Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) [includes ortho-, meta-, para- isomers or any combination thereof]

EC Number: 500-315-8; 500-024-6; 500-045-0; 500-209-1; 248-762-5; 243-816-4; 248-291-5; 687-833-9 and others

CAS Number: 127087-87-0; 9016-45-9; 26027-38-3; 68412-54-4; 27986-36-3; 20427-84-3; 27176-93-8; 1119449-38-5 and others

CLH-O-0000007028-75-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) [includes ortho-, meta-, para- isomers or any combination thereof]

EC Number:

500-315-8;
500-024-6;
500-045-0;
500-209-1;
248-762-5;
243-816-4;
248-291-5;
and others

CAS Number:

127087-87-0;
9016-45-9;
26027-38-3;
68412-54-4;
27986-36-3;
20427-84-3;
27176-93-8;
1119449-38-5
and others

Index Number: **none**

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Version number: 2.0 **Date: June 2020**

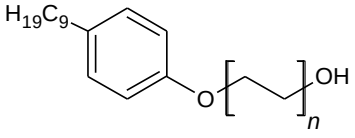
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON NONYLPHENOL, BRANCHED AND LINEAR, ETHOXYLATED (WITH AVERAGE MOLECULAR WEIGHT < 352 G/MOL) [INCLUDES ORTHO-, META-, PARA-ISOMERS OR ANY COMBINATION THEREOF]

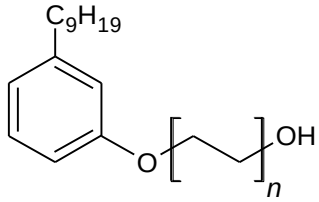
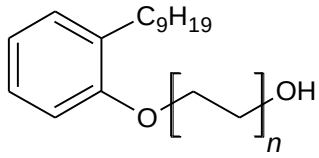
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) [includes ortho-, meta-, para- isomers or any combination thereof]
Other names (usual name, trade name, abbreviation)	
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	500-315-8 500-024-6 500-045-0 500-209-1 248-762-5 243-816-4 248-291-5 and others
EC name (if available and appropriate)	
CAS number (if available)	127087-87-0 9016-45-9 26027-38-3 68412-54-4 27986-36-3 20427-84-3 27176-93-8 1119449-38-5 and others
Other identity code (if available)	
Molecular formula	$(C_2H_4O)_n\ C_{15}H_{24}O$, with n = 1 to < 3 Where n = represents the number of ethoxylated group(s) to the phenolic group.
Structural formula	Representative structures: *para*- substitution 

	meta- substitution  *ortho*- substitution  n = represents the number of ethoxylated group(s) to the phenolic group n = 1 to < 3 ethoxy groups
SMILES notation (if available)	
Molecular weight or molecular weight range	264 g/mol ≤ average molecular weight < 352 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The major positional isomer is -para (≥90%), while the -ortho isomer is typically less than 10%*.
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	

*Naylor CG, 2004

1.2 Composition of the substance

Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) will be denoted as NPEn, where n describes the number of ethoxylated groups. This abbreviation is used to refer to a specific NPE substance. When referring to NPEs as a group, the reference short-chain NPE will be used.

Table 2: Constituents (non-confidential information)

Constituent Name	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Not relevant			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

Table 5: Test substances (non-confidential information)

Identification of test substance	The study(ies) in which the test substance is used	
Nonylphenol, branched, ethoxylated (NPEO)*	Hydrolysis in water Phototransformation in air Aquatic toxicy studies	Measured Calculated Measured
NPE-1	Biodegradation –screening studies Simulation studies Aquatic toxicity studies	Measured
NPE-1.5**	Biodegradation –screening studies Aquatic toxicity studies	Measured
NPE-2	Biodegradation –screening studies Simulation studies Bioaccumulation	Measured

* See Table 6 for the constituents of NPEO

** The term NPE 1.5 refers to a mixture of various oligomers where the mean number of ethoxylated groups per molecule is 1.5,

A registration dossier for nonylphenol, branched, ethoxylated (NPEO, CAS 68412-54-4) is available and was used as primary source for information with regard to the covered substances in this CLH report. The substance is registered as a UVCB substance, primarily comprising of one and two ethoxy groups (see Table 6). The relative position of the nonyl group on the aromatic ring was not defined.

Table 6: Information on the constituents of substance, nonylphenol, branched, ethoxylated (NPEO)

Constituent Name*	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Nonylphenol, branched, n=1	confidential	None	None
Nonylphenol, branched, n=2	confidential	None	None
Nonylphenol, branched, n=3	confidential	None	None
Nonylphenol, branched, n=4	confidential	None	None
Nonylphenol, branched, n=5	confidential	None	None

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON NONYLPHENOL, BRANCHED AND LINEAR, ETHOXYLATED (WITH AVERAGE MOLECULAR WEIGHT < 352 G/MOL) [INCLUDES ORTHO-, META-, PARA-ISOMERS OR ANY COMBINATION THEREOF]

Constituent Name*	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Nonylphenol, branched, n=6	confidential	None	None

n = represents the number of ethoxy group(s) to the phenolic group

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry		None									
Dossier submitters proposal	TBD	Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) [includes ortho-, meta-, para- isomers or any combination thereof]	500-315-8 500-024-6 500-045-0 500-209-1 248-762-5 243-816-4 248-291-5 and others	127087-87-0 9016-45-9 26027-38-3 68412-54-4 27986-36-3 20427-84-3 27176-93-8 1119449-38-5 and others	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=10	
Resulting Annex VI entry if agreed by RAC and COM	TBD	Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) [includes ortho-, meta-, para- isomers or any combination thereof]	500-315-8 500-024-6 500-045-0 500-209-1 248-762-5 243-816-4 248-291-5 and others	127087-87-0 9016-45-9 26027-38-3 68412-54-4 27986-36-3 20427-84-3 27176-93-8 1119449-38-5 and others	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=10	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*Hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*Hazard class not assessed in this dossier*	No
Oxidising gases	*Hazard class not assessed in this dossier*	No
Gases under pressure	*Hazard class not assessed in this dossier*	No
Flammable liquids	*Hazard class not assessed in this dossier*	No
Flammable solids	*Hazard class not assessed in this dossier*	No
Self-reactive substances	*Hazard class not assessed in this dossier*	No
Pyrophoric liquids	*Hazard class not assessed in this dossier*	No
Pyrophoric solids	*Hazard class not assessed in this dossier*	No
Self-heating substances	*Hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*Hazard class not assessed in this dossier*	No
Oxidising liquids	*Hazard class not assessed in this dossier*	No
Oxidising solids	*Hazard class not assessed in this dossier*	No
Organic peroxides	*Hazard class not assessed in this dossier*	No
Corrosive to metals	*Hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*Hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*Hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*Hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*Hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*Hazard class not assessed in this dossier*	No
Respiratory sensitisation	*Hazard class not assessed in this dossier*	No
Skin sensitisation	*Hazard class not assessed in this dossier*	No
Germ cell mutagenicity	*Hazard class not assessed in this dossier*	No
Carcinogenicity	*Hazard class not assessed in this dossier*	No
Reproductive toxicity	*Hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure	*Hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*Hazard class not assessed in this dossier*	No
Aspiration hazard	*Hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	Acute Category 1 (M factor =1) Chronic Category 1 (M factor = 10)	Yes
Hazardous to the ozone layer	*Hazard class not assessed in this dossier*	No

Labelling

Pictogram:	GHS09
Signal word:	Warning
Hazard statement:	H410 (Very toxic to aquatic life with long lasting effects)

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352 g/mol) [includes ortho-, meta-, para- isomers or any combination thereof] are not listed in Annex VI of the CLP Regulation.

RAC general comments

Nonylphenols ethoxylated (**NPEs**) are C9 alkylphenols with an ethoxylate chain of variable length and number of repeating units. NPEs have a broad range of uses as detergents, emulsifiers and wetting or dispersing agents. Uses in the textile industry are also reported in washing, dyeing and bleaching processes.

Nonylphenol, branched and linear, ethoxylated (with average molecular weight < 352g/mol) [includes ortho-, meta-, para- isomers or any combination thereof] is further referred to in this opinion as **short-chain NPEs** with from 1 to <3 ethoxylate units. This opinion is one of three related opinions, the other two concern medium-chain (3 to <11) and long chain (11-30) NPEs. The NPEs were divided into these 3 groups based on their aquatic toxicity since the aquatic toxicity is expected to decrease with the increase of the number of ethoxylate groups in the view of the DS.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Requirement for harmonised classification by other legislation or process.

Nonylphenol ethoxylates (NPEs) fall under the Prior Informed Consent Regulation (PIC, EC/649/2012). The PIC regulation manages the import and export of certain hazardous chemicals and places obligations on companies who intend to export these chemicals to non-EU countries. It aims to promote shared responsibility and cooperation in the international trade of hazardous chemicals, and to protect human health and the environment by providing developing countries with information on how to store, transport, use and dispose of hazardous chemicals safety.

As a result of the inclusion of NPEs within the PIC regulation, transportation of NPEs is restricted. This restriction also applies to mixtures containing NPEs above a concentration that leads to classification of the mixture as a result of the presence of NPEs. However, currently a harmonized classification is lacking for these substances. Further, self-classifications vary between the industries. As a result, classification of the mixtures is dependent on the self-classification of the suppliers and is therefore variable. This leads to lack of protection for human health or the environment and lack of clarity to law enforcement.

A harmonised classification for NPEs would result in clarification on the obligations for mixtures as falling under the PIC regulation. Law enforcement of this PIC regulation would be improved.

Most marketed NPE including registered forms are UVCBs containing NPEs varying in number of ethoxylate groups, linearity or branching of the nonyl group or the postion of the ethoxylate group(s) versus the nonyl group on the benzene ring (para-, meta- or ortho-isomers or any combination thereof). As the exact identity of the tested form is unknown, extrapolation to the other forms within the group for which

extrapolation is proposed is difficult. However, in line with the extrapolation applied for the inclusion of these substances in the PIC regulation and applied for the restriction of NPEs in textiles, such extrapolation is considered justified also here.

An approach using groups of NPE's instead of covering only individual UVCB substances is used because often NPEs are exported as mixtures from which it is difficult to determine which NPEs were included. Therefore, inclusion of all possible NPEs (including mono-constituent, multi-constituent and UVCB substances) would allow the use of the additivity approach for the most relevant endpoint being aquatic toxicity.

This CLH report is one of three proposals that cover various groups of NPEs. These groups were defined based on reliable (Klimisch scores 1 or 2) aquatic toxicity data for NPEs. These data indicate that aquatic toxicity decreases with the increase of the number of ethoxylate groups (see Table 8 for acute toxicity and Table 9 for chronic toxicity). This difference in toxicity within the whole range of ethoxylate groups was considered to be problematic for classification and labelling. For this reason the studies were grouped in short, medium and long ethoxylate groups according to the degree of toxicity. The borders of the groups are determined in such a way that most of the endpoints fit within the ranges of the group, although some endpoints make the exception.

The most logical ranges were determined as follows:

Short-chain group (NPEn where n = 1 to < 3 ethoxylate group(s)):
Acute and chronic data on NPE with 1 to 2 ethoxy groups are available for fish, invertebrates and algae. The (E)LC_{50} and NOEC values for fish and Daphnia are all < 1 mg/L. This led to the classification of this group of NPEs as Aquatic Acute Cat. 1 and Chronic Cat. 1. The EC_{50} for algae of >3.0 mg/L and the NOEC of 1.22 mg/L for the same species was the only value that did not fit into the short chain group.

Medium-chain group (NPEn where n = ≤ 3 to < 11 ethoxylate groups):
Acute data on NPE for 3, 4, 5, 6, 9 and 9.5 ethoxy groups are available for acute fish, invertebrates and algae with (E)LC_{50} values ranging between 1 and 14 mg/L.

Chronic data on NPE 4 and 9 were available for fish with the NOEC values between 0.114 and 0.54 mg/L and for Daphnia with the NOEC value of 10 mg/L. In addition, chronic algae toxicity NOEC data were between 1 and 3 mg/L for NPE of 3 and 6 ethoxy groups, respectively. For the medium chain group of NPEs between 3 and 10 ethoxy group where there is missing data on certain NPE chain length these data are assumed to be comparable with data of the same group. For chronic toxicity values, the division between the short and the medium-chain group is not sharp as the short chain group hold a NOEC of 0.122 mg/L while the medium-chain group holds a lower NOEC of 0.114 mg/L. This overlap is however minimal which makes the division between NPE-2 and NPE-3 acceptable. The medium-chain group was classified as Chronic Cat. 2 and no classification for acute toxicity.

Long-chain group (NPEn where n = ≤ 11 to ≤ 30 ethoxylate groups)
Acute data on NPE with 11 – 30 ethoxy groups is available only for NPE-12 (algae) and NPE-30 (fish), with the (E)LC_{50} values > 1 mg/L. Chronic data are only available for NPE-12 (algae) with a NOEC of 20 mg/L. For this long-chain group there is no acute toxicity classification proposed, while for chronic toxicity a conclusion on classification was not possible due to limited data.

Table 8: Overview of valid acute toxicity data and grouping of NPEn

Only relevant and valid studies (Klimisch scores 1 and 2) for nonylphenol ethoxylates have been listed. The reliablity and description of each study can be found in section 11.5.

Substance tested	**Method and species**	**Results (mg/L)** $L(E)C_{50}$	**Proposed NPEn-group**
Fish			
NPE-1	OECD TG 203 Fathead minnow (*Pimephales promelas)*	96h-LC_{50} = 0.218	Short-chain
NPE-2	OECD Guideline 203 Fathead minnow (*Pimephales promelas)*	96h LC_{50} = 0.323	Short-chain
NPE-4 NPE-5 NPE-9 NPE-9.5	Test guideline not mentioned Bluegill sunfish (*Lepomis macrochirus*)	96h LC50 = 1.3 96h LC50 = 2.4 96h LC50 = 7.9 96h LC50 = 7.6	Medium-chain
NPE-9	Test guideline not mentioned Fathead minnow *(Pimephales promelas)*	96h-LC50 = 4.6	Medium-chain
NPE-30	Test guideline not mentioned Bluegill sunfish (*Lepomis macrochirus*)	96h LC50 > 1000	Long-chain
Invertebrates			
NPE-1	*EPA guideline* *Cerodaphnia dubia*	48h-EC_{50} = 0.328	Short-chain
NPE-1.5	No guideline *Mysidopsis bahia*	48h-EC_{50} = 0.11	Short-chain
NPE-2	*EPA guideline* *Cerodaphnia dubia*	48h-EC_{50} = 0.716	Short-chain
NPE-9	Test guideline not mentioned *Daphnia magna*	48h EC50 = 14	Medium-chain
Algae			
NPE-2	TG201 *Pseudokirchneriella subcapitata*	72h $EC_{50,growth}$ > 3.0	Short-chain
NEP-3 NPE-6 NPE12	*Scenedesmus subpicatus* performed according to test guideline 201	72h-ErC50= 2.9 72h-ErC50=13 72h-ErC50=89	Medium-chain Medium-chain Long-chain

Table 9: Overview of valid chronic toxicity data and grouping of NPEn

Substance tested	**Method**	**Results (mg/L)**	**Proposed NPEn-group and remarks**
Fish			
NPE-1	Not a test guideline method 21 d exposure Rainbow trout (*Oncorhynchus mykiss*)	NOEC VTG[1] = 0.03	Short-chain
NPE-1	Not a test guideline method 100d exposure Medaka (*Oryzias latipes)*	NOECsurvival =0.105 NOEC SSC[2] = 0.035	Short-chain
NPE-1/ NPE-2	Not a test guideline method 90d exposure Medaka (*Oryzias latipes)*	NOEC = 0.05	Short-chain
NPE-2	Not a test guideline method	LOEC ≤ 0.038	Short-chain

	rainbow trout	VTG induction, GSI and germ cell stages	
NPE-1/ NPE-2	Not a test guideline 21 d exposure Rainbow trout (*Oncorhynchus mykiss*)	LOEC GSI[3] < 0.122 gonadal histology LOEC = 0.122 VTG[1]	Short-chain
NPE-4	Method not specified 100d exposure Medaka (*Oryzias latipes*)	0.114, survival 0.38, SSC[2]	Medium-chain
NPE-9	Method not specified 100 d exposure Medaka (*Oryzias latipes*)	0.54, survival 0.54, SSC[2]	Medium-chain
Invertebrate			
NPE-1	TG211 *Daphnia magna* 21-d exposure	NOEC reproduction = 0.1	Short-chain
NPE-1.5	EPA OTS 797.1950 *Mysisdopsis bahia* 28-d exposure	NOEC reproduction = 0.0077	Short-chain
NPE-9	EPA guideline 7d exposure *Daphnia magna*	10 (mortality) >10 (growth)	Medium-chain
Algae			
NPE-2	TG201 *Pseudokirchneriella subcapitata*	72 h NOEC $_{Growth}$ = 1.22	Short-chain
NEP-3 NPE-6 NPE-12	*Scenedesmus subpicatus* performed according to test guideline 201	72h-NOE_bC = 1 72h- NOE_bC = 3 72h- NOE_bC = 20	Short-chain Short-chain Long-chain

1: VTG = vitellogenin
2: SSC = second sex characteristics

Fate and behaviour

NPEs are expected to be hydrolytically stable. They have a moderate potential to absorb to organic matter. Due to their low vapour pressure and low Henry's law constant, evaporation into the atmosphere is expected to be neglible. In general, degradation of NPEs involves progressive shortening of the ethoxylate chain. Hydrolytic or biodegradative ether cleavage leads to the accumulation of NPE-1 and NPE-2.

Individual short-chain NPEs are expected to show common biodegradation properties and pathways. NPE-1 and NPE-2 are expected to degrade the slowest (Van Vlaardingen et al., 2003) and several studies show that in solution NPE-1/NPE-2 ethoxylates compounds degrade overtime to nonylphenol (European Chemical Agency, 2013; Metcalfe et al., 2001). Short-chain NPEs are considered as substances with potential to bioaccumulate.

Medium-chain NPEs are not readily biodegradable using the standard screening test methods (e.g. the application of the 10-day window criterion). However, significant levels of biodegradation (52 – 99%) are observed for all NPEs tested indicating they metabolize to some extent. This rate of degradation seems to rise with increasing number of ethoxylated group. The bioaccumulation potential for this group varies with increasing ethoxy group. Based on $logK_{ow}$ values, the lower end of the group have the potential to bioaccumulate whilst the upper end group a low potential to bioaccumulate.

Long-chain NPEs are considered rapidly degradable and as substances with low potential for bioaccumulation since the estimated LogKow values are above the CLP trigger of ≥ 4.

Conclusion

The grouping of the various lengths of ethoxylated NPEs in short-, mid- and long-chain groups was based on their acute and chronic toxicity. The majority of the endpoints given in Tables 8 and 9 fall within the choosen borders of the groups. Endpoints on the fate and behaviour of NPEs did not further influence this choice. Overall, the choice of grouping based on valid (E)LC_{50} and NOEC values and the corresponding classification are summarised in Table 10. It is shown that the choice of the ranges of each group resulted in distinctive differences in their classification.

Table 10: Grouping and classification of NPEs

Nonylphenol Ethoxylate	Proposed Classification	
	Acute	Chronic
Short-chain: 1 to < 3 ethoxy groups	Category 1 (M = 1)	Category 1 (M = 10)
Medium-chain: ≤ 3 to < 11 ethoxy groups	no acute classification	Category 2
Long – chain: ≤ 11 to ≤ 30 ethoxy groups	no acute classification	no chronic classification

5 IDENTIFIED USES

According to the chemicial safety report, the substance is only used by workers in industrial settings (manufacture and formulation of the substance). For example, industrial manufacture of NPE and industrial formulation of mining products (floating agents) containing NPE.

6 DATA SOURCES

The data presented in this CLH report is reproduced from several sources.

- Annex XV dossier – Identification of 4-nonylphenol, branched and linear, ethoxylated as SVHC. Germany, 2012.
- ECHA (2013), Support document for identification of 4-nonylphenol, branched and linear, ethoxylated.
- REACH registration dossiers for nonylphenol, ethoxylated (CAS 9016-45-9)
- REACH registration dossiers for nonylphenol, branched, ethoxylated (CAS 68412-54-4)
- Public literature

7 PHYSICOCHEMICAL PROPERTIES

NPEs belong to a larger family called alkyphenol ethoxylates (APE). APEs are not synthesized on an individual basis but are formed and processed as a mixture containing oligomers with varying number of ethoxy groups. Therefore physicochemical parameters for isomers will –in most cases- be estimated values (Van Vlaardingen et al., 2003).

Physical chemical data were calculated with EPI Suite (v4.11) and provided in Table 11. The following factors were taken into account in the calculations: the degree of ethoxylation , substitution position of the nonyl group on the phenol molecule (ortho-, meta-, and –para) and the structure of the nonyl group (linear or branched form). The estimated results are provided as single values since substitutions of the nonyl group on the phenol did not influence the physical chemical properties. The KowWin QSAR is not suitable for nonyl ethoxylate since it is a surfactant therefore the results should be used with caution.

Table 11: Summary of estimated physicochemical properties for nonylphenol, branched and linear, NPE-1and NPE-2.

	Linear	**Branched**
NPE-1	**Ortho, meta and para substitutions**	**Ortho, meta and para substitutions**
Physical Chemical Property		
Molecular weight (g/mole)	264.41	264.41
Log Kow v 1.68[2]	5.58	5.28
Water solubility at 25°C mg/L (WaterNT v1.01)[1]	0.54604	4.31
Vapour pressure mmHg at 25°C (Modified Grain method) (MPBPWIN v.1.43)	1.78E-07	2.85E-06
Henry's Law Constant (atm m3/mol) (HENRYWIN v3.20, Bond method)	1.65E-07	1.65E-07
	Linear	**Branched**
NPE-2	**Ortho, meta and para substitutions**	**Ortho, meta and para substitutions**
Physical Chemical Property		
Molecular weight (g/mole)	308.47	308.47
Log Kow v 1.68[2]	5.30	5.01
Water solubility at 25°C mg/L (WaterNT v1.01)[1]	1.01	7.97
Vapour pressure mmHg at 25°C (Modified Grain method) (MPBPWIN v.1.43)	9.14E-09	1.25E-07
Henry's Law Constant (atm m3/mol) (HENRYWIN v3.20, Bond method)	2.56E-09	2.56E-09

1: WaterNT is based on a new set of (larger) fragments which are optimized for water solubility. The set of fragments contains the whole molecule of nonylphenol ethoxylate. The estimation of water solubility is therefore reliable.
2: As nonyl ethoxylate is a surfactant the Kow estimation as well as the experimental determination is difficult as the border between the fractions water and octanol is disturbed by nonyl ethoxylate.

Data is also available for registration UVCB substance, nonylphenol, branched, ethoxylated (CAS 68412-54-4), see table 12.

Table 12: Summary of physicochemical properties reported in the registration dossier for nonylphenol, branched, ethoxylated (CAS 68412-54-4)*

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	A clear and colourless viscous liquid with a weak non-specific odour	Bodsch J, 2010a	Measured
Melting/freezing point	-54.80°C	Huber V, 2010a	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Boiling point	354°C	Huber V, 2010b	Measured
Relative density	0.9947 at 20°C	Bodsch J, 2010b	Measured
Vapour pressure	0.00043, 0.00066 and 0.0048 hPa at 20, 25 and 50°C, respectively	Smeykal H, 2010a	Measured
Surface tension	31.56 mN/m at 20°C	Bodsch J, 2010c	Measured The critical micelle concentrationwas determined to be 4.55 mg/L.
Water solubility	4.55 mg/L at 20°C (slightly soluble in water)	Bodsch J, 2010d	Measured The water solubility was determined by measuring the critical micelle concentration (CMC).
Partition coefficient n-octanol/water	5.39 at 20°C NPE-1: 4.17 at 20.5°C NPE-2: 4.21 ± 0.18 at 20.5°C	Bodsch J, 2010c Van Vlaardingen, et al, 2003	Estimated The reported value represents the weighted mean value log Pow value of the substance. Branched structures was not considered in the calculation Measured Using the shake flask method and a normal-phase HPLC, according to OECD guideline 107. A commercial mixture of NPE-n isomers was used in log *K*ow determination; isomers were separated by normal phase HPLC analysis and quantified individually. It is noted that for surface active substances such as nonylphenol ethoxylate the shake-flask methods is not the most suitable experimental method to determine the Log Kow due to micelle/emulsion formation.
Flash point	193.5°C at 1013 hPa	Huber V, 2010c	Measured
Flammability	non flammable	Smeykal H, 2010b	Measured
Explosive properties	non explosive		
Self-ignition temperature	410°C at 1013 hPa	Herbert V, 2010	Measured
Oxidising properties	non oxidising		
Stability in organic solvents and identity of relevant degradation products	No significant degradation (>10 %) of NPE in methanol and ethyl acetate was observed. NPE is therefore considered as stable in organic solvents	Lange J, 2010	Measured
Dissociation constant	Due to the low solubility and the UV/VIS absorption properties of NPE, an experimental determination of the	Huber V, 2010d	Measured

Property	Value	Reference	Comment (e.g. measured or estimated)
	dissociation constant was not possible		
Viscosity	692.55 mm²/s and 118.65 mm²/s at 20°C and 40°C, respectively	Smeykal H, 2010c	Measured

*Reported as a UVCB substance.

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier.

10 EVALUATION OF HEALTH HAZARDS

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Environmental fate and aquatic toxicity data on NPE with 1 to < 3 ethoxy groups are available and serve as the basis for their classification. In various studies, NPEs and nonylphenol were evaluated in the same test. Therefore in some instances, the results for nonylphenol are reported in the CLH dossier. These are provided only for information purposes, and the data on NP is not used to read-across for the classification of NPEs.

11.1 Rapid degradability of organic substances

Table 13: Summary of relevant information on rapid degradability

Test substance	Method	Results	Remarks	KS*	Reference
Ready biodegradability					
NPE-1 NPE-2 Purity 99%	OECD 301 F Manometric respiratory test No GLP	NPE-1: 25.9 ± 8.1 % at day 28 NPE-2: 0 % at day 28	Not readily biodegradable	2	Stasinakis *et al*. (2008)[2]
NPE-1.5	OECD 301 B CO2 evolution GLP Adapted inoculum	45.3 ± 18.4% at day 28 58.7% at day 35	10 day window was failed Not readily biodegradable	2	Anonymous, (1999a)[1] Staples *et al*. (2001)[1,2]
Hydrolysis					
NPEO**	OECD Guideline 111	<10% transformed after 120 hours at pH 4, 7 and 9 at	NPEO is considered hydrolytically	1	Anonymous, (2010a)[1]

		50°C	stable		
Phototransformation					
NPE-1 in water	Direct and indirect Photolysis Test Guideline: unknown GLP: unknown Sensitiser: Natural water	No photodegradation of NPE-1 was observed		2	Ahel *et al.* (1994a)[1]
NPE-2 in air	Phototransformation using AOPWin v1.92 program (EPIWEB v 4.0)	The estimated KOH and t1/2 was found to be 5.86 x10^{-11} cm^3/ molecule-sec and 0.189015 days, respectively		2	U.S. Environmental Protection Agency (2009)[1]
Simulation studies (sediment)					
NPE-1 98% aerobic	fresh water sediment	DT_{50} = 69.3 – 115.5 days	Primary degradation	2	Yuan *et al.*, (2004)[1]
NPE-1 anaerobic	fresh water sediment	$DegT_{50}$ = 49.5 – 77.0 days	Primary degradation	2	Chang *et al.*, (2004)

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.
** See Table 6 for the constituents of NPEO.
[1] As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.
[2] As summarised in the ECHA support document for identification of 4-nonylphenol, branched and linear, ethoxylated as substances of very high concern.

11.1.1 Ready biodegradability

Stasinakis et al, 2008
The biodegradability of several potential endocrine disrupting compounds, namely 4-n-nonylphenol (4-n-NP), nonylphenol monoethoxylate (NPE-1), nonylphenol diethoxylate (NPE-2), bisphenol A (BPA), triclosan (TCS), di-(2-ethylhexyl)-phthalate (DEHP), perfluorooctanoate (PFOA) and perfluorononanoate (PFNA) was evaluated in this study, using OECD method 301F (manometric respirometry test) and activated sludge as inoculum. Emphasis will be given to the results of NPE-1 and NPE-2.

Activated sludge was collected from a municipal WWTS (Mytilene, Lesvos). The inoculum was preconditioned to reach the endogenous respiration rate, by washing once with tap water, diluting to a concentration of 3 g l^{-1} dry matter and finally aerating for 2 days. This starved activated sludge suspension was further diluted to the inoculum concentration given in OECD protocol. Manometric respirometry tests were carried out in the Sensomat system (AQUALYTIC® ZN, Tintometer GmbH, Germany), which is based on a manometric principle and uses innovative piezo-resistive pressure sensor technology. Owing to microbial activity, oxygen is taken from the gas phase of the hermetically sealed reaction vessels, while carbon dioxide released from respiration is absorbed by KOH in a small tube and the resulting reduction in air pressure inside the closed system is measured.

Biodegradation experiments were performed in eight experimental cycles. Biodegradation of each target compound was studied in triplicate in AQUALYTIC® flasks. Initially, solution of each compound in methanol (1–2 ml) was poured into AQUALYTIC flasks and it was allowed to stand at room temperature for complete methanol evaporation. Afterwards, appropriate volumes of the mineral medium consisted of KH2PO4, K2HPO4, Na2HPO4· 12H2O, NH4Cl, MgSO4 · 7H2O, CaCl2 and FeCl3 · 6H2O (OECD, 1993) were added to each flask and the mixture was subjected to ultrasonication. The concentrations of the target compounds ranged between 10 and 50 mg l^{-1}, while the inoculum concentration was 30 mg l^{-1} dry matter. To prevent nitrification, allylthiourea was added in all flasks at concentration of 10 mg l^{-1}. Addition of

allylthiourea is not considered in the original OECD 301F, however it has been proved to be an effective inhibitor of nitrification processes and it has been often used in respirometric biodegradation tests.

Results

After a lag phase of 17.3 ± 0.7 days, NPE-1 was aerobically biodegraded with 25.9 ±8.1% at day 28. For NPE-2 no biodegradation was observed. This test is considered as a valid study, with reliability 2.

Anonymous, 1999a and Staples, et al. 2001

A study was conducted to evaluate the ready biodegradability of NPE-1.5 and NPE-9 by using activated sludge from a wastewater treatment plant as the microbial seed. In this dossier emphasis will be given to the results of NPE-1.5. The procedure followed OECD guideline 301B was performed under GLP. The test substance with the standard nutrient medium inoculated with inoculum (30 mg suspended solids/L), was kept in bottles (in darkness) at 22 ± 2 °C for 35 d. A blank control, reference material (Sodium benzoate) and a toxic control were run in parallel for validation purposes. Carbone dioxide traps were removed and analysed on days 1, 2, 4, 6, 9, 13, 18, 22, 28 and 35. Test substance concentration and dissolved oxygen concentrations for each test medium were determined on days 15 and 35.

Results

45.3 ± 18.4% CO_2 evolution was observed for NPE-1.5 after 28 days and 58.7% after 35 days. The 10-day window was failed. 33.4% suspended organic carbon was determined on day 35. This suggests that NPE incorporated into biomass or adsorbed to suspended material. The reference material attained 60% mineralization in 6 d and 95.4% after 35 d and passed the OECD '10-day window' criterion.

NPE-1.5 did not meet the pass level biodegradation of 60% within 28 days. Therefore it is considered not readily biodegradable under the conditions of the OECD Guideline 301B. This test is considered as a valid study, with reliability 2.

Conclusions on ready biodegradability

Reliable biodegradation studies for NPE with 1, 1.5 and 2 ethoxy groups are not readily biodegradable. NPE-1 biodegraded up to 26% at 28 days, NPE-1.5 biodegraded up to 45% at 28 days and NPE-2 showed no biodegradation (0%) at 28 days.

11.1.2 BOD_5/COD

No information is available.

11.1.3 Hydrolysis

Anonymous, 2010a

Hydrolysis as a function of pH was determined for nonylphenol, branched, ethoxylated (NPEO) according to OECD Guideline 111. NPEO contains about 80% NPE-1 and NPE-2, and the remaining being longer chain ethoxylates up to NPE-6. The study was conducted with test item concentrations of 1.5 mg/L in buffer solutions at pH 4, 7 and 9 at a test temperature of 50°C (preliminary test). Samples were taken at test start (0 h) and test end (120 h) and analysed via HPLC on a reversed phase column with FLD using an external standard. Buffer solutions were analysed at test start and test end and indicated no interference with the test item. The analytical method for determination of the test item was validated and tested with satisfactory results in regard to linearity, repeatability of injections, accuracy, precision and specificity. Degradation was calculated as the percentage loss of the test item over the time. In the preliminary test, the test item was found to be stable at pH 4, 7 and 9, respectively. No further testing was deemed necessary as less than 10 % of the applied test item were transformed after 120 h (5 days) at each of the three pH values. Reaction rate constants and half-lives could not be calculated because the test item undergoes no significant hydrolysis. With respect to the guidelines a half-life of > 1 year could be assumed for ambient temperature conditions. NPE is considered to be hydrolytically stable. This test is considered as a valid study, with reliability 1.

Conclusions hydrolysis
NPEO is considered hydrolytically stable. Hydrolysis is not a relevant pathway in the degradation process for short-chain NPEs in the aquatic environment.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant for C&L.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No information available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Biodegradation in sediment

Yuan et al., 2004
Yuan *et al*. (2004) sampled sediment from the same samples sites as Chang *et al*. 2004 and studied the aerobic degradation of NP and NPE-1. There is no mention of test method or GLP compliance. The half-lives $DegT_{50}$ (primary degradation) for NP ranged from 13.6-99.0 days and for NPE-1 they ranged from 69.3 to 115.5 days. These results suggest that microorganisms adapt in a site specific manner, and therefore vary in terms of biodegrading capacity. If the sediment was additionally acclimated with nonylphenol, NP was completely degraded by day 28 and NPE-1 by day 56. This test is considered as a valid study, with reliability 2.

Chang et al., 2004
Chang *et al*. (2004) studied the degradation of NP and NPE-1 by anaerobic microorganisms from NP acclimated river sediments. There is no mention of test method or GLP compliance. The $DegT_{50}$ (primary degradation) ranged from 49.5 to 77.0 days (30 °C). At day 8, NP was determined as intermediate product. The concentration of NP increased from day 8 to day 14 but was absent after an 84-day incubation period. Degradation rates for NPE-1 were enhanced by increasing temperature and inhibited by the addition of acetate, pyruvate, lactate, manganese dioxide, ferric chloride, sodium chloride, heavy metals, and phthalic acid esters. This test is considered as a valid study, with reliability 2.

Teurneu, 2004
The degradation of NPE-n (n=2, 4, 10, and 40) was studied under aerobic and anaerobic conditions at 27°C and 10°C. In this evaluation the focus is on the results for NPE-2. For the batch experiments sediment samples from the bottom of a sedimentation basin of an industrial site (production of NPE-n) were used. There is no mention of test method or GLP compliance. The initial concentration of NPE-n was 500 mg/L. The long-chain ethoxylates showed greater degradation than the short-chain ethoxylates. This was confirmed by screening of degrading organisms in the sediment. A higher presence of bacteria capable of 10 and 40 ethoxylate degradation was observed. The results of the sediment analysis indicate an accumulation of NP in the sediment. After 44 days degradation of NPE-2 was 4% at 27°C under aerobic conditions and 5% under anaerobic conditions. At 10°C degradation was 0 and 1% under aerobic and anaerobic conditions, respectively. This test is considered not reliable with Ri3 as the test was not GLP compliant.

Conclusions (an)aerobic degradation in sediment

The data shows that in sediment short-chain ethoxylated NPE-1 degrade slowly under aerobic and anaerobic conditions.The information on NPE-2 supports this conclusion.

Biodegradation in soil

Three biodegradation tests in soil are available for short-chain NPEs. The studies are reliable with a reliability score of 2. Although soil studies are not considered relevant for classification these studies are included in this report to give information on the behaviour of the active substance.

Marcomini et al., 1989[1]

The fate of a mixture of NPE-n (n= 1-3) in sludge amended soil was studied by Marcomini *et al*. 1989. The soil samples were collected from the upper 5 cm of planted grass land. This site was part of a long term field study and had received anaerobically digested sludge at an average application rate of 13.5 tonnes/ha year (dry weight). The sludge was applied to the surface soil as a liquid spread, four to six times per year.
The initial concentrations of NPE-1 and NPE-2 in the amended soil were 1.1 and 0.095 mg/kg (dry weight). 320 days after the last sludge application the residual mean concentrations were 0.11 and 0.013 mg/kg (dry weight) for NPE-1 and NPE-2, respectively. The disappearance of NPE-1 and NPE-2 were fast in the first two weeks followed by a slow disappearance from days 30-90; from day 150 no significant disappearance was noted and NPE-1 and NPE-2 was classed as being persistent. The estimated degradation half-lives of 4-NPE-1 in the soil in the initial phase was 7 days (NPE-2 = 8 days), 150 days for the transition phase (NPE-2 = 110 days) and >360 days after 150 days of application. These half-lives are for primary biodegradation and were calculated assuming pseudo first order kinetics. This test is considered as a valid study, with reliability 2.

Ejlertsson et al., 1999[1]

The degradation of a NPE-1-2 mixture (2, 60 and 308 mg/L) in landfilled municipal solid waste and landfilled sludge was determined under methanogenic conditions for 150 days (Ejlertsson *et al*., 1999). In both inocula at a concentration of 2 mg/L NPE-1-2 the added 4- 4-NPE-1-2 was transformed to 4-NP by anaerobic microorganisms. The background level of 4- NP in the landfilled municipal solid waste was measured and was so high that a transformation of 4-NPE-1-2 would only increase the indigenous 4-NP concentration with 5-10% (significant decrease of 4-NPE-1 and 4-NPE-2 was observed within 22 days). An increase to 81 % during 53 days was observed in samples with landfilled sludge. At a concentration of 60 mg/L 4-NPE-1-2 approximately 20 % 4-NP was formed during 40 days (landfilled municipal solid waste) and 80 days (landfilled sludge). The concentration of formed 4-NP remained constant until day 150. At 308 mg/L 4-NPE-1-2 less than 1% of the added 4-NPE-1-2 was transformed into 4-NP. This test is considered as a valid study, with reliability 2.

Gejlsbjerg et al., 2001[1]

The mineralization of ^{14}C-labelled 4-NPE-2 was investigated in different sludge-soil mixtures and soils (Gejlsbjerg *et al*., 2001). The mineralization of 4-NPE-2 was indirectly affected by the amount of sludge in the test mixtures. A higher content of sludge in the mixtures reduced the overall concentration of oxygen, which resulted in a decrease of the mineralization of 4-NPE-2. A higher water content resulted in lower concentrations of oxygen, thus in decrease of mineralization, too. Mineralization of 4-NPE-2 was not affected by the soil type since the percentage of compound mineralized (64.4 %) after two months was not different between any of the test mixtures. This test is considered as a valid study, with reliability 2.

[1] As summarised the ECHA Support Document on 4-nonylphenol, branched and linear, ethoxylated.

Conclusion degradation in soil

In summary, results show that the overall biodegradation of NPEs in soil is slow and depends on the amount of oxygen available. Results of Ejlertsson *et al*. (1999) show that nonylphenol is formed during this process. 81 % of the overall NPEs concentration at the end of the experiment (2 month) was 4-nonylphenol in a landfill with anaerobic sludge. Thus results indicate that nonylphenol ethoxylates may degrade to 4-nonylphenol. Because conversion is slow, it can be expected that the remaining ethoxylate concentration is a long-term source of 4-nonylphenol in soil. The studies of Marcomini *et al*., (1989), Ejlertsson *et al*., (1999) and Gejlsbjerg *et al*. (2001) show a slow degradation of short-chain NPEs in soil. Results also indicate that these may degrade to 4-nonylphenol.

11.1.4.4 Photochemical degradation

Table 14. Summary of relevant information on phototransformation

Test/ substance	Method	Results	Remarks	KS*	Reference
NPE-1 in water	Direct and indirect Photolysis Test Guideline: unknown GLP: unknown Sensitiser: Natural water	No photodegradation of NPE-1 was observed		2	Ahel *et al.* (1994a)[1]
NPE-2 in air	Phototransformation using AOPWin v1.92 program (EPIWEB v 4.0)	The estimated KOH and t1/2 was found to be 5.86 x10^{-11} cm^3/ molecule-sec and 0.189015 days, respectively		2	U.S. Environmental Protection Agency (2009)[1]

*Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

[1]As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

Ahel et al. (1994a)

A study was conducted to assess the rates of photochemical transformation of nonylphenol (NP), nonylphenol mono ethoxylate (NPE-1) in natural waters.

The phototransformation experiments were conducted both in the presence of sunlight and artificial light. In the sunlight phototransformation experiment, quartz tubes containing the solutions of NP or NPE-1 in filtered lake water were exposed to sunlight (average sun irradiation intensities were 0.705 kW/m2 and 0.760 kW/m2) at a depth of 20-25 cm (in Chriesbach creek) or at surface (in shallow flat-bottomed container filled with tap water) of water. In the laboratory experiment using a merry-go-round reactor (MGRR), the solutions of) NP and NPE-1 in distilled water (for direct photolysis) or filtered lake water (for sensitized photolysis) were exposed to the mercury lamp (700 W) light (10 times more light intensity than sunlight during a sunny summer day). Quantitative determinations of the analytes were performed by normal-phase HPLC after a simple extraction of the water samples with n-hexane.

The first-order rate constant of sunlight photolysis (kp) for NP was estimated to be 0.09 h^{-1}. This corresponds to a half-life of 10-15 h under continuous clear sky, noon and summer sunlight in the surface layer of natural waters. The photolysis rate in the deeper layers was strongly attenuated, being approximately 1.5 times slower (kp = 0.06-0.05 h^{-1}) at depths of 20-25 cm than at the surface. The photochemical degradation of NPE-1 was found to be insignificant. The laboratory experiments using artificial light showed that the

photochemical degradation of both NP and NPE-1 was due mainly to sensitized photolysis whilst direct photolysis was comparatively slow. The data generated with artificial light can only be considered to be of qualitative nature since the chosen conditions are different from natural sunlight and are not recommended for standard testing.

Based on this information aquatic photodegradation is considered not to have a relevant impact on the degradation of short-chain NPE in the aquatic environment. This test is considered as a valid study, with reliability 2.

U.S. Environmental Protection Agency (2009)
The hydroxyl radical reaction half-life estimation value was estimated for NPEO using the AOPWin v1.92 program of EPIWEB v 4.0. The hydroxyl radical reaction half-life estimation values were first calculated using the default values of 12-h day, hydroxyl radical concentration (1.5×10^{-6} molecules (radicals)/cm^3) and hydroxyl rate constant (49.2915 x 10^{-12} cm^3/molecule-sec) of the AOPWin v1.92 program. Then the final values of hydroxyl radical reaction half-life were calculated on a weighted-average basis using the mole fractions of the individual components.

The hydroxyl radical reaction half-life values were also estimated for three typical isomers (due to branching) of nonyl chain of NPEO (i.e. 1,2-dimethyl-1-ethyl-pentyl phenol E-2; 1,3-dimethyl-1-propyl-butyl phenol E-2; 1-ethyl-1-methyl-hexyl phenol E-2) component of test substance. The KOH values ranged from 58.01 x 10^{-12} to 58.29 x 10^{-12} cm^3/molecule-sec and the half-life ranged from 0.183-0.184 days taking into account a 12-h day and a mean OH radical concentration of 1.5 x10^6 OH radicals per cm^3 for the three isomers.

The estimated $K_{OH}$and $t_{1/2}$ for NPE-2 using AOPWin v1.92 program (EPIWEB v 4.0) was found to be 5.86 x$10^{-11}$$cm^3$/molecule-second 0.189015 days respectively. There was no significant impact of branching of nonyl chains (isomers) of NPEO on the photo-transformation estimations. Consequently, photodegradation in air is expected not to be a relevant path of degradation for NPEO. This test is considered as a valid study, with reliability 2.

Conclusions photodegradation
No photodegradation of NPE-1 in water has been observed. Photodegration is not a relevant pathway in the degradation process for NPEO in air and the aquatic environment.

11.1.4.5 Summary and discussion of degradation

NPE-1 is considered hydrolytically stable and photodegradation of NPE-1 in water has not been observed. Hydrolysis and photodegration are not relevant pathways of degradation process for NPE in the aquatic environment. Reliable biodegradation screening studies on short-chain NPEn (n = 1 or 2) show that these substances are not readily biodegradable. NPE-1 biodegraded up to 26% after 28 days, NPE-1.5 biodegraded up to 45% after 28 days and NPE-2 showed no biodegradation (0%) after 28 days. Biodegradation water/sediment studies are not available.

In the biodegradation sediment study, DT_{50} ranges of 69.3 – 115.5 days and 49.5 – 77 days was obtained for NPE-1under aerobic and anaerobic conditions, respectively. NPE-2 degraded up to 4% at 27°C and 0% at 10°C under aerobic conditions. Under anaerobic conditions NPE-2 degraded up to 5% at 27°C and 1% at10° C under. The degradation of the short chain ethoxylates in sediment is considered slow. Reliable biodegradation soil studies are not available. However, results indicate that 4-nonylphenol ethoxylates may degrade to 4-nonylphenol in soil.

This evaluation shows that NPEs with 1 or 2 ethoxy groups, degrade to some degree in water, however this biotransformation is not rapid. The position of the nonyl group (-ortho, -meta or -para) and the arrangement of the nonyl group (branched and linear) is not expected to have a significant impact on degradation.

Based on the findings from the ready biodegradable tests, short-chain NPEs, are considered not rapidly degradable for purposes of classification.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Adsorption/Desorption

An estimated Koc value of 2661 L/kg for NPE-2 was reported in the REACH registration dossier. The Koc was calculated using the MCI (Molecular Connectivity Index) approach of KOCWIN v2.00 program. The values of the Koc were calculated for the individual components of NPE-2. NPE-2 has a moderate potential to adsorb to organic matter.

Volatilisation

The vapor pressure of NPE was determined to be 0.043 Pa at 20°C. An estimated Henry's law constant for NPE-2 was reported. The average estimated Henry's law constant for NPE-2 using both bond and group method approach of the HENRYWIN v3.20 program (EPIWEB v 4.0) was found to be 3.21 x 10^{-7} atm-m^3/mole (3.25 x 10^{-2} Pa-m^3/mole). This suggests that the substance has a low potential to distribute into the atmospheric compartment.

Distribution modelling

The estimated percentage distribution of NPE-2 (the major component of the test substance) to air, water, soil and sediment, using Fugacity level III model (EPIWEB v 4.0) was found to be 0.32, 22.5, 75.4 and 1.77, respectively. Based on these results, NPE-2 is expected to distribute predominantly to water and soil.

11.4 Bioaccumulation

Table 15: Summary of available information on bioaccumulation

Method	Results	Remarks*	Reference
BCF study with mussel *Mytilus edulis* Aqueous (freshwater) Method: GC-MS NPE-1, NPE-2	BCF: > 100 — < 200 (NPE-1) BCF: > 50 — < 100 (NPE-2)	2 (reliable with restrictions)	Granmo *et al.* (1991)[1,2]
Field study with *Cladophora glomerata, Potamogeton crispus, Squalius cephalus* Heck, *Barbus barbus* and *Salmo gairdneri* aqueous and/or feed (freshwater) Details of method: - Measured/calculated log Pow:	BAF values NPE-1 (Staples *et al*. 1998) <u>Fish</u>: *Squalius cephalus*: 1 *Oncorhynchus mykiss*: 3 *Barbus barbus*: 19 <u>Algae</u>: *Cladophora glomerata*: 10 <u>Water plants</u> *Potamogeton crispus*: 2	2 (reliable with restrictions) BAF values are dimensionless (whole body w.w.) CSR only provided BAF	Ahel *et al.* (1993)[1]; Staples *et al.* (1998)[3]

4.03-4.39 at 20.5°C NPE-1 and NPE-2	BAF values NPE-2 (CSR) Fish: *Salmo gairdneri*: 0.8 *Squalius cephalus*: 2 *Barbus barbus*: 37 Algae *Cladophora glomerata*: 23 Water plants *Potamogeton crispus*: 10	data on NPE-2 Staples et al. 1998 also provided BAF data on NPE-1	
Field study with *Ambloplites rupestri, Lepomis macrochirus, Lepomis cyanellus, Micropterus dolomieui, Catostomus commersoni, Maxostoma macrolepidotum, Osmerus mordax* NPE-1 and NPE-2 were measured Method: exhaustive steam distillation with concurrent liquid extraction	Bioconcentration factors not established. NP was the predominant compound, with concentrations of NPEs less than those of NP	2 (reliable with restrictions)	Keith *et al.* (2001)[1]

*Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

[1]As summarised in the Chemical Safety Report for nonylphenol, branched, ethoxylated, EC number 500-209-1, November 2015 and April 2018.

[2] As summarised the open literature.

[3] Mentioned in Staples *et al*. (1998) in addition to the CSR.

11.4.1 Estimated bioaccumulation

The estimated octanol-water partition coefficient values for NPE-1 range from 5.28 (branched) - 5.58 (linear) and for NPE-2 from 5.01 (branched) - 5.30 (linear). Data reported for the registered UVCB substance nonylphenol, branched, ethoxylated (CAS 68412-54-4) state a value of 5.37. This value represents the weighted mean value log Pow value of the substance and not individual constituents. Branched structures were not considered in the calculation.

U.S.Environmental Protection Agency (2014)

The BCF of nonylphenol branched ethoxylated was estimated using the BCFBAF v3.01 program of EPIWEB 4.1 (Arnot Gobas method), based on the weighted average of the BCFs of the various constituents. A value of 648 was obtained. This value is above the CLP trigger value of BCF ≥ 500. The authors of the REACH registration dossier assign this with a Klimisch score of 2.

11.4.2 Measured partition coefficient and bioaccumulation test data

Ahel and Giger (1993)

A study was conducted to determine the n-octanol water partition coefficient (log K_{ow}) for NPE-1 and NPE-2, using the shake flask method and a normal-phase HPLC, according to OECD guideline 107. The log Kow of the test substance was determined to be 4.17 and 4.21 at 20.5°C for NPE-1 and NPE-2 respectively. This method is not the most suitable experimental method to determine the Log K_{ow} given the surface active

propteries of the registered substance and due to micelle/emulsion formation (see Table 11), hence affecting the reliability of the result.

Granmo et al. (1991)

An accumulation study was performed with caged mussels (*Mytilus edulis*) in the unpolluted waters of a fjord on the Swedish West Coast. Mussels (40-50 mm) taken from a cultivation in an unpolluted area in the Northern part of the coast were stored in submersed tanks with a controlled dosage of wastewater from the outlet of a chemical plant of the Swedish West Coast producing surface active agents. The wastewater was distributed to the tanks in a semi-static system where the water was changed every 4 h. The concentrations of wasterwater were 100, 10 and 1%, respectively. A total of 25 specimen were used per concentration. After 50 days, the mussels were brought to the laboratory and analysed for shell growth and condition. Ten specimen per concentration were prepared and stored at -50°C for chemical analysis. Mussel dry weight was estimated. The concentrations of NP and NP ethoxylates in the frozen samples were analysed by GC-MS. Results were reported based on mussel fresh weight and fat weight. The concentrations of NP, NPE-1, NPE-2 and NPE-3 in the wastewater (100%) were equivalent to 40, 60, 40 and 50 µg/L, respectively. The study results indicated that NP and its short chain ethoxylates bioaccumulated in mussels and that the degree of bioaccumulation was dependent on chain length, as expected based on water solubility. The average bioconcentration factor for the 100, 10 and 1% wastewater concentrations combined was between 300-400 for NP, 100 -200 for NPE-1, 50 - 100 for NPE-2 and approximately 50 for NPE-3. No information is provided on lipid normalization. The authors of the REACH registration dossier assign this study with a Klimisch score of 2 (reliable with restrictions). The overall quality and reliability of the reported BCF values could not be ascertained because essential information is missing from the study summary. Based on this, dossier submitter assigns the study a Klimisch score of 4 (non-assignable).

Ahel et al. (1993) and Staples et al.(1998)

A field study was conducted to evaluate the bioaccumulation of NP, NPE-1 and NPE-2 in algae, aquatic plants, fish and bird (Ahel *et al.*, 1993). Samples of macrophytic algae were collected manually from the Chriesbach and the Glatt River (Zurich, Switzerland), in the summer and autumn of 1984 and 1985. The samples were wrapped in aluminum foil and kept deepfrozen (-20°C) until analysis. Fish species of the area (*Squalus cephalus, Barbus barbus and Salmo gairnieri*) were collected in the Chriesbach Creek, dissected immediately and deep frozen until tissue analysis (muscle, gut, liver, gills, heart, roe and/or brain) was conducted. One wild duck (*Anas boscas*) was also caught, dissected and its tissues (liver, muscle, guts, stomach, heart and brain) stored for consequent analysis. Dry matter content was determined for each sample. Water samples were also taken (location and number not specified) for NP, NPE-1 and NPE-2 analysis. Steam distillation / cyclohexane extracts were quantified by HPLC. The extraction efficiencies for NP, NPE-1 and NPE-2 were 100, 96 and 82%, respectively. The limit of quantification of the HPLC method was 0.03 mg/kg dry weight (d.w.) based on 10 g of fresh tissue.

The highest concentrations were found in the algae *Cladophora glomerata*, with 38.0, 4.7 and 4.3 mg/kg dw (dry weight) for NP, NPE-1 and NPE-2, respectively. The concentrations in fish were lower (NP: <0.03 – 1.6 mg/kg dw, NPE-1: 0.06 – 7.02 mg/kg dw and NPE-2: <0.03 – 3.07 mg/kg dw). In the duck, the values ranged between <0.03 – 1.20 (NP), <0.03 – 2.10 (NPE-1) and <0.03 – 0.35 (NPE-2) mg/kg dw. The average water concentrations of NP, NPE-1 and NPE-2 in the Chriesbach Creek were 3.9, 24 and 9.4 µg/L (arithmetic mean of three determinations).

The authors reported the concentrations in fish muscle and several body organs and expressed results as a dry weight basis to concentrations in water, which is not a typical manner in which fish data is reported. Staples *et al.* (1998) considered this methodology to be incorrect and recalculations were carried out. When all data was expressed on a weight weight basis assuming that fish muscle (the edible portion of the fish) was 85% water /15% dry matter and that algae were 95% water / 5% dry matter, the non-lipid BAFs were below 50 for all species. The BAFs for the algae *Cladophora glomerata* were equivalent to 487 (NP), 10 (NPE-1) and 23 (NPE-2). The BAFs for the water plant species *Potamogeton crispus* 32 (NP), 2 (NPE-1) and 10 (NPE-2). The BAFs for fish ranged from 6 – 87 (NP), 1 – 19 (NPE -1) and 0.8 – 37 (NPE -2). No recalculated values were presented for duck.

The registrant considered the results as an indication of relatively low bioaccumulation potential in the aquatic environment for NPE-1 and NPE-2 and no significant biomagnification in the food chain. The dossier submitter considers that the quality and reliability of the reported BAF values cannot be ascertained. In general, BAF ratios derived from field studies do not include any information about ecosystem conditions, intake routes and relationships between concentration of the substances in the organism and water concentrations. It should also be noted that the total concentrations in the fish are not provided and the lipid normalisation lacks. The study is assigned a Klimisch score of 4 (non-assignable) by dossier submitter. Based on the aforementioned, the reported BAF values are not used for classification purposes.

Keith et al. (2001)

To evaluate bioaccumulation potential and identify potential related risks, concentrations of NP, NPE-1, NPE-2 and NPE-3 were determined in the tissues of fish inhabiting various waters in Michigan (USA), namely the Kalamazoo River Basin and Lake Michigan near the mouth of the Kalamazoo River. The Kalamazoo River flows through both urban and rural areas and receives secondary and tertiary WWTP effluents and industrial discharges, including those of paper manufacturing facilities. Sampling along the river was conducted up and downstream of WWTP, whenever possible. Fish were selected based on availability at sampling site, size (weight), migratory behaviour and placement in the food chain. Species analysed included rock bass (*Ambloplites rupestris*), bluegill sunfish (*Lepomis macrochirus)*, green sunfish (*Lepomis cyanellus*), smallmouth bass (*Micropterus dolomieui*), white suckers (*Catostomus commersoni*), longnose suckers (*Maxostoma macrolepidotum*) and rainbow smelt (*Osmerus mordax*). Fish were collected at three occasions between late June and early November 1999 and stored at -20°C for analysis. The digestive/excretory system was chosen for analysis, as this is the area where NP is likely to accumulate. The analysis method involved extraction of samples using exhaustive steam distillation with concurrent liquid extraction. No sampling of water was conducted. The detection limits for NP, NPE-1, NPE-2 and NPE-3 were 3.3, 16.8, 18.2 and 20.6 ng/g, respectively. Concentrations of NP among all sites and species ranged from <3.3 to 29.1 ng/g wet weight (ww) and varied little among sites. NPE-1 was detectable in some samples but at concentrations less than the method detection limit (16.8 ng/g). Concentrations of NPE-2 and NPE-3 in all samples were less than their respective minimum detection levels. Bioconcentration factors were not established. However, the study suggests the presence of nonylphenols in fish but at relatively small concentrations. NP was the predominant compound, with concentrations of NPEs less than those of NP. Fish collected near WWTP effluent discharge sites contained relatively greater concentrations than those collected from more remote areas. The authors of the REACH registration dossier assign this study with a Klimisch score of 2. The quality and reliability of the study could not be ascertained because essential information is missing from the study summary and BCF values were not determined.The study is assigned a Klimisch score of 4 (non-assignable) by the dossier submitter.

Summary

Experimentally derived BCF values for fish are not available for short-chain NPEs. Only measurements of NP, NPE-1, NPE-2 in freshwater fish in the field situation are available. The data indicated that concentrations of NPE-1 and NPE-2 were below minimum detection levels of 18.2 and 20.6 ng/g, respectively. BAF values from field studies were reported for NPE-1 between 1 and 19 and for NPE-2 between 0.8 and 37. To determine bioaccumulation, BCF values are used when available and compared to the critieria set out in the CLP regulation (Annex I, 4.1.2.8.1). The BAF values cannot be compared directly with bioaccumulation criteria and therefore their use for classifications purposes is limited. According to the CLP guidance, high quality BCFs determined for non-fish species (e.g. blue mussel, oyster and/or scallop) may be used directly for classification purposes if no fish BCF is available as a worst case value for fish. A BCF of > 100 — < 200 for NPE-1 and a BCF of > 50 — < 100 for NPE-2 was obtained with *Mytilus edulis*. The quality and reliability of the reported BCF values cannot be ascertained because essential information is missing from the study summary. Therefore, the BCF values based on *Mytilus edulis* were not used for classification.

Even though both experimental and predicted logK_{ow} data are less reliable, both indicate values of above > 4. It was therefore decided to base the bioaccumulation conclusion on these values, due to their consistency and lack of more reliable data. Short-chain NPEs can be considered as substances with potential to bioaccumulate, given the logK_{ow} values are above the CLP trigger value of ≥ 4.

11.5 Acute aquatic hazard

Table 16: Summary of relevant information on acute aquatic toxicity (only valid studies included)

Method	Substance tested	Results (mg/L) L(E)C_{50}	Remarks	KS*	Reference
Fish					
OECD Guideline 203 Fathead minnow (*Pimephales promelas)*	NPE-1	96h-LC_{50} = 0.218	Flow-through; freshwater	2	Anonymous (2007)[1]
OECD Guideline 203 Fathead minnow (*Pimephales promelas)*	NPE-2	96h LC_{50} = 0.323	Flow-through; freshwater Based on measured concentrations	2	Anonymous (2007)[1]
Crustaceae					
EPA guideline Cerodaphnia dubia	NPE-1	48h-EC_{50} = 0.328	Semi-static; Based on measured concentrations	2	Anonymous (2007)[1]
No guideline *Mysidopsis bahia*	NPE-1.5	48h-EC_{50} = 0.11	Static renewal; Natural salt water; Based on measured concentrations (not specified)	2	Hall *et al.* (1989)[1]
EPA guideline Cerodaphnia dubia	NPE-2	48h-EC_{50} = 0.716	Semi-static; Based on measured concentrations	2	Anonymous (2007)[1]
Algae					
OECD Guideline 201 *Pseudokirchneriella subcapitata*	NPEO	72h $EC_{50,growth}$ > 3.0	Static; Based on nominal concentrations	2	Anonymous, (2010b)[1]

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

[1] As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

11.5.1 Acute (short-term) toxicity to fish

Table 17. Summary of acute fish toxicity tests

Method	Substance tested	Results (mg/L)	Remarks	KS*	Reference
OECD Guideline 203 Fathead minnow (*Pimephales promelas)*	NPE-1	96 h LC_{50} = 0.218	Flow-through freshwater based on measured concentrations	2	Anonymous (2007)[1]
Medaka (Oryzias latipes)	NPE-1	48 h LC_{50} = 3.0	Static Based on nominal concentrations	3	Yoshimura (1986)[2]

			Supporting study		
OECD Guideline 203 Fathead minnow (*Pimephales promelas)*	NPE-2	96 h LC_{50} = 0.323	Flow-through freshwater test mat. Based on measured concentrations	2	Anonymous (2007)[1]

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

[1] As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

[2] summarized from publication retrieved from open literature

Anonymous (2007): NPE -1

A study was conducted to evaluate the acute toxicity of NPE-1 to *Pimephales promelas* under flow-through conditions in fresh water in accordance with OECD 203. Less than 4 day old *Pimephales promelas* larvae exposed to NPE-1 at the analytical measured concentrations of <10.4 µg/L (control); 39.4 ± 15.3 µg/L; 51.4 ±15.3 µg/L; 88.7 ± 22.8 µg/L; 164 ± 31 µg/L and 338 ± 10 µg/L for 96 h. Four replicates of the control and of each NPE-1 concentration were used during the test. The animals were not fed during the test and were observed daily for mortalities. Corresponding potassium chloride reference tests were conducted and found to be within acceptable limits. Survival in the controls for all of the replicates was >95%. Under the test conditions, the LC50 for *Pimephales promelas* was determined to be 0.218 mg/L (95% CL) after 96 h exposure of NPE-1. Reliability 2

Yoshimura (1986)

In a study by Yoshimura (1986), a static acute toxicity test was carried out to determine the 48h- LC50 of several NPEs. Medaka (*Oryzias latipes*) of 2 cm average length and 0.2 g of average weight were placed at random in groups of 10 in glass beakers containing 2L of each concentration of samples. After the preliminary range-finding test, the LC_{50} determinations were carried out by observing fish survival in single test solution prepared for each concentration. The 48-LC50 values of NPE-1 for medaka were determined to be 3.0 mg/L. The reliability of this study is 3 because of an absence in some essential information like the chemical analysis. This study can only be used as supporting information.

Anonymous (2007): NPE -2

A study was conducted to evaluate the acute toxicity of NPE-2 to *Pimephales promelas* under flow-through conditions in fresh water in accordance with OECD 203. Less than 4 day old *Pimephales promelas* larvae were exposed to NPE-2 at the analytical measured concentrations of <12 (control), 99.4 ± 37.2, 129 ± 43, 188 ± 47, 287 ± 75, and 630 ± 150 µg/L for 96 h. Four replicates of the control and of each NPE-2 concentration were used during the test. The animals were not fed during the test and were observed daily for mortalities. Corresponding potassium chloride reference tests were conducted and found to be within acceptable limits. Survival in the controls for all of the replicates was >95%. Under the test conditions, the LC50 for *Pimephales promelas* was determined to be 0.323 mg/L (95% CL) after 96 h exposure of NPE-2 (TenEyck *et al.*, 2007). Reliability 2.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Table 18. Summary of Crustacean acute toxicity tests

Method	**Substance tested**	**Results LC_{50} (mg/L)**	**Remarks**	**KS***	**Reference**
OECD Guideline 202 24 h exposure *Daphnia magna*	NPE-1	24 h LC50 = 0.39	Static; Not measured	3	Sun and Gu (2005)[1]
EPA guideline	NPE-1	48 h LC50 = 0.328	Semi-static;	2	Anonymous

48h exposure *Cerodaphnia dubia*			Based on measured concentrations		(2007)[1]
No guideline 48h exposure *Cerodaphnia dubia*	NPE-1-2	48 h LC50 = 1.04	Static; Concentrations not measured; Based on nominal concentrations	3	Ankley *et al*. (1990)[2]
No guideline 48h exposure *Mysidopsis bahia*	NPE-1.5	48 h LC50 = 0.11	Static renewal; Natural salt water; Based on nominal concentrations	2	Hall *et al*. (1989)[1]
EPA guideline 48h exposure *Cerodaphnia dubia*	NPE-2	48 h LC50 = 0.716	Semi-static; Based on measured concentrations	2	Anonymous (2007)[1]
OECD Guideline 202 24 h exposure *Daphnia magna*	NPE-2	24 h LC50 = 0.56	Static; Not measured	3	Sun and Gu (2005)[1]
ISO 6341 (1982) 48h exposure *Daphnia magna*	NPE-2	48 h LC50 = 0.148	Static; Not measured	3	Maki *et al*. (1998)[1]

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

[1] As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

[2] Summarized from publication retrieved from open literature.

Sun and Gu (2005)

A study was conducted to evaluate the acute toxicity of NPE-1, and NPE-2 to *Daphnia magna* under static conditions in fresh water in accordance with OECD Guideline 202. Ten *D. magna* (10 neonates per vessel) were exposed to NPE-1, and NPE-2 at five different concentrations for each chemical and to one solvent control (hexane) for 24 h. Each concentration was replicated. The organisms were not fed during the test and mortality was observed after 24 h. Under the test conditions, the 24h-LC50 for *Daphnia magna* was determined to be 0.39 and 0.56 mg/L for NPE-1, and NPE-2, respectively (Sun and Gu, 2005). Reliability of this study is considered as 3 because the test chemical was not measured and the test duration is shorter than than the typical 48 hours for daphinids.

Anonymous (2007)

Tests were conducted to evaluate the acute toxicity of NPE-1, and NPE-2 to *Cerodaphnia dubia* under semi-static conditions in fresh water according to the EPA Guideline. Less than 24 h old *C. dubia* were exposed to NPE-1, or NPE-2 for 48 h at the analytical measured concentrations of NPE-1 <10.4 µg/L (control); 27.4 ± 1.9 µg/L; 53.9 ± 5.6 µg/L; 114 ± 12 µg/L; 221 ± 25 µg/L and 486 ± 28 µg/L; NPE-2 <12 (control), 274 ± 35, 489 ± 41, 839 ± 83, 1800 ± 80, and 3640 ± 4 µg/L. Four replicates of the control and each of the five exposure concentrations were used, with each beaker containing five organisms. Organisms were not fed during the test and were observed daily for mortalities. Negative control: Plain stock solution Positive control: Potassium chloride. Survival in the controls for all of the *C. dubia* exposures was ≥ 90% and the result from the reference substance test was found to be within acceptable limits. Under the test conditions, the LC50 for *C. dubia* was determined to be 0.328 mg/L, and 0.716 mg/L after 48 h exposure of NPE-1 and NPE-2 (TenEyck *et al*., 2007). This study is considered as a valid study, with reliability 2.

Ankley et al. (1990)

The cladoceran, *Cerodaphnia dubia* was used in all static toxicity tests of NPE-1-2. Tests were performed at 25°C for 48 h in 10 ml volumes with five organisms per chamber, using a 50% dilution series. The laboratory dilution/control water (DMW) consisted of 10% mineral water in high purity water from a Millipore ® system. Definitive toxicity tests with each nonylphenol ethoxylates (1+2) were set up in

duplicate. No carrier solvents were used. All test concentrations were nominal. Under the test conditions, the LC50 for *Daphnia magna* was determined to be 1.04 mg/L after 48 h exposure to NPE-1-2. Reliability of this study is considered as 3 because the test chemical was not measured.

Hall et al. (1989)

Mysids (*Mysidopsis bahia*) were used to evaluate the acute toxicities of NPE-1.5. All tests were 48-hr static renewals (renewals at 24 hr) at 25 ± 1^0C under a light:dark photoperiod of 16-hr:8-hr. All mysids were 3 to 8 days old at the start of tests. Aged, natural saltwater of 25 to 28 %o salinity (25 micron filtered) was the control and dilution water in all experiments. All chemical exposures contained two replicates of four organisms per concentration and controls contained four replicates of four organisms. A modification of the gas chromatography following continuous distillation and extraction with octanol was used to measure NPE-1.5. *M. bahia* were fed live *Artemia* (<24-hr old) at the start of tests and after renewing solutions. Dissolved oxygen, pH, and salinity were monitored at the start, after 24-hr, at renewal, and at termination of experiments. Dissolved oxygen was measured in all solutions at the start, at 24-hr and 48-hr of all tests. Salinity and pH were monitored only in the controls and the two highest toxicant concentrations, since changes in these parameters were not observed as a result of addition of chemicals. Experiments with <20 percent control mortality were used in comparing the toxicity of different surfactants. Control mortality above the recommended 10% was deemed acceptable because this occurred on only a few occasions and lower levels of mortality occurred for mysids exposed to low levels of the surfactant. Under the test conditions, the 48h-LC50 values for *Mysidopsis bahia* was determined to be 0.11 mg/L for NPE-1.5 This study is considered valid, with reliability 2.

Maki et al. (1998)

An acute toxicity of NPE-2 to *Daphnia magna* was conducted after 48 h exposure according to ISO 6341 (1982). Five 24-h neonates were gently added to 10 of hard water containing the test chemical. The tests were carried out twice. Consequently, 10 *Daphnia* were used for each sample concentration. The vials were kept at 20 ºC in the dark. The number of surviving *Daphnia* was counted after 48 h. The 50% lethal concentrations (LC50s) and 95% confidence limits for test chemicals were calculated with Probit analysis. Under the test conditions, the LC50 for *Daphnia magn*a was determined to be 0.148 mg/L after 48 h exposure of NPE-2. Reliability of this study is considered as 3 because the test chemical was not measured.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Table 19. Summary of algae toxicity tests

Method	Substance tested	Results (mg/L)	Remarks	KS*	Reference
OECD Guideline201 72 h exposure *Pseudokirchneriella subcapitata*	NPEO**	$EC_{50,growth}$ > 3.0 $NOEC_{growth}$ = 1.5 $EC_{10,growth}$ = 1.22 $EC_{50,yield}$ = 2.02 $NOEC_{yield}$ =0.75	Static; Based on nominal concentrations	2	Anonymous (2010b)[1]

* Klimisch score indicated in the REACH registration dossiers.

** See Table 6 for the constituents of NPEO1:

[1] As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

For summary of the algae toxicity test results, please see section 11.6.3

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available for NPEs.

11.6 Long-term aquatic hazard

Table 20: Summary of relevant information on chronic aquatic toxicity (only valid studies included)

Method	Substance tested	Results (mg/L)*	Remarks	KS*	Reference
Fish					
Not a test guideline method 21 d exposure Rainbow trout (*Oncorhynchus mykiss*)	NPE-1	NOEC VTG[1] = 0.03	Flow-through; Based on measured concentrations	2	Dussault *et al*. (2005)[4]
Not a test guideline method 100d exposure Medaka (*Oryzias latipes)*	NPE-1	NOECsurvival =0.105 NOEC SSC[2] = 0.035	Static; Based on measured concentrations	2	Balch and Metcalfe (2006)[4]
Not a test guideline method 90d exposure Medaka (*Oryzias latipes)*	NPE-1/ NPE-2	NOEC = 0.05	Static; Based on measured concentrations of mixture of NPE-1 and NPE-2	2	Metcalfe *et al*., (2001)[4]
Not a test guideline method rainbow trout	NPE-2	LOEC ≤ 0.038 VTG induction, GSI and germ cell stages	Flow-though Fish were exposed to one nominal concentration = 0.03 mg/L. The mean measured was 0.038 mg/L.	2	Jobling *et al*. (1996)[4]
Not a test guideline 21 d exposure Rainbow trout (*Oncorhynchus mykiss*)	NPE-1/ NPE-2	LOEC GSI[3] < 0.122 gonadal histology LOEC = 0.122 VTG[1]	semi-static, based on nominal concentration	2	Le Gac *et al*. (2001)[4]
Crustaceae					
OECD Guideline 211 *Daphnia magna* 21-d exposure	NPE-1	NOEC reproduction = 0.1	Semi-static; Based on nominal concentrations	1	Anonymous (2010c)[5]
EPA OTS 797.1950 *Mysisdopsis bahia* 28-d exposure	NPE-1.5	NOEC reproduction = 0.0077	Flow-though Based on measured concentrations	1	Anonymous (1999b)[5]
Algae					
OECD Guideline 201 *Pseudokirchneriella subcapitata*	NPE-2	72 h NOEC Growth = 1.22	Static; Based on nominal concentrations	2	Anonymous (2010b)[5]

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

1: VTG = vitellogenin

2: SSC = second sex characteristics

3. GSI = Gonadosomatic index
4: Summarized from publication retrieved from open literature.
5: As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

11.6.1 Chronic toxicity to fish

Table 21. Summary of fish chronic toxicity tests

Method	Substance tested	Results (mg/L)	Remarks	KS*	Reference
Not a test guideline method 21 d exposure Rainbow trout (*Oncorhynchus mykiss*)	NPE-1	NOEC VTG[1] = 0.048	Flow-through Based on measured concentrations	2	Dussault *et al.*, (2005)[4]
Not a test guideline method 100 d exposure Medaka (*Oryzias latipes*)	NPE-1	NOEC survival = 0.105 NOEC sex ratio = 0.105 NOEC SSC[2] = 0.035	Static Based on measured concentrations	2	Balch and Metcalfe (2006)[4,5]
Not a test guideline method 90 d exposure Medaka (*Oryzias latipes*)	NPE-1/ NPE-2	NOEC survival = 0.1 (nominal) NOEC sex ratio = 0.1 (nominal) NOEC (gonadal histology) = 0.0155/0.0235	Static renewal Based on measured concentrations measured as NPE-1/NPE-2	2	Metcalfe *et al.* (2001)[4]
Not a test guideline method rainbow trout	NPE-2	LOEC VTG[1] induction, GSI[3] and gonadal histology ≤ 0.038	Flow-though Based on measured concentrations	2	Jobling *et al.* (1996)[4]
Not a test guideline method 21 d exposure Rainbow trout (*Oncorhynchus mykiss*)	NPE-1/ NPE-2	NOEC VTG[1] induction = 0.122 NOEC GSI[3] and gonadal histology < 0.122	semi-static, based on nominal concentration 0.122 is nominal. It is expected the measured concentration LOEC < 0.061	2	Le Gac *et al.*, (2001)[4]

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.
1: VTG = vitellogenin
2: SSC= secondary sex characteristics
3: GSI= Gonado-somatic index
4: Summarized from publication retrieved from open literature
5:As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018

Dussault et al. (2005)
Rainbow trout (*Oncorhynchus mykiss*) (45.1 ± 0.74 g) were randomly transferred to 50 L aerated glass aquaria (6 per aquarium, 2 replicate tanks) and exposed to nominal concentrations of 0.01% ethanol (carrier control), 1, 3, 10, 30, and 100 μg/L of NPE-1 in a flow-through system. Pooled water samples were taken

weekly for measurement of alkylphenol concentrations. After 21 days, fish were anaesthetized, meristic characters (for length and weight) were recorded, and a blood sample was taken by caudal puncture into heparinized Vacutainer tubes. Blood was centrifuged and the plasma was separated for vitellogenin analysis. Background concentrations during the NPE-1 experiments were below 1 μg/L. The average measured exposure concentrations determined to be 0.8, 3.9, 6.9, 48.3 and 281.3 µg/L for NPE-1. The NOECs for induction of vitellogenin (VTG) by NPE-1 were 0.048 mg/L. This test is considered as a valid study, with reliability 2.

Balch and Metcalfe (2006)

A study was conducted to evaluate the effects NPE-1 on growth and survival of the Japanese medaka (*Oryzias latipes*). Exposure to the fry began within 1 day of hatch and continued for 100 days under static conditions. The test water in individual exposure tanks was renewed every 48 h. Renewal was 100%, 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. Gentle aeration was applied to the tank water so that dissolved oxygen was at or near saturation. Survival and growth were monitored in each treatment after the first and second month of exposure by taking a digital image of the exposure tank and counting the number of fish. After counting, 20 fish were removed, euthanized and total body length and weight was measured to assess growth. Treatments that experienced greater than 20% mortality in the first month of exposure, or exceeded a total cumulative mortality of 30% prior to the termination of exposure were eliminated and not included in the analysis. NPE-1 of 10, 30, 100, and 300 µg/L, were tested. Each treatment was started with 150 fry to ensure at least 50 fish survived to the end of the 100-day exposure period. In addition, 40 fish from each treatment were removed and euthanized for growth measurements. None of the treatments were replicated. Fifty randomly chosen fish from each treatment were sacrificed at the end of the 100-day exposure period. Endpoints reported were secondary sex characteristics, total body length and weight, and development of gonadal intersex (i.e. testis-ova). Gonadal tissues were examined to verify the gonadal sex of the fish and to monitor for evidence of gonadal intersex. The secondary sex characteristics were assessed according to the shape of the urogenital papilla, dorsal and anal fins and the presence or absence of papillary processes on the anal fin. Concentrations of all test compounds declined over the period between test solutions renewals. The average measured exposure concentrations determined to be 3.5, 10.5, 35, 105 µg/L.Fish survival during the 100-day exposure period was greater than 70% in all treatments. The NOEC value for survival is 105 µg/L for NPE-1. Exposure of medaka to all test compounds did not change sex ratios at all tested concentrations. There were significantly elevated incidences of medaka with mixed secondary sex characteristics in the highest treatment with NPE1 (105 µg/L) in comparison to the incidence in the acetone control treatment. A low percentage (i.e., 4%) of the fish in the clean control also exhibited mixed secondary sex characteristics. However, this likely reflects a small number of errors in assessing male and female traits. Only 1 out of 29 males in the NPE-1 (105 µg/L) treatment had papillae on the anal fin. Papillary processes were present on the anal fins of males from all other treatments. The NOEC for SSC is 35 µg/L for NPE-1. This test is considered as a valid study, with reliability 2.

Metcalfe et al. (2001)

Exposure to the mixture of 54% NPE-1 and 44% NPE-2 was initiated with early life stages of medaka (*Oryzias latipes*) at 1d after hatch in a static-renewal system. Exposures took place in glass containers of progressively larger sizes (i.e., 1, 2, and 10 L) as the medaka grew and stopped at 90d post hatching. Concentrations in test containers were prepared by adding 1- to 5-ml volumes of stock solutions prepared in acetone to the water in the test containers. In the control treatment, acetone alone was added in the same volumes as the experimental treatments. Two experiments were conducted with the NPE-1/NPE-2 test mixture. The first experiment was conducted with 25, 50 and 100 µg/L of the test mixture, but because the sex of a large proportion of medaka from these treatments could not be determined because the gonad was not sectioned properly during histologic preparation (i.e., unknown sex), the experiment was repeated with only the 100-mg/L and control treatments. Each treatment included 60 fish at the start of the experiment. The fish were maintained in a light:dark cycle of 16:8 h and were fed a diet of newly hatched brine shrimp twice daily for the duration of the experiment. The NPE-1 and NPE-2 were analyzed by normal-phase high performance liquid chromatography with a fluorescence detector.

Mean concentrations of NPE-1 over 48 h were between 55 and 65% of nominal. Mean percent concentrations of NPE-2 declined from 109 to 95% over the 48-h exposure period (mean 104%). In the solution of NPE-1/NPE-2, NP was detected at relatively low concentrations (10% of nominal concentration of NPE-1/NPE-2) in samples collected at 48 h, indicative of some metabolic degradation over time of these ethoxylate compounds to nonylphenol. In both experiments, the mean lengths and weights of the medaka exposed to the NPE-1/NPE-2 mixture did not differ significantly from those of control medaka. In both experiments, the sex ratios of medaka exposed to the NPE-1/NPE-2 mixture were not statistically different from those of controls. Only one testis–ova was observed in the second experiment at 100 µg/L of the test mixture, a subtle change in testicular development characterized by a single oogonium observed in one section. Eosinophilic fluid was observed in the body cavity of one specimen exposed to 100 mg/L of the NPE-1/NPE-2 mixture in the second experiment. Based on the observation of alterations to gonadal development, the NOEC for medaka exposed to nonylphenol ethoxylates (NPE-1 and 2) was 50 µg/L corresponding to the mean of NPE-1/NPE-2 concentrations of 15.5/23.5 µg/L. This test is considered as a valid study, with reliability 2.

Jobling et al. (1996)

Groups of 2 year old adult male rainbow trout were exposed to a nominal concentration of 30 µg/L of NPE-2 for 3 weeks in a flow through system. Concentrations of NPE-2 measured throughout the experiment were 38.3 µg/L. Blood samples were taken from all fish both initially and at the end of a 3-week period of exposure. Blood plasma was assayed for vitellogenin 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. The middle portion of one of the testes was fixed for histological analysis. All of the fish survived and grew during the 3-week experiment. NPE-2 caused significant elevations in the concentrations of vitellogenin measured in the plasma of exposed fish. The pronounced increases in plasma vitellogenin concentrations were accompanied by concomitant significant decreases in the rate of testicular growth as showed by GSI. Histological examination of the testes revealed varying degrees of spermatogenic inhibition in exposed fish relative to the controls. This test is considered as a valid study, with reliability 2. Fish were only exposed to one concentration.

Le Gac et al, (2001)

Rainbow Trout (*O. mykiss*, 13-month old male, mean body weight of 306 ± 87 g) were exposed (4 replicates, 6 fish per tank) to Igepal CO-210 (80 % NPE-1 and 20 % NPE-2) for 3 weeks in a semi-static renewal culture. The test concentrations were nominally 450 and 1 800 nmol/L, plus an ethanol solvent control (0.004 % ethanol, 5 fish per tank). These were calculated, corresponding to nominal concentrations of 0.122 and 0.491 mg/L (assuming that the concentrations were based on the average molecular mass of the mixture). The test concentration was analysed for NPE-2 but NPE-1 and NPE-2 could not be resolved separately. The measured concentration at the high 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. 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.

Fish weight was unaffected by 3-week-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 low and high test 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 low test concentration (52 % in stages I–III and 48% in stages IV–VI). This effect was more obvious at the high concentration (68.2 % in stages II–III and 31.8 % in stages IV–VI). The LOEC is considered to be 0.122 mg/L (nominal); the measured concentration would be much lower (less than half this value, i.e. 0.061 mg/L), but the lack of reporting makes it difficult to estimate a time-weighted mean. No significant VTG induction was observed at the low concentration. In contrast, VTG was increased at the high concentration. This test is considered as a valid study, with reliability 2.

11.6.2 Chronic toxicity to aquatic invertebrates

Table 22. Summary of Crustacean chronic toxicity tests

Method	Substance tested	Results(mg/L)	Remarks	KS*	Reference
OECD Guideline 211 21d exposure *Daphnia magna*	NPEO**	NOEC reproduction = 0.1	Semi-static Based on nominal concentrations	1	Anonymous (2010c)[1]
EPA OTS 797.1950 28-d exposure *Mysisdopsis bahia*	NPE-1.5	NOEC reproduction = 0.0077	Flow-though Based on nominal concentrations	1	Anonymous (1999b)[1]

* Klimisch scores indicated in the REACH registration dossiers or evaluated by the dossier submitter for the publications retrieved from the open literature. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.

** See Table 6 for the constituents of NPEO

[1]:As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018

Anonymous (2010c)

The *Daphnia magna* reproduction test (semi-static, 21 d) of Nonylphenol, branched, ethoxylated was conducted according to OECD 211 (version 1998). Nonylphenol, branched, ethoxylated contains about 80% NPE-1 and NPE-2, and the remaining being loner chain ethoxylates up to NPE-6. For the test *Daphnia magna* STRAUS (Clone 5) was used. 10 test organisms, individually held, were used per concentration level and control. At test start they were 2 to 24 h old. The study was carried out under semi-static conditions with a three times per week renewal of the test solutions. Nominal concentrations of the test item were selected after a preliminary acute immobilization test (48 h, static) as follows: 10.0 - 32.0 - 100 - 320 - 1,000 µg/L. The tested concentrations levels of the test item and the control were analytically verified by HPLC of samples 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 biologically relevant test concentrations of 100 to 1,000 µg/L. Therefore, all effect values given are based on the nominal concentrations of the test item.

The average number of juveniles per parent alive at the end of the test in the control group was 122 after 21 days. The reproductive output was statistically significant reduced at the concentration level of 320 µg/L when compared to the control. The EC10-value for the reduction of the reproductive output was calculated to be 85.3 µg/L. An EC50-value for the reproductive output was not determinable because no effects $\geqslant$ 50 % (reduction or increase of the reproductive output) occurred within the tested concentration range. Due to mortality of all parental daphnids at the concentration level of 1,000 µg/L no reproduction was observed at this concentration. The coefficient of variation of the number of living offspring produced per parent was 7 % at the control. At the concentration levels of 10.0 and 32.0 µg/L the calculated coefficient of variation was 5 % and comparable to the control. At the concentration levels of 100 and 320 µg/L the coefficients of variation were 17 and 22 %. The LOEC and the NOEC after 21 days based on the reduction of the reproductive output as the most sensitive effect were determined to be 320 and 100 µg/L, respectively; the EC50 for reproduction could not be determined. The reliability of this study was considered as 1.

Anonymous (1999b)

A study was conducted to determine the long-term toxicity of the substance NPE-1.5 (except NPE-1.5 also contains 3.8% of NP), to aquatic invertebrates according to EPA OTS 797.1950 (Mysid Chronic Toxicity Test), in compliance with GLP. *Mysisdopsis bahia* was exposed to the test substance at concentrations of 0, 2.3, 4.7, 9.4, 19 and 37 µg/L (equivalent to measured concentrations 0, 2.2, 4.0, 7.7, 16 and 32 µg/L) for 28 days under flow-through conditions. Each exposure concentrations were analytically confirmed by HPLC on Days 0, 7, 14, 18, 21 and 28. Mortality (parents and offspring), appearance and behaviour were checked

daily. After mating and throughout the rest of the study, the reproductive success was assessed. At study termination, body length and weight were recorded. Water quality measurements (temperature, dissolved O2 concentration, pH and salinity) were also performed. Test substance concentrations were generally consistent (between 91.9 to 106%) and no visible sign of undissolved test substance was observed. After 28 days of exposure, survival of 85% was observed among organisms exposed to the control solution. Survival of 73, 80, 80, 78 and 68% were observed in animals exposed to the mean measured concentrations of 2.2, 4.0, 7.7, 16 and 32 µg/L, respectively. It was not different from the control group. Reproductive success in controls (1.1 offspring per female per day) exceeded minimum standards (i.e., >75% of the paired females produced young and the average number of young produced by the paired females was > 0.30). The reproductive success in the three lower concentrations (2.2 - 7.7 µg/L) was not different from the controls but it became significantly different from the control group for the two highest concentrations, i.e. 16 and 32 µg/L. Reproductive success for organisms exposed to the two highest treatment levels,16 and 32 µg/L, averaged 0.58 and 0.23 offspring per female per reproductive day, respectively, and was significantly different from the reproductive success of the control organisms. The total body length as well as total body weight in all tested concentrations (for males and females) was not different from the controls, except at the highest concentration tested, i.e. 32 µg/L. Under the study conditions, the 28 d LOEC and NOEC (for reproduction in *Mysidopsis bahia*) were determined to be 16 and 7.7 µg/L . The reliability of this study was considered as 1.

11.6.3 Chronic toxicity to algae or other aquatic plants

Table 23. Summary of algae toxicity tests

Method	Substance tested	Results (mg/L)	Remarks	KS*	Reference
OECD Guideline 201 72 h exposure *Pseudokirchneriella subcapitata*	NPEO**	$EC_{50,growth}$ > 3.0 $NOEC_{growth}$ = 1.5 $EC_{10,growth}$ = 1.22 $EC_{50,yield}$ = 2.02 $NOEC_{yield}$ =0.75	Static; Based on nominal concentrations	2	Anonymous (2010b)[1]

* Klimisch score indicated in the REACH registration dossiers. If the dossier submitter does not agree with the reliability assignment made by the dossier submitter, then this will be indicated in the study summaries below.
** See Table 6 for the constituents of NPEO.
[1] As summarised in the REACH Registration for nonylphenol, branched, ethoxylated, accessed on November 2015 and April 2018.

Anonymous (2010b)
The toxicity of Nonylphenol, branched, ethoxylated to the unicellular freshwater green algae *Pseudokirchneriella subcapitata* was determined according to the principles of OECD 201. Nonylphenol, branched, ethoxylated contains about 80% NPE-1 and NPE-2, and the remaining being longer chain ethoxylates up to NPE-6. The study was conducted under static conditions with an initial cell density of 5 x 10^3 cells/mL. Six concentrations were tested in a geometrical series with a dilution factor of 2: 0.0938 - 0.188 - 0.375 - 0.75 - 1.50 - 3.0 mg/L. Three replicates were tested for each tested concentrations and six replicates for the control. Environmental conditions were determined to be within the acceptable limits. The concentrations of NPE-2 were analysed at all concentration levels at test start and test end via HPLC-DAD analysis. The measured concentrations at test start and end were in the range of 94 - 98 % and 80 – 95 % of the nominal values, respectively. All effect values are given based on nominal test concentrations. NPE-2 demonstrated the following effects on the growth of algae (*P. subcapitata*): EC50 on growth rate (ErC50) > 3.0 mg/L (nominal); NOEC (growth rate) 1.5 mg/L (nominal); EC10 (growth rate) 1.22 mg/L (nominal); EC50 on yield (EyC50) 2.02 mg/L (nominal); NOEC (yield) 0.75 mg/L (nominal). This study is considered valid and Reliability 2 because the study report is relatively short and some details cannot be found.

11.6.4 Chronic toxicity to other aquatic organisms

No data available for NPEs.

11.7 Comparison with the CLP criteria

The classification proposal is based on data for NPE-1, NPE 1.5, NPE-2 and the mixture of NPE-1 and NPE-2.

11.7.1 Acute aquatic hazard

Valid acute aquatic toxicity data are available for all three trophic levels. The relevant endpoint values for fish, aquatic invertebrates and algae range are 0.318 (NPE-1), 0.11 (NPE-1.5) and > 3.0 (NPE-1/NPE-2) mg/L, respectively. Algae is the least senstitive species where for the other species the L(E)C50 values lie in the range 0.1 – 1 mg/L. The lowest L(E)C_{50} value obtained in acute aquatic toxicity studies is 0.11 mg/L in mysids. This value is lower than the classification threshold value of 1 mg/L. NPEs with 1 to < 3 g/mol ethoxy groups fulfil the criteria for classification as acute hazard to the aquatic environment, Acute Cat. 1. An M-factor of 1 is warranted based on the L(E)C50 between 0.1 and 1 mg/L.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

NPE-1 and NPE-2 are considered not rapidly degradable for classification purposes (see Section 11.1.4.5) and regarded as substances with potential to bioaccumulate.

Chronic aquatic toxicity information is available for all three trophic levels. The lowest long-term toxicity values for fish, aquatic invertebrates and algae are 0.105, 0.0077, and 1.22 mg/l, respectively. Based on the *Americamyis bahia* 28-day NOEC value of 0.0077 mg/L, NPEs covered under the group entry fulfill the criteria for classification as chronic category 1 hazard for the environment. An M-factor of 10 is warranted based on the NOEC between 0.001 and 0.01 mg/L

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARD

	CLP regulation	
	Classification	M-factor
Resulting harmonised classification	Aquatic Acute category 1 H400: Very toxic to aquatic life Aquatic Chronic category 1 H410: Very toxic to aquatic life with long lasting effects	M = 1 M = 10

RAC evaluation of aquatic hazards (acute and chronic)
Summary of the Dossier Submitter's proposal Regarding the available reliable data, the DS proposed to classify short chain NPEs as Aquatic Acute 1, H400 with an M-factor of 1 and Aquatic Chronic 1, H410 with an M-factor of 10.

With a surface tension lower than 60 mN/m (OECD TG 115), short chain NPEs are surface active, affecting the measurements various properties such as water solubility and octanol-water partitioning. For Short chain NPEs, a water solubility of 4.55 mg/L was determined by the critical micelle concentration and a Log Pow value of 5.39 at 20°C was estimated (Bodsch J., 2010c). Short chain NPEs have a moderate potential to absorb to organic matter. A Koc value of 2661 L/kg for NPE-2 is reported in the registration dossier. Due to their low vapour pressure and low Henry's law constant, volatilisation of short chain NPEs is expected to be negligible.

Rapid Degradability

A summary of the relevant information on rapid degradability is provided by the DS in the table 13 of the CLH report.

NPE with 1 to 2 ethoxy groups are hydrolytically stable at pH 4, 7 and 9 as less than 10% of NPE were transformed after 120h (Anonymous, 2010a).

The DS reported that no photodegradation in water had been observed (Ahel *et al*., 1994a).

Two reliable biodegradation screening studies on short-chain NPEs are reported by the DS. Using OECD TG 301F (manometric respirometry test) and activated sludge as inoculum, after a lag phase of 17.3 ± 0.7 days, NPE-1 was aerobically biodegraded with 25.9 ±8.1% at day 28 and for NPE-2 no biodegradation was observed. In OECD TG 301B with NPE-1.5, 45.3 ± 18.4% CO_2 evolution was observed after 28 days and 58.7% after 35 days. The 10-day window was not met. The DS concluded that NPE with 1, 1.5 and 2 ethoxy groups are not readily biodegradable.

No water simulation tests are available, but the DS reported two reliable sediment simulation tests. The DT_{50}s for primary degradation ranged from 69.3 to 115.5 days (Yuan *et al*., 2004) and 49.5 to 77.0 days at 30 °C (Chang et al., 2004). Three reliable biodegradation tests in soil are available for short chain NPEs and were reported by the DS for information on the behaviour on these substances although soil assays are not considered relevant for classification purpose where aquatic degradation studies are available.

Bioaccumulation

A summary of the available information on bioaccumulation is presented by the DS in table 15 of the CLH report.

The DS reported that the estimated octanol-water partition coefficient values for NPE-1 range from 5.28 (branched) to 5.58 (linear) and for NPE-2 from 5.01 (branched) to 5.30 (linear). Data reported for the registered UVCB substance nonylphenol, branched, ethoxylated (CAS 68412-54-4) state a value of 5.37 represented the weighted mean value log Pow value of the substance and not individual constituents. Branched structures were not considered in the calculation.

The BCF of nonylphenol branched ethoxylated was estimated to be 648 using the BCFBAF v3.01 program of EPIWEB 4.1 (Arnot Gobas method), based on the weighted average of the BCFs of the various constituents (US EPA, 2014).

The DS noted that experimentally derived BCF values for fish are not available for short-chain NPEs. Measurements of NP, NPE-1, NPE-2 in freshwater fish in the field are available. The data indicated that concentrations of NPE-1 and NPE-2 were below

minimum detection levels of 18.2 and 20.6 ng/g, respectively. BAF values from field studies were reported for NPE-1 between 1 and 19 and for NPE-2 between 0.8 and 37. The DS considered that the BAF values cannot be compared directly with bioaccumulation criteria and therefore their use for classification purposes is limited.

BCF values obtained with *Mytilus edulis* were between 100 and 200 for NPE-1 and between 50 and 100 for NPE-2. However, the quality and reliability of the reported BCF values cannot be ascertained due to the lack of information in the study summary.

Based on the estimated BCF value of 648, the DS considered that the short chain NPEs from 1 to 2 have the potential to bioaccumulate.

Aquatic acute toxicity

A summary of the relevant information on aquatic acute toxicity is presented by the DS in table 16 in the CLH report.

For acute aquatic toxicity, studies are presented for the three trophic levels, fish, invertebrates, and algae. An acute fish toxicity study conducted according to OECD TG 203 is available for fathead minnow (*Pimephales promelas*) with NPE-1 and NPE-2. Under the test conditions, the LC_{50} for was determined to be 0.218 mg/L and 0.323 mg/L after 96 h exposure of NPE-1 and NPE-2, respectively.

For invertebrates, two acute studies with *Ceriodaphnia dubia* and with the saltwater mysid *Mysidopsis bahia* were reported by the DS and considered as reliable. For *C. dubnia*, tests were conducted to assess the acute toxicity of NPE-1 and NPE-2 under semi-static conditions in freshwater according to an EPA Guideline. Under these test conditions, the LC_{50} for *C. dubia* was determined to be 0.328 mg/L, and 0.716 mg/L after 48 h exposure to NPE-1 and NPE-2, respectively.

Mysids (*Mysidopsis bahia*) were used to evaluate the acute toxicity of NPE-1.5 under semi-static conditions. Under the test conditions, the 48h LC_{50} values for *Mysidopsis bahia* was determined to be 0.11 mg/L.

For the primary producers, only one reliable study is available. The toxicity of Nonylphenol, branched, ethoxylated (containing about 80% NPE-1 and NPE-2, and the remaining being longer chain ethoxylates up to NPE-6) to the unicellular freshwater green algae *Pseudokirchneriella subcapitata* was determined according to the OECD TG 201. . The 72 h E_rC_{50} and E_yC_{50} values were determined to be > 3.0 mg/L and 2.02 mg/L, respectively.

According to these studies, algae are found to be less sensitive and for the other species the $L(E)C_{50}$ were in the same range (0.1 to 1 mg/L). The lowest $L(E)C_{50}$ is obtained with the mysids (0.11 mg/L) and as this value is lower than the threshold value of 1 mg/L for acute aquatic classification purpose. Then, the DS proposed to classify short chain NPEs (1- < 3 ethoxy groups) as Aquatic Acute Category 1, H400 with a M-factor of 1 based on the $L(E)C_{50}$ between 0.1 and 1 mg/L.

Aquatic chronic toxicity

The valid available data for chronic aquatic toxicity described by the DS are presented in table 20 of the CLH report.

Five long-term studies with fish, the rainbow trout and medaka were presented in the CLH report. In Dussault *et al*. (2005), Rainbow trout (*Oncorhynchus mykiss*) were

exposed to nominal concentrations of 0.01% ethanol (carrier control), 1, 3, 10, 30, and 100 µg/L of NPE-1 in a flow-through system. After 21 days, the NOECs for induction of vitellogenin (VTG) by NPE-1 were 0.048 mg/L.

Groups of 2 years old adult male rainbow trout were exposed to a nominal concentration of 30 µg/L of NPE-2 for 3 weeks in a flow through system (Jobling *et al*., 1996). NPE-2 was found to cause significant elevations in the concentrations of vitellogenin measured in the plasma of exposed fish. The pronounced increases in plasma vitellogenin concentrations were accompanied by concomitant significant decreases in the rate of testicular growth as showed by GSI. Histological examination of the testes revealed varying degrees of spermatogenic inhibition in exposed fish relative to the controls.

In Le Gac *et al.* (2001), Rainbow Trout (*O. mykiss*, 13-month-old males) were exposed to a mixture containing 80% NPE-1 and 20% NPE-2 for 3 weeks in a semi-static renewal culture. After a 3-week-exposure, 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 concentration, respectively. No significant VTG induction was observed at the low concentration but VTG was increased at the high concentration. The NOEC for vitellogenin induction was 0.122 mg/L and the NOEC for the Gonado somatic index and gonadal histology was less than 0.122 mg/L.

A study (Balch and Metcalfe, 2006) was conducted to evaluate the effects NPE-1 on growth and survival of the Japanese medaka (*Oryzias latipes*). Exposure to the fry began within 1 day of hatch and continued for 100 days under semi-static conditions. Endpoints reported were secondary sex characteristics, total body length and weight, and development of gonadal intersex (i.e., testis-ova). The NOEC value for survival and for the sex ratio were 105 µg/L for NPE-1. The NOEC for the secondary sex characteristics were 35 µg/L for NPE-1.

Metcalfe *et al*. (2001) exposed Japanese medaka at 1d after hatch to a mixture of 54% NPE-1 and 44% NPE-2 in a static-renewal system until 90d post hatching. Based on the observation of alterations to gonadal development, the NOEC for medaka exposed to nonylphenol ethoxylates (NPE-1 and 2) was 0.050 mg/L corresponding to the mean of NPE-1/NPE-2 concentrations of 0.0155/0.0235 mg/L.

For invertebrates, the DS reported two tests one performed with water fleas and one other with saltwater mysids. The *Daphnia magna* reproduction test (semi-static, 21 d) of Nonylphenol, branched, ethoxylated was conducted according to OECD TG 211. Nonylphenol, branched, ethoxylated contains about 80% NPE-1 and NPE-2, and the remaining being loner chain ethoxylates up to NPE-6. The reproductive output was statistically significantly reduced at the concentration level of 0.320 mg/L and the EC_{10}-value was calculated to be 0.0853 mg/L. The NOEC after 21 days based on the reduction of the reproductive output as the most sensitive effect were determined to be 0.100 mg/L.

A study was conducted to determine the long-term toxicity of the substance NPE-1.5 (except NPE-1.5 also contains 3.8% of NP), to aquatic invertebrates according to EPA OTS 797.1950 (Mysid Chronic Toxicity Test), in compliance with GLP. *Mysidopsis bahia* was exposed to the test substance at concentrations of 0, 2.3, 4.7, 9.4, 19 and 37 µg/L (equivalent to measured concentrations 0, 2.2, 4.0, 7.7, 16 and 32 µg/L) for 28 days under flow-through conditions. Under the study conditions, the 28 d NOEC (for reproduction in *Mysidopsis bahia*) was determined to be 0.0077 mg/L.

The toxicity of Nonylphenol, branched, ethoxylated (containing about 80% NPE-1 and NPE-2, and the remaining being longer chain ethoxylates up to NPE-6) to the unicellular freshwater green algae *Pseudokirchneriella subcapitata* was determined according to OECD TG 201. . The 72 h E_rC_{10} and NOEC (yield) values were determined to be 1.22 mg/L and 0.75 mg/L respectively.

Chronic aquatic toxicity information is available for all three trophic levels and the lowest long-term toxicity values for fish, aquatic invertebrates and algae are 0.105, 0.0077, and 1.22 mg/L, respectively. The DS considered, based on the *Mysidopsis bahia* 28-day NOEC value of 0.0077 mg/L, short chain NPEs fulfil the criteria for classification as Aquatic Chronic 1, H410 with an M-factor of 10 based on the NOEC between 0.001 and 0.01 mg/L.

Comments received during consultation

During the consultation, one MSCA and one other national authority commented on the classification proposal of the DS. Both support the classification proposal for short chain NPEs as Aquatic Acute 1, M=1 and Aquatic Chronic 1, H410, M=10 but the validity of the lowest acute endpoint for *Mysidopsis bahia* used to derive the Aquatic Acute classification was discussed. The national authority proposed to revise the Klimisch Score of 2 to 4 or 3 due to a mortality exceeding the validity criteria of 10% in the control, GLP compliance is not reported in the study, raw data were not documented and test concentrations were not mentioned. They also asked for clarifications on validity criteria in algal study and whether the endpoints were based on mean measured concentrations for Anonymous (2007), Hall *et al*. (1989), and Anonymous (2010b).

Regarding the control mortality of the study with *Mysidopsis bahia*, despite the validity criterion for ≤10% control mortality not being met, the DS considered the study acceptable because this occurred on only a few occasions. Nevertheless, the DS agreed that the test was not GLP compliant, that raw data were not documented and that actual test concentrations were not mentioned. In this case, assigning a Klimisch score of 4 because of the limited information could be warranted and the DS invited RAC to consider this option.

Assessment and comparison with the classification criteria

Aquatic Chronic classification

Degradation

The dataset presented indicates that short-chain NPEs are not affected by abiotic degradation. Indeed, in a valid OECD TG 111, less than 10% of NPE are transformed after 120 hours at pH4, 7 and 9 at 50°C, demonstrating that hydrolysis is not a relevant pathway for NPE degradation. Insignificant photochemical degradation was found for NPE-1 in phototransformation experiments conducted both in presence of sunlight and artificial light.

In valid 301F and 301B tests, NPE-1 was aerobically biodegraded with 25.9 ±8.1% at day 28, for NPE-2 no biodegradation was observed, and 45.3 ± 18.4% CO_2 evolution was observed for NPE-1.5 after 28 days and 58.7% after 35 days, indicating that neither NPE-1 and NPE-2, nor NPE-1.5 fulfilled the pass level biodegradation within 28 days to be

considered as readily biodegradable.

Additional supportive studies presented by the DS reported low primary degradation in sediment.

According to the criteria on rapid degradability defined in the section 4.1.2.9 in the CLP regulation and based on the valid and available data presented by the DS, and especially on the findings from the screening biodegradation tests, RAC agreed to consider short-chain NPEs as **not rapidly degradable** for classification purpose.

Bioaccumulation

No experimental study was available to derive BCF values for fish. As noted in the SEv Dossier (ECHA, 2018), BAF values suggested a relatively low bioaccumulation potential in the aquatic environment and no significant biomagnification in the food chain. However, due to uncertainties, uncertain relationship between the biota and water concentrations, very low sample numbers, data gap on whole fish concentrations (muscle will not necessarily contain the highest concentrations), lack of lipid normalisation, RAC concurred with the DS that field situation study and BAF values are not appropriate for a classification purpose because these values cannot be directly compared with the BCF threshold.

In the CLP guidance, high quality data on the BCF for some invertebrates (blue mussel, oyster, scallop are quoted) may be used instead of fish BCF, as a worst-case surrogate. In the CLH report, BCF values obtained with *Mytilus edulis* were between 100 and 200 for NPE-1 and between 50 and 100 for NPE-2. However, the DS and RAC considered that the quality and reliability of the reported BCF values cannot be ascertained due to the lack of information in the study summary.

The estimated BCF value of 648 for nonylphenol branched ethoxylated using the BCFBAF v3.01 program of EPIWEB 4.1 (Arnot Gobas method), based on the weighted average of the BCFs of the various constituents (US EPA, 2014) meet the CLP trigger value for indication of bioaccumulation (BCF ≥ 500). Nevertheless, according to CLP guidance and information requirements R7c, for surface active substances, the classification of the bioconcentration potential based on hydrophobicity measures such as log K_{ow} should be used with caution and due to the uncertainty, measured BCF values are preferred. Furthermore, for complex substances, estimating an average or weighted BCF value is not recommended. In this case, bioaccumulation should be assessed on the representative constituents, meaning for short chain NPE, NPE-1 and NPE-2 at least, separately.

In the absence of valid experimentally determined BCF value, RAC considered that the estimated octanol-water partition coefficient values for NPE-1 range from 5.28 (branched) - 5.58 (linear) and for NPE-2 from 5.01 (branched) - 5.30 (linear) are the values to take into account for classification purpose. Based on these estimated values, RAC concludes that the short chain NPEs have a potential to bioaccumulate.

Aquatic Toxicity

***Table 24**: Summary of the available acute and chronic toxicity data compared with the CLP criteria. mm = mean measured, n = nominal concentrations, m = initial measured concentration. Key endpoints used in acute and chronic hazard classification are highlighted in bold.*

Method	Substance tested	Results	Remarks	Reference
Acute toxicity				
Fish				
OECD TG 203 Fathead minnow (*Pimephales promelas)*	NPE-1 NPE-2	96h-LC_{50} = 0.218 mg/L (mm) 96h LC_{50} = 0.323 mg/L (mm)	Flow-through; freshwater RI = 2	Anonymous (2007)
Invertebrates				
US EPA 600/4-90/027F 48h exposure *Ceriodaphnia dubia*	NPE-1 NPE-2	48 h LC_{50} = 0.328 mg/L (mm) 48 h LC_{50} = 0.716 mg/L (mm)	Semi-static RI = 2	Anonymous (2007)
No guideline 48h exposure *Mysidopsis bahia*	NPE-1	48 h LC_{50} = 0.11 mg/L (m?)	Semi-static; Natural salt water; RI = 4	Hall *et al*. (1989)
Algae				
OECD TG201 72h exposure *Pseudokirchneriella subcapitata*	NPEO	$EC_{50,\ growth}$ > 3.0 mg/L $EC_{50,\ yield}$ = 2.02 mg/L (n)	Static; RI = 2	Anonymous (2010b)
Chronic toxicity				
Fish				
Not a test guideline method 21d exposure Rainbow trout (*Oncorhynchus mykiss*)	NPE-1	NOEC VTG = 0.048 mg/L (mm)	Flow-through; Plasma vitellogenin endpoint RI = 2	Dussault *et al*. (2005)
Not a test guideline method 100d exposure Medaka (*Oryzias latipes)*	NPE-1	NOEC survival =0.105 mg/L NOEC sex ratio =0.105 mg/L NOEC SSC = 0.035 mg/L (mm)	Static; Endpoint: mixed secondary sexual characteristics RI = 2	Balch and Metcalfe (2006)
Not a test guideline method 90d exposure Medaka (*Oryzias latipes)*	NPE-1/ NPE-2	NOEC survival = 0.1mg/L NOEC sex ratio = 0.1mg/L NOEC (gonadal histology) = 0.0155/0.0235 mg/L (mm)	Static; Endpoint: Testis-ova RI = 2	Metcalfe *et al*. (2001)
Not a test guideline method	NPE-2	LOEC VTG induction, GSI	Flow-though Endpoint:	Jobling *et al*. (1996)

rainbow trout males only 21 d exposure		and gonadal histology ≤ 0.038 mg/L (mm)	Plasma vitellogenin and decrease in testicular growth RI = 2	
Not a test guideline 21d exposure Rainbow trout (*Oncorhynchus mykiss*) Males only	NPE-1/ NPE-2	NOEC GSI gonadal histology < 0.122 mg/L NOEC VTG = 0.122 mg/L (n)	semi-static, endpoint: reduced testicular growth and development RI = 2	Le Gac *et al*. (2001)
Invertebrates				
OECD TG 211 *Daphnia magna* 21d exposure	NPE-1	NOEC reproduction = 0.1 mg/L (n)	Semi-static; RI = 1	Anonymous (2010c)
EPA OTS 797.1950 *Mysidopsis bahia* 28d exposure	NPE-1.5	NOEC reproduction = 0.0077 mg/L (m, n)	Flow-though RI = 1	Anonymous (1999b)
Algae				
OECD TG 201 *Pseudokirchneriella subcapitata* 72h exposure	NPE-2	E_rC_{10} = 1.22 mg/L NOEC growth = 1.5 mg/L NOEC yield = 2.02 mg/L (n)	Static; RI = 2	Anonymous (2010b)

VTG = vitellogenin, SSC = second sex characteristics

Aquatic acute classification

A full acute data set (fish, aquatic invertebrates, algae) is available for short-chain NPEs. For the DS, the most sensitive species is *Mysidopsis bahia.* This species is known to be very sensitive to different organic compounds. Nevertheless, the reliability of the key study (Hall *et al*., 1989) was questioned during the public consultation. As confirmed by the DS, the concentration NPE+1.5 was measured by gas chromatography but in the published article the period of this measurement was not reported. The most important point is the deviation from the 10% mortality as a validity criterion. In any control, it is assumed that control mortality reached 20%. The impact of this mortality excess is not possible to assess due to the lack of data reported in the article, the unavailability of the raw data and the vague description as followed: *"With the exception of one test, only experiments with <20 percent control mortality were used in comparing the toxicity of different surfactants. Control mortality above the recommended 10% was deemed acceptable because this occurred on only a few occasions and lower levels of mortality occurred for mysids exposed to low levels of surfactant. A toxicant dose-response was otherwise observed in these tests. Reference toxicant data generated during the study demonstrated that sensitivity of mysids in tests with 11 to 19% control mortality was the same as that for mysids used in experiments with ~10% control mortality."* In table 2 of the available report, a control mortality as high as 25% is quoted and used due to the lack of mortality in NPE+50 exposures that is a long chain NPEs, out of this CLH proposal scope. Due to this uncertainty where the mortality excess could lead to an endpoint

underestimation, RAC is of the opinion that this study is not reliable for use under CLP.

As the available L(E)C_{50}s for fish and aquatic invertebrates are 0.218 mg/L (NPE-1) and 0.328 mg/L (NPE-1), respectively, and as these values are lower than the classification threshold value of 1 mg/L, RAC agrees that short chain NPEs (1 to < 3 ethoxylate groups) fulfil the criteria for classification **as Aquatic Acute Category 1, H400**. According to CLP table 4.1.3, a **M-factor=1** is warranted for L(E)C_{50}s ranged from 0.1 to 1 mg/L.

Aquatic chronic toxicity

Chronic toxicity data are available for fish, aquatic invertebrates, and algae.

RAC noted that for fish studies, few of them presented results for endpoints normally used for classification purpose as growth and survival. Most results of these studies are related to endocrine disrupting effects. Survival and growth endpoints may not be the most sensitive. For example, from the Medaka studies, a NOEC for reproduction in the range 0.035-0.1 mg/L was suggested by the observed effects on secondary sex characteristics for NPE-1 as proposed in the SEv dossier. RAC considers the most sensitive species to be *M. bahia* with a 28-day NOEC value of 0.0077 mg/L. RAC agrees that as not rapidly degradable substances, Short chain NPEs (1 to < 3 ethoxylate groups) fulfil the criteria for classification as **Aquatic Chronic 1, H410. An M-factor of 10** is warranted based on the NOEC between 0.001 and 0.01 mg/L.

Conclusion

RAC agrees with the DS that **short chain NPEs (1 to < 3 ethoxylate groups) warrant classification as:**

Aquatic Acute 1 (H400), M=1

Aquatic Chronic 1 (H410), M=10

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No information available.

13 ADDITIONAL LABELLING

No additional information available.

14 REFERENCES

A full refernce list for selected studies are included in the confidential annex.

Anonymous	1999a	Nonylphenol and octylphenol and their ethoxylates – determination of the biodegradability by the CO2 evolution modified sturm test.
Anonymous	1999b	Chronic toxicity to misids (*Mysidopsis bahia*) under flow-through conditions (study report).

Anonymous	2006	Developmental effects in Japanese medaka (*Oryzias latipes*) exposed to nonylphenol ethoxylates and their degradation products. Chemosphere 62(8):1214-1223.
Anonymous	2007	Toxicity of Nonylphenol, Nonylphenol Monoethoxylate, and Nonylphenol Diethoxylate and Mixtures of these Compounds to *Pimephales promelas* (Fathead Minnow) and *Ceriodaphnia dubia*. Arch Environ Contam Toxicol 53, 599–606.
Anonymous	2010a	Berol 259 (CAS No. 68412-54-4) Hydrolysis as a function of pH.
Anonymous	2010b	Berol 259 (CAS No. 68412-54-4) Alga, Growth Inhibition Test with *Pseudokirchneriella subcapitata*, 72 h.
Anonymous	2010c	Berol 259 (CAS No.68412-54-4) *Daphnia magna* reproduction Test, Semi-Static, 21 d.
Ahel M, Giger W	1993	Partitioning of alkylphenols and alkylphenol polyethoxylates between water and organic solvents. Chemosphere 26: 1471-1478
Ahel M, McEvoy J, Giger, W	1993	Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. Env. Poll. 79: 243-248.
Ahel M, Scully FE, Hoigne J, Giger W	1994a	Photochemical degradation of nonylphenol and nonylphenol polyethoxylates in natural-waters. Chemosphere 28:1361–8
Ankley GT, Peterson GS, Lukaswycz MT and Jensen DA	1990	Characteristics of surfactants in toxicity identification evaluations. Chemosphere 21 (1-2): 3-12.
Bodsch J	2010a	Appearance: physical state, colour and odour. Testing laboratory: Dr. U. Noack-Laboratorien, Kathe-Paulus-Strasse 1, D-31157 Starstedt. Report no.: CAP13606N. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden Report date. 2010-04-09.
Bodsch J	2010b	Berol 259, determination of the density / relative density. Testing laboratory: Dr. U. Noack-Laboratorien, Kathe-Paulus-Strasse 1, D-31157 Starstedt. Report no.: CPD13606N. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden Report date. 2010-04-15.
Bodsch J	2010c	Determination of critical micelle concentration (CMC). Testing laboratory: Dr. U. Noack-Laboratorien, Kathe-Paulus-Strasse 1, D-31157 Starstedt. Report no.: CMC13606N. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle 3, 44485 Stenungsund, Sweden. Report date. 2010-04-14.
Bodsch J	2010d	Statement, Berol 259. Estimation of the partition coefficient (n-octanol/water). Testing laboratory: Dr. U. Noack-Laboratorien, Kathe-Paulus-Strasse 1, D-31157 Starstedt. Report no.: CES13606. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. CES13606. Report date. 2010-07-23.
Chang BV, Yu CH, Yuan SY	2004	Degradation of nonylphenol by anaerobic microorganisms from river sediment. Chemosphere 55(4):493-500

Dussault EB, Sherry JP, Lee HB, Burnison BK, Bennie DT and Servos MR	2005	In vivo estrogenicity of nonylphenol and its ethoxylates in the Canadian environment. Human and Ecological Risk Assessment 11(2):353-364.
European Chemical Agency (ECHA)	2013	Support document for identification of 4-nonylphenol, branched and linear as support of very high concern because due to their endocrine disrupting properties they can give cause probable serious effects to the environment which give rise to an equivalent level of concern to those of CMRs and PBTs/vPvBs. Adopted on 13 December 2012.
Ejlertsson J, Nilsson ML, Kylin H, Bergman A, Karlson L, Öquist M, Svensson BH.	1999	Anaerobic degradation of nonylphenol mono- and diethoxylates in digestor sludge, *landfilled municipal solid waste, and landfilled sludge.* Environmental Science and Technology 33(2):301-306.
Granmo Å *et al.*	1991	Toxicity of 4-nonylphenol to aquatic organisms and potential for bioaccumulation. Proceedings of the seminar on nonylphenol.
Hall WS, Patoczka JB, Mirenda RJ, Porter BA and Miller E	1989	Acute toxicity of industrial surfactants to *Mysidopsis bahia*. Arch Environ Contam Toxicol 18: 765-772.
Huber V	2010a	Final report, Berol 259, batch no.: S-000929. Melting point A.1. (OECD 102). Testing laboratory: Siemens AG, Prozess-Sicherheit, Industriepark Höchst, C487, D-65926 Frankfurt am Main, Germany. Report no.: 20100176.01. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3,44485 Stenungsund, Sweden. Company study no. 20100176.01. Report date. 2010-07-20.
Huber V	2010b	Final report, Berol 259, batch no.: S-000929. Boiling point A.2. (OECD 103). Testing laboratory: Siemens AG, Prozess-Sicherheit, Industriepark Höchst, C487, D-65926 Frankfurt am Main, Germany. Report no.: 20100176.01. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. 20100176.01. Report date. 2010-07-20.
Huber V	2010c	Final report, Berol 259, Batch No.: S-00929. Flash point A.9. Testing laboratory: Siemens AG, Prozess-Sicherheit, Industriepark Höchst, C487, D-65926 Frankfurt am Main, Germany. Report no.: 20100176.03. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. 20100176.03. Report date. 2010-07-20.
Huber V	2010d	Final report, Berol 259, Batch No.: S-000929. Dissociation constant pKa (OECD 112). Testing laboratory: Siemens AG, Prozess-Sicherheit, Industriepark Höchst, C487, D-65926 Frankfurt am Main, Germany. Report no.: 20100176.05. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. 20100176.05. Report date. 2010-07-20.
Jobling S, Sheahan D, Osborne JA, Matthiessen P and Sumpter JP.	1996	Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphnolic chemicals. Environomental Toxicology and Chemistry 15(2):194-202.

Keith TL, Snyder SA, Naylor CG, Staples CA, Summer C, Kannan K, Giesy JP	2001	Identification and quantitation of nonylphenol ethoxylates and nonylphenol in fish tissues from Michigan. Environ. Sci. Technol. 35(1):10-13.
Lange J	2010	Berol 259 (CAS number 68412-54-4). Stability in organic solvents. Testing laboratory: DR. U. NOACK-LABORATORIEN, Käthe-Paulus-Str. 1, D-31157 Sarstedt. Report no.: CSS13606N. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. CSS13606N. Report date. 2010-07-19.
Maki H *et al*.	1998	Halogenation and toxicity of the biodegradation products of a nonionic surfactant, nonylphenol ethoxylates. Environ. Toxicol. and Chem. 17(4): 650-654.
Marcomini A, Capel PD, Lichtensteiger T, Brunner PH, Giger W	1989	Fate of organic pollutants in sludge amended soil and sludge-only landfills: linear alkylbenzenesulphonates,nonylphenols and polychlorinated biphenyls. Organic contaminants in waste water, sludge and sediment Proc workshop, Brussels, 1988:105-123.
Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE and Potter T	2001	Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 20(2):297-308.
Naylor CD	2004	The environmental safety of alkypeol ehtoxylates demonstrated by risk assessment and guidelines for their safe use. In: Handbook of detergents. Part B: Environmental impact. New York, NY: Marcel Dekker. P. 429-445.
Servos MR	1999	Review of the aquatic toxicity, estrogenic repsonses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. Water Qual. Res. J. Canada 34: 23-177.
Smeykal H	2010a	Final report, Berol 259, batch no.: S-000929. Vapour pressure A.4. (OECD 104). Testing laboratory: Dr. U. Noack-Laboratorien, Käthe-Paulus-Str. 1, D-31157 Sarstedt. Report no.: 20100176.02. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. 20100176.02. Report date. 2010-07-16.
Smeykal H	2010b	Final report, Berol 259, batch no.: S-000929. Flammability (solids and liquids) / pyrophoric properties A.13. / UN test N.2. Testing laboratory: Siemens AG, Prozess-Sicherheit, Industriepark Höchst, C487, D-65926 Frankfurt am Main, Germany. Report no.: 20100176.07. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. 20100176.07. Report date. 2010-07-16.
Smeykal H	2010c	Testing laboratory: Siemens AG, Prozess-Sicherheit, Industriepark Höchst, C487, D-65926 Frankfurt am Main, Germany. Report no.: 20100176.06. Owner company: Akzo Nobel Surface Chemistry AB, Stenungealle' 3, 44485 Stenungsund, Sweden. Company study no. 20100176.06. Report date. 2010-07-20.
Staples CA, Weeks J, Hall JF, Naylor CG	1998	Evaluation of aquatic toxicity and bioaccumulation of C8- and C9-alkylphenol ethoxylates. Environ Toxicol Chem 17: 2470-2480

Staples CA, Naylor CG, Williams JB, Gledhill WE	2001	Ultimate biodegradation of alkylphenol ethoxylate surfactants and their biodegradation intermediates. Environ Toxicol Chem 20: 2450-2455.
Stasinakis AS, Petalas AV, Mamais D, Thomaidis NS	2008	Application of the OECD 301F respirometric test for the biodegradability assessment of various potential endocrine disrupting chemicals. Bioresour Technol 99(9):3458-3467.
Sun H and Gu X	2005	Comprehensive Toxicity Study of Nonylphenol and Short-Chain Nonylphenol Polyethoxylates on Daphnia magna. Bull. Environ. Contam. Toxicol. 75:677–683
Teurneu B	2004	Biodegradation of Nonylphenol Ethoxylates. Master thesis. Dept. of Biotechnology Lund University.
U. S. Environmental Protection Agency	2009	AopWin v1.92 (EPIWEB v 4.0). (EPIWEB v 4.0) US EPA. [2009]. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.00. United States Environmental Protection Agency, Washington, DC, USA.
Van Vlaardingen P, Posthumus R & Trass TP	2003	Environmental risk limits for alkylphenols and alkylphenol ethoxylates. RIVM Report 601501019/2003. http://www.rivm.nl/bibliotheek/rapporten/601501019.pdf.
Yoshimura K	1986	Biodegradation and fish toxicity of nonionic surfactants. JAOCS 63 (12) 1590-1596
Yuan SY, Yu CH, Chang BV	2004	Biodegradation of nonylphenol in river sediment. Environmental Pollution 127(3):425-430.

15 ANNEXES

Information on the constituents of substance is given separately as a confidential Annex.