Substance Name: 4-Nonylphenol, branched and linear, ethoxylated[1]

EC Number: -

CAS Number: -

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT FOR IDENTIFICATION OF

4-NONYLPHENOL, BRANCHED AND LINEAR, ETHOXYLATED

AS SUBSTANCES OF VERY HIGH CONCERN BECAUSE, DUE TO THEIR DEGRADATION TO SUBSTANCES OF VERY HIGH CONCERN (4-NONYLPHENOL, BRANCHED AND LINEAR) WITH ENDOCRINE DISRUPTING PROPERTIES, THEY CAUSE PROBABLE SERIOUS EFFECTS TO THE ENVIRONMENT WHICH GIVE RISE TO AN EQUIVALENT LEVEL OF CONCERN TO THOSE OF CMR[2] and PBT/vPvB[3] SUBSTANCES

Adopted on 12 June 2013

[1] Please note that the full name of the substance as it will appear in the Candidate List is: 4-Nonylphenol, branched and linear, ethoxylated *[substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof]*

[2] CMR means carcinogenic, mutagenic or toxic for reproduction

[3] PBT means persistent, bioaccumulative and toxic; vPvB means very persistent and very bioaccumulative

CONTENTS

TABLES

Substance Name(s): 4-Nonylphenol, branched and linear, ethoxylated[4]

EC Number(s): -

CAS number(s): -

- The substances covered by the entry 4-Nonylphenol, branched and linear, ethoxylated [substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof] are identified as substances meeting the criteria of Article 57 (f) of Regulation (EC) 1907/2006 (REACH) because (through their degradation) they are substances with endocrine disrupting properties for which there is scientific evidence of probable serious effects to the environment which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of REACH.

Summary of how the substances meet the criteria of Article 57(f) of REACH

4-Nonylphenol, branched and linear, ethoxylated [substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof] are identified as substances of very high concern in accordance with Article 57 (f) of Regulation (EC) 1907/2006 (REACH) because, due to their degradation, they are a relevant source in the environment of substances of very high concern (4-Nonylphenol, branched and linear (4-NP)). Therefore, there is scientific evidence of probable serious effects to the environment from these substances, through their degradation to 4-Nonylphenol, branched and linear, which gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of REACH.

This conclusion is based on the fact that 4-Nonylphenol, branched and linear, ethoxylated (4-NPnEO) degrade to 4-Nonylphenol, branched and linear, either already in wastewater treatment plants, or via further degradation processes in sediments (e.g. of aquatic bodies receiving the wastewater effluents) and soils (e.g. receiving sewage sludge). Available information for 4-NPnEO indicate that 4-NPnEO contribute to the 4-NP concentration in the environment. A significant amount is either degraded to 4-NP itself in waste water treatment plants or is released to rivers in a form which may undergo further degradation to 4-NP. According to available data from sewage treatment plants, 4-NP formed from degradation of 4-NPnEO is responsible for an increase of the 4-NP load to the environment (soil, sediment and water) by 54 to 758 %. Sediment organisms may be exposed to 4-NP, which results from the degradation of 4-NPnEO, either directly, downstream of the effluent, or in the longer term after its adsorption to sediment and soil. Similar holds true for pelagic organisms such as fish which may be exposed via remobilisation of 4-NP from sediment to the water body.

<u>Based on the above conclusion, evidence that these substances are of an equivalent level of concern includes:</u>

- 4-Nonylphenol, branched and linear have been identified as substances of very high concern and included in the Candidate List due to the endocrine disrupting properties which cause probable serious effects to the environment

[4] 4-Nonylphenol, branched and linear, ethoxylated [substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof]

- To be consistent with the approach implemented in Annex XIII of the REACH regulation for PBT substances, it seems reasonable to conclude that any substance which may result in relevant exposure to a SVHC (i.e. due to degradation to this substance under environmental conditions) should be considered as SVHC itself as it results in the same equivalent level of concern.
- Once released to the environment 4-NPnEO will remain a long-term source of 4-NP due to the tendency of short chain ethoxylates to bind to the sediment combined with a very slow degradation in anaerobic sediments of both the ethoxylates and their degradation product 4-NP. Therefore, 4-NP formed by degradation of its ethoxylates may accumulate in sediment.
- Especially due to the fact, that short term exposure to 4-NP may result in life time effects in aquatic organisms and due to the fact that sudden environmental events may increase short term exposure concentrations, such a sink (mainly of short chain ethoxylates) and long-term source for 4-NP is considered of very high concern.

The equivalent level of concern is based on the degradation to 4-NP. However for further considerations it is important to note that available information for NPnEO indicate that short chain ethoxylates (NP1EO and NP2EO) show endocrine activity themselves: Results for *Onchorhynchus mykiss* and *Oryzias latipes* with NP1EO and NP2EO indicate that the in vivo and in vitro endocrine activity is nearly as high (factor 10) or similar to the endocrine activity of 4-nonylphenol. These tests do not include adverse endpoints. Hence, it is not possible to conclude whether 4-NP1EO and 4-NP2EO are endocrine disruptors themselves, or not. However due to the similar in vivo endocrine activity and information available for 4-NP it seems possible that these substances may cause endocrine disrupting adverse effects.

Registration dossiers submitted for the substances: none

JUSTIFICATION

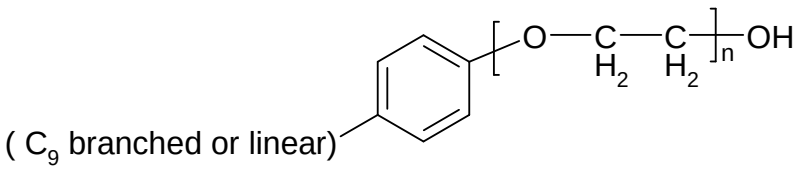
1 Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	-
EC name:	-
CAS number (in the EC inventory):	-
CAS number:	-
CAS name:	-
IUPAC name:	4-Nonylphenol, branched and linear, ethoxylated *[substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof]*
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	$(C_2H_4O)n\ C_{15}H_{24}O$, with n≥1
Molecular weight range:	-
Synonyms:	-

Structural formula:



1.2 Composition of the substance

Name: **4-Nonylphenol, branched and linear, ethoxylated *[substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof]***

Description: **group entry**

Degree of purity:-

Table 2: Constituents

Constituents	Typical concentration	Concentration range	Remarks
no information available			

Table 3: Impurities

Impurities	Typical concentration	Concentration range	Remarks
no information available			

Table 4: Additives

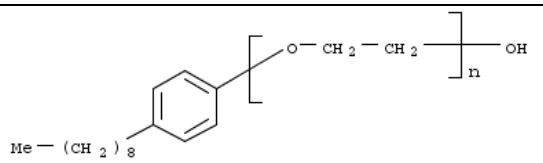
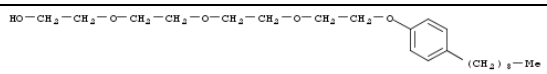
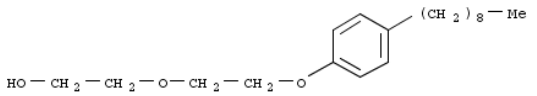
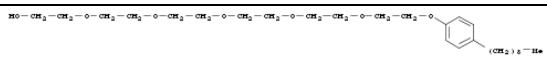
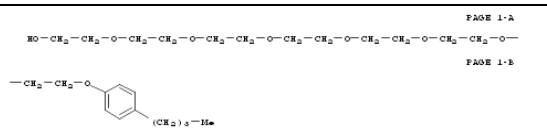
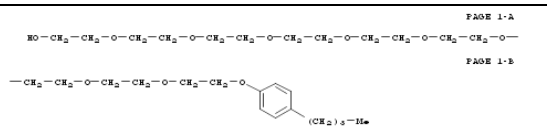
Additives	Typical concentration	Concentration range	Remarks
no information available			

No detailed composition of the substance can be given. The given identity 4-Nonylphenol, branched and linear, ethoxylated [*substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof*] shall cover the group of ethoxylates of 4-Nonylphenol, branched and linear.

In table 5 all substances are listed which are covered by the group entry and are pre-registered or for which a C&L notification has been submitted. No registration dossiers have been submitted for the substances which are listed in table 5.

Table 5 provides a non-exhaustive list of examples of substances covered by the group entry.

Table 5: Non-exhaustive list of substances covered by the group entry and for which there is information available in REACH-IT *

EC Name CAS Name: IUPAC Name:	EC – Nr.	CAS Nr.	Molecular formula	Structure
EC Name: - CAS Name: Poly(oxy-1,2-ethanediyl), α-(4-nonylphenyl)-ω-hydroxy- IUPAC Name: Poly(oxy-1,2-ethanediyl), α-(4-nonylphenyl)-ω-hydroxy-	-	26027-38-3	$(C_2H_4O)_n$ $C_{15}H_{24}O$	
EC Name: 2-[2-[2-[2-(4-nonylphenoxy)ethoxy]ethoxy]ethoxy]ethanol CAS Name: Ethanol, 2-[2-[2-[2-(4-nonylphenoxy)ethoxy]ethoxy]ethoxy]- IUPAC Name: 2-(2-(2-(2-(4-Nonylphenoxy)ethoxy)ethoxy)ethoxy)ethanol	230-770-5	7311-27-5	$C_{23}H_{40}O_5$	
EC Name: 2-[2-(4-nonylphenoxy)ethoxy]ethanol CAS Name: Ethanol, 2-[2-(4-nonylphenoxy)ethoxy]- IUPAC Name: 2-(2-(4-Nonylphenoxy)ethoxy)ethanol	243-816-4	20427-84-3	$C_{19}H_{32}O_3$	
EC Name: - CAS Name: 3,6,9,12,15-Pentaoxaheptadecan-1-ol, 17-(4-nonylphenoxy)- IUPAC Name: 17-(4-Nonylphenoxy)-3,6,9,12,15-pentaoxaheptadecan-1-ol	-	34166-38-6	$C_{27}H_{48}O_7$	
EC Name: 20-(4-nonylphenoxy)-3,6,9,12,15,18-hexaoxaicosan-1-ol CAS Name: 3,6,9,12,15,18-Hexaoxaeicosan-1-ol, 20-(4-nonylphenoxy)- IUPAC Name: 20-(4-Nonylphenoxy)-3,6,9,12,15,18-hexaoxaicosan-1-ol	248-743-1	27942-27-4	$C_{29}H_{52}O_8$	
EC Name: - CAS Name: 3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol, 26-(4-nonylphenoxy)- IUPAC Name: 26-(4-Nonylphenoxy)-3,6,9,12,15,18,21,24-octaoxahexacosan -1-ol	-	14409-72-4	$C_{33}H_{60}O_{10}$	

* This is a list of substances identified as covered by the generic substance description, however further substances not listed here may be covered as well.

The following abbreviations are used throughout the dossier:

4-NPnEO: para - nonylphenol ethoxylated with unknown or variable branching of the alkylgroup. If not indicated otherwise n describes the median grade of ethoxylation of the substance.

NPnEO nonylphenol (branched) ethoxylated with unknown or variable position and branching of the alkylgroup. If not indicated otherwise n describes the median grade of ethoxylation of the substance.

4-NP para - nonylphenol (branched) with unknown or variable branching of the alkylgroup

NP nonylphenol (branched) with unknown or variable position and branching of the alkylgroup

1.3 Physico-chemical properties

No physical and chemical properties could be found in accepted databases for the exemplary noted substances in Table 5. Furthermore no registration dossiers are available for these substances.

Hence no physical and chemical properties can be provided.

Due to this fact physical chemical data are calculated with EPI-Suite v4.10 to get a brief overview for the substance properties. As this information is only regarded as supportive no appraisal for the reliability is provided.

Table 6: Physical-chemical properties of a subset of 4-tert-nonylphenol ethoxylates with different grades of ethoxylation

Grade of ethoxylation	NP2EO	NP4EO	NP6EO	NP8EO	NP10EO
molecular weight (g/mole)	308.47	396.57	484.68	572.79	660.89
water solubility @ 25 °C (mg/l) (from WSKOW v1.41)	1.051	0.91	0.75	0.59	0.46
vapour pressure @ 25 °C (mm Hg)*	$9.14*10^{-9}$	$2.28*10^{-11}$	$4.02*10^{-14}$	$9.78*10^{-17}$	$8.71*10^{-24}$
Henry's Law constant (atm-m3/mol) (from HENRYWIN v3.20)	$2.56*10^{-9}$	$6.18*10^{-13}$	$1.49*10^{-16}$	$3.61*10^{-20}$	$2.11*10^{-19}$
Log Kow (from KOWWIN v1.67)	5.30	4.75	4.20	3.66	3.11

The values in the table above are predicted data. Therefore it should be considered that not all possible effects, e.g. steric effects, of the substances could be included in the used models.

2 Harmonised classification and labelling

4-nonylphenol ethoxylates are not classified according to Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation).

The degradation product 4-nonylphenol is a substance of very high concern included in the Candidate List because of its probable serious effects to the environment as a result of its endocrine disrupting properties, which give rise to an equivalent level of concern (European Chemicals Agency, 2012b). It is listed in Annex VI of Regulation (EC) No 1272/2008 as follows:

Table 7: Classification and labelling of 4-nonylphenol according to part 3 of Annex VI, Table 3.1 of Regulation (EC) No 1272/2008

Index-No	International Chemical Identification	EC-No	CAS-No	Classification		Labelling		Specific concentration limits, M-factors
				Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	
601-053-00-8	nonylphenol; [1] 4-nonylphenol, branched [2]	246-672-0 [1] 284-325-5 [2]	25154-52-3 [1] 84852-15-3 [2]	Repr. 2 Acute Tox.4* Skin Corr. 1B Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H314 H400 H410	GHS08 GHS05 GHS07 GHS09 Dgr	H361fd H302 H314 H410	

Table 8: Classification and labelling of 4-nonylphenol according to part 3 of Annex VI, Table 3.2 of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC-No	CAS-No	Classification	Labelling	Concentration limits
601-053-00-8	nonylphenol; [1] 4-nonylphenol, branched [2]	246-672-0 [1] 284-325-5 [2]	25154-52-3 [1] 84852-15-3 [2]	Repr. Cat. 3; R62-63 Xn; R22 C; R34 N; R50-53	C; N R: 22-34-62-63-50/53 S: (1/2-)26-36/37/39-45-46-60-61	

3 Environmental fate properties

3.1 Degradation

In the following chapter, degradation data are analyzed with respect to the question whether or not they indicate that 4-nonylphenol ethoxylates (4-NPnEO) may be of equivalent level of concern due to their degradation to 4-nonylphenol (4-NP). 4-NP is a substance of very high concern included in the Candidate List because of its probable serious effects to the environment as a result of its endocrine disrupting properties, which give rise to an equivalent level of concern. Information for 4-tert-octylphenol ethoxylates (4-tert-OPnEO) is included as supportive information. 4-tert-OPnEO were identified as substances of very high concern in December 2012 due to their degradation to 4-tert-octylphenol (4-tert-OP) which is a SVHC itself (European Chemicals Agency, 2012a). With regard to the endpoints of concern, 4-tert-OPnEO are considered close analogues to 4-NPnEO due to their similar chemical structure with the only difference being the alkylgroup differing by one C-Atom. Both alkylphenol ethoxylates are degraded by a stepwise degradation of the terminal ethoxygroup. Although the length of the alkylgroup might influence the degradation process, it is unlikely that the change by one C-atom only, will result in strong differences.

Most biodegradation data and distribution data available include NPnEO or 4-tert-OPnEO with an average chain length of up to 20 (NP20EO). Many studies were performed with the technical nonylphenol ethoxylate (NPnEO) and thus the excact composition of the test material is often unknown. As the technical nonylphenol consists of about 95% para-nonylphenol, it can be assumed that the main constituents of the technical nonylphenol ethoxylate (NPnEO) were para-nonylphenol ethoxylates (4-NPnEO). However, in order to be transparent, only in those tests clearly indicating that 4-NPnEO was used, the substance was labelled as 4-NPnEO and for all other studies the unspecific name NPnEO was used. If not indicated otherwise, the nonylphenol etoxylates tested consist of a gauss distribution of differen grades of ethoxylation and n describes the median degree of ethoxylation.

Two studies (Teurneau, 2004 and Rudling and Solyom, 1974) with NPnEO up to n = 40 show that biodegradation for longer chain ethoxylates is similar or even quicker compared to the shorter chain ethoxylates in sewage sludge and sediment (from the bottom of a settling basin from an industrial site) (see table 11 and 14). Although it is, in principle, possible that the degradation pathway could change for longer chain ethoxylates leading to other metabolites than the alkylphenol e.g. by cleavage of the alkylgroup ahead of the cleavage of the ethoxygroup, this is very unlikely based on the following facts:

- Rudling and Solyom (1974) clearly showed by GC analysis that degradation of NPnEO up to n=14 includes a sequential removal of the ethoxy-group leading to NP2EO.
- Data provided by Teurneau (2004) indicate that the same holds true for NPnEO with up to 40 ethoxy groups. Chromatograms using a HS-PEG column showed that in a batch experiment with STP sludge at 10 °C, degradation of NP10EO resulted in the formation of NP2EO both under aerobic and anaerobic conditions. For NP40EO the same holds true for aerobic conditions while under anaerobic conditions some undefined slightly more polar compounds occurred which the author suggests to be ethoxylates with an ethoxy chain length between 4 and 10. In the experiment with the sediment from an industrial site under anaerobic conditions NP was accumulating in the sediment.

The study by Teurneau (2004) is not a peer reviewed study but the findings are supported by further information about the mechanism involved in the degradation process:

- Data provided by Jonkers et al. (2001) (NPnEO ≤ 15) in river water samples show that degradation of the alkylgroup requires removal of the ethoxy-group down to n= 2 before the degradation of the alkylgroup starts via carboxylation. This indicated that carboxylation of the alkylgroup occurs only if the ethoxylgroup is degraded first.

- Biodegradation of the ethoxychain prior to degradation of the alkylchain is expected to be more energy efficient. As described by Karsa and Porter (1995), biodegradation of the alkylchain of surfactants would require ω-oxidation as a first step (which is an energy demanding process). In addition β-oxidation as a second step of the alkylchain degradation pathway is hampered by branching (Karsa and Porter, 1995).

In summary it can be concluded that although data are mainly available for ethoxylates with a chain length up to 20 ethoxy groups, enough evidence is available to conclude that the degradation pathway is the same for longer chain ethoxylates.

3.1.1. Abiotic degradation

3.1.1.1 Hydrolysis

It is expected that 4-nonylphenol ethoxylates will not be subject to abiotic degradation via hydrolysis. The nonyl group and the phenolic ring structure are chemically stable against hydrolysis. Also the ethoxylate chain is not suspected to be degraded via hydrolysis, but via biotic degradation.

In conclusion it is supposed that hydrolysis is not a relevant degradation process under environmental conditions.

3.1.1.2 Phototransformation/photolysis

3.1.1.2.1 Phototransformation in air

As there is no experimental information available from registration dossiers on phototransformation in air an estimation of half-lives was done with AOPwin (v1.92a)[5] for certain ethoxylation grades of 4-nonylphenol and under the supposition that photolytic degradation will occur due to the presence of OH-radicals. No further evaluation of the QSAR regarding the domain of applicability was conducted as the information on phototransformation in air is regarded not essential for the SVHC identification.

Table 9: Estimated half-lives in air for assorted ethoxylation grades of 4-nonylphenol

Grade of ethoxylation	1	2	4	6	8
Estimated half-life (hours)	2.66	2.06	1.42	1.08	0.88

Due to the low vapour pressure of the ethoxylates evaporation into the atmosphere is expected to be negligible. For example short chain 4-NP1EO has a vapour pressure of 2.38 * 10^{-5} Pa, the vapour pressure is expected to decrease with increasing length of the ethoxylate chain. Therefore photodegradation in air is expected not to be a relevant path of degradation for 4-nonylphenol ethoxylates.

3.1.1.2.2 Phototransformation in water

As described further in the chapters on biotic degradation processes, the main products being released directly or indirectly to the water body are un-degraded long chain ethoxylates (4-

[5] Environmental parameters used for calculation: temperature 25°C, 12-hr day, OH-radical concentration 1.5*10^6 /cm³

NPnEO with n > 2) as well as ethoxylates with a low grade of ethoxylation (4-NP1EO und 4-NP2EO) and its carboxylates (4-NPnEC) and – to a lesser extent – 4-nonylphenol.

Based on the expected adsorption behavior and distribution modelling summarized in chapter 3.2, long chain ethoxylates are expected to remain in the water body, while short chain ethoxylates and 4-nonylphenol have higher log Pow–values and are therefore expected to adsorb to suspended organic matter and sediment. Thus phototransformation might be a relevant route for ethoxylates with a high grade of ethoxylation only. However, photodegradation is a relevant degradation process in the first few centimetres layer of the water column only or in shallow clear waters. Thus aquatic phototransformation is considered not to have a relevant impact on the degradation of 4-nonylphenol ethoxylates in the aquatic environment.

3.1.1.2.3 Phototransformation in soil

Not assessed for the identification of this substance as SVHC in accordance with Article 57(f).

3.1.2 Biodegradation

With regard to biodegradation, several studies are available that provide information about degradation pathways of 4-nonylphenol ethoxylates (4-NPnEO) in sewage treatment plants, surface water, sediment and soils. They are analysed with regard to the question whether or not 4-NPnEO will contribute to the emission of 4-nonylphenol (4-NP) to the environment. Data are analysed with regard to the following aspects:

- Are 4-NPnEO released to the environment (and to which extent)?
- Does the degradation to 4-NP in sewage treatment plants contribute to the emission of 4-NP to the environment?
- Do 4-NPnEO released to the environment contribute to the environmental concentration of 4-NP due to their degradation in environment compartments?

3.1.2.1 Biodegradation in water

Some of the most important studies describing biodegradation in water are summarized in the subsequent chapter. In order to facilitate the discussion in chapter 6, available information on biodegradation in sewage treatment plants and surface water is analyzed separately.

Results suggest the following general pathway, as described in the European Risk Assessment Report (European Commission, 2002) and Jonkers et al. (Jonkers et al., 2001).

As a first step the ethylene oxide groups (EO) of longer chain 4-NPnEO (n>4) are rapidly removed resulting in ethoxylates with less than four ethoxyl units (usually one or two units, 4-NP1EO and 4-NP2EO). The rate of removal of the EO chain increases with increasing chain length. Under aerobic conditions the shorter chain 4-NPnEO (n<4) will be further oxidised to the corresponding carboxylic acids (for example nonylphenoxyacetic acid (4-NP1EC) or nonylphenoxyethoxyacetic acid (4-NP2EC)) and carboxylated alkylphenol ether carboxylates (CAmPEnC with m=5-9 and n=0 or 1) (Jonkers et al., 2001). Under anaerobic conditions the shorter chain 4-NPnEO will be degraded to nonylphenol diethoxylate (4-NP2EO) and nonylphenol monoethoxylate (4-NP1EO). Finally the 4-NP1EC and 4-NP1EO will be converted into 4-nonylphenol (4-NP), especially under anaerobic conditions (Environment Agency UK, 2005; European Commission, 2002; Van Vlaardingen et al., 2003).

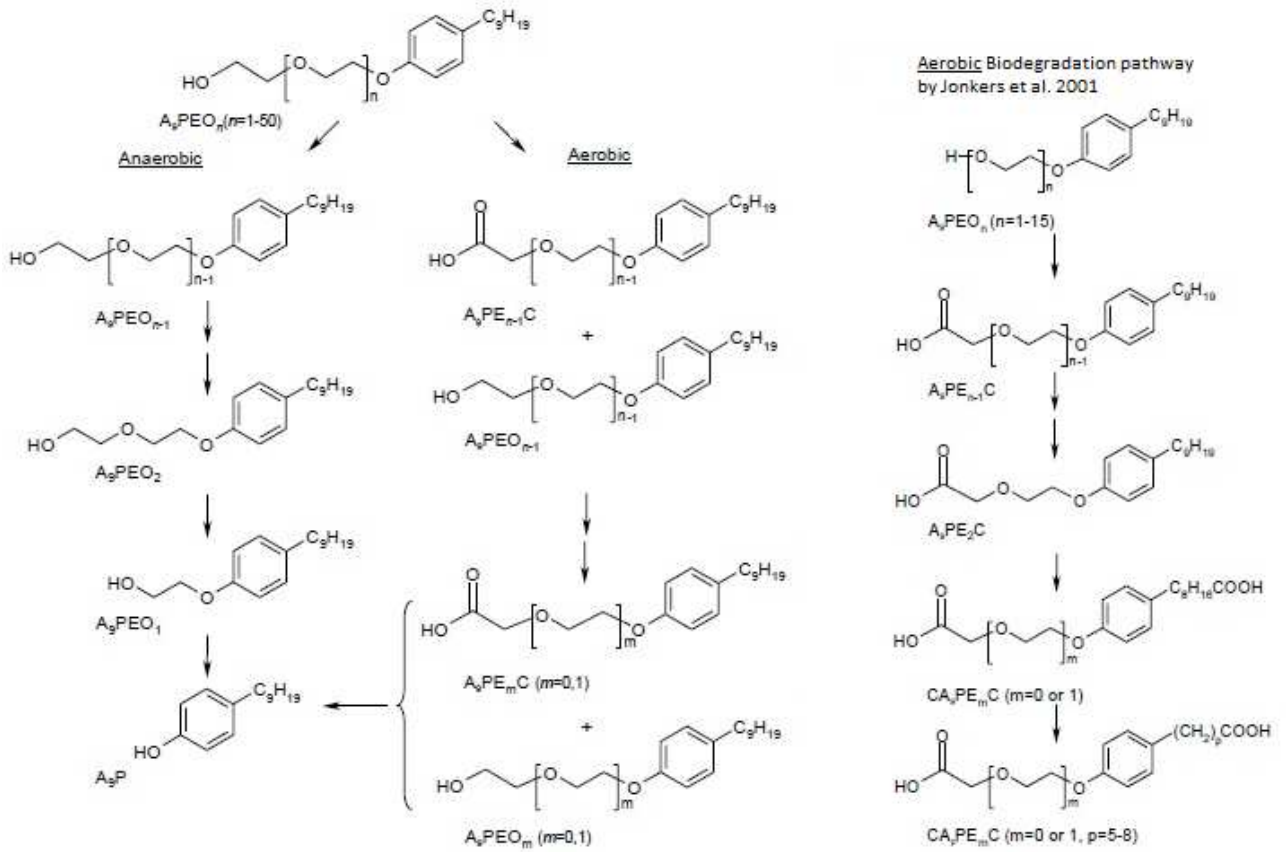


Figure 1: Biodegradation scheme for nonylphenol ethoxylates (Van Vlaardingen et al., 2003)

A more recent study shows that 4-NP1EC may degrade to 4-NP under oxic condition (Montgomery-Brown et al., 2008). The degradation pathways of 4-NP1EC were studied in oxic microcosms with organic carbon-poor soil and organic-rich sediments. The results show that the availability of dissolved oxygen determined the dominant degradation pathway. As observed in other studies, NP1EC was mainly degraded to CAP1EC via alkyl chain ethoxylation under microxic conditions (trace amounts of oxygen). On the other hand, under oxic conditions ether cleavage occurred and 4-NP was formed. However, under these conditions further rapid conversion of the 4-NP to nonyl alcohols occurred. This might be the reason why this degradation pathway was not observed in previous studies (Environment Agency UK, 2008).

3.1.2.1.1 Biodegradation in sewage treatment plants

Different types of studies are available to analyze the biodegradation of 4-nonylphenol ethoxylates (4-NPnEO) in sewage treatment plants. Five screening studies for 4-NPnEO and two studies for 4-tert-octylphenol ethoxylates (4-tert-OPnEO) provide information about the degree of degradation for long and short chain ethoxylates, without providing information about degradation products. In addition three simulation tests for 4-NPnEO and two tests with 4-tert-OPnEO are available which provide information about the degree of degradation as well as about the type of metabolites formed and the rate of degradation.

Screening tests

Table 10: Summary of Screening tests

Test substance	Method	Result	Reliability	Reference
4-NPEO				
NP9EO NP1.5EO CAS Nr. 9016-45-9	OECD 301 B Adapted inoculum	NP9EO: 74.8 ± 1.92 % CO_2 evolution in 28 days NP1.5EO: 45.3 ± 18.4 % CO_2 evolution in 28 days 10 day window was failed	2	(Gledhill, 1999; Staples et al., 2001)
NP9EO CAS Nr. 9016-45-	ISO 14593 headspace CO2 biodegradation test	after 28 days: 69.5 % CO2 (unaclimmated microbial seeds) 70.2 % CO2 (acclimated microbial seeds)	2	(Staples et al., 2001)
NP1EO CAS Nr. 27986-36-3 4-NP2EO CAS Nr. 20427-84-3	OECD 301 F	NP1EO: 25.9 ± 8.1 % NP2EO: 0 %	2	(Stasinakis et al., 2008)
NP9EO	OECD 301 B	53-58 % CO2 evolution in 28 days	4 (no details of the test)	(European Commission, 2002)
NP12EO	Closed bottle test OECD 301 B Gledhill test (US-EPA method 835.3120)	30 % ThOD 65% ThCO2 42% ThCO2 (acclilmated inoculum) 45% ThCO2 (unacclimated inoculum)	4 (secondary literature)	(Hughes et al., 1989; Environment Agency UK, 2008)
OPEO				
OP9EO OP1.5EO CAS Nr. 9036-19-5	OECD 301 B Adapted inoculum	OP9EO: 79.8 ± 1.59 % CO_2 evolution in 28 days OP1.5EO: 61.1 ± 0.98 % CO_2 evolution in 28 days 10 day window was failed	2	(Gledhill, 1999; Staples et al., 2001)
poly(oxyethylene) octylphenyl ether n=7-11(average of 9) CAS Nr. 9036-19-5	OECD 301 C	22 % degradation (measured by BOD) in 28 days	2	(National Institute of Technology and Evaluation, 2002)

The biodegradation of nonylphenol ethoxylates with a high number of ethoxyl groups (NP9EO) and its biodegradation intermediate NP1.5EO was measured using OECD 301B (Gledhill, 1999; Staples et al., 2001). The test was run with adopted inoculum from a waste water treatment plant. 45.3 % (NP1.5EO), and 74.8% (NP9EO) CO_2 evolution was observed after 28 days (58.7% and 79.5% after 35 days respectively). The 10 day window was failed. 33.4% (NP1.5EO) and 17.5% (NP9EO) suspended organic carbon was determined on day 35. This suggests that NPEO incorporated into biomass or adsorbed to suspended material. Staples et al. calculated first order half-lives (primary degradation) of 18.9 days (NP1.5EO; lag time = 9 days) and 13.6 days (NP9EO; lag time = 1 day). This study was also performed with OP9EO and OP1.5EO which showed similar results (79.8% (OP9EO) and 61.6 % (OP1.5EO) CO2 evolution after 28 days). The 10 day window was failed in either case. The calculated first order half-lives (primary degradation) were approximately 10 days (10.2 days OP9EO, 10.7 days OP1.5EO) with a lag time of 4 days.

Furthermore Staples et al. investigated the biodegradability of NP9EO with an ISO headspace

CO2 biodegradation test (Staples et al., 2001). Sludge from the same wastewater treatment plant as for the OECD 301B test was used. Each day for 7 days, activated sludge was settled for 30 min, 100 ml of supernatant were removed, and fresh sludge was added to the two semicontinuous activated sludge units. One unit received 10 mg C(NP9EO)/L each day and the other unit remained unacclimated. The prepared inocula were added to the test medium. After 28 days 69.5% (unacclimated) and 70.2% CO_2 (acclimated) were measured. 12.9% or 11.7% of the carbon remaining was in the dissolved phase and 17.6% or 18.1% was detected as suspended organic carbon.

In an OECD 301F test with NP1EO and 4-NP2EO additional 10 mg/L allythiourea was added for preventing nitrification (Stasinakis et al., 2008). After a lag phase of 17.3±0.7 days, NP1EO was aerobically biodegraded with 25.9±8.1% at day 28. For 4-NP2EO no biodegradation was observed.

The study of Hughes et al. (Hughes et al., 1989) was discussed in a unpublished report of the Environment Agency UK, which has been copied here in italic letters (Environment Agency UK, 2008):
Hughes et al. (1989) compared the ready biodegradability of NPE12 in different standard test systems: the modified Sturm test (OECD 301B), the Gledhill test (US EPA method 835.3120) and the closed bottle test. In all tests, ultimate biodegradation ranged between 30 and 65% as measured by conversion to carbon dioxide. Using the Gledhill test, the effect of using both acclimated and unacclimated microbial seed on biodegradation of NPE12 to carbon dioxide was investigated. However, no significant differences were noted: 45% ThCO2 mineralisation was reached with the unacclimated seed versus 42% ThCO2 mineralisation with an acclimated inoculum.

In a 28-day ready biodegradability test (OECD 301C) using 100mg/L of the poly(oxyethylene) octylphenyl ether (OPnEO with n=7-11, average of 9) and 30 mg/L sludge 22% degradation was measured by BOD (National Institute of Technology and Evaluation, 2002).

In summary, the results (based on 4-NPnEO and NPnEO) show, that both long and short chain 4-NPnEO are not readily biodegradable using standard test methods. However, the results do not allow any conclusion about degradation products. They provide some evidence, that 4-NPnEO are metabolized to some extent and that degradation may involve some stable metabolites.

Simulation tests

Table 11: Summary of biodegradation tests for nonylphenol ethoxylates in waste water treatment plants

Test substance	Type of test/ conditions	Result	Reliability	Reference
NPnEO (n = average of 9) (68412-54-4)	Sewage sludge; anaerobic	Increase of NP1EO and NP2EO concentration during decrease of NPnEO concentration (peak on day 14), NP1EO and NP2EO degrade to NP (peak on day 21) 30% dissipation of total NPnEO after 3 d	2	(Lu et al., 2008a)
NPnEO (n = average of 9) (68412-54-4)	Sewage sludge; anaerobic (sulphate-reducing conditions)	Increase of NP2EO (peak on day 7), NP1EO and NP (peak on day 21, maximum NP about 8 µM) concentration during decrease of NPnEO concentration 50% dissipation of total NPnEO after 3 d	2	(Lu et al., 2008b)
4-NP1-2EO mixture (0.15% NP, 70% NP1EO, 28% NP2EO, 2 % NP3EO)	Sewage sludge; anaerobic	10% digester sludge: 31 % NP was formed during 150 days 100% digester sludge: 57 % NP was formed during 150 days	2	(Ejlertsson et al., 1999)
NPnEO (n = 8-10,14,16,30)	Laboratory scale activated sludge system; aerobic	Degradation > 80% after 30 days (metabolites were not analysed) Metabolites were analyzed by gas chromatography in a previous screening test: Main product of NPnEO (n=8,10,14) was NP2EO after 4 days (20°C); Further degradation of NP2EO: 20°C: 50% after 28 days 15°C: 0 % after 28 days	2	(Rudling and Solyom, 1974)
NPnEO (n=4,10,40)	Batch experiment, sewage sludge; aerobic and anaerobic	Within 44 days: theoretical calculations: Aerobic: NP10EO degradation 29 % (27°C) and 25 % (10°C) NP40EO degradation 63% (27°C) and 21 % (10°C) Anaerobic: NP10EO degradation 40 % (27°C) and 0 % (10°C) NP40EO degradation 79% (27°C) and 30 % (10°C) Formation of NP2EO and unknown products of a size between 4 and 10 ethoxylates	2	(Teurneu, 2004)

Results by Lu et al. (Lu et al., 2008a; Lu et al., 2008b) showed under anaerobic and sulphate reducing conditions constant degradation of the longer chain ethoxylates with NP1EO and NP2EO being the most prominent ethoxylates from day 7 to day 60. After 3 days about 30 - 50% of the total NPnEO concentration dissipated to undefined products. Under both conditions (Lu et al 2008a and 2008b) NP concentration increased from 0 to about 8 µM during the rapid degradation of NP9EO (until day 21) and slowly decreased during the following phase of low NPnEO degradation, indicating, that its formation exceeds its transformation if its ethoxylates

are available as a source of NP.

The degradation of a 4-NP1-2EO mixture (2, 60 and 308 mg/L) in digester sludge (10% and 100%), landfilled municipal solid waste and landfilled sludge was determined under methanogenic conditions for 150 days (Ejlertsson et al., 1999). In this chapter results with regard to the digester sludge are reported while results for landfills are described in chapter 3.1.2.3. The background levels of 4-NP, 4-NP1EO and 4-NP2EO were high in the inocula. In all inoculates using a concentration of 2 mg/L 4-NP1-2EO the short chain ethoxylates were slowly transformed to 4-NP by anaerobic microorganisms. Transformation was highest in the 100% sludge sample compared to the diluted sample (57 and 31 % of the total 4-NP/4-NPnEO concentration respectively at day 150). 4-NP was not further degraded and incubation with radiolabelled 4-NPnEO showed that the phenol ring remained intact (no $^{14}C0_2$ or $^{14}CH_4$ production). Results with 60 and 308 mg/L 4-NPnEO indicate that degradation is concentration dependent. At 60 mg/L 4-NP1-2EO was slowly transformed to 4-NP1EO in 100% digester sludge but no transformation occurred in the 10% sludge sample and at 308 mg/L 4-NP1-2EO less than 1% of the added 4-NP1-2EO was transformed into 4-NP.

Rudling and Solyom investigated the biodegradability of longer chain ethoxylates (NPnEO up to n=30) with a laboratory scale activated sludge system (Rudling and Solyom, 1974). The initial concentration of the nonylphenol ethoxylates in the influent of the system was approximately 5.5 mg/L. The test was performed with presettled municipal sewage under treatment plant conditions. All NPnEO derivates (n=8, 10, 14, 16, and 30) show a similar degradation of more than 80% after 30 days. Further tests show that the removal is caused by degradation and not by adsorption to sludge.

The degradation of NPnEO (n=4, 10, and 40) was studied under aerobic and anaerobic conditions at 27°C and 10°C (Teurneu, 2004). For the batch experiments sludge samples from a waste water treatment plant were used. The initial concentration of NPnEO was 500 mg/L. The degradation rates were higher at 27°C than at 10°C. For the lower temperature a longer lag phase was observed. The degradation under anaerobic conditions resulted in a longer lag phase, too.

The study found an unusual high NP4EO removal rate (83 % at 27°C and 50% at 10 °C) under aerobic conditions for which the author considered further experiments would be needed. HPLC analysis showed removal percentages of 90-100% for NP10EO and NP40EO based on theoretical calculations using the coefficient of the reaction equation and the oxygen demand. At 10 °C, degradation of NP10EO resulted in the formation of NP2EO both under aerobic and anaerobic conditions. For NP40EO the same holds true for aerobic conditions while under anaerobic conditions some undefined slightly more polar compounds occurred which the authors suggest to be ethoxylates with a chain length between 4 and 10.

In summary available sewage treatment plant simulation tests for 4-NPnEO (based on studies carried out with 4-NPnEO and l NPnEO) substantiate the degradation pathway described above. Four Studies show that NP1EO and NP2EO are formed from longer chain NPnEO (n≤ 40) under aerobic and anaerobic conditions. Formation of NP under anaerobic conditions is substantiated in two studies. In addition, studies provide evidence about the degradation time of the different metabolites. In two studies (Lu et al 2008a and 2008b) dissipation of total NPnEO (including long chain and short chain NPnEO) was 30 and 50% after 3 days. The study by Ejlertsson et al, 1999 however indicates that the final step to 4-NP formation might be slower and that NP is a stable metabolite under these conditions (31 – 57 % formation of 4-NP during 150 d for 4-NP1-3EO.).

<u>Supporting information for 4-tert-OPnEO</u>

Results available for the close analogues 4-tert-octylphenol ethoxylates (4-tert-OPnEO) further substantiate the degradation pathways in anaerobic sewage sludge.

Table 12: Summary of biodegradation tests for octylphenol ethoxylates in waste water treatment plants

Test substance	Type of test/ conditions	Result	Reliability	Reference
Sewage, sewage sludge				
Tert-octylphenol polyethoxylate (13% OP1EO, 40% OP2EO, 29% OP3EO, 14% OP4EO, 4% OP5EO)	activated sludge inoculation (aerobic)	Rapid transformation from OPnEO to OPnEC (n=1-3) within 24 hours 30% degradation to undefined products	2	(Ball et al., 1989)
	primary sewage inoculation (aerobic)	Transformation of OPnEO to OPnEO (n=1-3) within 2 days Nearly no further degradation until day 17 (4% formation of undefined products) 80% degradation to undefined products until day 36 with an adaption time of 5 and 17 days for OP1EO and OP2EO		
	anaerobic bioassay	Transformation OPnEO (n≥2) to OP1EO within 10 days (no further degradation) 18% conversion to OP after 66 d		
P, tert.-octylphenoxynonaethoxyethanol (OPE10)	Shake culture tests (aerobic, acclimated sludge) Bench-scale activated sludge tests (aerobic) Continuous model septic tank (anaerob) with subsequent percolation field (acclimated) (^{14}C and ^{3}H labelling)	> 90% primary degradation within 7 days 90-95 % primary degradation after 11 days (acclimatization time 5-11 days) 63-66% loss of ^{14}C (degradation of the ethoxy-group) after 20 days acclimatization 58 % primary degradation in the septic tank (anaerob) (average until day160) 93 % primary degradation after percolation (average until day160) 7% loss of ^{14}C (degradation of the ethoxy-group) in the septic tank (average until day 170) ≈ 65 % loss of ^{14}C (degradation of the ethoxy-group) after percolation (at day 170) No loss of ^{3}H (no degradation of the phenol ring)	2	(Lashen et al., 1966)

Ball et al. studied the biotransformation of tert-octylphenol polyethoxylate under aerobic and anaerobic conditions (Ball et al., 1989). The test substance mixture of tert-octylphenol polyethoxylates and the corresponding carboxylic acids was inoculated with activated sludge (4-tert-OPnEO residues were previously detected), primary sewage and anaerobic bacteria.
The tests with activated sludge showed a rapid complete transformation of 4-tert-OPnEO within 24 hours. 70 % of the initial 4-tert-OPnEO dissipated to 4-tert-OPnEC (n=1-3) (4-tert-OP2EC predominant product and 30% dissipated to unidentified products).
Primary sewage as inoculum resulted in dissipation of 4-tert-OPnEO (n=4-5) within 2 days and an increase of 4-tert-OP2EO until day 17. Only 4 % of the initial input was degraded to undefined products. After an adaption time of 5 and 17 days for 4-tert-OP1EO and 4-tert-

OP2EO they degraded to unidentified products. Hence, results show that 4-tert-OPnEO (n > 3) quickly degrade to ethoxylates with lower grade of ethoxylation while further degradation of these products is much slower. After 127 days more than 99% were dissipated to products different from 4-tert-OP, 4-tert-OPnEO (n=1-5) and 4-tert-OPnEC (n=1-2).

Under anaerobic conditions 4-tert-OPnEO (n=2-5) nearly completely dissipated to 4-tert-OP1EO within 10 days. No further degradation occurred. After this, 4-tert-OP1EO converted slowly into octylphenol and, to a less extent, 4-tert-PnEC and undefined products. After 66 days 18% of the original 4-tert-octylphenol ethoxylates were converted into 4-tert-octylphenol, 6% were transformed to 4-tert-OPnEC (mainly OP2EC). Subsequent degradation of 4-tert-octylphenol appeared to be slow (7.9 % 4-tert-octylphenol at day 190). 89% of the input was degraded to undefined products after 190 days.

The biodegradation of radiolabelled (^{14}C in the ethoxylate chain and ^{3}H in the phenol ring) p,tert.-octylphenoxypolyethoxyethanol (OPnEO, n =10) was carried out by Lashen et al. (Lashen et al., 1966). The experiment included a) an aerobic shake culture test using acclimated bacterial culture from a laboratory continuous activated sludge unit, b) a bench-scale activated sludge test with 3 and 6 hours retention time and inoculated with fresh sludge and c) a (anaerobic) model septic tank percolation field system (retention time = 67 hours).

In the shake culture test and the bench scale test primary degradation was > 90 % within 7 days and after 11 days (3 and 6 hours retention time) respectively. An acclimation period was observed if fresh, not acclimated sludge was used. Dissipation of ^{14}C incorporated in the ethoxylate chain in the bench scale test indicate that primary degradation was mainly due to a transformation of the ethoxyl group (63-66%) while no degradation of the phenol was observed (no dissipation of ^{3}H incorporated in the phenol ring).

In the model tank-percolation field system primary degradation in the anaerob model tank was lower (58%) but reached 93% after transfer through the percolation field. Again this was mainly due to a degradation of the ethoxylate group (65% degradation) and no mineralization of the phenol group was observed.

Based on measurements of degradation products it can be concluded that 4-tert-OPnEO are quickly degraded to short chain 4-tert-OPnEO (n= 1-3) within 2 days in primary sewage with nearly no further degradation. Especially 4-tert-OP2EO (the main product) remains stable until day 17.

In summary, under aerobic conditions based on simulation studies it can be assumed that about 70% of the input is rapidly transformed to 4-tert-OPnEC (100% transformation after 24h) and 30% are further degraded.

Under anaerobic conditions results seem to depend on the test conditions. Results from a static test with anaerobic bacteria indicate that 100 % of the input is transformed to 4-tert-OP1EO after 10 days and 18% is further transformed to 4-tert-OP after 66 days. But results of the continuous model tank indicate that in a flow through system transformation might be slower (only 58% primary degradation and only 7% transformation of the ethoxy-group).

Overall, between 70 and 100% of the 4-tert-OPnEO is not mineralized under sewage treatment plant conditions.

These data support and further substantiate the findings for 4-NPnEO.

3.1.2.1.2 Biodegradation in surface water

Table 13: Summary of biodegradation tests in surface water

Test	Type of	Result	Reliability	Reference

substance	test/ conditions			
Fresh water				
NP4EO (with an ethoxylate range of 2-9 and NP10EO (with an ethoxylate range of 4-15)	aerobic	Primary degradation > 99 % after 100 hours; Metabolites: NPnEC No change in initially NP concentration (31days)	2	(Jonkers et al., 2001)
4-NP9EO (97.8% 4-NP9EO, 2.2% 2-NP9EO)	aerobic	After 128 days: Primary degradation 87-97% (adaption time: 28 days) 40.5 % $^{14}CO2$ 40.2 % of the initial radioactivity remaining in aqueous phase 20.8 % of the initial radioactivity incorporated into biomass Non-labelled test system: 0.4 % 4-NP as metabolite of initial 4-NPnEO; < 2% 4-NPnEC	2	(Naylor et al., 2006)
Estuarine water				
NPnEO (n=1-18, average =10)	Die-away test, aerobic	DisT50 = 23-69 days (winter 13°C) DisT50 = 2.5-35 days (summer 22.5°C) Main intermediate NP2EO	2	(Kveštak and Ahel, 1995)
NPnEO (n=7-24, average 18)	Die-away test, aerobic	Primary degradation 100% after 4-24 days (adaption time: 0-12 days) Maximum concentration of NP2EO in 4-16 days Maximum concentration of NP2EC in 20-76 days NP was not detected	2	(Potter et al., 1999)

Aerobic biodegradation of NPnEO was investigated in a laboratory-scale bioreactor filled with river water (Jonkers et al., 2001). The bioreactor was spiked with two different technical mixtures of NPnEO (NP10EO, NP4EO) at concentration of 10 mg/L. Small amounts of OPnEO and decylphenol ethoxylated were present in the mixtures. After 4 days 99% of the NPnEO mixtures were dissipated (primary degradation). Nonylphenol carboxylates (NPnECs) were identified as the main group of metabolites. The concentration of NPnECs increased until day 5 and subsequently decreased. No change in initial NP was observed during the experiment (31 days). Further degradation of NP1-2EC by a carboxylation of the alkylchain was observed in this experiment (CAmPEnC with m=5-9, n= 0 or1). Both short-chain NPEC and CAPEC metabolites were still present in the bioreactor after 31 days.

Aerobic Biodegradation of [^{14}C] 4-NP9EO was examined and changes in the oligomer distribution and mineralization to $^{14}CO_2$ were monitored for 128 days (Naylor et al., 2006). 87-97% of the initial 4-NPnEO was degraded to metabolites other than 4-NP, 4-NPnEO and 4-NPnEC after 128 d. Only 0.4% 4-NP was detected (non-labelled test system), suggesting that NP is a minor metabolite under aerobic conditions in river water. After 128 days 40.5% of [^{14}C] 4-NP9EO converted to $^{14}CO_2$ but an acclimation period of 28 days was needed.

Biotransformation of NPnEO by estuarine mixed bacterial cultures was analyzed under laboratory conditions by using a static die-away method (Kveštak and Ahel, 1995). The experiments were performed with autochthonous bacterial cultures from the brackish water and saline water. Biotransformation kinetics of mixed bacterial culture from the brackish water layer was faster than that from the saline water layer at all temperatures examined and at both concentrations of NPnEO (0.1 and 1 mg/L). This was probably due to a better pre-adaptation of the brackish water bacteria to NPnEOs in their natural habitat. Under winter temperature conditions (13°C) the estimated DisT50 ranged from 23-69 days, while the DisT50 under summer temperature conditions (22.5°C) ranged from 2.5-35 days.

Transformation to NPnEC was not followed and the main intermediate formed during the experiment was NP2EO.

A further static die-away test was performed with estuarine water from four sampling sites in Tampa Bay, FL (Potter et al., 1999). Depending on sampling site (middle of the Bay, port area and tidal river) the temperature ranged from 27.5 to 31.0 °C. The concentration of NP2EO, NP1EO, NP2EC, NP1EC, NP and total surfactant were monitored at intervals of 4-8 days for 89 days and at 30-day interval thereafter until 183 days. Due to the different sampling locations the following results are stated as a range. Complete primary degradation of NPnEO (n =18) was detected after 4-24 days with an adaption time between 0 and 12 days. The formation of NP2EO reached its maximum concentration in 4-16 days. NP2EC increased until day 20-76 with little or no decrease until the end. Smaller amounts were detected for NP1EO (<0.1 mg/L) and NP1EC (maximum concentration 20% of NP2EC). NP was not measured (detection limit 0.01 mg/l). Potter et al. estimated that approximately 36 to 56 % of the surfactants converted to CO_2 and H_2O or other metabolites. However, this was not confirmed analytically.

In summary three tests support the hypothesis, that under aerobic conditions in fresh water long-chain 4-nonylphenol ethoxylates (based on studies carried out with 4-NPnEO and NPnEO) will be rapidly degraded to 4-NPnECs (99% primary degradation after 100 hours (Jonkers et al., 2001) and formation of the corresponding alkylphenol is of minor relevance. However results by Kvestak and Ahel with a mixed culture of bacteria from brackish water indicate that transformation to 4-NP2EO may occur in brackish water and that degradation may be much slower during winter (DisT50 between 23 and 69 days) (Kveštak and Ahel, 1995). Furthermore, only 40% of 4-NPnEO mineralized to CO_2 in 128 d (Naylor et al., 2006).

3.1.2.2 Biodegradation in sediments

Table 14: Summary of biodegradation tests in sediment

Test substance	**Type of test/ conditions**	**Result**	**Reliability**	**Reference**
Estuarine water sediment				
NP4EO (with an ethoxylate range of 0-9)	aerobic	DisT50 = 85 days	2	(Ferguson and Brownawell, 2003)
	anaerobic	DisT50 = 289 days		
Fresh water sediment				
4-NP1EO	aerobic	DegT50 = 69.3 – 115.5 days (primary degradation)	2	(Yuan et al., 2004)
4-NP1EO	anaerobic	DegT50 = 49.5 – 77.0 days (primary degradation)	2	(Chang et al., 2004)
Sediment from an industrial site (sedimentation bassin)				
NPnEO (n=2,4,10,40)	aerobic and anaerobic	Within 44 days theoretical calculations: aerobic: NP2EO degradation 4 % (27°C) and 0 % (10°C) NP4EO degradation 10 % (27°C) and 7 % (10°C) NP10EO degradation 24 % (27°C) and 19 % (10°C) NP40EO degradation 31% (27°C) and 12 % (10°C) anaerobic: NP2EO degradation 5 % (27°C) and 1 % (10°C) NP4EO degradation 21 % (27°C) and 0 % (10°C) NP10EO degradation 36 % (27°C) and 26% (10°C) NP40EO degradation 49% (27°C) and 10 % (10°C)	2	(Teurneu, 2004)

		Formation of NP		

The degradation of radiolabelled NP4EO mixture (NPnEO n=0-9) in estuarine sediment was investigated under aerobic and anaerobic conditions in batch sediment slurry experiments (Ferguson and Brownawell, 2003). The sampling site (Jamaica Bay, NY, USA) has been extensively studied with regard to the NPnEO fate. It is situated near to the outfall of a major waste water treatment plant (NPnEO concentration in sediment >40 µg/g dry weight, mostly NP and NP1EO) and represents a highly polluted site (high contamination with heavy metals and organic contaminants). The total NPnEO mixture dissipated significantly faster under aerobic conditions (DisT50 = 85 days) than under anaerobic conditions (DisT50 = 289 days). Even under aerobic conditions only 1.7 % CO2 of the initial added [^{14}C6]- NP4EO was formed. This is contrary to other studies that have been reported that NPnEO converted to CO_2 under aerobic conditions. The authors stated various reasons, for example: reduced bioavailability of NPnEOs due to sorption to the highly organic-rich sediment; inhibition of mineralization by high concentrations of toxicants (sediment is known to be toxic to microorganisms in Microtox™ assays). Nonylphenol was present at low levels (~5%) in the [^{14}C6]- NP4EO spiking material and was observed to persist at these low levels throughout the degradation experiment in both oxic and anoxic treatments. At the end of the experiment, NP accounted for only approximately 3% of the initially added ^{14}C activity in both the aerobic and anaerobic treatments. The authors mentioned that this might be due to a small amounts of NP formed and removed at similar rates or that the time scale of the experiment was not long enough. Even if there are some concerns about this study due to the toxicity and high organic carbon content of the sediment, the study provides some indication what could happen if a site is very polluted.

Chang et al. studied the degradation of 4-NP1EO by anaerobic microorganisms from NP-acclimated river sediments (Chang et al., 2004). The $DegT_{50}$ (primary degradation) ranged from 49.5 to 77.0 days (30 °C). After day 8, 4-NP was determined as intermediate product. The concentration of 4-NP increased from day 8 to day 14. Degradation rates for 4-NP1EO 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.

Yuan et al. sampled sediment from the same samples sites as Chang et al. 2004 and studied the aerobic degradation of 4-NP1EO (Yuan et al., 2004). The half-lives DegT50 (primary degradation) 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, NP1EO was completely dissipated after 56 days.

The degradation of NPnEO (n=2, 4, 10, and 40) was studied under aerobic and anaerobic conditions at 27°C and 10°C (Teurneu, 2004). For the batch experiments sediment samples from the bottom of a sedimentation basin of an industrial site (production of NPnEO) were used. The initial concentration of NPnEO 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.

In summary, only little information is available for biodegradation of 4-NPnEO in sediment. These data show that in sediments 4-nonylphenol ethoxylates (based on studies carried out with 4-NPnEO and NPnEO) degrade to 4-nonylphenol under aerobic and anaerobic conditions. Degradation of the short chain 4-nonylphenol ethoxylates (n = 1-4) is slow and depends on temperature with dissipation half-lives of 49-115.5 d and even longer (289 d at a highly polluted site). Although results in aerobic sewage treatment plants indicate that under aerobic conditions dissipation of 4-nonylphenol ethoxylates and formation of the corresponding

carboxylates is a fast process, results by Ferguson and Brownawell (Ferguson and Brownawell, 2003) indicate that in pre-contaminated sediment this process may be hindered. Overall results indicate that 4-nonylphenol ethoxylates may degrade to 4-nonylphenol in sediment. Because degradation may be slow, it can be expected, that they are a long-term source for 4-nonylphenol in sediment.

3.1.2.2.1 Biodegradation in soil

Table 15: Summary of biodegradation tests in soil

Compound	**Result**	**Reliability**	**Reference**
NP12EO	90-99% dissipation within first week Biphasic kinetic 1. $DisT_{50}$ = 0.3 – 5.2 days 2. $DisT_{50}$ = 11.40 – 48.0 days	2	(Sjöström et al., 2008)
Mixture of 4-NP1EO, 4-NP2EO and 4-NP3EO Imbentin -N/7A	4-NP1EO: 90 % dissipation after 322 days triphasic kinetics: 1. Initital period (1-14 days): $DisT_{50}$ = 7 days 2. Transition time (30 – 90 days): $DisT_{50}$ = 150 days 3. Long-term persistence (> 150 days): $DisT_{50}$ > 360 days 4-NP2EO: 86 % dissipation after 322 days triphasic kinetics: 1. Initital period (1-14 days): $DisT_{50}$ = 8 days 2. Transition time (30 – 90 days): $DisT_{50}$ = 110 days 3. Long-term persistence (> 150 days): $DisT_{50}$ > 360 days	2	(Marcomini et al., 1989)
Linear 4-NP2EO	Mineralization after 2 months: sludge-soil ratio 1:20 (40% water content) = 61.4 % sludge-soil ratio 1:20 (80% water content) = 12.4 % sludge-soil ratio 1:100 (40% water content) = 70.2 % sludge-soil ratio 1:100 (80% water content) = 43.4 % sludge only = 14.8 % soil only = 64.4 %	2	(Gejlsbjerg et al., 2001)
4-NP1-2EO mixture (0.15% 4-NP, 70% 4-NP1EO, 28% 4-NP2EO, 2 % 4-NP3EO)	landfilled sludge: 81 % 4-NP was formed during 53 days; > 53days concentration remained constant	2	(Ejlertsson et al., 1999)
NP4EO, NP9EO	NP4EO: 12-29 % mineralization within 150 days NP9EO: 17-28 % mineralization within 150 days	2	(Dettenmaier and Doucette, 2007)

Sjöström et al. examined degradation of NP12EO in four contrasting agricultural soils (Sjöström et al., 2008). A biphasic dissipation kinetic was observed. The rapid initial dissipation with DisT50 = 0.3 – 5.2 days were followed by a slower dissipation phase (DisT50 = 11.4 - 48.0 days). After 30 days results showed the formation of NP from NP12EO. NP remained nearly stable at the end of the experiment. No detectable NP12EO remained in the soils after 105 days and no intermediate degradation products were found.

The fate of a mixture of 4-NPnEO (n= 1-3) in sludge amended soil was studied by Marcomini et al. (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 filed 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 4-NP1EO and 4-NP2EO 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 4-NP1EO and 4-NP2EO, respectively. The disappearance of

4-NP1EO and 4-NP2EO 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 4-NP1EO and 4-NP2EO was classed as being persistent. The estimated degradation half-lives of 4-NP1EO in the soil in the initial phase was 7 days (4-NP2EO = 8 days), 150 days for the transition phase (4-NP2EO = 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.

The mineralization of ^{14}C-labelled 4-NP2EO was investigated in different sludge-soil mixtures and soils (Gejlsbjerg et al., 2001). The mineralization of 4-NP2EO 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-NP2EO. A higher water content resulted in lower concentrations of oxygen, thus in decrease of mineralization, too. Mineralization of 4-NP2EO 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.

The degradation of a 4-NP1-2EO 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 4-NP1-2EO the added 4-NP1-2EO was transformed to 4-NP by anaerobic microorganisms. The background level of 4-NP in the landfilled municipal solid waste was so high that a transformation of 4-NP1-2EO would only increase the indigenous 4-NP concentration with 5-10% (significant decrease of 4-NP1EO and 4-NP2EO 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-NP1-2EO 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-NP1-2EO less than 1% of the added 4-NP1-2EO was transformed into 4-NP.

Dettenmaier and Doucette conducted microcosm experiments to evaluate the mineralization of NPnEO (n= 4, 9) in a soil/biosolids (99.5:0.5 w/w) environment planted with crested wheatgrass (Dettenmaier and Doucette, 2007). The microcosms were located in a greenhouse with a 18:6-h light:dark photoperiod and a day/night temperature of 20±1/16±1 °C. Three inital concentrations (6, 24, 47 mg/kg dw) of NPnEO were tested. 12-29 % of NP4EO and 17-28% of NP9EO mineralized to $^{14}CO_2$ within 150 days. No statistical difference was shown between planted and unplanted systems.

In summary results (based on studies carried out with 4-NPnEO and NPnEO) show, that the overall biodegradation of 4-nonylphenol ethoxylates in soil is slow and depends on the amount of oxygen available. Results by Sjöström et al. (Sjöström et al., 2008) and Ejlertsson et al. (Ejlertsson et al., 1999) show that nonylphenol is formed during this process. While it was only a minor pathway in agricultural soil (Sjöström et al., 2008), 81 % of the overall 4-nonylphenol ethoxylates concentration at the end of the experiment (2 month) was 4-nonylphenol in a landfill with anaerobic sludge (Ejlertsson et al., 1999). Thus results indicate that 4-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.

Results from 4-NP studies show dissipation or primary degradation with DisT50 = 2.1-51 days. 4-Nonylphenol degrades as well as 4-NPnEO biphasic (fast inital phase (DegT50 < 16.7 days and a following slower degradation phase (DegT50> 40 days). Two studies investigated mineralization with 5 % CO_2 after 58 days and 7% CO_2 after 150 days (European Chemicals Agency, 2012b). These results show that the rate of 4-NP-removal is not faster than the rate of 4-NP-formation from 4-NPnEO.

3.1.3 Summary and discussion on degradation

In summary data on degradation of 4-nonylphenol ethoxylates (4-NPnEO; based on studies carried out with 4-NPnEO and NPnEO) indicate the following:

Ready biodegradability tests with short and long-chain NPnEO provide evidence, that 4-NPnEO are metabolized to some extent and that degradation may involve stable metabolites.

In sewage treatment plants it can be expected that– depending on the test conditions - between 70 and 100% of the 4-NPnEO is not mineralized; 4-NPnEO will therein be converted into short chain ethoxylates and be further degraded to the corresponding carboxylates or to 4-nonylphenol (4-NP), during aerobic and anaerobic phases respectively. Transformation to 4-NP (during sewage treatment) is actually expected to occur to a low extent, due to slow degradation rates of the short chain ethoxylates. Thus, in summary it is expected that 4-NPnEO in sewage will be basically transformed to short chain nonylphenol ethoxylates and their corresponding carboxylates, which will be the main compounds released to the aquatic environment.

Even if photodegradation might occur in water, the overall contribution to the whole degradation process is negligible. The low vapour pressure of long chain 4-NPnEO indicates that photodegradation in air is only a minor degradation path but it might be of some relevance for 4-NP1EO.

In aerobic surface water, further biodegradation of the short chain 4-NPnEO to its corresponding carboxylates (4-NPnEC) is expected to be the predominant pathway. While such transformation may be quick in summer (DisT50 = 2.5-35 days for NPnEO), results for a brackish bacteria community indicate that it may be slower in winter (DisT50 between 23 and 69 days). Further degradation of the short chain 4-NPnEC may occur through carboxylation of the alkylchain (Jonkers et al., 2001). However, as summarized in Van Vlaardingen et al. evidence of complete degradation under natural conditions is scarce (Van Vlaardingen et al., 2003).

Once transferred into sediment, it can be expected that the 4-NPnEO are transformed to the stable 4-NP. Degradation half-lives indicate that this is a slow process under anaerobic conditions (Dis/DegT50 = 49-77 or even 289 days (NPnEO)). While some data for activated sludge indicate that under aerobic conditions formation of nonylphenol carboxylates is the dominant process, data in a pre-contaminated sediment indicate, that this might be hindered in contaminated sediments (DisT50 = 69.3 – 115.5 days). Overall, sediments are expected to be a continuous source of 4-NP formed from 4-NPnEO due to the slow degradation rate. Due to the even slower degradation of 4-nonylphenol compared to 4-NP1-2EO (DegT50 46.2 days (primary degradation) to no elimination after 703 day in anaerobic freshwater sediment - depending on linear or branched isomers), it can be assumed that the formation of 4-nonylphenol exceeds its degradation. But available information does not allow to calculate steady state concentrations for 4-nonylphenol based on the degradation of its ethoxylates.

Processes in soil are similar to those observed in sediment but primary degradation seems to be even slower. Results indicate that, after a quick first degradation, further biodegradation of 4-NPnEO will be slow. Thus, similar to sediment, once contaminated with 4-NPnEO, soils are expected to be a continuous source for 4-NP in the environment. Information provided in the biodegradation tests for NPnEO indicate that the formation of NP exceeds its degradation as complete mineralization of NPnEO was low and nonylphenol was continuously formed and thus the overall degradation rate of 4-NP decreased (Sjöström et al., 2008).

In summary, available studies indicate that 4-NPnEO degrade to 4-NP, especially under anaerobic conditions. Hence, 4-NPnEO are relevant precursors for the substance of very high concern 4-NP.

4-NP itself is a stable metabolite which strongly adsorbs to soil, sludge and sediment. In sediment a DegT50 of 46.2 days (primary degradation) to no elimination after 703 days (depending on linear or branched isomers) was observed under anaerobic conditions (European Chemicals Agency, 2012b).

3.2 Environmental distribution

3.2.1 Adsorption/desorption

According to Leisewitz and Schwarz (Leisewitz and Schwarz, 1997), the affinity to the organic phase (soil, sediment, organic material) increases with decreasing length of the ethoxylate side-chain (which is subject to processes of biotic degradation).

No experimental information from registration dossiers is available yet. Therefore QSARs were used to estimate the adsorption potential for a subset of ethoxylation grades (Table 16). No further evaluation of the QSAR regarding the domain of applicability was conducted as the information on adsorption is regarded not essential for SVHC identification.

Table 16: Adsorption potential for assorted ethoxylation grades of 4-nonylphenol

Grade of ethoxylation	log Koc (KOCWIN v2.00[a], log K_{OW}-method)	log Koc (KOCWIN v2.00[a], MCI-method)	loc Koc @ pH 7.4 (ACD/Labs[b] @ pH 7.4)
1	3.66	3.48	4.51
2	3.41	3.39	4.46
4	2.93	3.22	4.20
6	2.44	3.04	3.95
8	1.96	2.86	3.69

Explanation of footnotes:
[a] calculation was conducted with KocWIN v2.00 which is an integral part of the QSAR suite EPIweb v4.1 (2008)
[b] calculation was conducted on the ChemSpider-website (www.chemspider.com; available 19.04.2012). The QSAR for the calculations are included in the ACD/PhysChem Suite.

The QSAR-predicted values for the adsorption potential underline the conclusions made by Leisewitz and Schwarz. The relatively high log Pow of 4-NPnEO with low grades of ethoxylation argues for accumulation in the compartments soil, sediment and organic material.

3.2.2 Volatilisation

The calculation of the Henry-Constant with QSAR HenryWIN v3.20 (Sept. 2011; group estimation method) revealed a value of 0.127 Pa*m³/mole for mono-ethoxylated 4-nonylphenole, indicating a moderate tendency for volatilisation from aqueous media. No further evaluation of the QSAR regarding the domain of applicability was conducted as the information on volatilisation is regarded not essential for SVHC identification.

Since the vapour pressure further decreases with increasing grade of ethoxylation volatilisation is not expected to be a relevant path of removal or environmental distribution for 4-nonylphenol ethoxylates.

3.2.3 Distribution modelling

Distribution modelling according to Mackay Level III

The data on distribution in the environment in this subsection is based on QSAR-predicted substance properties calculated within EPIsuite v4.10. No further evaluation of the QSAR regarding the domain of applicability was conducted as the information on adsorption is regarded not essential for SVHC identification.

Table 17: Adsorption potential for assorted ethoxylation grades of 4-nonylphenol

Grade of ethoxylation	Distribution to:			
	Air (percent)	Water (percent)	Soil (percent)	Sediment (percent)
1	0.59	22.5	74.8	2.13
2	9.16E-06	92.7	1.24E-03	7.32
4	6.09E-14	91.6	2.63E-08	8.43
6	2.37E-22	94.3	1.23E-13	5.69
8	9.75E-31	95.7	8.83E-19	4.33

As a result of the low tendency for evaporation from aqueous media, the predicted adsorption behaviour, and the outcome of the Level III distribution modelling, it could be concluded that, for 4-NPnEO released in water, higher ethoxylated molecules will remain in the water phase whereas low ethoxylated ones will adsorb to organic suspended matter or the sediment.

3.2.4 Measured distribution data

Information from studies about the behaviour of nonylphenol ethoxylates in surface water and during waste water treatment are summarised in the following tables.

Table 18: Summary of behaviour of NPnEO during waste water treatment

Test substance	**Result**	**Reliability**	**Reference**
NPnEO (n=1-20), NP1EC, NP2EC, NP	*Average value from 11 waste water treatment plants (Switzerland):* Primary effluent: NPnEO (n=3-20)= 82.4 % NP1EO + NP2EO = 11.5 % NP1EC + NP2EC = 3.1 % NP = 3 % Secondary effluent: NPnEO (n=3-20)= 28.2 % NP1EO + NP2EO = 21.8 % NP1EC + NP2EC = 46.1 % NP = 3.9 % Increase of NP mass compared to influent in two selected waste water treatment plants: 181 - 758 % (comparison of raw seawage mass (mol/day) with mass in digested sludge and secondary effluent (mol/day). 96.7% and 92 % of mass efflux respectively are adsorbed to sludge 60-65% of all nonylphenol compounds that have entered sewage treatment are released into the environment: NPnEC = 19 % NP1EO + 4-NP2EO = 11 % NP = 25 %	2	(Ahel et al., 1994a)

	NPnEO (untransformed) = 8 % 60 % of total load (NPnEO und NPnEC) are discharged into receiving waters via secondary effluent; 40 % of the total load (> 90 % NP) disposed to the environment via digested sludge		
NPnEO (n=1-12), NP	*Tanguu WWTP, Tianjin* Influent NP = 0.93 – 6.0 µg/L Effluent NP = 1.32 – 5.22 µg/L Removal (average) Total NPnEO (n=1-12) = 70 % NPnEO (n>6) = 82.6 - >99 % NP5EO = 43.2 % NPnEO (n=1-4) = 62.4-74.6 % NP = 70.8% increase in effluent compared to influent; NP was accumulated in all effluent samples (except April 2004)	2	(Yu et al., 2009)
NPnEO (n= 1-12), NPnEC (n= 1-3), NP	carbonaceous treatment: total removal NPnEO (NPnEO, NP1-3EC, NP) = 36.9 % Increase of NP concentration by 25.5 % in effluent compared to influent carbonaceous/nitrification treatment: total removal NPnEO (NPnEO, NP1-3EC, NP) = 59 % NP removal = 42.6 ± 30.4 % carbonaceous/nitrification/denitrification treatment: total removal NPnEO (NPnEO, NP1-3EC, NP) = 26.8 % Increase of NP concentration by 54.1 % in effluent compared to influent	2	(McAdam et al., 2011)
NPnEO (n=1-2), NP	Calculation based on measured data: 50% (molar base) of NPnEO in influent was transformed to NP and accumulated in digested sludge	2	(Brunner et al., 1988)
NPnEO (n=9)	20.8 % of the influent radioactivity removed as CO2 55.9 % was found in effluent as NP/NPnEO (6.9 %), NPnEC (26 %) and highly degraded metabolites (23.1 %) 6 % adsorbed to sludge (3.5 % as NP/NPnEO and 2.5 % as biomass) 8.35 % remained in aqueous part of the system 0.72 % removed from the system in sludge 8.23 % of the radioactivity was unaccounted for Increase of NP = 112.5 %	4 (secondary literature)	(Varineau et al., 1996; European Commission, 2002)

The behaviour of NPnEO in several full-scale mechanical-biological waste water treatment plants in the Glatt Valley, Switzerland was investigated by Ahel et al. (Ahel et al., 1994a). The concentration of NPnEO (n=3-20) decreases from primary to secondary effluent (82% to 28%), while the concentrations of the metabolites NPnEO (n=1-2, 12% to 22%), NPnEC (n=1-2, 3% to 46%) and NP (3% to 4%) increase. 60-65% of all 4-nonylphenol compounds that have entered the waste water treatment plants are released into the environment, approximately 25% released to the environment in the form of NP and 11% in the form of NP1EO and NP2EO. Almost all of the released NPnEO and NPnEC, as well as the majority of NP1EO and NP2EO, are discharged into receiving waters via secondary effluents (60% of the total input into the environment). NP (>90%) is disposed to the environment via digested sludge (40% of the total input into the environment). Analysis of mass flux in two waste water treatment plants revealed that the overall NP concentration compared to the influent increased by 181 – 758 % due to the degradation of NPnEO to NP with most of the NP being adsorbed to the sludge.

Yu et al. monitored NPnEO and their metabolites in waste water treatment plants of Tianjin (Yu et al., 2009). 70% of NPnEO (n=1-12) was removed. In all waste water treatment plants effluent samples (except the sample from April 2004) NP was accumulated (average 70.8%) with a mean value of 2.92 µg/L.

The fate of NPnEO during different activated sludge treatments (carbonaceous treatment,

carbonaceous/nitrification treatment, carbonaceous/nitrification/denitrification treatment) was investigated by Mc Adam et al. (McAdam et al., 2011). Based on mass balance, overall biodegradation efficiencies for NPnEOs, NPnEC (n=1-3) and NP were 37%, 59%, and 27% for the carbonaceous, carbonaceous/nitrification, and carbonaceous/nitrification/denitrification activated sludge plant, respectively. Beside short chain ethoxylates and carboxylates (n=1-3) NP was also formed at the carbonaceous (25.5%) and carbonaceous/nitrification/denitrification activated sludge plant (54.1%). In contrast, NP removal of 42.6±30.4% was observed at the carbonaceous/nitrification activated sludge plant.

The behaviour of NPnEOs and their biodegradation intermediates during sewage treatment procedure were investigated (Shao et al., 2003). Compared with concentrations of NP and NP2EO, the concentration of NP1EO was significantly low, suggesting that once NPnEOs were degraded into NP1EO, they would be easily transformed into NP. The removal of NPnEOs has a tendency to increase with the increase of EO chain length. The removals of NP2EO, NP3EO and NP4EO were below 60%, significantly low in comparison with those of NPnEOs at n>9 (>70%, exception n=7 with 59.6%). The removal of NPnEO was contributed by two paths: biodegradation of NPnEOs from longer ones to shorter ones, and sorption of NPnEOs to sludge. For NP sorption was the primary path. The relatively low removals of NPnEOs with short EO chains were perhaps due to the simultaneous occurrence of decomposition and formation of these compounds.

Brunner et al. determined NP, NP1EO and NP2EO in raw sewage, secondary effluent and stabilized sewage sludge of 29 Swiss sewage treatment plants (Brunner et al., 1988). They showed that NP1EO and NP2EO were formed by degradation from NPnEO. NP1EO and NP2EO were partly attached to the sludge and partly discharged to the effluent. By sludge treatment under anaerobic conditions large amounts of NP were formed by NP1EO. They calculated (based on measured data) that 50% on a molar base (17% w/w) of the NPnEO in raw water finally ends up as NP in sewage sludge.

The study of Varineau et al. was discussed in the Risk Assessment Report of 4-nonylphenol, which has been copied here in italic letters (European Commission, 2002):
The biodegradation of ^{14}C ring-labelled NPnEO (average n=9) has been studied in a semicontinuous activated sludge treatment system. The activated sludge was derived from the mixed liquors from the aeration basin of a wastewater treatment plant. The water used in the test was the primary effluent from the settling basin at the wastewater treatment plant, supplemented with nutrient broth. The background concentration of nonylphenol and NPnEO (range n=1-17) were 43.6 µg/l and 978 µg/l respectively. Before the test was started, the activated sludge was acclimated for 14 days by exposure to the primary effluent. After 14 days 300 ml of the activated sludge was placed into the degradation reactor and primary effluent containing 2 mg/l of the ^{14}C labelled NPnEO was fed into the reactor. A semi-continuous fill and draw procedure was used such that around 200 ml of the liquid in the reactor was drawn off and replaced by the primary effluent containing the ^{14}C-labelled substance every 2.3 days. This gave a sludge retention time and hydraulic retention time of 52 and 3.45 days respectively in the system. The total sampling time was 30 days. Based on radioactivity measurements, 20.8% of the influent radioactivity was removed as CO_2, 55.9% was found in effluent as nonylphenol/NPnEO (6.9%), NPnEC (26%) and highly degraded metabolites (23.1%), 6% remained in the test system adsorbed to sludge (3.5% as nonylphenol/NPnEO and 2.5% as biomass), 8.35% remained in the aqueous part of the system (1.03% as nonylphenol/NPnEO, 2.88% as NPnEC, and 3.45% as highly degraded metabolites), 0.72% of the radioactivity was removed from the system in sludge (0.09% as nonylphenol/NPnEO, 0.34% and NPnEC and 0.3% has highly degraded metabolites) and 8.23% of the radioactivity was unaccounted for. Overall, there was a 93% removal of the NPnEO from the influent. Specific analysis for nonylphenol showed that from the total influent concentration of nonylphenol/NPnEO compounds (total 204 µg, of which around 8 µg was nonylphenol), around 4 µg of nonylphenol was discharged in effluent, 5 µg was adsorbed on sludge and 8 µg was retained in the system. Thus there appears to have been a net generation of nonylphenol in the system (i.e. 8 µg was added to the system, 17 µg present in the system - if it is assumed

that no degradation of nonylphenol occurred then around 4.6% of the NPnEO was converted to nonylphenol) (Varineau et al., 1996). Based on the data net generation of NP accounted for an increase of the overall NP by 112.5% compared to the influent.

Soares et al. analysed data of approximately 300 sewage treatment plants in 14 countries (Soares et al., 2008). Nonylphenol occurs frequently as a stable intermediate in effluents and sewage sludge.Different results (removal of NP 11-99%) have been found, due to varying factors like treatment process, temperature or region. Nonylphenol has a higher occurrence in waste water of industrial or highly populated regions (urban areas). Higher concentrations of nonylphenolic compounds were found during working days and late afternoons than at weekends and during the night. However, the results indicate that sewage treatment plants are only partially efficient in removing nonylphenolic compounds (Environment Agency UK, 2008).

Table 19: Summary of behaviour of NPnEO in surface water

Test substance	Result	Reliability	Reference
Surface water			
NPnEO, NP1EO + NP2EO, NP1EC + NP2EC, NP	*Glatt River, Switzerland:* Total Input (n=10): NPnEO (n=3-20)= 21.6 % (23.4 mol/day) NP1EO + NP2EO = 22.5 % (24.3mol/day) NP1EC + NP2EC = 51 % (55.2 mol/day) NP = 4.9 % (5.3 mol/day) Output NPnEO (n=3-20)= 3.4 % (2.8 mol/day) NP1EO + NP2EO = 8.8 % (7.2 mol/day) NP1EC + NP2EC = 85.4 % (70.1 mol/day) NP = 2.4 % (2.0 mol/day) Dissipation nonylphenolic compounds = 24 %	2	(Ahel et al., 1994b)

The behaviour of NPnEO and their metabolites in surface water (Glatt River, Siwtzerland) was studied by Ahel et al. (Ahel et al., 1994b). Several sampling sides along the river were analysed. Discharge of secondary effluents from municipal sewage treatment plants into the river was the predominant source of nonylphenol ethoxylates. Concentration varied substantially depending upon sampling location, season and time of the day. The concentrations of nonylphenolic compounds were significantly lower in summer than in winter. The overall dissipation efficiency on the river section was 24 %. While 88 % of the total NPnEO input into the river based on effluents form sewage treatment plants was eliminated only 62 % of nonylphenol disappeared and the concentration of the short chain carboxylates (NP1-2EC) increased. Results indicate that although some mineralization occurred, most of the nonylphenolic compounds remained in the river- during summer predominantly as NP1-2EC and in winter as NP1-2EO. Degradation toward NP occurs, but degradation to short chain NP1-2EO and NP1-2EC were of higher relevance. However, although NP usually belongs to the less abundant surfactant-derived nonylphenolic compounds in the Glatt River, a majority of the concentration values were higher than 1 µg/L. In sediment NP was the predominant nonylphenolic compound.

3.2.5 Summary distribution

The fate of 4-nonylphenol ethoxylates (4-NPnEO; based on studies carried out with NPnEO) in waste water treatment plants varies because of: different source water, operating conditions and treatment technologies. Measured data in sewage treatment plants are difficult to interpret with regard to the question whether or not degradation of 4-NPnEO to 4-nonylphenol (4-NP) results in a relevant contribution to the overall 4-NP concentration in the environment; as usually already the influent contains alkylphenols and both formation of the alkylphenol and its degradation contribute to the overall concentration. However results for NPnEO reveal some

general aspects which are in line with results of the biodegradation experiments:

Overall results show, that the concentration of long chain ethoxylates in sewage treatment plants decrease as expected based on degradation data. Data substantiate that the ethoxylates are not subject to complete mineralization. The ultimate degradation of the ethoxylates (mineralization and degradation to metabolites other than short chain ethoxylates, carboxylates and alkylphenol) was between 27 and 45%. Results by Ahels et al. substantiate that the formation of nonylphenol carboxylates may be the predominant route of transformation, as NP1-2EC were the most dominant metabolites in the waste water treatment plant effluent (Ahel et al., 1994a). Net load data indicate that the contribution to the overall 4-NP concentration released to the environment may be high: All studies suggest that the net loads of nonylphenol increase during sewage treatment. Varineau et al (Varineau et al., 1996) described a net increase (difference between influent and effluent) of about 112.5% (17 µg/L compared to 8 µg/L at the influent), while the net increase was 71 % in the study by Yu et al (Yu et al., 2009) and 181- 758% in the study by Ahel et al. (1994a). Data by McAdam suggest, that 4-NP formation is mainly a result of the denitrification step (54% in this study) (McAdam et al., 2011).

Results by Ahel et al. and Varineau et al. suggest that the majority of the remaining NPnEO will be released into receiving waters via secondary effluent while sludge will be a significant pathway for NP (Ahel et al., 1994a; Varineau et al., 1996). Brunner et al. calculated that about 50% (molar base; 17% w/w) of the NPnEO in the raw sewage were transformed to NP, which then accumulated in the digested sludge (Brunner et al., 1988).

Based on such data reasonable worst-case assumptions for the fate of 4-NPnEO during anaerobic waste water treatment were estimated in the environment risk assessment report for nonylphenol (European Commission, 2002). According to this calculation 45% of the 4-NPnEO would be mineralized, 33 % would be released via effluent as 4-NP1EO, 4-NP2EO and 4-NPnEC (25%) and as 4-NPnEO (n>3) (8%) and 21.5% would leave the waste water treatment plant as 4-NP (19% via anaerobically digested sludge and 2.5 % via effluent). Additionally, 2.5 % of the released 4-NPnEO could be further degraded to 4-NP in the environment. This worst-case assumption was based on data collected during the 1990s. An unpublished report from 2008 show that this worst-case assumption still appears to be reliable based on newer data (Environment Agency UK, 2008).

Although only part (~21.5%) of the ethoxylates may be ultimately degraded to 4-NP during sewage treatment (which is probably due to the fact, that degradation of the short chain ethoxylates to 4-NP is a very slow process), this transformation contributes, as described in the chapters above, relevantly to the overall 4-NP output of sewage treatment plants (increase by 25 – 758 %).

3.3 Bioaccumulation

Not assessed for the identification of this substance as SVHC in accordance with Article 57(f).

3.4 Secondary poisoning

Not assessed for the identification of this substance as SVHC in accordance with Article 57(f).

4 Human health hazard assessment

Not assessed for the identification of this substance as SVHC in accordance with Article 57(f).

5 Environmental hazard assessment

The following sections summarize available ecotoxicity information for 4-nonylphenol ethoxylates (4-NPnEO). Information showing that the degradation product 4-nonylphenol is a substance of very high concern, due to its endocrine disrupting properties which cause probable serious effects in the environment, is summarized in the SVHC supporting document for 4-Nonylphenol (European Chemicals Agency, 2012b).

5.1 Aquatic compartment (including sediment)

5.1.1 Toxicity data

Available toxicity data for nonylphenol, branched and linear, ethoxylated (4-NPnEO and NPnEO) are roughly summarized in order to analyze whether or not they may give rise to an equivalent concern compared to 4-nonylphenol, branched and linear (4-NP) with regard to their endocrine properties. Only endpoints relevant with regard to the endocrine properties are analyzed.

5.1.1.1 In vitro

In vitro results may provide information about a specific mechanism of action, in this case estrogen receptor binding. They may also provide information about the potency of this mechanism but do not consider whether or not effects may occur in intact organisms and do not provide information on the potency *in vivo* as this is influenced by pharmaco-kinetic processes such as uptake distribution, accumulation and excretion.

Data for NPnEO and one of its degradation products (short chain nonylphenol carboxylates (NP1-2EC) are summarized in

Table 20 supplemented with data for octylphenol ethoxylates which are used as supportive information.

Table 20: Summary of *in vitro* test results for nonylphenol branched and linear ethoxylates and – as supporting information - for 4-tert-octylphenol ethoxylates using cells from aquatic organism VTG = vitellogenin; E2 = 17ß-estradiol; EE2 = Ethinylestradiol, RP (relative potency) = EC_x E2/EC_x NPnEO, RIE (relative inductive efficiency) =maximal induction compared to maximal E2 induction):

Test substance	**Cell type**	**Test condition / parameter**	**Effect concentrations**	**Potency (relative to 17ß-estradiol and/ or OP)**	**Reference**
NPnEO					
NP1EO/NP2EO mixture	Yeast cells	YES assay,	EC_{20}: E2 0.022 µg/L NP = 246 µg/L NP1EO = 10 000 µg/L	RP: (EC_{20} E2/EC_{20} NP(nEO):): NP: 0.000089 NP1-2EO: 0.0000023	(Metcalfe et al., 2001)
NP1-2EO NP6EO NP10EO (technical mixtures)	Yeast cells	YES assay,	$EC_{50:}$ E2 = 2.8 $^{-5}$ mg/L NP= 9.3 $^{-4}$ mg/ NP6EO = 40% effect at 10 mg/L NP10EO = 30% effect at 6.6 mg/l	RP: (EC_{50} E2/EC_{50} NP(nEO):): NP:.003 NP6EO: ND NP10EO: ND RIE: NP:.72 % NP6EO: 40 % NP10EO: 30%	(Isidori et al., 2006)

NP2EO	Yeast cells	Recombinant yeast (strain BJ2168) heterogously expressing rtER. Induction of β-galactosidase activity:	LOEC (E_2) = 10^{-8} to 10^{-9} M LOEC (4-NP) = 10^{-6} M N2EO = No estrogen activity up to 10^{-4} M (	RP (LOEC(E_2)/LOEC(4-*n*-NP)) NP : 1 x 10^{-2} - 1 x 10^{-3}	(Madigou et al., 2001)
NP2EO NP7EO	Yeast cells	Recombinant yeast (strain BJ-ECZ) heterogously expressing rtER. ß-galoctisidase activity	LOEC (E_2) = 10^{-9} M LOEC (4-NP) = 10^{-6} M EC_{max} (E_2) = 10^{-8} M EC_{max} (4-NP) = 10^{-5} M	RP LOEC(E_2)/LOEC(4-NP(nEO)) NP = 1 x 10^{-3} RIE: NP: 92% NP2EO: 39 – 64 % NP7EO: 21-34 %	(Petit et al., 1997)
NP10EO	YES Assay,	28 mg/L in biodegradation assay using inocolum from Helsinki and Jyväskylä City WWTP		increased YES response with increasing degradation toward shorter chain NPnEO	(Pessala et al., 2009)
NP2EO	MCF cells	Induction of the transcriptional activity of the estrogen receptor	E2: 6 fold induction at 10^{-8}M	Stimulation at 10^{-5} M, higher than for NP at 10^{-5} M	(White et al., 1994)
NP2EO	*O.mykiss*, Primary hepatocytes	VTG induction		VTG induction at 10^{-5} M for both NP2EO and NP but less pronounced for NP2EO	(White et al., 1994)

NP2EO NP9EO	*O.mykiss* Primary hepatocytes derived from male, (mostly) immature fish	Expression of vitellogenin protein (rtVgt)	EC_{50} (E_2) = 1.81 x 10^{-9} M EC_{50} (NP) = 16.15 x 10^{-6} M EC_{50} (NP2EO) = 17.27 x 10^{-6}M EC_{50} (NP9EO) = 82 x 10^{-6}M LOEC (E_2) = 1 x 10^{-11} M EC_{max} (E_2) = 1 x 10^{-7} M	RP (ED_{50} (E_2) / ED_{50} 4-*n*-NP(nEO)): NP: 3.3 x 10^{-6} NP2EO: 6 x 10^{-6} NP9EO: 2 x 10^{-6}	(Jobling and Sumpter, 1993)
NP2EO	*O. mykiss,* Primary hepatocytes derived from male fish	Expression of vitellogenin mRNA (rtVgt mRNA)	EC_{max} (E_2) = 1 x 10^{-6} M EC_{max} (4-*n*-NP) = 1 x 10^{-5} M	RIE ((maximal) Vtg mRNA expression level induced by 4-NP relative to that induced by E_2.): NP ≈ 25 % NP2EO: No estrogen potency up to 10^{-4} M	(Madigou et al., 2001)
NP2EO NP7EO	*O. mykiss,* Primary hepatocytes derived from male fish	Expression of vitellogenin mRNA (rtVgt mRNA)		RIE: NP: 25.9% NP2EO: 156 % N7EO: 1%	(Petit et al., 1997)

NP10EO	*O. mykiss,* Primary hepatocytes	VTG induction and EROD activity 28 mg/L in biodegradation assay using inocolum from Helsinki and Jyväskylä City WWTP		No response	(Pessala et al., 2009)
OPnEO					
OP2EO OPnEO (n = 3,4,5,12)	MCF cells	Induction of the transcriptional activity of the estrogen receptor	E2: 6 fold induction at 10^{-8}M OP: 4 fold induction at 10^{-6}M OP2EO: 2 fold induction at 10^{-6}M OPnEO (n=3,4,5,12): < 1fold induction at 10^{-6}M	OP: 0.01 compared to E2 (comparison of concentrations inducing similar induction) OP2EO: half fold induction compared to OP OPnEO: negligible effects	(White et al., 1994)
OP3EO OP9-10EO (technical mixture)	Yeast cells, human ER receptor hERα	YES assay, EC50:concentration giving 50% of the maximal response induced by 17ß estradiol	EC_{50} E2 = 2.8 $^{-5}$ mg/L OP = 2.5^{-2} mg/L OP3EO = 19 mg/L OP9-10EO = n.d. (19% effect at 5 mg/L	RP: (EC_{50} E2/EC_{50} OPnEO): OP:.001 OP3EO: 0.0000014 OP9-10EO: ND RIE: OP: 61% OP3EO: 53% OP9-10EO: 19%	(Isidori et al., 2006)
NPnEC					

NP1EC	O.mykiss Primary hepatocytes derived from male, (mostly) immature fish	Expression of vitellogenin protein (rtVgt)	EC_{50} (E_2) = 1.81 x 10^{-9} M EC_{50} (NP) = 16.15 x 10^{-6} M EC_{50} (NP1EC) = 15.3 x 10^{-6}M LOEC (E_2) = 1 x 10^{-11} M EC_{max} (E_2) = 1 x 10^{-7} M	RP (ED_{50} (E_2) / ED_{50} 4-*n*-NP(nEO)): NP: 3.3 x 10^{-6} NP1EC: 6.3 x 10^{-6}	(Jobling and Sumpter, 1993)
NP1EC	O.mykiss, Primary hepatocytes	VTG induction		VTG induction at 10-5 M for NP1EC, similar to NP2EO	(White et al., 1994)

With regard to the short chain nonylphenol ethoxylates, information for a mixture of NP1EO and NP2EO and NP2EO alone are available from 6 studies using three different study types (MCF, primary hepatocytes form O.mykiss and the YES assay). In all except one study (Madigou et al., 2001) short chain nonylphenol ethoxylates showed estrogen activity:

- Based on EC_{50} and EC_{20} values the relative potency in the YES assay was 0.025 and 0.00012 compared to nonylphenol (Isidori et al., 2006; Metcalfe et al., 2001) while Petit et al. (Petit et al., 1997) showed that the activity was about half of the activity of nonylphenol at 10^{-4} M and Madiguo et al. (Madigou et al., 2001) found no activity at all up to 10^{-4} M.
- Based on the level of VTG induction at similar test concentrations, the relative induction efficacy in primary hepatocytes from *O.mykiss* was similar or even higher compared to nonylphenol in three of the four studies while one study (Madigou et al., 2001) showed no induction up to 10^{-4}M.
- Similarly, an even higher induction compared to nonylphenol was also observed in the sole MCF assay by White et al, (White et al., 1994).

Thus in summary, results for short chain nonylphenol ethoxylates support the finding that such ethoxylates exhibit some estrogen activity in vitro. Results with primary hepatocytes from *O.mykiss* indicate that the relative binding efficacy may be similar or even higher compared to nonylphenol.

Less data are available for longer chain nonylphenol ethoxylates. Three studies compared short chain ethoxylates and longer chain ethoxylates, two of these in the YES assay (Isidori et al., 2006; Petit et al., 1997) and one using primary hepatocytes (Jobling and Sumpter, 1993). In addition one study (Pessala et al., 2009) analyzed NP10EO. In all studies estrogen activity decreased with increasing chain length. However, while some showed nearly no estrogen activity for the longer chain ethoxylates, others revealed estrogen activity although with low efficacy.

Results for octylphenol ethoxylates support these findings:
Two studies are available using MCF cells (White et al., 1994) and the Yeast YES assay (Isidori et al., 2006). Both tests included short chain ethoxylates (OP2EO and OP3EO respectively) as well as longer chain ethoxylates (OP3EO, OP5EO, OP12EO and OP9-10EO respectively).

In both tests short chain ethoxylates were estrogenic active but with a lower potency compared to OP (half fold induction and 0.0088 potency compared to OP respectively). Longer chain ethoxylates showed only very weak estrogenic activity.

Overall studies for octylphenol ethoxylates and nonylphenol ethoxylates show that the short chain ethoxylates still possess an estrogen activity in vitro while this activity decreases with increased chain length.

In vitro studies for nonylphenol carboxylates with a low degree of ethoxylation (NP1EC) show that their in vitro potency is similar to those of the nonylphenol ethoxylates with low degree of ethoxylation (see Table 20).

5.1.1.2 Long-term toxicity to fish

Long term toxicity studies are summarized in order to analyze whether or not 4-nonylphenol branched and linear ethoxylates may result in endocrine mediated adverse effects in fish. Thus only studies evaluating endocrine related endpoints are considered. Eight studies for three species are available. Results are summarized in Table 21:

Table 21: Summary of in vivo data for fish exposed with nonylphenol ethoxylates

Reference	**Life stage/ duration**	**Test substance**	**Concentration / test condition / tested substance / solvent**	**Vitellogenin**	**Histology**	**Fertility/ Fecundity**	**Sex – ratio**	**Sec. sex characteristics**	**Other effects**	**Reliability**
O.latipes										
(Metcalfe et al., 2001)	Sex-development 1 d posthatch for 90d	NPE1O/NPE2O mixture (54% NP1EO, 44% NP2EO)	Semi-stat; 25; 50; 100	-	Only 1 slight testis-ova at 100 µg/L		No changes in sex-ratio		No effects on growth	2
(Balch and Metcalfe, 2006)	FSDT (with deviations) Starting from hatch within 1d, Exposure: 100d	NP1EO (high purity)	3.5; 10.5; 35; 102 µg/L (m); 3 - 10 - 30 – 100-300 µg/L (n) Semi-static, renewal of test water every 48 h		No testis-ova (NP LOEC 29µg/L (18 of 22 phenotypic males had testis-ova);			Mixed sec. sex char. (MSC): LOEC 105 LOEC NP: 8.7µg/L (20%), Papillary processes, LOEC 105 µg/L only one out of 29 fish had papillary processes at the anal fin		2
(Balch and Metcalfe, 2006)		NP4EO (technical)	3.8; 11.4; 38; 114; 380 Semi-static, renewal of test water every 48 h					No mixed secondary sex characteristic, no changes in sex-ratio no intersex up to 380 µg/L		**2**
(Balch and Metcalfe, 2006)	FSDT (with deviations) Starting from hatch within 1d, Exposure: 100d	NP9EO (technical)	16.2; 54; 162; 540 Semi-static, renewal of test water every 48 h					No mixed secondary sex characteristic, no changes in sex-ratio no intersex up to 540 µg/L		**2**
O.mykiss										
(Dussault et al.,	Adults, 21 d	NP1EO (purity >	Flow-through; 0.8; 3.9; 6.,9;	LOEC 281 µg/L					Relative potency	2

Reference	Life stage/ duration	Test substance	Concentration / test condition / tested substance / solvent	Vitellogenin	Histology	Fertility/ Fecundity	Sex – ratio	Sec. sex characteristics	Other effects	Reliability
2005)		95%)	48; 281 µg/L real	(induction comparable to 0.1 µg/L E2, in all fishes observed					compared to NP 0.22	
(Ashfield et al., 1998)	Posthatched females / 22 and 35 d)	NP2EO (technical)	Flow-through/ 1.0; 10; 30; 50 µg/L (nominal)						Growth (weight) LOEC < 1 µg/L (only transident) NP: LOEC 10 µg/L	2- nom. Conc.
(Jobling et al., 1996)	2-year old male rainbow, Experiment in May Exposure: 3 weeks	NP2EO (technical)	38 µg/L (m)(Limittest) Flow-through	VTG induction slightly less than NP induction	Spermatogenesis: cell type Spermatogonia A was significantly elevated, similar to NP (38 µg/L)				GSI significantly reduced gonadal growth similar to NP	**2**
P.promelas										
(Nichols et al., 2001)	Adults, 42 d	NP9EO (technical, < 1% NP, NP1EO, NP2EO))	Static, 0.21; 0.65; 2,1; 7.9 µg/L	No significant changes in male and females (increase in males at low concentration but no dose-response, not significant		No significant changes in fecundity, only 1 chamber with actively laying nd fertilizing eggs at 2.1 and 7.9 µg/L			No changes in mortality, no changes in egg viability	**2**

Reference	Life stage/ duration	Test substance	Concentration / test condition / tested substance / solvent	Vitellogenin	Histology	Fertility/ Fecundity	Sex – ratio	Sec. sex characteristics	Other effects	Reliability
(Miles-Richardson et al., 1999)	Reproduction assay sexually mature fish (12 – 18 months) paired 42 d	NP9EO (technical)	Two experiments (data from experiment 2 not usable, egg production was totally inhibited by solvent control): First experiment was conducted July to August. 0.15; 0,43; 1.45; 4.5 µg/L (m)		No effects on the relative proportion of eggs in any of the stages of follicles for NP (≥ 3.4µg/L) or NPnEO No testicular lesion (based on sertoli cell proliferation and percentage of seminiferous tubules), measured as severity score (effects for NP started at 1.6 µg/L)			No changes in gross appearance of the fatpad in males for NP (up to 3.4 µg/L) and NPnEO, no changes in secondary sex-characteristicsin males for NP and NPnEO		**2**
(Bistodeau et al., 2006)	Larvae (< 72h post-hatch) for 64 d, Competition assay (formerly exposed males and unexposed males competing for reproduction) at the age of 6 month (without exposure)	Mixture of NP/OP/NPnEO/OPnEO/NPnEC (mainly (78,6 % 4NP1EC and NP2EC)*	Flow-through 148; 73.9, 38.1 µg/L total concentration	No VTG induction for mixture and NP (measured after competition study i.e. nearly 6 month after end of exposure	No changes in phenotypic males			Reduced prominence of tubercles and dorsal pad at 148 µg/L but not for NP and other treatments	High larval mortality (78%) at 148µg/L, no effects for NP Reduced ability to hold and defend a nest site from control fishes at 38.1 µg/L and above,)	2 High mortality in controls (> 40% in all treatment, very high at 74 µg/L

For *O.latipes* two fish sexual development studies are available.

Balch and Metcalfe exposed *O.latipes* larvae to nonylphenol, NP1EO, NP4EO and NP9EO for 100d starting from hatch (Balch and Metcalfe, 2006).

With regard to NP1EO, fish showed similar effects on secondary sex characteristics compared to nonylphenol but at higher test concentrations. The LOEC for so called mixed secondary sex characteristics (individuals that showed both male and female sec) was 102 µg/L compared to 8.7 µg/L for nonylphenol. Similar to nonylphenol fish which were considered males based on their gonads did not show any papillary processes (a dominant male secondary sex characteristic) but again the LOEC was higher (102 µg/L compared to 29 µg/L). While most of the phenotypic males showed testis ova at a LOEC of 29 µg/L after exposure to nonylphenol no such effects were observed for NP1EO. Exposure to NP4EO and NP9EO did not result in any effects up to 380 µg/L and 540 µg/l respectively.

Findings by Metcalfe et al support the finding that exposure to NP1EO does not result in significant induction of testis-ova if exposure starts after hatch (Metcalfe et al., 2001). After exposure of newly hatched fish for 90d to a mixture of NP1EO and NP2EO only 1 testis-ova was observed at the highest test concentration (100 µg/L). No changes in the sex-ratio based on gonads were observed.

Thus in summary, results provide evidence for an in vivo endocrine activity of NP1EO in *O. latipes* due to changes in secondary sex characteristics. No data are available whether such activity may result in endocrine mediated apical effects. With regard to *O.mykiss* two screening assays and one fish sexual development test are available for the short chain nonylphenol ethoxylates (NP1EO and NP2EO). Results by Jobling at al. (1996) of a screening assay for NP2EO again indicate that short chain alkylphenol ethoxylates may induce endocrine activity in vivo (Jobling et al., 1996). However, in this case effect concentrations were similar to those observed for nonylphenol. NPnEO induced vitellogenin in adult males, increased the proportion of early sperm stages and reduced gonadal growth at 38 µg/l. Effects are similar to those observed for 36µg/L nonylphenol. A similar sensitivity of *O.mykiss* to short chain nonylphenol ethoxylates and nonylphenol was substantiated by Ashfield et al. (Ashfield et al., 1998) who found similar effects on growth during a sexual development test with the LOEC being even factor 10 lower than for nonylphenol (LOEC 1 and 10 µg/L respectively). Results by Drussalt et al. support an estrogen mode of action but at higher concentrations (LOEC 281 µg/L with a relative potency compared to nonylphenol of 0.22 (Dussault et al., 2005).

Results observed by Nichols et al. (Nichols et al., 2001) and Miles-Richardson et al. (Miles-Richardson et al., 1999) with a longer chain ethoxylate (NP9EO) and *Pimephales promelas* support in vitro findings that longer chain ethoxylates do not exhibit endocrine activity. No changes in vitellogenin level, fecundity and egg viability were observed after exposure of adults for 42 d (Nichols et al., 2001) and no changes in secondary sex characteristics were observed by Miles-Richardson et al. (Miles-Richardson et al., 1999).

Thus in summary, data available for NP1EO and NP2EO for *O.latipes* and *O.mykiss* show that nonylphenol ethoxylates may induce in vivo endocrine activity. Based on data for *O.latipes* short chain nonylphenol ethoxylates are about factor 10 less potent than nonylphenol while data for *O.mykiss* indicate that the potency may be comparable. As no data about clearly endocrine mediated adverse effects are available, it cannot be assessed whether such activity may result in endocrine mediated apical effects.

In vivo studies for nonylphenol carboxylates with a low degree of ethoxylation (NP1EC and NP2EC) are summarized in Van Vlaardingen et al (2003). They indicate that they also may cause in vivo endocrine activity. In *O.mykiss* vitellogenin was produced in juvenile males and changes in testis and in spermatogenesis was observed, however at rather high concentrations (EC50 31.8 mg/L)

6 Conclusions on the SVHC Properties

6.1 PBT, vPvB assessment

Not assessed for the identification of this substance as SVHC in accordance with Article 57(f).

6.2 CMR assessment

Not assessed for the identification of this substance as SVHC in accordance with Article 57(f).

6.3 Substances of equivalent level of concern assessment

As described in chapter 3, 4-nonylphenol ethoxylates (4-NPnEO) are a relevant source for 4-nonylphenol (4-NP) in the environment due to their degradation to 4-NP in wastewater treatment plants, surface water, sediments and soils. 4-NP has been identified as a substance of very high concern and included into the Candidate List due to its endocrine disrupting properties which cause probable serious effects to the environment. Any precursor of this substance which may contribute to its occurrence to a relevant degree should be regarded as a substance of very high concern, too. The rationale for the identification of 4-NPnEO as SVHC is substantiated below by first discussing some general aspects and secondly describing the specific concern for the ethoxylates in detail.

6.3.1 Principle rationale for the identification of a substance as SVHC due to its degradation to a substance of very high concern

Substances are identified as "substances of very high concern" due to their intrinsic properties leading to very high concern. For such substances regulatory measures such as inclusion into the Candidate List and further measures to account for the risk arising from these properties are considered necessary. As the measures are based on the intrinsic properties of these substances it seems to be rational that all substances that may contribute to the occurrence of such substances due to their degradation under realistic conditions should be regarded as substances of very high concern themselves.

Indeed such a rationale is already included in the new Annex XIII for substances being of very high concern due to their PBT or vPvB properties. Annex XIII states that transformation / degradation products should be taken into account when assessing the PBT properties of a substance. This implies that a substance may be considered as substance of very high concern due to the PBT properties of its transformation product.

Recently the European Commission suggested a similar rationale to identify substances as SVHC according to Art 57(f) of REACH which may degrade to a substance having CMR properties. It is straightforward that such an approach should account for all substances degrading /transforming to any substances of very high concern.

With regard to substances transforming to a substance which is of very high concern due to its endocrine disrupting properties and subsequent probable serious effects to the environment, such an approach is further substantiated by the type of concern of the degradation product. As described in the support document for the identification of 4-NP as SVHC, one aspect contributing to the very high concern is the difficulty to accurately describe and analyse the risk of such a substance. If substances increase the overall

environmental concentration of such SVHC due to their degradation to the SVHC, this increases the possibility to underestimate the risk for the substance of very high concern.

6.3.2 Rationale for the identification of 4-nonylphenol branched and linear ethoxylated as substances of very high concern due to their degradation to 4-nonylphenol, branched and linear

The rationale provided below is based on available degradation data for 4-NPnEO and NPnEO. Further information on 4-tert-octylphenol ethoxylates (4-tert-OPnEO) is included as supporting evidence and for comparison. 4-tert-OPnEO are already identified as SVHC because of their degradation to the SVHC 4-tert-OP (European Chemicals Agency, 2012a). They are considered close analogues due to their similar chemical structure with the only difference being the alkylgroup differing by one C-atom. Both alkylphenols are degraded by a stepwise cleavage of the terminal ethoxygroup.

Although data are mainly available for ethoxylates with an average grade of ethoxylation of up to 20, it is assumed that the chain length – up to a specific grade – does not influence the degradation process and thus that available data may be extrapolated to longer chain ethoxylates (see chapter 3.1).Many studies were performed with the technical nonylphenol ethoxylate (NPnEO) and thus the exact composition of the test material is often unknown. As the technical nonylphenol consists of about 95% para-nonylphenol, it can be assumed that the main constituents of the technical nonylphenol ethoxylate (NPnEO) were para-nonylphenol ethoxylates (4-NPnEO). Data provided in chapter 3 show that 4-NPnEO may degrade to 4-NP in sewage treatment plants and thus increase the overall 4-NP load in the environment. Degradation in waste water treatment plants is not complete. 4-NPnEO are also released from waste water treatment plants. Due to their further degradation to 4-NP in sediment and soil, 4-NPnEO distributed to those environmental compartments contribute to the overall concentration of 4-NP in the environment, too.

6.3.2.1 Emission from sewage treatment plants

As analyzed in simulation studies (chapter 3.1.2.1.1) and substantiated by quantitative measurements in sewage treatment plants (chapter 3.2.4) primary degradation of 4-nonylphenol ethoxylates is fast. Degradation is not complete but involves formation of NP2EO, NP1EO and NP under anaerobic conditions in sewage sludge.

Degradation of 4-nonylphenol ethoxylates (based on studies carried out with 4-NPnEO and NPnEO) in sewage treatment plants

- 87 % primary dissipation during sewage treatment (mean of 11 plants (Ahel et al., 1994a))
- Formation of NP1EO and NP2EO during degradation of NPnEO under anaerobic conditions with a peak on day 7-21 (Lu et al., 2008a; Lu et al., 2008b)
- Formation of NP during decreased NPnEO (n =9) concentration under anaerobic conditions with a peak on day 21 (Lu et al., 2008a; Lu et al., 2008b)
- 31 -57% formation of 4-NP from 4-NP1-3EO after 150 days under anaerobic conditions (Ejlertsson et al., 1999)
- Formation of NP2EO and unknown products of a size between 4 and 10 ethoxy units from NPnEO (n up to 40) under anaerobic conditions (Teurneu, 2004)

Only few data are available for the aerobic degradation pathway for 4-NPnEO. However data for 4-tert-OPnEO indicate that under aerobic conditions both short chain carboxylates as well as short chain ethoxylates may be rapidly formed (Ball et al, 1989).

Results provided in chapter 3 and summarized in the next box indicate that the degradation products are more stable and thus overall mineralization or degradation to metabolites other than those described above is generally very slow.

Overall dissipation /degradation of transformation products (based on studies carried out with tNPnEO) in sewage treatment plants:

- Only 30-50% disappearance of total NPnEO (including NPnEC and NP) in 3 days in anaerobic sewage sludge (Lu et al., 2008a; Lu et al., 2008b)
- Overall dissipation of NPnEO (including NPnEC and NP) between 27 and 45% in several waste water treatment plants (Ahel et al., 1994a; McAdam et al., 2011; Varineau et al., 1996; Yu et al., 2009)

The data are supported by data for 4-tert-OPnEO which showed nearly no degradation of 4-tert-OPnEC in aerobic sludge after 24 hours and only minor degradation of 4-tert-OP1-2EO within 17 days in primary sewage (Ball et al. 1989) and no degradation of the radiolabeled phenol moiety within 7 days in activated sludge and in an anaerobic percolation field system (170 days) (Lashen et al., 1966)

The data indicate, that a relevant amount of 4-NPnEO is released to the environment from sewage treatment plants either as short chain ethoxylates or as 4-nonylphenol: Measurements in sewage treatment plants by Ahel et al., McAdam et al., Varineau et al. and Yu et al. (Ahel et al., 1994a; McAdam et al., 2011; Varineau et al., 1996; Yu et al., 2009) indicate, that about 55 - 73% of the NPnEO influent in primary sewage treatment plant will be released to the environment. Data provided by Ahel et al and Varineau et al. (Ahel et al., 1994a; Varineau et al., 1996) show that about 4.6 % to 25% of the NPnEO influent is released as nonylphenol, mainly via sludge. Main degradation products in the effluent are NPnEO and NP. According to the environment risk assessment report for 4-nonylphenol (European Commission, 2002; Varineau et al., 1996) the data provided by Varineau et al, 1996 can be used as a reasonable worst case assumption for 4-NPnEO.

Summary release of 4-nonylphenol ethoxylates (based on studies carried out with NPnEO) from waste water treatment plants into the environment

(Ahel et al., 1994a)	(Varineau et al., 1996)
60-65 % of nonylphenolic compounds in influent released to the environment as: 19 % NPnEC 11% NPnEO 25 % NP (> 22.5 % in sludge, <2.5% in effluent)* 40% of total load released via sludge	36% of influent released to the environment as NP/NPnEO or NPnEC: 26% NPnEC in effluent 7 % NP/NPnEO in effluent 3.5 % NP/NPnEO adsorbed to sludge (overall 4.6 % of the NPnEO converted into NP)

* based on the calculation that > 90% of NP is adsorbed to sludge

Based on these data it becomes obvious, that degradation of 4-NPnEO to 4-NP in sewage treatment plants may be a relevant direct source of 4-NP for soil via sludge accounting for 3.5 – 22.5% of the overall 4-NPnEO influent. Undegraded 4-NPnEO and short chain ethoxylates (4-NP1-2EO) released via effluent may be a potential source of 4-NP in surface water as they may further degrade to 4-NP in the environment (see next paragraphs). Based on the assumption by Ahel et al (1994a) for NPnEO, that 60% of the not further degraded NPnEO, short chain ethoxylates and NP are released via effluent, 21.6 – 36% of the overall NPnEO influent would be released to surface water via effluent. Release of NP from sewage treatment plants as a result of NPnEO degradation in sewage treatment plants seems to be low at a first glance. However as described in chapter 3.2.4, this results in a relevant increase of the overall release of NP to surface water. The degradation of NPnEO

may result in a 54 – 758 % increase of the NP load released to the overall environment.

Summary formation of 4-NP (based on studies carried out with NPnEO) during waste water treatment:

- Ahels et al. (Ahel et al., 1994a): 181 and 758 % increase of overall NP mass (mol/day) in two waste water treatment plants
- Varineau et al. (Varineau et al., 1996): 112.5 % increase of overall NP mass during sewage treatment compared to influent
- Yu et al. (Yu et al., 2009): 70% concentration increase in effluent compared to influent
- McAdam et al. (McAdam et al., 2011): 54 % increase in effluent compared to influent in the carbonaceous/nitrification/denitrification activated sludge plant

6.3.2.2 Further degradation in the environment

Once released to the environment via wastewater treatment effluent it can be expected that 4-NP and short chain 4-NPnEO will distribute into sediment while longer chain 4-NPnEO and 4-NPnEC remain in the water phase, as indicated by the distribution properties of 4-NP and its ethoxylates described in chapter 3.2.1 - 3.2.3.

In the water column long chain 4-NPnEO are expected to further degrade to short chain 4-NPnEO or 4-NPnEC depending on the environment condition. As the short chain 4-NPnEO are expected to distribute into sediment, they may contribute to the overall sediment load.

Biodegradation in surface water (based on studies carried out with technical NPnEO):

- > 99% primary degradation in 100hours under aerobic conditions (main product NPnEC) (Jonkers et al., 2001)
- DisT50 23-69 days (winter) and 2.5-35 days (summer) in an aerobic die away test with estuarine bacteria ; main intermediate NP2EO (Kveštak and Ahel, 1995)

Results from sediment tests indicate that – as expected from anaerob sewage sludge studies - 4-nonylphenol is formed under anaerobic conditions with a DegT50 of 49 – 77 days. Degradation may be even slower if highly polluted sediments are used.

Biodegradation in sediment (based on studies carried out with 4-NPnEO and technical NPnEO):

- Aerob: DegT50 (primary degradation) in river samples 69.3-115.5 days; increased with temperature (Yuan et al., 2004)
- Anaerob: DegT50 (primary degradation) in anaerobic river samples 49-77 days; increased with temperature, 4-NP formation (Chang et al., 2004)
- Aerob: DisT50 = 85 days (NPnEO including short chain NPnEO and 4-NP), only 1.9 % mineralization after 120 days in highly polluted sludge (Ferguson and Brownawell, 2003)
- Anaerob: DisT50 = 289 days (NPnEO including short chain NPnEO and NP), formation of NP1EO during decrease of NP2-5EO, no formation of NP- during 120 days in highly polluted sludge at a highly polluted site (Ferguson and Brownawell, 2003)

Thus, in summary once released to the environment, 4-NPnEO will undergo further degradation to 4-NP in anaerobic sediments and in river water during winter conditions. Degradation half lives are low and 4-NP is a very stable product in sediment (DegT50 46.2 days (primary degradation) to no elimination after 703 day in anaerobic freshwater sediment - depending on linear or branched isomers) (European Chemicals Agency, 2012b). Thus once released to surface water and distributed to sediment, degradation of 4-NPnEO will remain a long lasting source for 4-NP.

Release to soil via sewage sludge may be an additional relevant source of 4-NP and short chain ethoxylates due to the high adsorption of 4-NPnEO to sludge. Results described in chapter 3.1.2.3 indicate that short chain ethoxylates may degrade to 4-NP in soil but slowly (DisT50 between 48 days (Sjöström et al., 2008 for NPnEO) and > 360 days (Marcomini et al, 1989)). Thus, once released to soil, short chain 4-NPnEO may contribute to the overall concentration of 4-NP in soil. Because conversion is slow, it can be expected that these ethoxylates are a constant source of 4-NP in soil. Results by Sjöstrom et al (2008) indicate that 4-nonylphenol formation exceeds its transformation.

4-NP itself is a stable metabolite which strongly adsorbs to soil, sludge and sediment.

6.3.2.3 Equivalence of concern

Besides the rationale that all relevant precursors of SVHCs should be considered as SVHCs themselves, some specific aspects with regard to 4-NP and its ethoxylates substantiate the equivalent level of concern for 4-NPnEO:

As degradation of the ethoxylates in sediments is a very slow process, it can be expected, that sediments will remain a relevant source for 4-NP long after the cessation of exposure of 4-NP and its ethoxylates. This is of high importance as degradation of 4-NP in sediments is very slow (DegT50 46.2 days (primary degradation) to no elimination after 703 day in anaerobic freshwater sediment - depending on linear or branched isomers) and thus 4-NP formed by degradation of its ethoxylates may accumulate in sediment.

4-NP adsorbed to sediment may be an unpredictable relevant source of 4-NP in surface water due to environmental events such as flood or dredging. Effects observed for 4-NP on aquatic organisms indicate, that short term exposure during sensitive life stages may increase their susceptibility and may lead to effects during the entire life stage. Any environmental event leading to a higher release of 4-NP produced by degradation of its ethoxylates may coincide with such sensitive life stages resulting in unpredictable high effects.

In addition to the concern based on the degradation to 4-NP, available information indicates that short chain ethoxylates (4-NP1EO and 4-NP2EO) and carboxylates (4-NP1EC and 4-NP2EC) may show endocrine activity themselves: results for *O.mykiss* and *O.latipes* indicate that the in vivo and in vitro endocrine activity of those substances is nearly as high (factor 10) or similar to the endocrine activity of 4-NP. These tests do not include adverse endpoints and thus it is not possible to conclude whether or not 4-NP1EO and 4-NP2EO are endocrine disruptors themselves. However due to the similar endocrine activity and information available for 4-NP it seems possible that they may cause endocrine disrupting adverse effects.

6.3.3 Conclusion on the equivalence of concern for 4-nonylphenol ethoxylates

In consistence with the approach used for PBT substances it seems reasonable to conclude that any substance which may result in exposure to an SVHC (i.e. due to degradation to this substance) should be considered as SVHC itself as it has an equivalent level of concern.

Available information for 4-NPnEO indicates that 4-NPnEO contribute to the 4-NP concentration in the environment. A significant amount is either degraded to 4-NP itself in waste water treatment plants or is released to rivers in a form which may undergo further degradation to 4-NP. Available data show that 4-NP formed from degradation of 4-NPnEO may increase the 4-NP load to the environment (via sludge and effluent) by 54 to 758 % and may thus contribute to the 4-NP concentration in the environment.

Once released to the environment 4-NPnEO will remain a long-term source for 4-NP due to the tendency of short chain ethoxylates to bind to the sediment combined with a very slow degradation in anaerobic sediments of both the ethoxylates and their degradation product 4-NP. This long-term source results in additional exposure of both sediment and pelagic organisms such as fishes (via remobilisation) to 4-NP.

Especially due to the fact that short term exposure to 4-NP may result in life time effects in aquatic organisms and due to the fact that sudden environmental events may increase short term exposure concentrations, such a sink (mainly of short chain ethoxylates) and long-term source for 4-NP is considered of very high concern. The possible endocrine activity of short chain ethoxylates (4-NP1EO and 4-NP2EO) add to the concern.

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