NP (CAS no. 104-40-5), OP (CAS no. 140-66-9), NPEO (CAS no. 68412-54-4) and OPEO (CAS no. 9036-19-5). A concentration-dependent increase in relative liver VTG mRNA transcripts expression compared with the controls was observed for both species. However, actual purity and ethoxylate chain length distributions are not provided. In the discussion part of the paper, it is stated that "only NP, OP, and one-carbon ethoxylates (four compounds) were used." The eMSCA considers that the reliability of this study is unassignable: the test substance identity is unclear, the reporting of results is confusing (exposure concentrations are inconsistent) and there may have been a contribution from the alkylphenols even though they seem to have been present at relatively low concentrations.

Similarly, Xie et al. (2005) detected a vitellogenic response in an *in vivo* juvenile Rainbow Trout VTG assay following a 7-d exposures to two commercial NPEO-containing surfactants (R-11 and Target Prospreader Activator (TPA)). However, the actual test substance identity is not clear from the paper (results are expressed in terms of nonylphenol). This study attracted critical comment (Kramer et al., 2008) and a response from the authors (Schlenk, 2008), but this did not relate to the NPEO portion of the study.

[18] This is referred to as NP2EO in the paper, but the eMSCA thinks it would be more appropriate to identify it as NP1.5EO.

renewed once per day), with ethanol as a co-solvent. The test concentrations were nominally 450 and 1 800 nmol/L, plus a solvent control (0.004 % ethanol); assuming that the concentrations were based on the average molecular mass of the mixture, the two nominal test concentrations were presumably 0.122 and 0.491 mg/L (this information is not provided in the paper). The test concentration was analysed but NP1EO and NP2EO could not be resolved separately. The measured concentration at the highest dose varied around 580 nmol/L (32 % of nominal) thirty minutes after renewal (in three tanks) and around 120 nmol/L (7 % of nominal) one day later (in three tanks). The concentration at the lower exposure varied around 150 nmol/L (33 % of nominal) thirty minutes after renewal (in three tanks) and there was no determination after 24 h. Water quality was checked every day and temperature, oxygen, pH, ammonia, nitrate and nitrite were "adequate".

Four 200 L tanks were used for each treatment (five for the solvent control), with six fish per tank. Blood was collected for VTG analysis at the end of the exposure, and fish were sacrificed 4.5 weeks after the end of exposure for histological examination of the testes (85 % of the control fish gonads were still immature at the end of the exposure, so this period allowed further development to take place).

Fish weight was unaffected by exposure. However, a significant inhibitory effect of the substance on testicular growth and development was observed. When compared to the solvent control group, the mean gonado-somatic index (GSI) values decreased by 18% and 40% at the lowest and highest test substance concentration, respectively. Histological analysis showed that the control testes had reached more advanced stages of spermatogenesis (39.5 % in stages I–III and 60.5 % in stages IV–VI) than in fish exposed to the lowest test concentration (52 % in stages I–III and 48% in stages IV–VI). This effect was more obvious at the highest concentration (68.2 % in stages II–III and 31.8 % in stages IV–VI). The eMSCA therefore assumes that the LOEC was ≤0.122 mg/L (nominal); the measured concentration would be much lower (less than half this value), but the lack of reporting makes it difficult to estimate a time-weighted mean.

No significant VTG induction was observed at the lowest test concentration, with levels remaining close to or under the limit of detection. However, in both a preliminary 9-day exposure trial with juvenile fish and the main experiment, the highest concentration induced 200–300-fold increases in mean blood plasma VTG concentration. Effects were similar in juvenile females in the preliminary experiment. The eMSCA therefore assumes that the LOEC for VTG lies in the range ≤0.122-0.491 mg/L (nominal); the measured concentration would be much lower, but the lack of reporting makes it difficult to estimate a time-weighted mean.

The *in vitro* effects on basal and insulin-like growth factor-1 (IGF-I) stimulated DNA synthesis by early germ cells were also studied. Testicular cells obtained at different stages of spermatogenesis were cultured for 4.5 days in the presence of the test substance with and without IGF-I. 3H-thymidine (3H-Tdr) incorporation was measured and 125I-IGF-I specific binding was determined. Basal and IGF-I-stimulated 3H-Tdr incorporation (i.e. DNA synthesis) was decreased by the test substance (at 30 µmol/L, but not 10 µmol/L) whilst 1-100 nmol/L 17β-estradiol had no effect. The study authors suggested that beside effects on sex steroid production or action, the substance could act on germ cells by disrupting cell membrane receptivity to peptide hormones like growth factors. However, the eMSCA estimates that 30 µmol/L is about 8.2 mg/L, which exceeds the critical micelle concentration of NP1-2EO as well as the acute fish LC_{50}. The observed "effect" in the *in vitro* part of the study might therefore be irrelevant so is not considered further in this evaluation.

The eMSCA considers the *in vivo* part of this study to be reliable with restrictions, because it did not follow a formal test guideline and there was a significant loss of test concentration.

Discussion

Recommendation: The Registrants should include RSS for the five additional fish studies, and provide all relevant effect data in the RSS for the Balch and Metcalfe (2006) study.

The Registrants select the 91-d NOEC of 0.006 mg/L for 4-NP with Rainbow Trout (*O. mykiss*) to represent the long-term toxicity to fish for the registered substance. The eMSCA recognises that the selected value is the lowest long-term fish NOEC used in the registration dossiers for 4-NP and also the Risk Assessment Committee opinion for the restriction of NPEO in textiles (ECHA, 2014). However, the ECHA decision for 4-NP under Substance Evaluation includes a request to consider other effects on fish (e.g. egg hatchability) that may reduce the fish NOEC further.

Further discussion is presented in Section 7.10.

7.8.1.2. Aquatic invertebrates

Short-term toxicity to invertebrates

The registration dossier on the ECHA dissemination website includes the following information[19]:

- The acute toxicity of NP1EO and NP2EO was investigated in the cladoceran *Ceriodaphnia dubia* under semi-static conditions (TenEyck and Markee, 2007). There is no information about whether the study was performed according to GLP. Initial test concentrations were measured analytically. The procedures followed US EPA guideline 600/4-90/027F and the studies are considered reliable with restriction by the Registrants (eMSCA note: the RSS does not explain why they are not fully valid). The 48-h LC_{50} values were 0.328 and 0.716 mg/L for NP1EO and NP2EO, respectively.
- Two further acute studies for NP2EO have been performed on the cladoceran *Daphnia magna*. In a static study said to be equivalent or similar to OECD TG 202, Sun and Gu (2005) reported a 24-h LC_{50} of 0.56 mg/L. The eMSCA notes that the normal test duration for this species is 48 hours, so this study could under-estimate the toxicity. Maki et al. (1998) reported a 48-h LC_{50} of 0.148 mg/L in a study performed to ISO 6341 (1982). The RSS does not mention the exposure conditions, and the eMSCA has not checked the original reference. Both studies are considered reliable with restriction by the Registrants.
- Hall et al. (1989) reported a 48-h LC_{50} of 0.11 mg/L for a test substance referred to as "NP1.5EO" with the saltwater mysid *Mysidopsis* [*Americamysis*] *bahia* in a semi-static study following US EPA Guideline OPP 72-2. Test concentrations were measured according to the RSS, and the study is rated as reliable with restrictions by the Registrants.

In addition, Coady et al. (2010) mention one additional study (not included in the REACH registrations): England (1995a) reported a 96-h EC_{50} for NP1.5EO of 0.626 mg/L for immobilisation in the cladoceran *Ceriodaphnia dubia*. The reliability cannot be evaluated by the eMSCA as the original report is not available, but Coady et al. (2010) appear to

[19] Servos (1999) and Vlaardingen et al. (2003) mention some older data reporting effects at higher concentrations (e.g. Ankley et al., 1990). These have not been considered by the eMSCA.

have had access to the report because they provide the composition of the test substance as 41.5 % NP1EO, 37.3 % NP2EO, 11.1 % NP3EO, 3.8 % NP4EO & 3.8 % NP. In addition, the usual test duration for this species is 48 h for acute end points.

Discussion

The Registrants conclude that invertebrate acute LC_{50} values range from 0.11 to 0.716 mg/L, which is comparable to the fish data included in the registration dossier. The eMSCA notes that the apparent lack of analytical monitoring during some of the studies means that it is not possible to know whether the nominal (or initial) concentrations were maintained. The reported $L(E)C_{50}$ values might therefore under-estimate acute toxicity to invertebrates. One study suggests that NP2EO is about half as acutely toxic to invertebrates as NP1EO, although concentration losses might affect the comparison.

The Registrants select the *D. magna* 48-h LC_{50} of 0.148 mg/L for NP2EO (Maki et al., 1998) for PNEC derivation. The eMSCA notes that this is not necessarily the most sensitive end point. NP1EO would be expected to be more toxic to *D. magna* than NP2EO, but a study is not available. The eMSCA has therefore estimated the 48-h LC_{50} for NP1EO using two methods[20]:

a) The preferred 48-h LC_{50} for 4-NP with *D. magna* cited by the REACH Registrants of that substance is 0.085 mg/L; the value for NP1EO would therefore be expected to lie in the range 0.085 – 0.148 mg/L. Based on a simple interpolation comparing predicted log K_{OW} (KOWWIN v1.67 estimate) with LC_{50} values on a molar basis, the *D. magna* 48-h LC_{50} for NP1EO is predicted to be around 0.11 mg/L.

b) From the study with *C. dubia*, NP1EO is 2.2 times more acutely toxic than NP2EO (for fish, the ratio is 1.5; see Section 7.8.1.1). Applying the same factor to the NP2EO data for *D. magna* suggests that the 48-h LC_{50} for NP1EO would be around 0.07 mg/L for this species, although this would be an over-estimate of toxicity based on the 48-h LC_{50} reported for 4-NP.

The eMSCA therefore suggests that an appropriate *D. magna* acute value for NP1EO is 0.11 mg/L.

It is possible to use the additivity equation provided in the CLP Regulation to estimate a D*. magna* 48-h LC_{50} for the commercial substance:

$$ATE_{mix} = 100 / \sum_{n} (C_i / ATE_i)$$

where:

ATE_{mix}= Acute Toxicity Estimate of the mixture

ATE_i = Acute Toxicity Estimate of ingredient i

C_i = concentration of ingredient i (% w/w) [40 % w/w]

i = the individual ingredient from 1 to n

n = the number of ingredients

Assuming that the 48-h LC_{50} for NP1EO and NP2EO is 0.11 and 0.148 mg/L, respectively, and that these two constituents are present in the registered substance in equal amounts of around 40 % w/w, with additive toxicity and ignoring any contribution from other constituents, the combined mixture LC_{50} would be around 0.16 mg/L using the additivity equation provided above.

[20] The eMSCA has not used quantitative structure-activity relationships to predict toxicity as these generally rely on the log K_{OW} value, which is of uncertain reliability for this type of substance.

The additivity equation can also be used to compare the measured *C. dubia* data for NP1EO and NP2EO with that for the mixture tested by England (1995a). Using the reported composition of 41.5 % NP1EO, 37.3 % NP2EO & 3.8 % NP, a 96-h EC_{50} for 4-NP of 0.069 mg/L (England, 1995b) and assuming that the other constituents were not toxic to *C. dubia*, the mixture 96-h EC_{50} can be calculated to be 0.43 mg/L. This is the same order of magnitude as the measured value (it is about 1.5 times lower, so it is possible that NP3-4EO also have relevant toxicity).

The presumed commercial mixture has a 48-h LC_{50} of 0.11 mg/L for *Americamysis bahia*. Since NP1EO is likely to be the most toxic constituent, it will presumably have a lower LC_{50}, but it is not possible to provide a reliable estimate. However, an approximation can be made using the additivity equation and the same composition assumptions as above, together with the ratio of acute *C. dubia* toxicities for NP1EO and NP2EO of 2.2. This would give a 48-h LC_{50} of 0.064 mg/L for NP1EO and 0.128 mg/L for NP2EO. The 48-h LC_{50} for this species with 4-NP reported in the risk assessment report prepared under the Existing Substance Regulation (EC) No. 793/93 was 0.043 mg/L (Ward and Boeri, 1990), which is broadly consistent with these values.

Long-term toxicity to invertebrates

The registration dossier on the ECHA dissemination website includes one long-term toxicity study for the cladoceran *Daphnia magna* (Unpublished), which was performed in accordance with GLP and is considered reliable without restriction by the Registrants. The RSS indicates that the test substance identity was the same as the substance in Section 1 of the registration dossier. The Registrants have clarified that the test substance was Berol 259, which contained around 80 % NP1EO and NP2EO, the remaining 20 % being longer chain ethoxylates up to NP6EO.

This OECD TG 211 study used semi-static conditions, with test solution renewal three times per week. Nominal concentrations were 0.01, 0.032, 0.1, 0.32 and 1 mg/L. Test concentrations were analytically verified by HPLC on days 0, 5 and 16 (fresh media, 0 h) and on days 2, 7 (old media, 48 h) and 19 (old media, 72 h). The recoveries in the fresh media (0 h) and in the old media (48 or 72 h) were determined to be within ± 20 % of the nominal values throughout the test at the nominal test concentrations of 0.1 to 1 mg/L, so all effect values were based on the nominal concentrations.

All parental daphnids died at 1 mg/L. Reproductive output was statistically significantly reduced at 0.32 mg/L compared to the control. The percentage of dead juveniles relative to the total number produced was in the range of 2 to 3 % at 0.01 to 0.1 mg/L, and 14 % at 0.32 mg/L. The coefficient of variation of the number of living offspring produced per parent was 7 % in the control, which was comparable to the two lowest test concentrations. At 0.1 and 0.32 mg/L the coefficients of variation were 17 and 22 % (consistent with the maximum variation allowed for in the controls (≤25 %)).

The 21-d LOEC, NOEC and EC_{10} based on the reduction of the reproductive output as the most sensitive effect were 0.32, 0.1 and 0.0853 mg/L, respectively.

Sousa (1999) performed a GLP-compliant Chronic Mysid Toxicity Test (EPA OTS 797.1950) using the mysid shrimp *Mysidopsis [Americamysis] bahia.* [21] The study was

[21] This study was missing from the REACH registrations when the SEv work began but mentioned in Coady et al. (2010). The initial output of this SEv was therefore a draft decision asking the Registrants to either perform a study of long-term toxicity to mysid shrimps using the registered substance (or NP1EO as the likely most toxic constituent) or obtain access to the Sousa (1999) test report. This was considered necessary to support both hazard classification and PNEC derivation. The Registrants were able to gain access to the original study report during the commenting period

performed for the APE Research Council and the Registrants rate this study as reliable without restriction. This is a key study, but the eMSCA has not reviewed the study report[22]. The Registrants indicate that the test substance composition closely resembled that of the registered substance but also contained 3.8 % of 4-NP[23]. The organisms were exposed to nominal test substance concentrations of 0, 0.0023, 0.0047, 0.0094, 0.019 and 0.037 mg/L for 28 days under flow-through conditions. Exposure concentrations were analytically confirmed by HPLC on day 0, 7, 14, 18, 21 and 28, and were between 91.9 and 106 % of nominals. No effects were observed on mortality, but reproductive success was significantly different from the control group for the two highest concentrations. Body length/weight was also affected at the highest concentration. The 28-d LOEC and NOEC were 0.016 and 0.0077 mg/L, respectively, for parental (F_0) survival, growth and reproduction and F_1 survival (based on mean measured concentrations).

In addition, Coady et al. (2010) mention one additional study (not included in either the REACH registrations or ECHA, 2013 & 2014):

- England (1995a) reported a 7-d $NOEC_{reproduction}$ of 0.285 mg/L for NP1.5EO with the cladoceran *Ceriodaphnia dubia*. The reliability cannot be evaluated by the eMSCA as the original report is not available, but Coady et al. (2010) appear to have had access to the report because they provide the composition of the test substance as 41.5 % NP1EO, 37.3 % NP2EO, 11.1 % NP3EO, 3.8 % NP4EO and 3.8 % NP.

The eMSCA has not assessed any studies with NP>4EO, but notes that Oliveira-Filho et al. (2009) reported a NOEC for fecundity of < 0.01 mg/L for the freshwater snail *Biomphalaria tenagophila* during an 8-week trans-generation exposure to NP9.5EO. Based on the eMSCA's experience with snail testing of bisphenol-A under the Existing Substances Regulation, snail reproduction can be highly variable and influenced by several factors. Further information on mollusc toxicity has been requested for 4-NP under Substance Evaluation, and the eMSCA considers that those data should be evaluated before deciding whether similar information might be useful for short chain NPEO.

Discussion

The Registrants conclude that the 21-d NOEC (reproductive output) for *D. magna* with the registered substance is 0.1 mg/L. The 21-d EC_{10} is lower at 0.0853 mg/L but the Registrants do not select this as the preferred measure from this study. The ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b (Version 3.0, February 2016) indicates that an EC_{10} is generally preferred to the NOEC as it is statistically derived from the entire dataset, and less dependent on test design considerations than the NOEC.

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and included a robust study summary in their registration update as part of their response. The request was therefore deleted.

[22] Coady et al. (2010) indicate that it was performed in the same laboratory and used the same method as a study with 4-NP that gave a 28-d NOEC of 0.0039 mg/L (Ward & Boeri, 1991). This latter study was considered to be reliable in the risk assessment of 4-NP performed by the eMSCA under the Existing Substances Regulation (EC) No. 793/93, so the study with NP1.5EO is likely to be similarly reliable.

[23] Coady et al. (2010) presumably had access to the test report because they give the test substance composition as 41.5 % NP1EO, 37.3 % NP2EO, 11.1 % NP3EO, 3.8 % NP4EO and 3.8 % NP.

As discussed above, the eMSCA predicts that the 48-h LC_{50} for *D. magna* for the whole substance is likely to be around 0.16 mg/L. The LOEC from the *D. magna* reproduction test (0.32 mg/L) is approximately twice this predicted acute LC_{50}, and it also exceeds the reported acute LC_{50} for NP2EO cited by the Registrants. This could imply a problem with either this long-term study or the acute data for NP2EO. There could also be clone differences between the studies.

For comparison, the lowest long-term cladoceran 21-d NOEC for 4-NP cited in ECHA (2014) is 0.053 mg/L for *D. magna*, and the acute:chronic ratio is 1.6 (0.085:0.053). If this is applied to the acute LC_{50} for NP2EO, the equivalent estimated chronic NOEC would be 0.09 mg/L (0.148/1.6). Based on a simple interpolation comparing log K_{OW} (KOWWIN v1.67 estimate) with these NOEC values on a molar basis, the *D. magna* 21-d NOEC for NP1EO is predicted by the eMSCA to be around 0.07 mg/L.

There will be uncertainty in these estimates because NOECs depend on the spacing of test concentrations used in a particular test (and the control variability) (a comparison using EC_X values might be more reliable). Nevertheless, using the same assumptions and additivity equation as above, the mixture NOEC would be 0.098 mg/L based on the NOECs for NP1EO and NP2EO. This is very similar to the reported measured value, but demonstrates that the NOEC for NP1EO is likely to be below 0.1 mg/L.

The eMSCA therefore considers that the available study potentially underestimates long-term *Daphnia* toxicity for the main constituents of the registered substance. The estimates made above rely on the acute study for NP2EO being reliable, and assume that toxicity is linked to narcosis (hydrophobicity). However, since NPEOs are surfactants this relationship may not be reliable. Separate chronic *Daphnia* tests with NP1EO and NP2EO may be useful to clarify the level of toxicity they cause, although this is not essential for risk management purposes.

The cladoceran *Ceriodaphnia dubia* is slightly less chronically sensitive than *Daphnia*. The acute:chronic ratio for NP1.5EO with the former species is 2.2 (i.e. 0.626/0.285). Applying this factor to the available acute *C. dubia* studies for NP1EO and NP2EO would suggest that reproduction NOECs for these two constituents would be around 0.15 and 0.33 mg/L, respectively, for this species.

The study by Sousa (1999) on mysid reproduction for NP1.5EO is the most sensitive long-term invertebrate result, with a 28-d NOEC of 0.0077 mg/L. Mysids were the most sensitive taxon in the 4-NP data set used in ECHA (2014) with a 29-d NOEC of 0.0039 mg/L[24], although this was the same order of magnitude as the most sensitive fish NOECs. 4-NP would appear to be twice as toxic as NP1.5EO on a concentration basis, or 1.6 as toxic on an approximate molar basis; this comparison is uncertain since the actual NOEC depends on the choice of test concentrations in each test. Considering biological variability, the eMSCA suggests that NP1.5EO could be almost as potent as 4-NP to mysids. In addition, NP1EO and NP2EO may be individually more toxic than suggested by this mixture NOEC, but it is not possible to estimate this with any certainty.

[24] The 4-NP data set in ECHA (2014) contains two other NOECs for invertebrates that are lower than the *Daphnia* NOEC (0.040 mg/L for the oligochaete *Caenorhabditis elegans*, and 0.042 mg/L for the insect *Chironomus tentans*). Additional testing with these species for NP1-2EO could also produce lower invertebrate NOECs than the one selected by the Registrant.

7.8.1.3. Algae and aquatic plants

The registration dossier on the ECHA dissemination website summarises one algal toxicity study on NPEO (Unpublished), which was conducted in accordance with GLP and is considered reliable without restriction by the Registrants. The RSS indicates that the test substance identity was the same as the substance in Section 1 of the registration dossier. The CSR uses the term "NPE-2" (i.e. NP2EO) in some parts of the description of this study, but the eMSCA believes that this is a typographical error. The Registrants have confirmed to the eMSCA that the test substance composition was the same as that for the long-term *Daphnia* study of (Unpublished) (see Section 7.8.1.2).

The toxicity of NPEO to the green alga *Pseudokirchneriella subcapitata*[25] was determined according to OECD TG 201 under static conditions. Nominal test concentrations were 0.0938, 0.188, 0.375, 0.75, 1.50 and 3.0 mg/L. The measured concentrations at the beginning and end of the test were in the range of 94 - 98 % and 80 – 95 % of the nominal values, respectively. All effect values are therefore based on nominal concentrations. The 72-h E_rC_{50} was > 3.0 mg/L and the 72-h NOE_rC was 1.5 mg/L (nominal). The 72-h E_rC_{10} was 1.22 mg/L (nominal).

Discussion

The Registrants conclude in their CSRs that a 72-h E_rC_{50} of 3 mg/L and 72-h NOE_rC of 1.5 mg/L can be used for PNEC derivation for NPEO.

The eMSCA agrees that this information is acceptable. The study indicates that algae are less sensitive in chronic studies than fish or invertebrates. No data are available for either NP1EO or NP2EO separately; NP1EO would be expected to be slightly more toxic than NP2EO since it is slightly more hydrophobic.

The Registrants also present three algal studies for 4-NP in their registration dossier, but do not discuss this information. One study is available for the same species as used for the NPEO study (*P. subcapitata*), giving a 96-h E_rC_{50} of 0.41 mg/L. Another study for *Desmodesmus subspicatus* gives a 72-h E_rC_{50} of 0.323 mg/L and a 72-h E_rC_{10} of 0.025 mg/L. This is the lowest long-term algal result for 4-NP cited in ECHA (2014), and is more sensitive than the long-term *Daphnia magna* data, but less sensitive than long-term fish and mysid data for 4-NP. Additional testing with *Desmodesmus subspicatus* for NP1-2EO may therefore produce a lower algal E_rC_{50}/NOEC than the one selected by the Registrant. However, it is still likely to be higher than the short-term data for fish and invertebrates (i.e. since NP1EO would not be expected to be more toxic than 4-NP, the 72-h E_rC_{50} for NP1EO would be >0.323 mg/L). Due to the lack of reliable long-term data for fish and invertebrates for NP1-2EO, it is possible that such a test with *Desmodesmus subspicatus* may be useful for assessing chronic ecotoxicity. This is considered further under Section 7.8.4.

7.8.1.4. Sediment organisms

Not evaluated.

7.8.1.5. Other aquatic organisms

Not evaluated.

[25] Formerly *Selenastrum capricornutum* but sometimes referred to as *Raphidocelis subcapitata*.

7.8.2. Terrestrial compartment

Not evaluated.

7.8.3. Microbiological activity in sewage treatment systems

Not evaluated.

7.8.4. PNEC derivation and other hazard conclusions

The surface water PNECs derived by the Registrants are presented in Table 11.

Table 11

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS			
Compartment	**PNEC (µg/L)**	**Justification**	**eMSCA remarks**
Freshwater	0.8	An assessment factor (AF) of 10 is applied to the lowest chronic value of 0.0077 mg/L for NP1-2EO	See main text below
Marine water	0.8	As for freshwater	
Intermittent releases to water	1.48	An assessment factor of 100 was applied to the lowest available valid acute value of 0.148 mg/L (48-h LC_{50} for *Daphnia magna*).	See main text below

Note: When this evaluation began, the Registrants had derived a $PNEC_{water}$ of 1.6 µg/L for freshwaters, based on data for 4-NP, divided by a Toxicity Equivalency Factor for of 0.37 (based on Coady et al., 2010). The marine $PNEC_{water}$ was the derived by dividing the freshwater value by a factor of 10.

When the SEv process began, the PNEC derivation was based on data for 4-NP and a Toxicity Equivalency Factor (TEF) to extrapolate to NPEO. The registration dossier indicated that ecotoxicity declines with increasing ethoxylate chain length, due to declining hydrophobicity (indicated by a decrease in the predicted log K_{OW}). Since 4-NP is more toxic than the NPEO constituents of the registered substance (based on narcotic activity), the Registrants argued that data on 4-NP could be used to predict the hazard properties of NPEO as a worst case. 4-NP is a data-rich substance so in principle the eMSCA thinks that this would be an acceptable approach provided that the selected 4-NP information is appropriate and the TEF is reliable. The eMSCA had the following observations:

- *4-NP information*: The Risk Assessment Committee opinion on the proposed restriction of NPEO in textiles (ECHA, 2014) concluded that based on traditional apical endpoints, the $PNEC_{water}$ for 4-NP is 0.004 mg/L [0.4 µg/L], but noted that this might not take due account of endocrine disruption (ED) effects. Applying the TEF of Coady et al. (2010) to this value, the $PNEC_{freshwater}$ for the registered NPEO substance would become ≤1.1 µg/L, which is slightly lower than that originally proposed by the Registrants.

  However, ECHA (2014) noted that additional information could lower the $PNEC_{water}$ (e.g. it might be around 0.1 µg/L – see appendix of ECHA, 2014). Due to these concerns, the eMSCA performed a Substance Evaluation for 4-NP in 2014/5. A decision has been issued to the 4-NP Registrants to request information for several fish and aquatic invertebrate toxicity end points which may reduce the $PNEC_{water}$ further in due course. A final conclusion should therefore await the evaluation of this information.

- *TEF reliability*: The TEF value of 0.37 is based a review by Coady et al. (2010), who concluded that NP1EO and NP2EO are a factor of 2.7 times less toxic to aquatic organisms on average than 4-NP, which supports the use of a more conservative TEF of 0.5 as used by Environment Canada.

  Coady et al. (2010) derived their TEF based on an analysis of ecotoxicity data obtained in the same laboratory and with the same methods for both 4-NP and NP1.5EO. However, only seven study 'pairs' are available, of which three consider acute end points only, which are not relevant for a PNEC derivation based on long-term end points. The remaining four studies are:

- Balch & Metcalfe (2006) for secondary sexual characteristics in Japanese Medaka (*Oz. latipes*) exposed for 100 days,
- Sousa (1999)/Ward & Boeri (1991a) for mysid shrimp reproduction,
- England (1995a&b) for reproduction in *Ceriodaphnia* (a less sensitive species than mysids) and
- Dussault et al. (2005) for VTG induction in male Rainbow Trout (*O. mykiss*) after 21 days exposure.

The ratio of 4-NP to NPEO toxicity in each case (based on NOECs) is 0.09, 0.51, 0.31 and 0.27, with an average of 0.295 (i.e. "NP1.5EO" is around 3.4 times less toxic than 4-NP). However, the reliability of NOEC comparisons depends on the concentration spacing – if the two studies in a pair used different test concentrations, the comparison may be misleading (comparisons based on EC_{10} values may be preferable). Similarly, comparisons may be confounded by concentration losses in some of the studies, so whether the comparison is based on nominal or measured concentrations might be important. The test substances may also have had differing purities, creating a further source of uncertainty. In addition, only two of the study pairs actually provide data on relevant apical end points. Consequently, the eMSCA believes that these data are not sufficient for deriving a robust TEF.

Coady et al. (2010) supplemented their analysis with QSAR estimates of ecotoxicity using ECOSAR v1.00 (in US EPA EPISuite v.4.0) and log K_{OW} values of 4.48, 4.2 and 4.2 for 4-NP, NP1EO and NP2EO, respectively. However, these K_{OW} values are different to those cited by the Registrants, and as pointed out in Section 7.4, the eMSCA considers that predictions based on the K_{OW} may be unreliable for a surface active substance (for which K_{OW} cannot be measured in the conventional sense). The eMSCA has therefore not considered this information further as it would receive a low weighting in the assessment.

In addition, Coady et al. (2010) considered mixture studies, but since these only concerned acute end points, they are not relevant for a PNEC derivation based on long-term end points.

A final consideration is whether an "average" TEF from different taxonomic groups should be applied to data for a single species. Mysids have very similar sensitivity towards NP1.5EO and 4-NP in long-term tests (i.e. a 28-d NOEC of 7.7 and 3.9 µg/L, respectively) and some of the available studies suggest that both substances may actually have similar levels of long-term toxicity towards fish (e.g. Jobling et al., 1996). If a 4-NP fish NOEC is used as the basis of the PNEC for the registered substance, the eMSCA does not think that it is appropriate to apply a TEF derived from other taxonomic groups. Nevertheless, the only long-term fish data considered in Coady et al. (2010) are from the study of Balch and Metcalfe (2006), and as discussed in Section 7.8.1.1, there is some doubt about the data comparison due to the loss of concentration in this study.

For all of these reasons, the eMSCA does not consider the TEF derivation to be sufficiently reliable for PNEC derivation.

It may be possible to produce a more reliable TEF if further ecotoxicity data were collected on the registered substance (and/or its more toxic constituents) for direct comparison with similar long-term data for 4-NP. However, this is not proportionate given the resource requirements and possible animal welfare considerations.

The Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.10: Characterisation of dose [concentration]-response for environment of May 2008) proposes that data on freshwater or marine fish, crustacea and algae can be used interchangeably for evaluation of the risks to both the fresh and saltwater compartments, and ECHA (2014) considered that it was appropriate to merge the freshwater and marine data sets for aquatic PNEC derivation for 4-NP. There is no reason to expect that

NP1-2EO will behave significantly differently to 4-NP in terms of relative ecotoxicity between taxonomic groups. The 28-d NOEC for mysids with NP1.5EO (0.0077 mg/L) is the lowest effect concentration in the registered substance data set. Since reliable chronic fish data are not available, an assessment factor of 50 could be considered for PNEC derivation; however, the resulting PNEC would be lower than that derived for 4-NP, which is not supported by the data demonstrating that 4-NP is the more toxic substance for most organisms. The eMSCA notes that a chronic mysid NOEC is actually the lowest value in the current data set for 4-NP, and is very similar to the lowest fish NOEC currently available for that substance.

The eMSCA therefore considers that it is appropriate to use an assessment factor of 10 with the mysid NOEC for NP1.5EO, giving a $PNEC_{water}$ of 0.77 µg/L for the registered substance for both fresh and marine water compartments. An alternative could be to apply the TEF for mysids of 1.97 (i.e. 7.7/3.9)[26] to the 4-NP PNEC derived with a much larger data set, which would give an almost identical value of 0.78 µg/L. The eMSCA therefore considers that a $PNEC_{water}$ of 0.8 µg/L would be appropriate for risk assessment purposes, which is half the value originally selected by the REACH Registrants, but has since been adopted by them in response to this SEv.

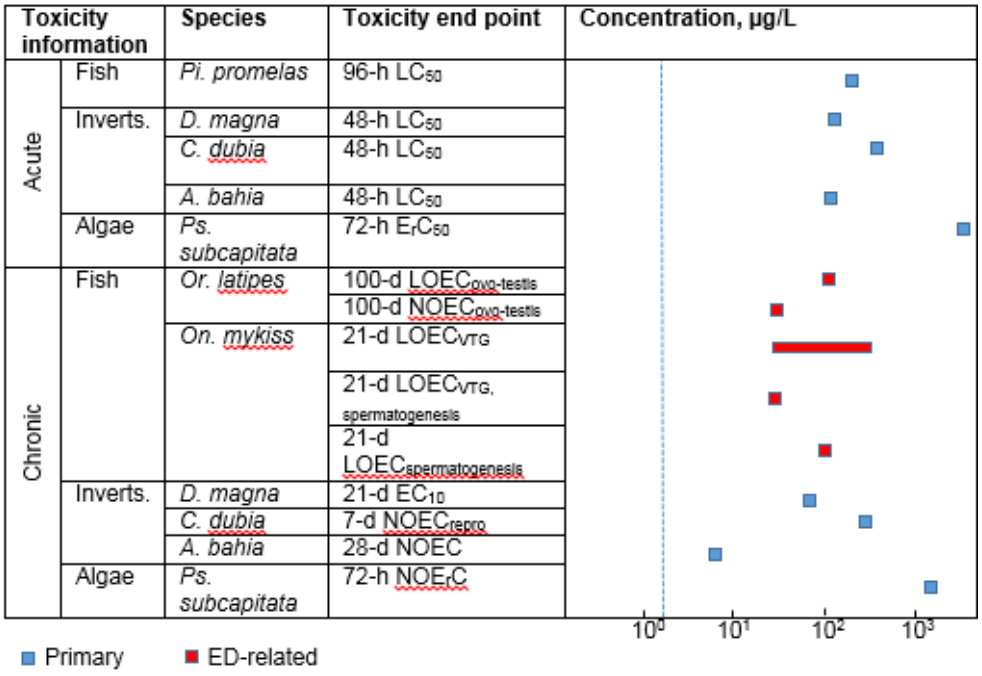
Another factor to consider is whether a PNEC is appropriate for endocrine disrupting substances. Some regulatory groups propose that no safe level should be assumed (e.g. Duis et al., 2014), whereas others believe that risk assessment is appropriate provided certain conditions are met (e.g. Leopold et al., 2017). The European Commission has proposed that for authorisation purposes, no safe level should be assumed unless the applicant can demonstrate otherwise[27]. Since NP1EO and NP2EO appear to interact with the fish endocrine system, it is possible that the PNEC might not be appropriate for risk management purposes since the level of protection it offers against endocrine effects is unclear. This is presented graphically in Figure 1 and considered further in Section 7.10.

In summary, the eMSCA believes that the $PNEC_{water}$ derived by the REACH Registrants for the registered NPEO substance (0.8 µg/L) is broadly acceptable, although the level of protection against endocrine effects is unclear.

[26] Insufficient data are available to derive PNECs for NP1EO and NP2EO separately, but neither is expected to be more toxic to mysids than 4-NP (28-d NOEC: 3.9 µg/L). Therefore, the PNEC for these two constituents would be expected to lie in the range 0.4 – 0.8 µg/L.

[27] Endocrine Disruptors REACH Review, Doc. CA/25/2014 for the 14th Meeting of Competent Authorities for REACH and CLP (CARACAL) on 2 - 3 April 2014. European Commission, Brussels, 28 March 2014.

Figure 1 Ecotoxicity data for NP1-2EO



Dotted blue vertical line is the Registrants' PNEC

For intermittent releases, the eMSCA notes that the acute toxicity of NP1.5EO to mysids is 0.11 mg/L. Applying an assessment factor of 100 to this value would give a $PNEC_{intermittent}$ of 0.0011 mg/L (1.1 µg/L), which is lower than that derived by the Registrants by a factor of 1.3.[28] Nevertheless, the assumption behind a PNEC for intermittent discharges is that when exposure stops rapidly, populations can tolerate higher concentrations than when it is long lasting. There is some indication that the substance may interfere with endocrine systems (see Section 7.10). Exposure during a critical window of sensitivity could therefore have a significant effect, and so the assumption might not be appropriate. The eMSCA therefore considers that the $PNEC_{intermittent}$ and $PNEC_{water}$ should be the same (0.8 µg/L).

[28] NP1EO will be more toxic to mysids, with a predicted LC_{50} of around 0.064 mg/L. Applying an assessment factor of 100 to this value would give a PNEC of 0.0006 mg/L, which is lower than that derived for continuous releases by the eMSCA.

7.8.5. Conclusions for classification and labelling

A mixture of ecotoxicity data is available. For some end points (e.g. algal and long-term invertebrate toxicity), tests have been performed with the whole substance only. For others, data are available for one or both of the main constituent NPEO groups. The substance may vary in composition between suppliers, which also complicates conclusions on hazard classification. The eMSCA therefore considers that it might be better to classify the main constituents of the registered substance (NP1EO and NP2EO) separately, and then classify the registered substance as a mixture.

The lowest reported data for the most relevant constituents of the registered substance are presented in Table 12.

Table 12

ECOTOXICITY DATA RELEVANT FOR HAZARD CLASSIFICATION				
End point	**Taxonomic group**	**Substance**		
		NP1EO	**NP2EO**	**Whole substance**
Short-term (acute) L(E)C_{50} (mg/L)	Fish	0.218	0.323	-
	Invertebrates	*Ceriodaphnia*: 0.328 [*Daphnia*: 0.11 predicted by eMSCA] [*Americamysis*: 0.064 predicted by eMSCA]	*Ceriodaphnia*: 0.716 *Daphnia*: 0.148 [*Americamysis*: 0.128 predicted by eMSCA]	*Ceriodaphnia*: 0.626 [*Daphnia*: 0.16 predicted by eMSCA] *Americamysis*: 0.11
	Algae	-	-	3
Long-term (chronic) NOEC/EC_{10} (mg/L)	Fish	≥0.105	-	-
	Invertebrates	[*Daphnia*: 0.07 predicted by eMSCA] - -	[*Daphnia*: 0.09 predicted by eMSCA] - -	*Daphnia*: 0.1 *Ceriodaphnia*: 0.285 *Americamysis*: 0.0077
	Algae	-	-	1.5

7.8.5.1 Aquatic acute classification

On the basis of the available measured data, both NP1EO and NP2EO have acute fish and invertebrate L(E)C_{50} values in the range 0.1 – 1 mg/L, with *Daphnia magna* the most sensitive species; algae are less acutely sensitive. One test with *Americamysis bahia* for "NP1.5EO" provides slightly more sensitive data though still in the same range. Both substances are therefore classifiable as Aquatic Acute 1. The M-factor would be 1 for NP1EO and NP2EO based on the information for *D. magna*. The same conclusion can be reached for the whole substance based on the data for *A. bahia.*

This is consistent with the self-classification provided by the REACH registrants for the whole substance. However, by analogy with 4-NP, NP1EO and NP2EO may be more acutely toxic to non-tested fish and invertebrate species. For example, the 96-h LC_{50} for *Pleuronectes americanus* could be around 0.03 and 0.04 mg/L for NP1EO and NP2EO, respectively (see Section 7.8.1.1). A study for NP1EO with *A. bahia* might also result in an LC_{50} in the same range (since the eMSCA tentatively estimates that it could be around

0.06 mg/L; see Section 7.8.1.2). If these data were confirmed, the acute M-factor would become 10.

7.8.5.2 Aquatic chronic classification

NP1EO and NP2EO are unlikely to be degraded abiotically at a significant rate, and the available data indicate that neither is readily biodegradable. Although they are inherently degradable, mineralisation is not fast, and there is insufficient evidence to show that either substance undergoes primary degradation with a half-life of ≤16 days to form non-classified substances (the REACH Registrants of NP1EC, a significant primary degradant of NP1-2EO, self-classify it for environmental hazard, and NP2EC also appears to be classifiable: see Appendix 1). On this basis, the whole substance can be considered not rapidly degradable. Definitive information on bioaccumulation is lacking, although this is less relevant for hazard classification in view of the conclusion on rapid degradation.

The long-term (chronic) toxicity data set is incomplete, with no standard test guideline data for fish with the whole substance, for *Daphnia* or algae with NP1EO, or for any taxonomic group with NP2EO:

- The long-term fish NOEC for NP1EO concerns survival and growth end points for Japanese Medaka (*Ozyrias latipes*). As discussed in Section 7.8.1.1, these may not be the most sensitive end points for this species, and effects on secondary sex characteristics, although not used themselves for classification purposes, suggest that a NOEC for reproduction could be in the range 0.035 – 0.1 mg/L. Studies with Rainbow Trout (*Oncorhynchus mykiss*) do not provide suitable end points for classification purposes but indicate a variety of effects below 0.05 mg/L for NP1EO.
- There is some uncertainty about the reliability of the reported *D. magna* NOEC for the whole substance, in view of the acute data for this species. The eMSCA predicts that *Daphnia* 21-d NOECs for NP1EO or NP2EO may fall in the range 0.01 – 0.1 mg/L. However, the mysid *Americamysis bahia* is significantly more sensitive than *D. magna* with a 28-d NOEC in the range 0.001 – 0.01 mg/L.
- Algal toxicity data for the whole substance suggest that both NP1EO and NP2EO will be less toxic to algae than invertebrates. Since NP1EO and NP2EO would not be expected to be more toxic than 4-NP, the 72-h E_rC_{10} for NP1EO would be >0.025 mg/L for the most sensitive species in the 4-NP data set (*Desmodesmus subspicatus*). This is in the same range as the predicted *Daphnia* NOECs, so algae are very unlikely to be the most sensitive trophic group.

Based on the *Americamysis* 28-d NOEC of 0.0077 mg/L, the whole substance is classifiable as Aquatic Chronic 1, with an M-factor of 10 as it is not rapidly degradable. No long-term mysid data are available for NP1EO or NP2EO as individual substances, but neither would be expected to be more toxic than 4-NP, which has a NOEC in the same range (0.0039 mg/L). They would therefore also be classified in the same way. The eMSCA notes that there is a lack of sufficiently reliable data for long-term fish toxicity for either the registered substance or its main constituents; the M-factor would be 1 if the surrogate approach for fish is used, which is not as stringent.

The self-classification proposed by the Registrants is consistent with this analysis, with an M-factor of 10 (0.001 < NOEC ≤ 0.1 mg/L), although this appears to be based on the fish NOEC for 4-NP as a worst case.

7.8.5.1 Environmental classification summary

The registered substance, NP1EO and NP2EO should all be classified as:

Aquatic Acute 1, M-factor of 1

Aquatic Chronic 1, M-factor of 10

This is consistent with the self-classification in the registration dossiers, although as noted above, it is possible that the acute M-factor could be 10 if more data become available.

A wide variation in self-classifications is reported on the CLP Inventory (see Section 7.6.2). Whilst this might reflect a wider variety of chain lengths than the specific ones covered by the registration dossiers (including polymeric substances), it might also indicate divergent interpretations of the available data. A harmonised classification proposal for NP1-2EO could provide more certainty and ensure that all classifications are based on the same basic data set.

7.9. Human Health hazard assessment

Not evaluated – the focus of this evaluation is on endocrine activity in fish, since this is the basis for the existing Candidate Listing entry.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

The World Health Organisation/International Programme on Chemical Safety working definition of an endocrine disruptor (WHO/IPCS, 2002) is:

> "*an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.*"

The evaluation of whether or not a substance is an endocrine disruptor in fish is based on *in silico*, *in vitro* and *in vivo* data in accordance with the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (OECD, 2012), using a weight-of-evidence approach. This is supplemented with information from other guidance documents (e.g. OECD, 2010) and information from the literature (e.g. IPCS, 2002; Kendall et al., 1998; Knacker et al., 2010; OECD, 2004). Effects on other taxa (including mammals) have not been considered.

The assessment of *in vivo* data focuses on adverse 'apical' population-relevant effects (e.g. on growth, development, survival or reproductive potential), and whether these are related to a presumed endocrine mode of action based on *in vitro* tests (rather than being a consequence of systemic toxicity).

Two different types of effects are considered and analysed separately:

- Indicators of an endocrine mode of action and
- Effects on apical endpoints that are considered to provide evidence that a substance exerts adverse population-relevant effects owing to its endocrine mode of action.

Indicators of endocrine mode of action

Indicators of an endocrine mode of action may be provided by biomarkers that are known to indicate a specific mode of action as well as by histological changes that are likely to be a direct response to an estrogenic mode of action.

One of the most common biomarkers indicating an estrogenic or androgenic endocrine mode of action is vitellogenin (VTG). Vitellogenin is naturally produced by female fish as a precursor of yolk proteins that are incorporated in eggs (IPCS, 2002). Induction of vitellogenin in female and (more pronounced) in male fish is a known indicator of an estrogen agonist mode of action. Induction in females is also an indicator for an androgen antagonist mode of action (IPCS, 2002; Kendall et al., 1998; Knacker et al., 2010; OECD, 2004, OECD 2012).

According to the OECD TG 229 for the fish short term reproduction assay (OECD, 2009) and the guidance document on the diagnosis of endocrine related histopathology in fish gonads (OECD, 2010), the following histological endpoints are diagnostic for endocrine activity:

- Male: increased proportion of spermatogonia (early sperm cells), presence of testis-ova (estrogenic response especially in juvenile and adult Japanese Medaka, but also in other differentiated gonochorist species), increased testicular degeneration, interstitial (Leydig) cell hyperplasia/hypertrophy, retained peritoneal attachments/ gonadal duct feminization of the testis (estrogenic response in juvenile Fathead Minnow and Zebrafish).

- Female: increased oocyte atresia, perifollicular cell hyperplasia/hypertrophy, decreased yolk formation (aromatase inhibition), changes in gonadal staging.

Other effects such as decreased proportion of spermatogonia, altered proportions of spermatozoa (mature sperm cells) and gonadal staging in males are of secondary diagnostic interest as they may also be influenced by other modes of action.

Changes in the gonadosomatic index (GSI) may provide additional information about the gonad maturation and spawning readiness (OECD, 2004). It describes changes in the relation of gonad to whole body mass and thus may be an indicator of the reproductive effort of organisms (Helfman et al., 1997). Although GSI might be influenced by other modes of action too, reduction of GSI in male fish is regarded as a sensitive parameter in reproductive studies with estrogenic substances (OECD, 2004). However, care must be taken as the GSI is highly dependent on the individual fish (frequent spawners) or seasonal gonadal stage (seasonal breeders).[29]In addition, the following apical endpoints are considered to be indicators of an estrogen agonist or anti-androgen mode of action according to the OECD guidance document (OECD, 2012):

- Depression of male secondary sex characteristics in Fathead Minnow or Medaka
- Female biased phenotypic sex ratio during sexual development

Decreases in *secondary sex characteristics* in males may indicate an estrogenic mode of action but should be interpreted with caution and based on weight of evidence according to (OECD, 2009). Induction of female secondary sex characteristics in males such as urogenital papillae in male Zebrafish was shown to be significant after exposure to estrogenic substances (Kendall et al., 1998; OECD, 2004).

Change of sex ratio towards females is a known result of estrogen exposure during sexual development (IPCS, 2002; Kendall et al., 1998; OECD, 2004). In aquaculture this phenomenon is frequently used to generate all female or partial female populations by exposing fishes to exogenous estrogen active substances (Baroiller et al., 1999; Piferrer, 2001).

[29] The size of the gonads (testis and ovaries) increases when gonads mature prior to spawning. Depending on the spawning strategy of fish species (total spawners, spawning only once in a breeding season or lifetime versus repeated, batch or serial spawners) the gonadal size and thus the GSI may substantially increase during a spawning season, reaching maxima just before spawning (Helfman et al., 1997). In repeated spawners, this process recurs and, as their spawning is usually not synchronized, individual gonadal growth differs in time.

Whether or not endocrine mediated effects are observable depends on the life stage tested. For example, testis-ova might be induced in adult males as at least in some species gonads remain bipotent, but sensitivity is usually highest during sexual development (e.g. Nakamura et al., 1998). Differences in development of fish species must be considered. *O. latipes* for example is a differentiated gonochorist that naturally develops either male or female gonads and sex is naturally not changed after gonadal development. Hormonal influence (especially of female hormones) in this species starts very early during pre-hatch development (OECD, 2004) and thus the life stage(s) under exposure need to be considered carefully while interpreting test results. Especially if effects on gonadal staging are analysed the reproductive cycle of a species should be considered. In particular for total spawners having only one breeding season such as *O. mykiss* effects may be observed only during the process of maturing prior to spawning and may be missed at other times of the year.

Indicators of endocrine-mediated adverse effects

Alterations of the endocrine system may cause adverse effects that are endocrine specific but may also influence endpoints that are not endocrine specific (Kendall et al., 1998; Knacker et al., 2010; OECD, 2004).

Secondary sex characteristics and sex ratio are apical endpoints that are considered to be estrogen or anti-androgen specific.

Other endpoints such as growth, sexual maturity, reproduction and behaviour are known to be sensitive to estrogens (IPCS, 2002; OECD, 2004; OECD, 2012). Fertility rate, growth, time to first spawn, sex ratio shift toward females (Medaka and Fathead Minnow) and delay of male sexual development (Zebrafish) evolved to be the most sensitive endpoints for estrogen agonists in fish full life cycle tests (Knacker et al., 2010). Thus, in combination with indicators of endocrine activity they provide evidence of estrogen mediated effects but alone they are not diagnostic for this mode of action as they might also be influenced by other modes of action.

Table 13 summarizes endpoints that are considered indicators of estrogenic activity and may be affected as a result of this activity *in vivo*.

Table 13

Summary of endpoints that are considered during analysis of fish data	
Endpoints indicating an estrogen agonist (or anti-androgen) mode of action	**Apical endpoints considered to be sensitive to an estrogenic mode of action *in vivo***
• Vitellogenin induction in males and females • Increased proportion of spermatogonia (early sperm cells), presence of testis-ova, increased testicular degeneration, interstitial (Leiydig) cell hyperplasia/hypertrophy, gonadal duct feminization of the testis/ retained peritoneal attachments in males • Increased oocyte atresia, perifollicular cell hyperplasia/hypertrophy, decreased yolk formation (aromatase inhibition), changes in gonadal staging in females • Depression of male secondary sex characteristics in Fathead Minnow or Medaka and induction of female secondary sex characteristics such as urogenital papillae in Zebrafish • Female biased phenotypic sex ratio during sexual development	• Female biased phenotypic sex ratio during sexual development especially in Medaka • Reproduction (fecundity, fertility, number of males or females with reproductive success) • Spawning behaviour • Growth of offspring

7.10.1.1 In vitro *data*

In vitro results fall within **Level 2** of the OECD Conceptual Framework (OECD, 2012), and may provide information about a specific mechanism of action, e.g. estrogen receptor binding. They may also provide information about the potency of this mechanism, but do not consider whether or not effects may occur in intact organisms and do not provide information on the potency *in vivo* as this is influenced by pharmacokinetic processes such as uptake, distribution, accumulation and excretion.

The REACH registrations do not provide any information on *in vitro* studies. ECHA (2013) summarises the available *in vitro* data for NP1-2EO mixtures and NP1EO and NP2EO alone:

- ***Yeast Estrogen Screening (YES) assay***:

  Metcalfe et al. (2001) determined an EC_{20} of around 10 mg/L for both a commercial NP1EO (Imbentin-N7A, "100 % w/w") and a commercial NP1-2EO mixture (54 % NP1EO, 44 % NP2EO and 2 % NP3EO), with a relative potency of 0.025 compared to 4-NP.

  Isidori et al. (2006) obtained an EC_{50} of 7.75 mg/L for an NP1-2EO mixture (purchased from Chebios, Rome, Italy; purity not stated), with a relative potency of 0.00012 compared to 4-NP.

  Petit et al. (1997) showed that the activity for "NP2EO" (actually Igepal® CO-210 with a composition of 80 % NP1EO and 20 % NP2EO[30]) in a yeast assay was about half of the activity of 4-NP at 10^{-4} M (equivalent to around 30 mg/L), whereas Madiguo et al. (2001) found no activity in a yeast assay for a specially synthesised straight chain NP2EO (purity unstated) up to the same concentration.

  The concentrations in all of these studies exceeded the critical micelle concentration, so the dissolved concentrations may have been lower (and the relative potencies therefore higher).

  In another study not included in ECHA (2013), Routledge and Sumpter (1996) found that NP2EO (a gift from ICI, Cleveland, UK; composition not stated) was around seventy times less potent than 4-NP in a recombinant yeast screen for estrogenic activity. The test concentration was not explicitly reported, but was said to be in the range 50 µg/L to 100 mg/L.

- ***Rainbow Trout (*O. mykiss*) primary hepatocytes***:

  Jobling and Sumpter (1993) reported an EC_{50} for VTG expression of 17.27×10^{-6} M (equivalent to 5.3 mg/L) for NP2EO (source: ICI, Cleveland, UK; composition not stated), with a relative potency of 0.94 compared to 4-NP. Simultaneous exposure of the hepatocytes with Tamoxifen (an estrogen antagonist) caused an inhibition of the estrogenic effect, suggesting that the action of the test substances was mediated by the estradiol receptor.

  White et al. (1994) found that VTG was induced by both NP2EO (a gift from ICI, Cleveland, UK; composition not stated) and 4-NP at 10^{-5} M (i.e. 3.1 mg/L for NP2EO), though it was less pronounced for NP2EO (less than two-thirds the amount).

  Petit et al. (1997) found that "NP2EO" (actually 80 % NP1EO and 20 % NP2EO as mentioned above) induced the expression of VTG mRNA at 10^{-4} M (equivalent to

[30] As reported by Le Gac et al. (2001).

around 30 mg/L, which is above the likely critical micelle concentration), but this was around twenty-five times less effective than 4-NP.

Madigou et al. (2001) found no induction of VTG mRNA by a specially synthesised straight chain NP2EO (purity unstated) up to 10^{-4} M.

- ***Human breast cancer cell lines***:

White et al. (1994) found that NP2EO (source as above) caused a greater stimulation of transcriptional activity of the estrogen receptor than 4-NP in two estrogen-responsive human breast cancer cell lines (MCF-7 and ZR-75), at the same concentration of 10^{-5} M (equivalent to 3.1 mg/L for NP2EO).

Broadly similar results were obtained when the studies compared the potency of OP2EO and OP3EO to 4-octylphenol (4-OP) (White et al., 1994; Isidori et al., 2006).

The US EPA's ToxCast™ programme (http://epa.gov/ncct/toxcast/) uses over 700 high-throughput screening assays that cover a range of high-level cell responses and approximately 300 signalling pathways. The eMSCA searched the ToxCast Dashboard (https://actor.epa.gov/dashboard/) for information in July 2016, but it does not contain any data for NP1-4EO.

The US EPA's Endocrine Disruptor Screening Program (EDSP) uses a two tiered approach to screen chemicals for their potential effect on estrogen, androgen and thyroid hormone systems. The eMSCA searched the EDSP21 Dashboard (http://actor.epa.gov/edsp21/) for information in July 2016, but it does not contain any data for NP1-4EO.

In correspondence with the eMSCA, the Registrants have investigated the potential for estrogen receptor binding of 4-NP and NP1EO to NP9EO using the models in the OECD Toolbox v 3.3.5 (falling within **Level 1** of the OECD Conceptual Framework (OECD, 2012)). Only 4-NP triggered alerts for estrogen receptor binding ('strong binder') and this was linked to the phenolic hydroxyl group which is not present in NPEO. The Registrants believe that this would support the lower toxicity, including for endocrine endpoints, of NPEO versus 4-NP.

Discussion

Recommendation: The Registrants should provide RSS for these *in vitro* studies to provide additional context for the long-term fish studies.

Since standard guidelines were not followed and a variety of end points and test substances have been examined, it is very difficult to compare the results from these different studies directly. The test substance is often a mixture of NP1EO and NP2EO, and purity is not always clear. The degradation of test substances in the test systems has not been considered either. Nevertheless, all but one of the studies indicate that NP1-2EO may exhibit weak estrogenic activity *in vitro*.

Whilst the YES assay suggests that these substances have a much lower potency compared to 4-NP, there is some uncertainty in the actual NPEO exposure concentrations since the majority of tests appear to have been conducted above the critical micelle concentration meaning that truly dissolved concentrations would have been lower. This may mean the potency difference is less pronounced in reality. The trout primary hepatocyte data indicate that NP1-2EO can induce VTG expression. Different studies report different relative binding efficiencies compared to 4-NP: in a human breast cancer cell line, NP2EO caused a greater stimulation of transcriptional activity of the estrogen receptor than 4-NP at the same concentration (no information is available for NP1EO in this test system). It is also worth noting that the reported effect concentrations for NP1-2EO generally appear to exceed 1 mg/L, close to the acute fish LC_{50} (see Section 7.8.1.1). It is unclear whether tissue concentrations could approach this level so their actual relevance is unclear.

The estrogen receptor binding modelling mentioned in the Registrants' correspondence is not presented in the registration dossiers. The eMSCA agrees that 4-NP is likely to bind more strongly to the estrogen receptor than NPEO based on structural considerations. However, the eMSCA notes that NPEO may potentially metabolise to 4-NP *in vivo*, and that other interactions could also affect steroid formation/metabolism (e.g. aromatase inhibition).

The eMSCA considers that further *in vitro* studies using high purity NP1EO and NP2EO following validated test guidelines and using measured test concentrations below the critical micelle limit might be useful to clarify the relative potency of the two substances in comparison with 4-NP. However, the *in vivo* fish studies give rise to a concern for fish that means that *in vitro* studies are not essential (see below). The Registrants could still choose voluntarily to conduct additional *in vitro* studies with the registered substance and/or its constituents to confirm or refute the published findings.

7.10.1.2 In vivo *data*

The registration dossiers do not include a standard test guideline study for long-term fish toxicity (which is an Annex IX requirement for substances manufactured or imported in quantities of 100 tonnes or more). However, as discussed in Section 7.8.1.1, several non-standard long-term studies indicate that NP1EO and NP2EO can interact with the fish endocrine system, with NOECs in the range 0.01 – 0.1 mg/L.

The available information provides evidence for *in vivo* endocrine activity of NP1EO in a study roughly equivalent to **Level 4** of the OECD Conceptual Framework (OECD, 2012), i.e. changes in secondary sex characteristics in Japanese Medaka (*Oz. latipes*) following exposure post-hatch (LOEC: 0.105 mg/L; NOEC: 0.035 mg/L (based on measured concentrations)) (Balch and Metcalfe, 2006). NP1EO appeared to be a factor of around ten times less potent than 4-NP in the same test system, although there were significant concentration losses during the semi-static exposure regime which might confound such a comparison. No data are available to indicate whether such activity may result in endocrine-mediated *apical* effects for NP1EO. In addition, 4-NP was present in the test solutions, and although concentrations were below the EC_{10} from a full life cycle test for this substance in the same species, it may possibly have contributed to the effects. One instance of testis-ova was observed in 30 male fish in a second study (Metcalfe et al., 2001) that used a single concentration of 0.1 mg/L nominal (approximately 0.057 mg/L mean measured), although the test substance was a mixture of NP1EO and NP2EO (no 4-NP was detected in the test solutions in this study).

To consider this further, the eMSCA notes that the database for 4-NP in ECHA (2012) contains several studies with *Oz. latipes*. The most sensitive end points were reported in a full fish life cycle test (FFLCT; **Level 5** of the OECD Conceptual Framework (OECD, 2012)) by Yokota et al. (2001), rated reliable with restrictions. These are compared in Table 14 to the data for the same substance obtained in the non-standard 100-d FSDT by Balch and Metcalfe (2006).

It can be seen that both studies gave similar results for testis-ova, but the most sensitive result in the FFLCT (survival in the F0 generation) was lower than that in the FSDT by an order of magnitude. Conversely, secondary sex characteristics were affected at a lower concentration in the FSDT than the FFLCT. Sex ratio might be as sensitive as survival in the FFLCT, but the reporting of the data make this difficult to establish with certainty. Conclusions about the relevance of end points in this particular FSDT study (which also investigated NP1EO) therefore cannot be made. However, based on the FFLCT, testis-ova occurrence may be a marker of relevant apical effects (e.g. sex ratio) at comparable concentrations. For NP1EO, there are indications that testis-ova may be induced at a concentration of 0.1 mg/L nominal (approximately 0.057 mg/L mean measured) (Metcalfe et al., 2001) and the 100-d NOEC for secondary sex characteristics in the Balch and Metcalfe (2006) study was 0.035 mg/L (LOEC: 0.105 mg/L). It therefore cannot be ruled out that NP1EO could induce relevant adverse apical effects in a more comprehensive study with *Oz. latipes* in a concentration range of 0.035 – 0.1 mg/L.

Table 14

Japanese Medaka data for 4-nonylphenol (based on measured concentrations)		
End point	**Yokota et al. (2001) Full fish life cycle study**	**Balch and Metcalfe (2006) 100-d fish sexual development study**
Testis-ova occurrence	LOEC: 0.0177 mg/L EC_{10}: 0.0082 mg/L	LOEC: 0.029 mg/L NOEC: 0.0087 mg/L
Survival	LOEC: 0.0177 mg/L NOEC: 0.0082 mg/L	LOEC: - NOEC: ≥0.029 mg/L
Secondary sex characteristics	LOEC: 0.0515 mg/L NOEC: 0.0177 mg/L	LOEC: 0.0087 mg/L NOEC: 0.0029 mg/L
Sex ratio in the F1 generation	LOEC: 0.0177 mg/L? NOEC: 0.0082 mg/L? [Unclear as statistics not provided]	-
Effects on F0 fertility/ fecundity	LOEC: 0.0515 mg/L NOEC: 0.0177 mg/L	-

No comparable information is available for NP2EO, but the long-term study of Metcalfe et al. (2001) – in which testis-ova was observed – involved a mixture of 54 % NP1EO and 44 % NP2EO. NP2EO might therefore possibly contribute to the induction of effects.

For a commercial mixture called "NP4EO" (actually 1 % NP1EO, 11 % NP2EO, 19 % NP3EO, 18 % NP4EO, 13.5 % NP5EO, 10.5 % NP6EO and 17 % NP>6EO), the 100-d NOEC was ≥1 mg/L nominal (≥0.380 mg/L (based on measured concentrations) for all effects in *Oz. latipes* (Balch and Metcalfe, 2006).

Toxicity of NP1-2EO towards other fish species also needs to be considered. The 4-NP data set in ECHA (2014) includes two fish species that are more sensitive than *Oz. latipes* in long-term tests; NOECs of 0.0074 and 0.006 mg/L were obtained for Fathead Minnow (*Pimephales* promelas) and Rainbow Trout (*Oncorhynchus mykiss*), respectively (the latter value is cited in the registration dossier for NPEO). The eMSCA has reviewed the available data for these species for the registered substance:

- No long-term ecotoxicity data appear to be available for NP1-2EO with *P. promelas*.[31]
- Four studies are available for *O. mykiss*, although it is difficult to place them in the OECD Conceptual Framework (OECD, 2012) because they were non-standard studies (**Level 3** might be appropriate). As a seasonal spawner, care needs to be taken over the interpretation of effects since this could depend on the breeding condition of the fish at the time of testing[32]. In addition, the

[31] A study of long-term toxicity to Fathead Minnow (*Pimephales promelas*) which compared the toxicity of 4-NP to that of a commercial NPEO product (Miles-Richardson et al., 1999) is not relevant in the view of the eMSCA because the test substance (Solfonic N-95) consisted primarily of NP7-11EO, with approximately 0.58% w/w of NP, NP1EO and NP2EO.

[32] For example, Jobling et al. (1996) found that fish exposed during May were much more sensitive to the effects of 4-NP than when they were exposed in August and November.

studies in which concentrations were measured tend to show significant loss of test substance. All results reported on the basis of nominal concentrations may therefore significantly under-estimate toxicity. The effects were as follows:

i. NP1EO induced the female egg protein vitellogenin (VTG, a biomarker for estrogenic activity[33]) in male fish, with a 21-d LOEC of 0.281 mg/L and 21-d NOEC of 0.048 mg/L, based on measured concentrations (Dussault et al., 2005).

ii. NP1EO (containing 20 % NP2EO) had a significant effect on testicular growth and development in 13-month old male fish exposed for 21 days, with a LOEC of ≤0.122 mg/L (nominal); measured concentrations cannot be estimated with confidence but would have been less than half this value. The LOEC for VTG induction was in the range ≤0.122 - 0.491 mg/L (nominal), and VTG induction was also observed in juvenile females after 9 days' exposure (Le Gac et al., 2001).

iii. A limit test over 21 days suggested that NP2EO induced VTG formation in adult males and affected sperm development and gonadal growth at a concentration of 0.038 mg/L (mean measured) (0.03 mg/L nominal) (Jobling et al., 1996). These observations were very similar to those caused by 4-NP in the same test system (and concentration). The purity of the substance is unknown.

iv. NP2EO of unstated purity appeared to affect juvenile female growth following exposure for up to 35 days, at similar concentrations to 4-NP, with a LOEC of 0.001 mg/L (nominal) (Ashfield et al., 1998). However, the dose response in this study was inconsistent and the eMSCA does not believe that this LOEC is reliable.

On the basis of this limited information, *O. mykiss* appears to have a broadly similar sensitivity to NP1-2EO as *Oz. latipes*, although the end points are different.

The induction of VTG in male fish indicates that NP1-2EO have estrogenic activity. The two studies that measured VTG induction were both of relatively short duration (21 days exposure). It is possible that VTG could have been induced at much lower concentrations if the exposure had been longer (e.g. as demonstrated by Ackermann et al. (2002) for 4-NP).

The effects on testicular growth and development in *O. mykiss* might be linked to this since the fish testis is a known target for estrogens, but no data are available for either NP1EO or NP2EO to allow a definitive conclusion to be drawn about whether endocrine-mediated population-relevant apical effects (i.e. female biased phenotypic sex-ratio during sexual development, reproduction (fecundity, fertility, number of males or females with reproductive success), spawning behaviour or growth of offspring) occur at relevant concentrations in fish. Actual LOECs and NOECs are confounded by loss of test concentration in some of the studies, but it appears that potentially significant biological effects could occur below 0.1 mg/L.

The eMSCA notes that the lack of detailed information on test composition in most of the studies may mean that other constituents (e.g. 4-NP) contributed to the observed effects, although where measured in test solutions, 4-NP levels seem to be below the NOEC for effects. There may also have been a contribution from degradants in those studies that experienced significant losses of nominal concentration.

[33] The gene for the production of VTG, a yolk precursor, is estrogen-responsive, and expression is dependent on the interaction of estrogen with estrogen receptors in the liver. In male Rainbow Trout, the VTG gene is normally silent but exposure to exogenous estrogens will cause expression.

7.10.2. Endocrine disruption - Human health

Not evaluated. The Candidate List entries for 4-NP and NPEO are based on effects on aquatic organisms only.

7.10.3. Conclusion on endocrine disrupting properties

The eMSCA considers that there is good *in vivo* evidence that NP1EO and NP2EO have estrogenic activity in fish, at concentrations below 0.1 mg/L, and this is supported by *in vitro* evidence. However, there are currently no data to demonstrate that these lead to adverse population-relevant apical effects (i.e. female biased phenotypic sex-ratio during sexual development, reproduction (fecundity, fertility, number of males or females with reproductive success), spawning behaviour or growth of offspring).

With the exception of imported articles and use as a chemical intermediate, the risk management of NPEO (including the registered substance subject to this evaluation) will in future be reliant on the authorisation procedure under REACH. NPEO was included on the Candidate List since it is a source of 4-NP due to environmental transformation; 4-NP was added to the Candidate List as a Substance of Equivalent Concern on the basis of its environmental ED effects. An applicant for authorisation of NPEO will therefore only be obliged to consider the potential risks arising from the impurity content/formation of 4-NP for their use(s) of the substance. This ignores any other adverse ED effects arising from NPEO itself (and also other transformation products like NPECs – see Appendix 1). It is therefore possible that authorisations may be granted on the basis of a risk assessment that is not adequately protective of environmental risks. In addition, future restriction proposals (e.g. for imported articles) should take the additive nature of estrogenic effects from NP1EO, NP2EO, 4-NP and NPECs into account, but the existing data set does not permit this.

From a traditional risk assessment perspective, the $PNEC_{water}$ of 0.8 µg/L derived from the mysid 28-d NOEC (see Section 7.8.4) does not lead to the identification of risks for surface waters (see Section 7.13). However, it is not clear what margin of safety is provided by this PNEC to protect against endocrine effects in fish. It is possible that the NOEC for long-term fish toxicity could be lower than the Registrants currently assume (e.g. similar to fish NOECs for 4-NP). If NP1-2EO itself was identified as an endocrine disruptor according to Article 57(f) of REACH because of its effects in fish (rather than because of a degradant), the Candidate List entry could be updated to ensure that these properties are fully taken into account by applicants for authorisation. In addition, depending on future EU policy, risk management might be based on the assumption of no safe threshold. Such an identification could also be relevant for prioritisation of NP1-2EO under the Water Framework Directive.

The eMSCA therefore considers that a further fish toxicity study at Level 4 or 5 of the OECD Conceptual Framework for endocrine disruption testing (OECD, 2012) would provide definitive information to allow conclusions to be drawn about the potential for ED-mediated adverse population-relevant effects in fish, with a clear $NOEC/EC_{10}$. The initial draft decision arising from this SEv therefore asked the Registrants to perform a Fish Sexual Development Test (OECD TG 234) on the registered substance (or NP1EO as the constituent that is likely to be the most toxic), using five test concentrations (plus relevant controls), flow-through conditions and analytical monitoring of test concentrations for the test substance, 4-NP and NP1EC. In their response, the Registrants both gave a written commitment to cease manufacture and use of this substance by 31 December 2020 and not to apply for authorisation. Given the high cost in terms of resources and vertebrate animal lives, the eMSCA decided that it would be disproportionate to request such a test from the two Registrants under these circumstances. If new uses or registrations occur in future, the substance may need to be put on the CoRAP again to clarify the endocrine disruption concern.

SEv does not apply to the polymeric forms of NPEO, since there is no registration requirement. A trade body representing the interests of the NPEO suppliers, or a national authority, could consider the voluntary performance of a Fish Sexual Development Test according to OECD TG 234 for NP1EO to clarify whether this type of substance has endocrine disrupting properties in its own right. This would be a demonstration of responsible care, and may be useful in jurisdictions outside the EU. Additional *in vitro* studies according to modern standard protocols could also be conducted voluntarily to confirm or refute the published findings.

Further evaluation of NPECs could also be considered, since these have separate registrations under REACH. It may therefore be appropriate for them to be screened for inclusion on the CoRAP.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

Not evaluated in any detail. The reliability of the exposure assessment would be considered by ECHA's Risk Assessment Committee in due course, if the Registrants seek an authorisation, which they have given a written undertaking not to do.

The Norwegian Competent Authority provided a summary of Norwegian monitoring data in June 2016, but this is not relevant to the exposure scenario in the REACH registrations[34].

7.13. Risk characterisation

Not evaluated.

The eMSCA considers that, based on the available data, a PNEC of 0.8 µg/L as derived by the Registrants does not result in risks to surface waters for either of the Registrants. However, **it is not clear what margin of safety is provided to protect against endocrine effects in fish**, and the reliability of the exposure assessment has not been evaluated by the eMSCA.

In response to the eMSCA's initial conclusion that a long-term fish toxicity test should be conducted to establish the endocrine disrupting properties of the substance, the Registrants proposed to use the $PNEC_{water}$ for 4-NP directly as a worst case. As discussed in Section 7.8.4, the $PNEC_{water}$ established by the RAC was 0.4 µg/L but could be lower (e.g. 0.1 µg/L) (ECHA, 2014). Both values would suggest a risk for one Registrant with releases to freshwater. This concerns a single specific site and is based on monitoring data in the receiving environment, so cannot be refined. Additional risk management measures would need to be applied.

The local regulatory authority for the user site may therefore wish to review the conditions of any permit to ensure that best available techniques are being applied to

[34] Email from Marius Gudbrandsen of the Norwegian Environment Agency to Steve Dungey, Sara Martin and Ian Doyle of the Environment Agency, 23 June 2016.

minimise environmental emissions until such time as use ceases. They could also consider a survey to evaluate whether fish in the local receiving environment are showing signs of endocrine disruption (this could involve non-lethal sampling of the fish epidermal mucus with a swab followed by analysis using a vitellogenin enzyme-linked immunosorbent assay (ELISA)). The eMSCA has contacted the relevant REACH Competent Authority contact in the Member State involved to highlight this issue.

7.14. References

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The focus of this evaluation has been on short-chain length NPEOs. References retrieved but found not to be relevant on review are listed below for completeness:

Leguen I and Prunet P (2001). *In vitro* effect of various xenobiotics on trout gill cell volume regulation after hypotonic shock. Aquatic Toxicology, 53, 201–214.
Reason: Contains information on lethality of NP2EO (and longer chain NPEO) to fish gill cells *in vitro*.

7.15. Abbreviations

%	Percentage
3H-Tdr	3H-Thymidine
4-NP	4-Nonylphenol, branched
BCF	Bioconcentration factor
BAFs	Bioaccumulation factors
AF	Assessment factor
C&L	Classification & Labelling
CAmPEnC	Carboxylated alkylphenol ether carboxylate
CLP	Classification, labelling and packaging (of substances and mixtures)
cm	Centimetre
CMC	Critical micelle concentration
CoRAP	Community Rolling Action Plan
CSR	Chemical Safety Report
d	Day
dw	Dry weight
E2	17β-estradiol
ECHA	European Chemicals Agency
ED	Endocrine disruption
EDSP	Endocrine Disruptor Screening Program
eMSCA	Evaluating Member State Competent Authority
EO	Ethylene oxide

ES	Exposure Scenario
EPA	Environmental Protection Agency
EU	European Union
FFLCT	Full fish life cycle test
FSDT	Fish sexual development test
g	Gramme
GSI	Gonadosomatic index
h	Hour
HPLC	High performance liquid chromatography
IGF-I	insulin-like growth factor-1
K_{OW}	Octanol-water partition coefficient
L	Litre
LC/MS	Liquid chromatography/Mass spectrometry
Log	Logarithmic value
LOEC	Lowest Observed Effect Concentration
M	Molar
m	Metre(s)
µg	Microgram
mg	Milligram
min	Minute
mL	Millilitre
mN	Millinewton
mol	Mole
MSCA	Member State Competent Authority
MSSC	‘‘Mixed’’ secondary sex characteristics
NOEC	No Observed Effect Concentration
NPEO	(4-)Nonylphenol ethoxylate
NPnEO	(4-)Nonylphenol n-ethoxylate
NP1EO	(4-)Nonylphenol mono-ethoxylate
NP2EO	(4-)Nonylphenol di-ethoxylate
NP1-2EO	Mixture of NP1EO and NP2EO
4-NP	4-Nonylphenol, branched and linear
OECD	Organisation for Economic Co-operation and Development

OPEO	4-tert-Octylphenol ethoxylates
OSI	Ovosomatic index
$PNEC_{water}$	Predicted No Effect Concentration for surface water
QPRF	QSAR Prediction Reporting Format
RCR	Risk characterisation ratio
RMM	Risk Management Measures
RSS	Robust study summary
SCAS	Semi-continuous activated sludge
SEv	Substance Evaluation
SVHC	Substances of Very High Concern
t	Tonne
TEF	Toxic equivalence factor
TG	Test Guideline
UK	United Kingdom
UVCB	Undefined or Variable composition, Complex reaction products or Biological materials
UV/VIS	Ultra-violet/visible
VTG	Vitellogenin
WHO	World Health Organisation
wt	Weight
YES	Yeast Estrogen Screening

7.16. Summary of recommendations

Recommendations

1. The Registrants should clarify the level of degradation observed after 112 days in the OECD TG 301D closed bottle biodegradation test, and assess the reliability of this result.

2. The Registrants should provide the full reference for the Cravedi et al. (2001) fish toxicokinetic study in the RSS.

3. The Registrants should include RSS for five additional fish toxicity studies (Jobling et al., 1996; Ashfield et al., 1998; Metcalfe et al., 2001; Le Gac et al., 2001; and Dussault et al., 2005), and provide all relevant effect data in the RSS for the Balch and Metcalfe (2006) study.

4. The Registrants should provide RSS for the *in vitro* studies of Jobling and Sumpter (1993), White et al. (1994), Routledge and Sumpter (1996), Petit et al. (1997), Madigou et al. (2001), Metcalfe et al. (2001) and Isidori et al. (2006) to provide additional context for the long-term fish studies. The eMSCA does not think that further *in vitro* studies are necessary given the observations in *in vivo* studies. However, the Registrants may choose voluntarily to conduct additional *in vitro* studies with the registered substance to confirm or refute the published findings.

APPENDIX 1 INFORMATION ON NONYLPHENOL ETHER CARBOXYLATES (AS NPEO TRANSFORMATION PRODUCTS)

As discussed in the main report, nonylphenol mono- and di-ether carboxylates (NP1EC and NP2EC) can be significant transformation products of NPEO in the environment under both aerobic and anaerobic conditions. This appendix provides a brief overview of relevant information for these degradants, since their hazard classification under the CLP Regulation and endocrine disruption potential could be relevant factors for the risk management of the registered substance. However, since NP1EC is separately registered under REACH, this Substance Evaluation of NPEO cannot be used to request additional information for that substance.

Unless otherwise stated, the following information is available on the ECHA dissemination web site (accessed on 22 July 2016).

Identity

Name	EC no.	CAS no.
(4-Nonylphenoxy)acetic acid (NP1EC)	221-486-2	3115-49-9
[2-(4-Nonylphenoxy)ethoxy]acetic acid (NP2EC)	631-246-2	106807-78-7

Hazard classification under the CLP Regulation

There is no harmonised classification, but the REACH Registrants self-classify NP1EC as:

Aquatic Acute 1, M-factor: 1
Aquatic Chronic 1, M-factor: 1

The eMSCA has not checked the underlying data that form the basis of this classification.

Although there is no REACH registration of NP2EC, it has entries on the CLP Inventory suggesting that is not classifiable for the environment, although this may be due to lack of data. For example, Maki et al. (1998) reported a 48-h LC_{50} for *Daphnia magna* of 0.99 mg/L, which means it may meet the criterion for Aquatic Acute 1.

Endocrine disruption

Several of the studies performed for NP1-2EO have also investigated the effects of NPECs[35]. For example:

- White et al. (1994) reported that vitellogenin (VTG) gene expression was stimulated by NP1EC[36] in Rainbow Trout (*O. mykiss*) hepatocytes *in vitro* in a dose-dependent manner in the concentration range 10^{-7} – 10^{-5} M [0.0276 - 2.76 mg/L]. The amount of VTG produced was slightly higher than NP2EO and about two-thirds that of 4-NP in the same test system at 10^{-5} M. Mitogenic effects were also observed in two estrogen-responsive human breast cancer cell lines (MCf-7 and ZR-75), with NP1EC stimulating cell growth above that of controls at 10^{-6} and 10^{-5} M (exceeding that caused by 4-NP at the same test concentrations). A time course for the effect at 10^{-6} M indicated that NP1EC, like octylphenol, stimulated the rate of growth and not just the saturation density. NP1EC also

[35] This list includes those studies listed in Vlaardingen et al. (2003), with the exception of acute studies.

[36] NP1EC was bought from Aldrich, UK. No purity information was provided.

stimulated transcription of a reporter gene for the estrogen receptor in MCF-7 human breast cancer cells at 10^{-5} M, to a level close to that caused by octylphenol (and higher than both NP2EO and 4-NP). In tests with cells lacking or expressing the receptor, transcriptional stimulation depended on the presence of cotransfected mouse estrogen receptor, demonstrating that the action was mediated by the estrogen receptor. NP1EC also displaced ^{3}H-labelled 17β-estradiol (E2) from the Rainbow Trout estrogen receptor in a competitive manner, with a similar potency to 4-NP (approximate K_d of 5 x 10^{-5} M). Similar results were obtained with the mouse estrogen receptor, although the data are not shown in the paper.

Other *in vitro* studies that have investigated NP1EC and/or NP2EC include Jobling and Sumpter (1993), Jobling et al. (1996), Routledge and Sumpter (1996), Metcalfe et al. (2001) and Burnison et al. (2002). The eMSCA has not reviewed this information, but notes that some effects were reported.

- Jobling et al. (1996) subsequently reported that NP1EC (purchased from Aldrich, Dorset, UK; purity not stated) was as estrogenic as 4-NP in a screening assay which exposed groups of two-year-old male *O. mykiss* to a single concentration of 30 µg/L (nominal; measured concentration was 31.82 ± 6.50 µg/L) for 21 days. It induced a plasma VTG concentration of around 1 000 ng/mL, and inhibited testicular growth and spermatogenesis.

- Dussault et al. (2005) found that NP1EC (purity > 99.9 %) induced plasma VTG above the detection limit in seven out of twelve immature *O. mykiss* after exposure to a nominal concentration of 1 000 µg/L [1 mg/L] (mean measured concentration 1.448 ± 0.136 mg/L (n=3)) for 21 days. No plasma VTG was detected at a nominal concentration of 300 µg/L [0.3 mg/L] and below. The effect was confirmed to be due to NP1EC rather than NP from chemical breakdown in the test solutions. The mean measured VTG concentration (± standard error) for all fish was 1.7×10^{5} ($\pm1.24\times10^{5}$) ng/mL. The relative potency compared to NP1EO in the same test system was reported to be 0.13, although the eMSCA cannot ascertain how this was calculated. VTG was induced at the same nominal concentration of both substances, although the measured concentrations differed, and slightly fewer fish were induced by NP1EC (the mean VTG concentration was also lower, although this might be because non-detects were included in the calculation). NP1EC concentrations in the water control were around 0.0025 mg/L.[37]

- Balch and Metcalfe (2006) investigated the effects of NP1EC on Japanese Medaka (*Oryzias latipes*) fry over a 100-d exposure period. Unlike NP1EO, there was no evidence of estrogenicity from NP1EC in this test system, up to a maximum measured test concentration of 2 mg/L.

- Further studies that investigated the effects of NP1EC are available (e.g. Metcalfe et al., 2001), but have not been evaluated by the eMSCA.

Overall, the studies suggest that both NP1EC can have *in vivo* endocrine activity, with VTG produced in juvenile male Rainbow Trout (*O. mykiss*). Whilst some studies suggest that NP1EC is less potent than NP1EO, others suggest similar or possibly even higher potency. It is therefore difficult to draw a firm conclusion about the relative potency of NPECs without a further critical review of the available data. The potency differences in

[37] 4-NP was also detected in test solutions in the range 0.0008 – 0.0047 mg/L (it is not clear which treatments these values relate to); in the NP experiment, no plasma VTG was induced at a nominal concentration of 0.001 mg/L (mean measured concentration of 0.0062 mg/L). NP1EO was detected in the solvent control at 0.0008 mg/L.

these studies may reflect differing chemical purity, differences in ability to maintain test concentrations, or the developmental stage of the fish.

On the basis of this information it would appear that NPECs might be less endocrine active than equivalent chain length NPEOs. A worst case assumption is that they are as active as NP1EO. Until a more reliable no effect concentration for endocrine disruptive properties of NP1-2EO has been established, the eMSCA does not believe it is necessary to perform any further review of NPECs for the purposes of this evaluation. **However, NP1EC could be a suitable candidate for the CoRAP as it is a registered substance.**