Proposed Environmental Quality Standards for Nonylphenol in Water

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This report reviews the available data on the use, fate/behaviour and aquatic toxicity of nonylphenol. EQSs have been proposed for the protection of aquatic life which will assist Agency staff in assessing the effect of this substance on water quality.

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FOREWORD

This report, which proposes Environmental Quality Standards (EQSs) for nonylphenol for the protection of fresh and saltwater life and for water intended for human consumption, is one of a series of nine produced under the Environment Agency's (formerly NRA) Phase IV EQS contract.

The other reports propose EQSs for aluminium, cyanide, chlorophenols, octylphenol, dioxins, sheep dip chemicals, chlorine dioxide and fluoride.

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EXECUTIVE SUMMARY

WRc has been contracted by the Environment Agency to propose Environmental Quality Standards (EQSs) for a number of chemicals following concerns about their potential effects on the aquatic environment. This report is one of a series produced in fulfilment of the contract. Nonylphenol has been identified by the Environment Agency as a problem chemical. This report reviews the properties and uses of nonylphenol, its fate, behaviour and reported concentrations in the environment, and critically assesses available data on its toxicity and bioaccumulation (see Sections 2 to 5 and Appendices A to D). The available data have been examined and used to derive EQSs for the protection of fresh and saltwater life as well as for water abstracted to potable supply. The proposed standards are presented in Table S1.

Table S1 Proposed EQS values for nonylphenol ($\mu g\ l^{-1}$)

Use	AA	MAC	Notes
Protection of freshwater life	1.0	10.0	
Protection of saltwater life	1.0	5.0	
Abstraction to potable supply	-	-[1]	[2]

Notes
AA = Annual average
MAC = Maximum allowable Concentration
1. There are insufficient data to propose a guideline value
2. A taste threshold of 1 $\mu g\ l^{-1}$ has been reported in the literature

Nonylphenol (NP) is used extensively in the production of other substances such as non-ionic ethoxylate surfactants. It is through the incomplete anaerobic biodegradation of these surfactants that most nonylphenol reaches the aquatic environment in effluents, e.g. from sewage treatment works and certain manufacturing operations. It was explicitly stated by the Environment Agency that the EQS was to be derived for NP and not nonylphenol ethoxylates. However, since NP is unlikely to be present in the aquatic environment in the absence of other nonylphenol ethoxylate (NPE) degradation by-products, the toxicity, fate and behaviour of some of these (i.e. nonylphenol mono- and diethoxylates (NP1EO and NP2EO), mono- and di-nonylphenoxy carboxylic acids (NP1EC and NP2EC) have also been considered in this report.

In the aquatic environment and during sewage treatment, NPEs are rapidly degraded to NP under aerobic conditions. NP may then be either fully mineralised or may be adsorbed to sediments. Since NP cannot be biodegraded under anaerobic conditions it can accumulate in sediments to high concentrations.

NP is of high toxicity to freshwater life. Effect concentrations as low as 135 $\mu g\ l^{-1}$ and 14 $\mu g\ l^{-1}$ have been reported for fathead minnow in acute and chronic tests, respectively. Marine organisms are also sensitive, with the mysid shrimp (*Mysidopsis bahia*) being particularly sensitive (effect concentration of less than 10 $\mu g\ l^{-1}$). The observed bioconcentration for NP in fish is moderately high with BCF factors typically around 300.

Nonylphenolic compounds have been found to be weakly oestrogenic and it has been suggested that their presence in sewage effluents is linked to the feminisation of fish exposed to such effluents. However, currently available data indicates that potentially adverse effects on survival and growth of fish occur at lower effect concentrations than those giving rise to oestrogenic responses, such as induction of female egg protein (vitellogenin) and therefore the proposed standard is based on 'conventional' toxicity endpoints, although at a level where any oestrogenic effects should be avoided. Nevertheless, if new evidence emerges about oestrogenic effects at lower concentrations then a future revision of the proposed standards may be warranted.

The freshwater standards are based on toxicity of NP to fathead minnow, *Pimephales promelas*. The lowest reported acute effect is a 96 hr LC_{50} of 135 $\mu g\ l^{-1}$; applying a safety factor of approximately 10 results in a proposed standard of 10 $\mu g\ l^{-1}$, expressed as a maximum acceptable concentration (MAC). A safety factory of approximately 100 to the same estimate of toxicity results in an annual average (AA) of 1 $\mu g\ l^{-1}$ which is consistent with a safety factor of 10 applied to the MATC from a study into survival of fathead minnow in a 33-day test (MATC of 10.2 $\mu g\ l^{-1}$).

The saltwater standards are based on effects of NP to the mysid shrimp, *Mysidopsis bahia*. The saltwater MAC is based on survival over 96 h and the corresponding AA on the reported growth effects following chronic exposure. The proposed AA of 1.0 $\mu g\ l^{-1}$ NP is derived by applying a safety factor of approximately 10 to the 28 day MATC for fecundity in this species (7.8 $\mu g\ l^{-1}$). The use of acute data and a larger safety factor for deriving the saltwater AA is considered inappropriate in this case because the saltwater standard would, in our view, be over-stringent. The proposed AA and MAC reflect the acute:chronic ratio of 5 evident from the acute and chronic studies with *Mysidopsis*.

Although the lowest reported effects suggest that marine organisms are more sensitive to NP than freshwater organisms, it seems unlikely that freshwater organisms are intrinsically more tolerant to the effects of NP than saltwater organisms. Probably, the higher effects concentrations seen with the freshwater organisms simply reflect the species and endpoints selected for testing.

Mammalian toxicity data are insufficient to propose an EQS for the protection of water for abstraction to potable supply although a concentration of 30 $\mu g\ l^{-1}$ would probably be protective of human health over a short exposure period.

NP appears to be non-bioavailable once sorbed to sediment but currently available analytical methods do not discriminate between dissolved and sorbed NP. Since NP is a mixture of many different isomers there could be analytical problems with respect to validating claimed limits of detection. Therefore, despite claimed limits of detection of 0.2 $\mu g\ l^{-1}$ and below for some analytical methods, it is suggested that it may be problematical to monitor with sufficient accuracy and validity the proposed standard of 1.0 $\mu g\ l^{-1}$ for total, dissolved, NP using current techniques.

KEY WORDS

Environmental Quality Standard, EQS, Nonylphenol, Freshwater, Saltwater, Toxicity, Bioaccumulation.

1. INTRODUCTION

This report reviews the properties, uses, fate, behaviour and reported concentrations in the environment for nonylphenol and critically assesses data on its aquatic toxicity, bioaccumulation and mammalian toxicology. This information has been used to derive, where appropriate, EQSs for the protection of aquatic life, and for waters abstracted for potable supply.

Technical grade nonylphenol (NP) is a mixture of monoalkylphenols, predominantly para-substituted. The isomer or mixture used in studies reported in the literature is not always specified. In this report, NP (mixed isomers) and unidentified isomers are discussed as NP, but when identified in the literature, the particular isomer is indicated.

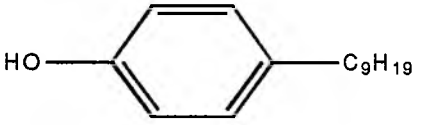
Since the primary source of nonylphenolic compounds in the aquatic environment is due to the incomplete biodegradation of nonylphenol ethoxylate (NPE) surfactants during sewage treatment, sewage effluents discharged to rivers can contain a range of nonylphenolic degradation by-products. Since NP is unlikely to be present in the aquatic environment in the absence of other NPE degradation by-products, the toxicity of these (i.e. nonylphenol mono- and di-ethoxylates (NP1EO and NP2EO), mono-ethoxy carboxylate and di-ethoxy carboxylate (NP1EC and NP2EC) have also been considered wherever possible in this report since their activities are likely to be at least additive.

2. NONYLPHENOL IN THE ENVIRONMENT

2.1 Physico-chemical properties

Nonylphenol is a mixture of isomers. The nonyl (C_9H_{19}) group may be branched or straight and may be located at either the 2, 3 or 4 position of the benzene ring. There are therefore hundreds of different potential nonylphenol isomers. The physical and chemical properties of nonylphenol given in Table 2.1 are either for a mixture or the 4-(or para) isomer.

Table 2.1 Chemical and physical properties of nonylphenol.

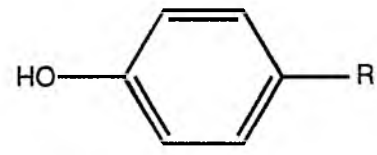
IUPAC CHEMICAL NAME	phenol, nonyl
OTHER CHEMICAL NAMES	Isononylphenol, p-nonylphenol, n-nonylphenol, 2,6-dimethyl-4-heptylphenol
SYNONYMS	NP, para-nonylphenol, Hydroxyl No. 253, Prevostel VON-100,
CAS REGISTRY NUMBER	4-nonylphenol (branched): 84852-15-3 Nonylphenol (mixed isomers): 25154-52-3 p-nonylphenol/ 4-nonylphenol: 104-40-5 o-nonylphenol/ 2-nonylphenol: 136-83-4
MOLECULAR FORMULA	$C_{15}H_{24}O$
MOLECULAR STRUCTURE	
MOLECULAR WEIGHT	220.36
COMPOSITION	C(82%), H(11%), O(7%)
APPEARANCE	Highly viscous, clear, pale yellow liquid with a slightly phenolic odour
MELTING POINT (°C)	-8 to -10 (1)
BOILING POINT (°C)	290-302 (may decompose before boiling) (1)
VAPOUR PRESSURE/VOLATILITY	23 mm Hg at 181 °C (3) Mean (at 25 °C) 4.6 x 10^{-3} mPa Standard. Dev. 3.5 x 10^{-3} (1)
DENSITY (g cm^{-3})	0.95 (1)
WATER SOLUBILITY (mg l^{-1}) (at pH 7)	11 (at 20 °C), 6.24 (at 25 °C) (1) 3.9 at 25 °C (in sea water)
DISSOCIATION CONSTANT (pKa)	4.53 (1)

HENRY'S LAW CONSTANT ($Pa\ m^3\ mol^{-1}$)	3714 (1) 1.23×10^{-5}, 5.9×10^{-6} ($atm\ m^3\ mol^{-1}$)(4)
Log Kow	3.28 (at 20 °C); 3.8-4.8 (at 25 °C) (1) NP1EO, NP2EO: 4.17, 4.21 (2)
Log Koc	3.1
FLASH POINT (°C)	149-155 (1)

References:
1. IUCLID (1996)
2. Ahel (1987)
3. Etnier (1985)
4. WRc calculations (1996)

2.2 Manufacture

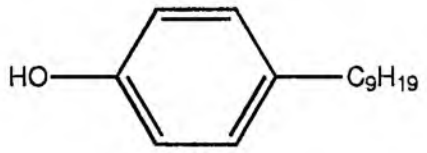
Industrial manufacture of alkylphenols is by liquid-phase alkylation of phenol with mixed isomeric nonenes (propylene trimer in the manufacture of NP) in the presence of an acid catalyst. Today production usually uses heterogeneous catalysts which replace liquid mineral catalysts. Premixed phenol and nonene are fed into an agitated tank reactor where they react at 50 to 15 °C for 5 to 120 min. The process yields a mixture of isomers, mostly *para*-, (see Figure 2.1) with some *ortho*- and 2,4-dinonyl substitution. The crude product is washed several times and heated under vacuum to remove traces of reactants and water. In the final step, NP is separated by vacuum distillation at 10 to 20 mm Hg. The yield of NP is about 75-99% (Etnier 1985, BUA 1988).



para-alkylphenol

Figure 2.1 The structure of para-substituted alkylphenols

The R group consists of an alkyl group which can occur in a multitude of highly branched isomers for all alkylphenols. By far the most important alkylphenol is nonylphenol in which case the R group is C_9H_{19} (Figure 2.2).



para-nonylphenol

Figure 2.2 The structure of para-substituted nonylphenol

Figures are not available for production and consumption figures specifically for the UK. However, in 1994, 77 505 tonnes of NP were produced in the European Union although, only 63 934 tonnes were actually consumed within the European Union, the remainder being exported. NP production is decreasing annually (CESIO, 1996).

2.3 Uses

Technical nonylphenol is almost exclusively used as an intermediate chemical in the production of other chemicals, such as stabilisers, resins, plastics, softeners, anti-static agents and dyestuffs. Around 43% of NP is estimated to be used for nonylphenol ethoxylate (NPE) production.

Alkylphenol ethoxylates, including nonylphenol ethoxylates, are an important group of non-ionic surfactant which over the last 40 years have been used in both industrial and domestic detergents, in paints, agrochemical emulsions, in metal working fluids and in the textile and dyeing industries. Nonylphenol polyethoxylates (NPnEOs) are the most common alkylphenol ethoxylates used in laundry detergents with 4-nonylphenol polyethoxylates being the most predominant class. However, smaller amounts of 2-nonyl-, 4-decyl- and 4-octyl- phenol polyethoxylates are also present in formulations and overall nEO chain lengths range between 3 and 20.

Another large use of NP is in the manufacture of rubber and plastics such as polyvinyl chloride (PVC) and polystyrene. In such instances it is used as an intermediate in the manufacture of phosphite anti-oxidants. The principal form being as tris-4-nonylphenol phosphite (TNPP). NP is also reacted with formaldehyde to form compounds useful as oil additives and synthetic lubricants. A relatively small amount is also used as a component in the formulation of foliar-applied agrochemicals, fungicides, bactericides, stabilising, emulsifying and dispersing agents because they improve wetting, penetration, absorption and water solubility characteristics of solutions.

In the UK, the major manufacturer of NPEOs is ICI Chemicals and Polymers at Wilton, near Middlesborough, Teeside. Only around 4% of NP is used as an end product (CESIO, 1996).

2.4 Entry into the aquatic environment

The primary source of nonylphenol detected in surface waters is the biodegradation of NP ethoxylates. During conventional mechanical-biological sewage treatment, at least 60-65% of nonylphenol polyethoxylates $(C_9H_{19}.C_6H_4O).(CH_2CH_2O)_nH$ are discharged to the environment as nonylphenol and short chain nonylphenol polyethoxylates (Ahel *et al*, 1994c).

During sewage treatment, under aerobic conditions, NPEs are split by microbial action into NP and a polyglycol chain. Due to the high surface activity of these compounds they are adsorbed to suspended solids and thus reach the anaerobic sludge digestion stage. While the latter is rapidly degraded, NP is not biodegraded under anaerobic conditions. Therefore, as the sludge is digested, some NP flows back into the sewage treatment plant with the surplus water. Consequently nonylphenol is frequently found in waters receiving inputs from sewage effluents and higher concentrations from works receiving certain trade wastes may be expected. The relative abundance of different metabolites is highly dependent on the treatment conditions, and temperature. Ahel *et al* (1994c) estimated the average composition of nonylphenolic compounds from a range of sewage treatment plants and the relative proportions of nonylphenolic compounds discharged into the environment. NP was predominantly associated with digested sludges, whilst NPEOs and NPECs were the predominant species in effluents.

2.5 Occurrence

The reported levels of nonylphenolic compounds detected in UK surface waters and effluents are presented in Table 2.2.

2.5.1 UK Surface Waters and Effluents

In a survey of levels of alkylphenols in the UK aquatic environment, six rivers in England and Wales were sampled at five or six sites each, together with 15 sewage treatment work effluents and six estuaries. The highest concentration of 330 $\mu g\ l^{-1}$ total extractable NP (TENP) was found in an effluent from a sewage treatment works receiving wastewaters from a textile-based industrial area in Yorkshire and discharging into the river Aire. Concentrations in the river Aire reached 180 $\mu g\ l^{-1}$ TENP. However, excluding the River Aire the majority of river samples (21 out of 23 samples taken from four different rivers) contained below 3.0 $\mu g\ l^{-1}$ TENP. Estuarine concentrations were considerably lower. The maximum level recorded was 5.2 $\mu g\ l^{-1}$ in the Tees estuary. Over 80% of estuarine samples contained <0.1 $\mu g\ l^{-1}$. In all surface waters dissolved concentrations of NP were much lower. The highest concentration was 53 $\mu g\ l^{-1}$, which was detected in the river Aire. In the other four rivers studied 22 out of 23 samples contained below 2.0 $\mu g\ l^{-1}$ NP (Blackburn and Waldock 1995).

Table 2.2 Levels of nonylphenols detected in UK surface waters and effluents

Region/River	NP		NP(1 or 2)EO		Ref.
	Effluent conc ($\mu g\ l^{-1}$)	Surface Water conc ($\mu g\ l^{-1}$)	Effluent conc ($\mu g\ l^{-1}$)	Surface Water conc ($\mu g\ l^{-1}$)	
South West EA (R. Dart)	2.4	0.055	-	-	1
South East (R. Lea)	-	12 (TE) 9 (Diss)	-	-	2
South East (R. Thames)	-	2.2 (TE) 0.7- <1.3 (Diss)	-	-	2
East Anglia (R. Gt. Ouse)	-	5.3 (TE) 1.3- <1.9 (Diss)	-	-	2
River Aire	-	180 (TE) 53 (Diss)	150	-	2
River Aire	330 (Total NP)		-	-	2
Yorkshire EA (R. Aire)	64 (STW influent)	5.9	-	-	3
Thames EA	<0.02 - 1.1	<0.02 - 0.45	-	-	4
North West EA	0.005 - 0.01	-	-	-	5
Scotland	<0.84 - 59.3 Av. = 9.2[a]	-	<6.8 - 275 Av. = 55.5[b]	-	6
ESTUARINE					
Outer Tees	-	5.2	-	-	2
Mersey	-	0.32	-	-	2

Key: TE = Total Extractable
Diss = Dissolved
STW = Sewage treatment works
[a] - Av. of 22 effluents from STW or Long Sea Outfalls
[b] - Av. of 21 effluents from STW or Long Sea Outfalls

References:

1. Warhurst (1995)
2. Blackburn & Waldock (1995)
3. D Gallagher (1996) Pers. Comm. NE EA
4. D Britnell (1995)
5. C Jarvis (1996) Pers. Comm. NW Environment Agency
6. D Pirie (1996) Pers. Comm. SEPA West

The South Western region of the Environment Agency (formerly the NRA) analysed levels of nonylphenol at an outfall from a sewage works discharging into the River Dart in Devon in the summer of 1994. This sewage works, which has secondary treatment, receives trade effluent from a wool processing factory which uses alkylphenol polyethoxylates surfactants. Levels of nonylphenol in the sewage outfall were found to be up to 2.4 $\mu g\ l^{-1}$ The maximum concentrations found in the mixing zone of the river downstream from the sewage outfall were 0.055 $\mu g\ l^{-1}$ of nonylphenol (Warhurst 1995). Since this survey, the use of alkylphenol ethoxylates at the factory has been discontinued.

Several projects are underway in the various Environment Agency regions to determine the levels of NP or alkylphenol ethoxylates in surface waters. i.e. North West and Midlands regions (Jarvis 1996, Pers. Comm. and Reardon 1996, Pers. Comm.). In addition, the Scottish Environmental Protection Agency (SEPA) are currently conducting a country-wide survey of alkylphenol ethoxylates comprising around 94 samples (Dr Gerry Best, 1996 SEPA, Pers. Comm.). The results are not available at the time of writing.

Waldock and Thain (1991) analysed for 4-NP in effluents and anaerobically digested sewage sludges from treatment plants in the UK. Concentrations of nonylphenols in effluents from several treatment works were generally low ranging from <2-21 ng l^{-1}. However, more recently Blackburn and Waldock (1995), reported levels of TENP in UK sewage effluents of up to 330 $\mu g\, l^{-1}$. Dissolved concentrations were much lower, the maximum level recorded was 5.4 $\mu g\, l^{-1}$.

2.5.2 Levels detected in surface waters and effluents in other countries

The levels of nonylphenols detected in surface waters and effluents in other countries are presented in Table 2.3.

Nonylphenol has been detected in river waters in several countries at levels of between 0.04 and 1000 $\mu g\, l^{-1}$. The highest level was detected in a river in the USA below a wool laundry (BUA 1988). During 1989, US rivers considered likely to contain NPEs were monitored for NP and NPE. Each site was just downstream from one or more outfall of industrial or treated municipal wastewater. Thirty of 500 suitable sites were considered sufficient to give national statistical validity to the survey. NP concentrations were greater than the detection limit of 0.1 $\mu g\, l^{-1}$ in 30% of samples. The highest concentration was 0.64 $\mu g\, l^{-1}$ with an average of 0.12 $\mu g\, l^{-1}$. NP1EO and NP2EO were above the detection limit in 33 and 41% of the samples, respectively, with mean concentrations of 0.09 and 0.1 $\mu g\, l^{-1}$, respectively (Naylor *et al* 1992). Other studies in Canada have revealed concentrations of 0.8-15 $\mu g\, l^{-1}$ nonylphenol in final effluent arising from a sewage treatment works (Lee and Peart 1995).

Detailed studies have been carried out on the levels of nonylphenolic compounds in Swiss rivers by Ahel and co-workers (1985, 1987, 1991, 1994b) and by Stephanou and Giger (1982). Generally, concentrations of NP, $NP_{1\text{-}2}EO$ were ten times higher in treated wastewaters than in river water. Both the NPEC and NPEO groups often exceeded 10 $\mu g\, l^{-1}$ in the river water. Only one nonylphenol result was above 10 $\mu g\, l^{-1}$ whereas over 84% of samples contained levels greater than 1 $\mu g\, l^{-1}$ (Ahel *et al* 1994b). Reported values are presented in Table 2.4.

Table 2.3 Levels of nonylphenols detected in surface waters and effluents in other countries

Region/River	NP		NP(1 or 2)EO		NPE(1 or 2)C		Ref.
	Effluent conc ($\mu g\ l^{-1}$)	Surface Water conc ($\mu g\ l^{-1}$)	Effluent conc ($\mu g\ l^{-1}$)	Surface Water conc ($\mu g\ l^{-1}$)	Effluent conc ($\mu g\ l^{-1}$)	Surface Water Conc ($\mu g\ l^{-1}$)	
Glatt/Switzerland	-	<0.3 - 7.9	-	<0.3 - 20/21	-	<0.1 - 29/59	1
5 rivers/Switzerland	-	<0.1 - 55.4	-	<0.1 - 32/37	-	-	1
Glatt/Switzerland	-	-	-	-	-	2 -116	2
Glatt/Switzerland	-	3.5 - 25	-	1.3 - 80	-	-	3
Glatt/Switzerland	-	<0.5 - 1.5	-	<0.5 - 16/18	-	-	4
Savannah/USA	-	1000 (downstream of wool scouring plant).	-	-	-	-	5
Savannah/USA	-	2	-	-	-	-	5
Tennessee R./USA	-	325	-	-	-	-	5
Delaware R./USA	-	0.04 - 2	-	-	-	-	5
30 rivers/ USA	-	<0.11 - 0.64	-	<0.06 - 0.6/1.2	-	-	5
Zurich, Switzerland	0 - 35	-	-	-	-	-	6
Toronto, Canada	0.8 - 15	-	-	-	-	-	7

References:

1. Ahel *et al* (1991)
2 Ahel *et al* (1987)
3. Ahel *et al* (1993)
4. Ahel *et al* (1985)
5. Naylor *et al* (1992)
6. Stephanou and Giger (1982)
7. Lee and Peart (1995)

Table 2.4 Concentrations of nonylphenolics reported in Swiss rivers (Ahel *et al* 1994b)

Chemical	Conc. range in Swiss rivers ($\mu g\ l^{-1}$)
nonylphenol	<0.3-45
nonylphenoxycarboxylic acid (NP1EC)	<1-45
nonylphenoxycarboxylic acid (NP2EC)	2-70
nonylphenol ethoxylate (NP1EO)	<3-69
nonylphenol ethoxylate (NP2EO)	<0.3-30

2.5.3 Sewage Sludge

Sweetman *et al* (1991) quantified levels of 4-NP in sewage sludges from two UK sewage treatment works (STWs). The levels in sludges of the STW receiving a large industrial input contained levels in the range 520 - 823 (average 637) mg kg^{-1} dry weight. These levels were approximately double those of the STW receiving predominantly domestic wastewaters (range 255 - 414, average 326 mg kg^{-1} dry weight). High levels of 4-NP have also been reported in other studies of anaerobically digested sewage sludges.

4-Nonylphenol has been detected at an average concentration of 1000 mg kg^{-1} and a maximum of 2500 mg kg^{-1} dry weight in the anaerobically stabilised sludge of 30 communal sewage treatment plants. Levels determined in eight aerobically stabilised sludges were significantly lower suggesting that 4-nonylphenol is persistent under mesophilic anaerobic conditions. The highest concentration in the aerobic stabilised sludge was 500 mg kg^{-1} with a mean of 280 mg kg^{-1}. Activated sludge and mixed primary and secondary sludge also showed lower concentrations of 4-NP with levels up to 140 mg kg^{-1} and similar concentrations of nonylphenol mono- and diethoxylates. No adverse effects on sewage bacteria were reported at these high concentrations of NP (Giger *et al* 1984).

In another Swiss study anaerobically digested sewage sludge from a sewage treatment plant, contained average concentrations of NP, NP1EO and NP2EO of 1200, 220 and 30 mg kg^{-1} dry weight, respectively (Marcomini and Giger 1987).

These data contrast with those from the US which show far lower levels of <2.8 and 10 mg kg^{-1} in wet and dry weight sludges, respectively. It has been suggested that the differences in levels determined in the US and in other countries may be due to the different analytical methods used (Naylor *et al* 1992). Alternatively, it could indicate better NPE removal efficiency by sewage treatment plants in the US, maybe reflecting higher average operating temperatures or different NPE loadings or isomers in influents to US sewage treatment works.

2.5.4 River Sediments

Nonylphenol and nonylphenol mono-and diethoxylates show significant association with sedimentary material. A survey of Swiss rivers found that the majority of sediments contained nonylphenol as the predominant nonylphenolic compound. In the most polluted sediments, taken from below effluent discharges, concentrations of up to 13.1 mg kg^{-1} dry weight of NP and up to 25 mg kg^{-1} dry weight of total NP, NP1EO and NP2EO were detected (Ahel *et al*, 1991, 1994b). Sediments rich in organic matter contained significantly higher concentrations than sand collected at the same locations.

During 1989, US rivers considered likely to contain NPEs were monitored for NP and NPEO. NP concentrations in sediments from thirty sites, each taken from just downstream of industrial or treated municipal wastewater outfalls, ranged from not detectable (<0.0029 mg kg^{-1}) in 28% of samples to 2.96 mg kg^{-1} (Naylor *et al* 1992).

3. ANALYSIS

3.1 Analytical requirements for EQS monitoring

The adequate monitoring of EQSs requires a suitably accurate analytical method. The accepted approach for the derivation of the accuracy requirements of an analytical system (when monitoring to a particular water quality standard) is described in WRc Report NS30 (Cheeseman *et al* 1989).

For an EQS of X units, the error on a single analytical result should not be larger than X/10 concentration units or 20% of the concentration in the sample, whichever is the greater. Following the convention of dividing the tolerable error equally between random and systematic sources, this implies:

- a maximum tolerable standard deviation of X/40 concentration units or 5% of the concentration in the sample, whichever is the greater; and
- a maximum tolerable bias of X/20 concentration units or 10% of the concentration in the sample, whichever is the greater.

It is recommended that the target limit of detection should be set at X/10 concentration units. For example, for a proposed EQS of 0.5 $\mu g\ l^{-1}$:

- the limit of detection should be 0.05 $\mu g\ l^{-1}$ or less,
- the total error should not exceed 0.05 $\mu g\ l^{-1}$ or 20% of the determinand concentration (whichever is the greater);
- the systematic error or bias should not exceed 0.025 $\mu g\ l^{-1}$ or 10% of the determinand concentration (whichever is the greater); and
- the total standard deviation of individual results should not exceed 0.0125 $\mu g\ l^{-1}$ or 5% of the determinand concentration (whichever is the greater).

3.2 Analytical techniques

Since NP is a mixture of many different isomers the monitoring of the EQS value poses a particular problem. In particular, the limit of detection for each analytical method for total NP can only be a best estimate since analytical standards are not available for the majority of NP isomers, and therefore assumptions must be made regarding the behaviour of many isomers during analysis. For instance, it is assumed that the percentage recovery and the limit of detection for the isomers for which standards are available are the same for every other NP isomer. Since this is unlikely to be the case, it is recommended that each of the limits of detection quoted for the following analytical techniques is considered as a tentative best estimate, and probably over-estimates their true detection limits. All the methods described are expected to include dissolved NP and a proportion (although not necessarily all) of any NP which is sorbed onto suspended solids.

A 'Blue Book' method for the analysis of nonylphenol and other alkylphenol ethoxylate telomers (with one or more ethoxylate units) in: sewage, sewage effluents, surface waters, and biodegradation and toxicity test liquors has been produced by the Standing Committee of Analysts (SCA) (HMSO 1990). The method is described below.

The surfactants are extracted from a known volume of aqueous sample by the Wickbold sublation procedure. The resulting sublation extracts are ion-exchanged under non-aqueous conditions to remove any ionic surface active materials which may interfere in the subsequent analysis. This is by high performance liquid chromatography with fluorescence detection. For a one-litre sample a limit of detection of 5 $\mu g\, l^{-1}$ was achieved. When spiked at 1000 $\mu g\, l^{-1}$ the mean recovery was 92 % with a relative standard deviation (RSD) of 2.6% (number of samples (n) = 5).

At WRc, nonylphenol (NP) is analysed by extracting a one-litre sample with hexane using steam distillation. The extract is then concentrated to 0.1 ml before being analysed by gas chromatography - mass spectrometry (GCMS) in the positive ion electron impact (+EI) mode. The limit of detection is 1 $\mu g\, l^{-1}$ but this could be lowered to 0.2 $\mu g\, l^{-1}$ by increasing the sample volume to five litres and using liquid-liquid extraction at pH 2 or less.

A method has also been reported for the analysis of nonylphenol and nonylphenol ethoxylates in water, sewage sludge and biota by Wahlberg *et al* (1990). A 250-ml water sample is acidified to pH 2 or less and extracted with dichloromethane. Sodium chloride was added before the extraction to prevent the formation of emulsions. After concentration the substances are converted into their pentafluorobenzoyl derivatives and quantified by GCMS or GC-ECD (electron capture detection). For waste waters the detection limits using GCMS were estimated to be less than 0.1 $\mu g\, l^{-1}$ for NP and 0.2-1 $\mu g\, l^{-1}$ for the ethoxylates. Typical recoveries were 98% for NP and 82-100% for the ethoxylates with an RSD of 2-7% (n = 5). This method was used by SEPA in their recent (1996) survey of Scottish surface waters, to quantify levels of nonylphenol and nonylphenol ethoxylates with five or less ethoxylate groups (D. Pirie (1996) SEPA West. Pers. Comm.). This method also appears to have been the basis of the analytical method used by Thames Environment Agency region during their 'Survey of alkylphenols in Thames region rivers and sewage effluents' (Britnell 1995). They report a limit of detection for NP of 0.02 $\mu g\, l^{-1}$ for their specific analytical method when using a two-litre sample. However, the method was only minimally validated and therefore the results of their analysis should be regarded as guideline values only.

A method has also been developed by Ahel *et al* (1987) for the analysis of nonylphenol carboxylic acids in sewage effluents. After acidification a one-litre sample was extracted with chloroform and then concentrated to dryness followed by reconstitution into dichloromethane. The extracts were then cleaned up using a silica column and then concentrated and methylated before determination by either HPLC or GC with flame ionisation detection (FID). Recovery experiments were performed using 4-octylphenoxy acetic acid giving 95% recovery with an RSD of less than 3% for both HPLC and GC. The reproducibility of nonylphenoxy acetic acid and nonylphenoxy ethoxy acetic acid were found to be 6.4% (GC) and 7.6% HPLC. The limit of detection for both techniques is approximately 1 $\mu g\, l^{-1}$ but the GC method is more effective at separating the nonylphenol isomers and other alkyl homologues whereas many of the isomers co-eluted using HPLC.

To achieve the necessary level of accuracy and statistical validity for monitoring the lowest proposed EQS value of 1 $\mu g\, l^{-1}$ a limit of detection ten times lower than the value would be required i.e. 0.1 $\mu g\, l^{-1}$. This appears to be at the limit of the various analytical techniques available. The limit of detection reported in the literature is around 0.2 $\mu g\, l^{-1}$ NP. However, the Thames Environment Agency region reported a limit of detection of 0.02 $\mu g\, l^{-1}$. Since NP is not a single chemical isomer, it would be necessary to ensure that either these co-eluted during chromatography or different peaks were combined in determinations of nonylphenol.

4. SUMMARY OF FATE AND BEHAVIOUR IN THE ENVIRONMENT

The primary source of NP in the aquatic environment is due to the incomplete biodegradation of NPE surfactants during sewage treatment. In laboratory biodegradability studies, such as the OECD screening and confirmatory tests, both linear and branched NPEs undergo substantially complete primary biodegradation following acclimation. However, NPEs are generally not fully biodegraded under normal sewage treatment plant operating conditions. This results in the discharge of nonylphenolic compounds (primarily associated with particulate matter) in sewage effluents. The biodegradation pathway of NPEs which leads to the production of NP is presented in Figure A2.1.

During sewage treatment at least 55% of influent NPEs are expected to be converted to NP and short-chain $NP_{(1-2)}EOs$ and NPECs. The metabolites of NPE biodegradation are less water soluble and more resistant to biodegradation than the parent NPEs. Consequently, sewage effluents contain a range of nonylphenolic degradation by-products, including nonylphenol mono- and diethoxylates (NP1EO and NP2EO), nonylphenoxy carboxylic acids (NPEC) and nonylphenol (NP). The majority of the NP formed during sewage treatment however remains associated with sewage sludge and therefore does not reach the aquatic environment. Temperature is also important in the biodegradation of NPEs at low temperatures for instance below around 10-15 °C, biodegradation is much slower and this can affect the levels and proportions of NPEs discharged in effluents.

Under aerobic conditions, such as in the water column and in soils, ethoxylated and carboxylated metabolites of NPE biodegradation are rapidly degraded to NP which may then go on to be fully mineralised. Under anaerobic conditions however, such as in anoxic sediments or settled sewage sludge, the biodegradation of metabolites to NP is much slower. This is because the basic reaction process is oxidation which is less favoured under anaerobic conditions. Since NP cannot be further biodegraded under anaerobic conditions and is hydrophobic, it tends to sorb strongly to sediments, particularly those containing organic carbon, where it can accumulate to high concentrations.

Under environmental conditions volatilisation from surface waters is not likely to be a significant loss processes for nonylphenol or its ethoxylates. There is, however, evidence that NP can be lost from the water column by photolysis. However, since the rate of photolysis is highly dependent on turbidity, temperature and dissolved organic carbon concentrations, it is likely that the rate of photolysis of nonylphenol under environmental conditions will be significantly longer than the reported half-life of 10-15 hours, obtained at between 14 and 17 °C.

5. SUMMARY OF AQUATIC AND MAMMALIAN TOXICITY

5.1 Aquatic Toxicity and Bioaccumulation

The primary source of nonylphenolic compounds in the aquatic environment is the incomplete biodegradation of NPE surfactants during sewage treatment. Consequently, sewage effluents discharged to rivers can contain a range of nonylphenolic degradation by-products, including NP. Since NP is unlikely to be present in the aquatic environment in the absence of other NPE degradation by-products, the toxicity of nonylphenol mono- and diethoxylates (NP1EO and NP2EO), and nonylphenoxy carboxylic acids (NPEC) have also been considered in this report.

There are limited toxicity data on NP1EO, NP2EO, NP1EC or NP2EC, although generally the toxicity of NPEs decreases with increasing ethylene oxide units. This is consistent with their decreasing fat solubility. NP is the most lipophilic of the NPE metabolites and appears to be the most toxic. For example the 48 hr LC_{50} of NP to Japanese medaka (*Oryzia latipes*) is 1400 $\mu g\ l^{-1}$, whilst for NP1EC and NP2EC the values are 9600 and 8900 $\mu g\ l^{-1}$, respectively (Yoshimura 1986). Similarly, the 48 hr LC_{50} of NP to *Ceriodaphnia dubia* is 470 $\mu g\ l^{-1}$ whilst for a mixture of both NP1EO and NP2EO it is 1040 $\mu g\ l^{-1}$ (Ankley *et al* 1990).

In acute toxicity tests, the most sensitive organism to NP was the marine shrimp, (*Mysidopsis bahia)* for which the 96 hr LC_{50} was 43 $\mu g\ l^{-1}$. In fish 96 hr LC_{50} values ranged from 135 $\mu g\ l^{-1}$ for fathead minnow (*Pimephales promelas*) to 3000 $\mu g\ l^{-1}$ for cod (*Gadus morhua*). NP was generally toxic to algae at concentrations of greater than 500 $\mu g\ l^{-1}$, although the lowest 96 hr EC_{50} (for growth) was 27 $\mu g\ l^{-1}$ in the marine diatom, *Skeletonema costatum.*

In chronic tests the most sensitive endpoint and species was growth of developing rainbow trout. The study is yet to be published in full and therefore it has not been possible to undertake a full evaluation. Furthermore the results are slightly anomalous and therefore their significance difficult to interpret: following a 35-day exposure period, 1 and 10, but not 30 $\mu g\ l^{-1}$ NP1EC accelerated growth and NP2EO caused a growth reduction at 1 and 10, but not 30 $\mu g\ l^{-1}$. However, NP caused a dose-dependant growth suppression at concentrations down to 10 $\mu g\ l^{-1}$, the NOEC for NP being reported as 1 $\mu g\ l^{-1}$ (Ashfield *et al* 1995). These effects persisted for one year after exposure was terminated but the magnitude of the response is unknown.

Apart from the rainbow trout study, for which few test details are available, the most sensitive freshwater species was the fathead minnow. A NOEC and LOEC of 7.4 and 14 $\mu g\ l^{-1}$ were determined, respectively, using survival over 33 days as the toxic endpoint. In chronic tests, marine species, the mysid shrimp was again the most sensitive species. In a 28-day test NOECs of 3.9 and 6.7 $\mu g\ l^{-1}$ were reported, respectively, for body length of the F1 generation and fecundity. Although effects on growth occurred at lower concentrations than those giving rise to effects on fecundity, the magnitude of the growth response at the LOEC was only 8% compared with the control, introducing some doubt about the ecological significance of this response.

Algae and fish have both been shown to bioaccumulate NP and NP1EO and NP2EO. Algae bioaccumulated NP to the greatest degree, but this may have been due to adsorption to their surface. In fathead minnow, BCFs of 271 and 344 were determined for exposure to 4.9 and 22.7 $\mu g\ l^{-1}$, respectively. The lower exposure concentration is the more relevant environmental concentration. Other BCF values reported in the literature for salmon (*Salmo salar*) (280) and heck (*Squalius cephalus*) (246) are similar. The rate of clearance of NP, after transferring fish to clean water, is rapid with half-lives of 1.2-1.4 days. The highest reported BCF values are 1300 for fish and 3400 for mussels using radio-labelled NP (Ekelund *et al* 1990). Other authors have reported much lower values. McLeese *et al* (1980) reported a BCF in mussels of ten, with a half-life of 0.3 days. In general, the observed bioconcentration of NP is moderate (around 300), which is below that which would be indicated by its octanol water partition coefficient of around four. This is probably due to the fact that NP will exist predominantly in its ionic form (and therefore be less lipophilic) at physiological pH.

NP dosed into sediments does not appear to be readily bioavailable. Much higher levels of NP are required in sediment than in water to cause adverse effects to the sensitive midge larvae (*Chironomus tentans*). The 14 day LC_{50} of NP in water was 119 $\mu g\ l^{-1}$, whilst the equivalent 14 day LC_{50} required a dose in sediment of greater than 34 000 $\mu g\ kg^{-1}$.

5.1.1 Oestrogenicity

Several recent field studies have implicated APEs, including NP, in the oestrogenic responses (vitellogenin production) in male fish exposed to sewage effluents. Further evidence for a role of NP in such responses comes from a number of laboratory studies. For example, a testis-ova intersex condition was observed in Japanese medaka (*Oryzias latipes*) exposed to 50 $\mu g\ l^{-1}$ NP (Gray and Metcalfe 1997).

Egg-protein (vitellogenin) production in rainbow trout is under oestrogenic control, and can be significantly increased by 20.3 $\mu g\ l^{-1}$ NP, and a threshold concentration of approximately 10 $\mu g\ l^{-1}$ has been reported (Jobling *et al.* 1996). These authors also describe the effects of NP and related compounds on testicular growth in rainbow trout where exposure for three weeks during sexual development resulted in a NOEC and LOEC of 20.3 and 54.3 $\mu g\ l^{-1}$, respectively. No impact on testicular growth was observed in sexually mature fish. In addition to these effects, exposure of sexually maturing male fish to 30 $\mu g\ l^{-1}$ of NP, NP1EC and NP2EO altered the histology of testes relative to controls, suggesting an effect on spermatogenesis. Another study by Ashfield *et al* (1995) reported no effect on sperm counts of juvenile rainbow trout exposed for 35 days to these compounds at the same concentration, although changes to the gonadosomatic index were evident. However, these were not dose-dependant, being increased following exposure to 30 $\mu g\ l^{-1}$ NP but decreased at lower concentrations. The authors of this study concluded that, when oestrogenic response are taken into account, exposure to NP, NP1EC and NP2EO had a positive effect on the reproductive status of rainbow trout.

Although responses such as induction of vitellogenin production are undoubtedly biomarkers of exposure to oestrogenic substances, and some of the observed responses denote adverse physiological changes, the ecological significance of such changes on reproduction and populations remains unclear. To date, oestrogenic responses have all been observed at concentrations higher than those giving rise to adverse effects on survival or reproduction in other fish and invertebrate species.

5.2 Mammalian Toxicity

Nonylphenol is readily absorbed and excreted by rats with around 90% of administered NP being excreted over a period of four days (Knaak *et al* 1966). NP appears to be of low oral acute toxicity in laboratory animals with average rat LD_{50} values of around 1500 mg kg^{-1} (BUA 1988). NP is also of low sub-chronic toxicity. In a 28-day feeding study in rats the adverse effects, which comprised increases in mean relative kidney, liver and testes weights, were noted at 400 mg NP kg^{-1} body weight. The No Observed Adverse Effect Level (NOAEL) was determined to be 100 mg kg^{-1} bodyweight. NP is of low reproductive toxicity. It has only shown adverse effects on developing foetuses at maternally toxic doses. In such a developmental toxicity and teratogenicity test, rats were given NP by gavage. The NOAEL for maternal effects was determined to be 75 mg kg^{-1} bodyweight whilst the NOAEL for teratogenic effects was determined to be 300 mg kg^{-1} body weight. p-Nonylphenol was not found to be mutagenic in any mutagenicity assay either *in vivo* or *in vitro* (IUCLID 1996).

No other data were available on the chronic toxicity, carcinogenicity, or reproductive toxicity of nonylphenol. However a 90-day sub-chronic feeding study and a multi-generation reproduction study in rats are currently underway in the USA. The results are not yet available.

In *in vivo* bioassays for oestrogenicity NP is between 2000 and 40 000 times weaker than ethinyl oestradiol. A NOAEL of 9.5 mg kg^{-1} per day and a LOAEL of 47.5 mg kg^{-1} have been determined for increases in absolute uterine weights and uterine to bodyweight ratios in rats given NP via the oral route (IUCLID 1996).

A taste threshold of 1 µg l^{-1} for NP has been reported in Austern *et al* (1975). This value appears to be very low but it has not been possible to obtain the original paper from which this value is cited in order to verify the citation. Dietz and Traud (1978) listed an odour threshold of 1000 µg l^{-1} for p-NP.

6. DERIVATION OF EQSs

6.1 Standards in other countries

Concentrations of NP which are considered to be protective of aquatic life have recently been estimated, either to derive water quality standards (Denmark, US EPA) or as a component of a risk assessment for NP (Nordic Council of Ministers, Building Research Establishment). In all these cases, the proposals are recent drafts and it is not clear whether any have yet been implemented. All the studies recommend broadly similar standards/PNECs (Table 6.1).

Table 6.1 Proposed standards/PNECs for NP for the protection of aquatic life

US EPA CMA, 1996)	Denmark (Samsoe-Petersen and Pedersen, 1995)	Nordic Council of Ministers (1996)	BRE (1997)
Proposed 'concern concentration' of 1.0 µg l^{-1}	Proposed water quality criterion of 1.0 µg l^{-1}	PNEC of 0.24 µg l^{-1}	PNEC in freshwater of 0.74 µg l^{-1} PNEC in seawater of 0.39 µg l^{-1}

Where a standard for nonylphenol ethoxylates is proposed, the same concentration as for NP is recommended (Nordic Council of Ministers, 1996). There are no drinking water standards set for NP in the EU Drinking Water Directive, by the World Health Organisation or by the US EPA.

6.2 Other controls on exposure

Other information relating to controls of NP in any environmental medium include an agreement between the Swedish Water and Wastewater Association, the Farmers organisation and the Swedish Environment Agency to limit the level of NP in sludge for spreading on farmland to 100 mg kg^{-1} total solids (TS) (Pers. Comm. Westerlund H. (1996) Swedish Water and Wastewater Association). However, no information is available on the basis for this standard. In addition, a number of wool scouring plants in Yorkshire, UK, have also phased out the use of alkylphenol ethoxylates (ENDS, March 1997) which would also have the effect of reducing inputs of NP.

The Convention on the protection of the marine environment of the North Atlantic (OSPAR, combining the activities of the 1974 Paris Convention on pollution from land-based sources to the North Sea and the 1972 Oslo Convention on waste dumping at sea) is concerned that, despite the fact that NPEs have been in use for over 40 years, there is little known about the consequences of their continued use. In view of this, contracting Parties to the Convention have made the following proposals:

- To study all uses of NPEs and similar substances, which lead to the discharge of these substances to sewer or to surface waters with a view to a reduction of such discharges.

- The use of NPEs as cleaning agents for domestic use should be phased out by the year 1995.

- The use of NPEs as cleaning agents for industrial use should be phased out by the year 2000.

- Care should be exercised to ensure that replacement materials for the current uses of NPEs are less damaging to the aquatic environment.

These proposals are designed to reduce emissions of NPEs and hence exposure to aquatic organisms in the medium to long term (CES 1993).

6.3 Protection of freshwater life

The available toxicity data indicate that NP is of high acute toxicity to freshwater species tested, with the majority of effect concentrations below 1000 $\mu g\ l^{-1}$. Acute toxicity data are available for most major taxonomic groups and there are also good quality chronic data for some species.

The lowest reported acute effect is a 96 hr LC_{50} of 135 $\mu g\ l^{-1}$ determined from studies with the fathead minnow, *Pimephales promelas*. A water column test using larvae of the midge (*Chironomus tentans*) yielded similar effect concentrations (96 h LC_{50} of 160 $\mu g\ l^{-1}$). Applying a safety factor of approximately 10 to the LC_{50} from the fathead minnow test results in a proposed standard of 10 $\mu g\ l^{-1}$, expressed as a maximum acceptable concentration (MAC). Although a lower effect concentration (69 $\mu g\ l^{-1}$) was reported from a 96-h test using *Ceriodaphnia dubia* (CMA 1995), the end point appears to have been miscellaneous abnormalities such as 'paleness', 'surfacing' and 'trailing extraneous material'. These end points are not considered a sufficiently robust basis on which to derive a standard.

The lowest reported chronic toxicity data relate to growth suppression of juvenile rainbow trout following a 35-day exposure period (see Section B1.6). The NOEC and LOEC for NP in this study were 1 and 10 $\mu g\ l^{-1}$, respectively although the magnitude of this growth inhibition is unknown. In this study, both NP2EO and NP1EC appeared to be more potent inhibitors of growth which contrasts with the higher toxicity exhibited by NP in other toxicity tests. Furthermore, the authors of this study considered that, when oestrogenic responses (e.g. vitellogenin levels, fecundity and progeny survival to hatch) are taken into account, exposure of the fish to the compounds at the concentrations tested had a positive influence on their reproductive status (Ashfield *et al* 1995). These factors - especially the lack of information on the extent of growth inhibition and the fact that the details of the study are not yet published - introduce some uncertainty into the interpretation of the growth inhibition seen with NP and reduce confidence in this study as a basis for setting a standard.

Applying a safety factor of approximately 100 to the lowest acute LC_{50} (135 $\mu g\ l^{-1}$) would result in an AA of 1 $\mu g\ l^{-1}$. This is also consistent with a safety factor of 10 applied to the MATC from a study into survival of fathead minnow in a 33-day test (MATC of 10.2 $\mu g\ l^{-1}$). This flow-through test was conducted for the Chemical Manufacturers Association (CMA, 1991) to GLP standards, and toxicant concentrations were measured throughout the study. A standard of 1 $\mu g\ l^{-1}$ would also confer a degree of protection to possible effects on growth of juvenile rainbow trout, if those effects were to confirmed. On this basis, an AA of 1 $\mu g\ l^{-1}$ is proposed. Acute and chronic studies with the fathead minnow indicate that a ratio of 10 between the proposed AA and MAC is appropriate.

Nonylphenol adsorbs strongly to sediments, and is therefore unlikely to remain in the water column for long periods. The limited sediment toxicity data indicate that once adsorbed to sediments NP is non-bioavailable. However, the available analytical methods are not capable of discriminating dissolved and sorbed NP.

6.4 Protection of saltwater life

By comparison with the studies on freshwater organisms, there are relatively few toxicity data available for saltwater organisms but both acute and chronic toxicity studies again reveal high toxicity.

A maximum allowable concentration (MAC) of 5 $\mu g\ l^{-1}$ is proposed, derived by applying a safety factor of 10 to the 96 hr LC_{50} of 43 $\mu g\ l^{-1}$ obtained for survival of *Mysidopsis bahia.* The marine diatom (*Skeletonema costatum*), appears to be a particularly sensitive species for which a 24 hour EC_{50} of 34 $\mu g\ l^{-1}$ has been reported. Applying a safety factor of 10 to this value would result in a similar EQS of 3.4 $\mu g\ l^{-1}$. For the same species, a 96 hr MATC of 14 $\mu g\ l^{-1}$ and an EC_{10} for growth of 12 $\mu g\ l^{-1}$ have been reported which are well above the proposed MAC. However, the exposure period for this test was longer than the life-cycle of the diatom and so the MATC and EC_{10} values have been regarded as measures of chronic toxicity. The proposed standard of 5 $\mu g\ l^{-1}$ should be adequate to protect against acute exposure of unicellular algae.

A chronic NOEC of 6.7 $\mu g\ l^{-1}$ has been reported for reduced fecundity of the mysid shrimp, *Mysidopsis bahia*, when exposed to NP in a 28-day test. The corresponding LOEC was 9.1 $\mu g\ l^{-1}$ and a MATC of 7.8 $\mu g\ l^{-1}$ can be calculated. Although a lower chronic NOEC and LOEC were reported for growth (body length) of the F1 generation of the mysid shrimp (3.9 and 6.7 $\mu g\ l^{-1}$, respectively) in the same study, the magnitude of the measured response was small: at the LOEC, growth of animals was reduced by only 8% compared to the controls. The biological significance of such a small effect is unclear but is certainly of less importance than the reported effects on fecundity. Consequently, in deriving a standard, greater emphasis has been placed on the effects of NP on fecundity.

Taking these chronic, sub-lethal data into account, a safety factor of approximately 100 applied to the lowest acute LC_{50} would result in an over-stringent standard (an AA of 0.5 $\mu g\ l^{-1}$). A more realistic standard, and one which still provides a safety margin of 5-fold for the reported growth effects in *Mysidopsis*, is derived by applying a safety factor of approximately 10 to the 28 day MATC for fecundity in this species (7.8 $\mu g\ l^{-1}$), resulting in a proposed AA of 1.0 $\mu g\ l^{-1}$ NP. The proposed AA and MAC reflect the acute: chronic ratio of 5 evident from the acute and chronic studies with *Mysidopsis*.

6.5 Comment on the proposed standards

The available evidence suggests that the MAC for the protection of freshwater life does not need to be as stringent as that for the protection of marine species and that a larger acute:chronic is warranted. Although the lowest reported effects suggest that marine organisms are more sensitive to NP than freshwater organisms, it seems unlikely that freshwater organisms are intrinsically more tolerant to the acute effects of NP than saltwater organisms. Indeed, the distribution of reported acute effects data for freshwater and marine invertebrates and fish are broadly similar (Figure 6.1). Possibly, the higher effects concentrations seen with the freshwater organisms simply reflect the species and endpoints selected for testing. Although freshwater mysids (Order: Mysidaceae) are rare - only *Mysis relicta* is native to the UK and then is confined to Ennerdale Water and some Irish lakes - some ecologically important detritivores are related to the mysid shrimps, notably the freshwater hoglouse *Asellus aquaticus* (Order: Isopoda) and *Gammarus pulex* (Order: Amphipoda). Further work with one of these species may be useful in validating the proposed standards for the protection of freshwater life.

As far as oestrogenic responses to NP are concerned, the reported effects concentrations are higher than those giving rise to adverse effects on survival, growth and fecundity of fish and invertebrates. Therefore the proposed standards should be protective of possible oestrogenic effects although this position may need to be reviewed as more data are generated.

6.6 Abstraction of water to potable supply

The data available on the mammalian toxicity of NP are sparse and the full toxicological significance of oestrogenic activity of compounds is not certain. For instance it appears possible that oestrogenic substances may interact synergistically.

However, from the limited data available it is possible to suggest a very conservative level of 30 $\mu g\ l^{-1}$ which is likely to be protective of human health over a short exposure period. This is based on the NOEL for increased uterine weight of 9.5 mg kg^{-1} bodyweight determined in oral rat studies. It is possible that aesthetic problems may occur in drinking water at levels below this since a taste threshold of 1 $\mu g\ l^{-1}$ for NP has been reported in the literature (Austern *et al* 1975). However, the confidence in this value is low and therefore an EQS for the protection of water abstracted to potable supply has not been derived.

The proposed standards are summarised in Table 6.2.

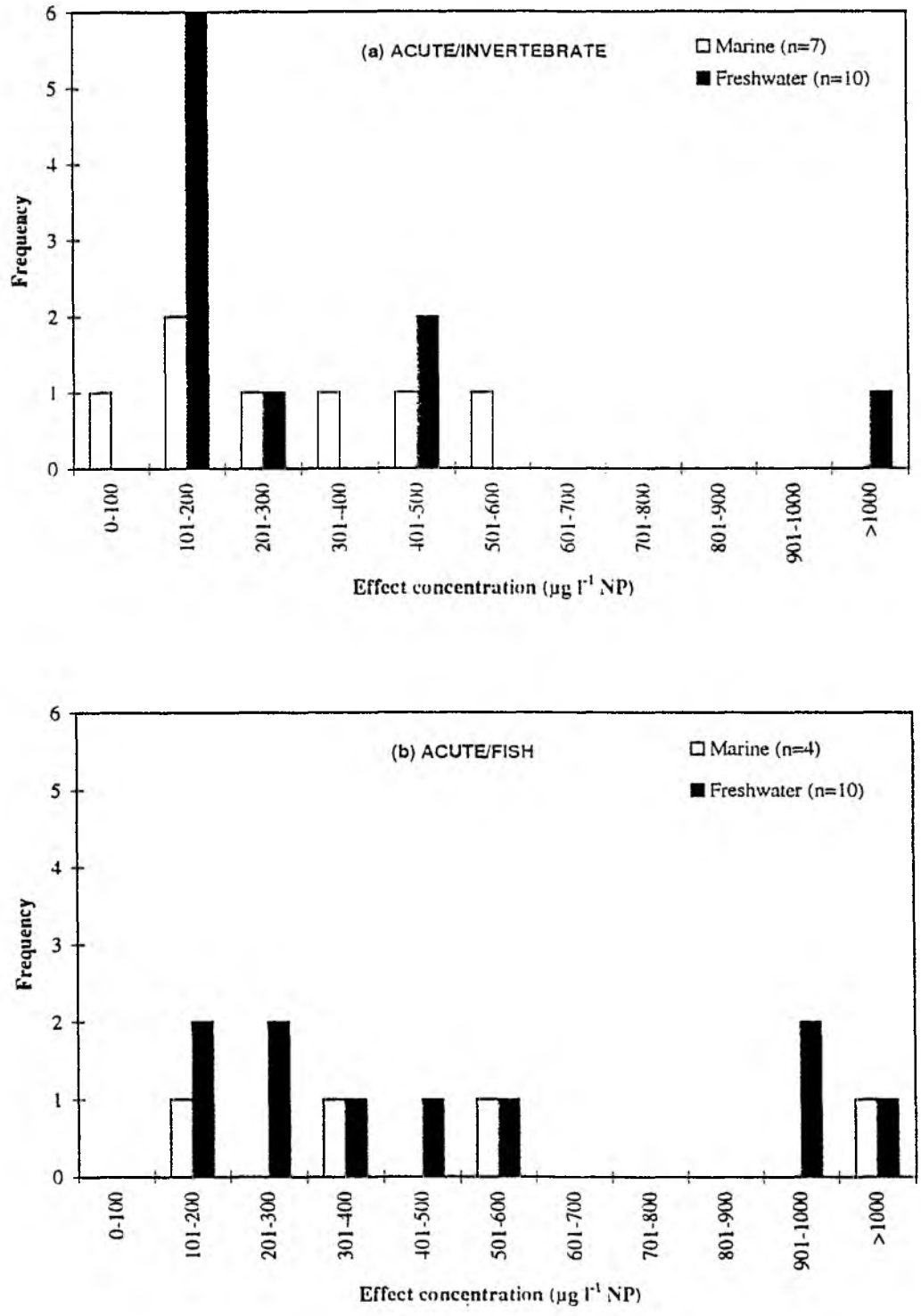


Figure 6.1 Distribution of effect concentration (EC_{50} or LC_{50}) using nonylphenol in acute studies with (a) fish and (b) invertebrates

Table 6.2 Proposed standards for nonyl phenol

	Proposed standards ($\mu g\ l^{-1}$)	
	Annual Average (AA)	Maximum Acceptable Concentration (MAC)
Protection of freshwater life	1.0	10.0
Protection of saltwater life	1.0	5.0
Potable supply	-	-

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APPENDIX A FATE AND BEHAVIOUR IN THE ENVIRONMENT

Sorption to sediments and biodegradation are the predominant mechanisms determining the fate of nonylphenolic compounds in rivers. Nonylphenol and nonylphenol mono- and diethoxylates show significant association with sedimentary material due to their hydrophobicity. The physico-chemical processes of hydrolysis, volatilisation and photolysis are not likely to be significant loss processes for nonylphenol or its ethoxylates in surface waters. Since NP is of low solubility and low volatility it is immobile in soils, even in those of low organic carbon content. As a consequence its potential for leaching and uptake by crop plants is very limited.

A1. FATE AND BEHAVIOUR IN SOIL AND SLUDGE

Since NP occurs in the stabilised sludges of sewage treatment works it is important to ascertain the ultimate fate of these compounds following sludge disposal. Several methods of disposal are currently used, the most common being incineration, landfill, application to agricultural land and discharge to the sea.

A1.1 Sludge

A level of 50 mg carbon l^{-1} of p-nonylphenol in sludge under anaerobic conditions has been reported not to be degraded over a period of 60 days (Battersby and Wilson 1989).

A1.2 Sludge Amended Soils

Nonylphenol is degraded under the aerobic conditions found in soil. A level of 275 mg kg^{-1} nonylphenol in soil was found by Trocme *et al,* (1986) to be 95% degraded in 48 days. In other trials 100 ppm nonylphenol in soil showed 89% degradation within 40 days. However, at a level of 1000 ppm only 60% of the nonylphenol was degraded. At this level respiration was inhibited after four days indicating an effect on the soil micro-organisms (Trocme *et al*, 1988).

Sweetman *et al* (1991) quantified the level of 4-NP in two soils. One a sludge amended soil, and the other a control soil to which no sludge had been applied. The levels in the sludge amended soil (range 340-252, average 296 μg kg^{-1} dry weight) were approximately twice that of the control site (range 117-216, average 166 μg kg^{-1} dry weight). A study to determine the uptake of 4-NP into plants from sludge amended soil was also conducted by Sweetman *et al* (1991). The initial 4-NP soil concentration was 8000-9000 μg kg^{-1}. After six weeks the 4-NP had been extensively degraded, in 25% of the soil samples the concentrations were below the detection limit of 200 μg kg^{-1}, although in one sample it was still at 1000 μg kg^{-1}. Uptake by the plants was very limited. A later study by Sweetman *et al* (1994) determined a half-life of 22 days for the disappearance of 4-NP from sludge amended soil in a 'pot' scale experiment.

The degradation of NP, NP1EO and NP2EO in a sludge-treated experimental plot was monitored over a period of one year by Marcomini *et al* (1989). The initial concentrations of the three metabolites in the soil were 4.7, 1.1 and 0.1 mg kg^{-1} (dry weight). One year later the residual concentrations were 0.5, 0.1 and 0.01 mg kg^{-1}, respectively. Greater than 80% of the biodegradation took place within the first month.

The Swedish Institute of Environmental Medicine recently completed a risk evaluation of the organic contents of sewage sludge. It was noted that NP did not accumulate in soil nor leach to groundwater. Furthermore, the uptake of NP by crops and animals appeared to be low. In Sweden, NP together with toluene, PAH and PCBs are the four organic indicator substances that are used on biosolids (sewage sludge of acceptable quality). An agreement between the Swedish Water and Wastewater Association, the farmers organisation and the Swedish Environment Agency limits the level of NP in sludge for spreading on farmland to 100 mg kg^{-1} Total Solids(TS). The average NP content of sludge in Sweden is 65 mg kg^{-1} TS with 84% of sewage sludges having mean annual values that meet the required level for spreading to land (Pers. Comm. H. Westerlund (1996) Swedish Water and Wastewater Association).

A1.3 Landfill

NP and NP1EO were detected in the leachate from a landfill containing tannery sludge. The authors suggested that conditions favourable to the mobilisation of NP may occur during the initial transient acetogenic stage of a landfill (Ballarin *et al* 1989). In another study, a number of sludge-only landfills ranging from 0.5 to 30 years old were investigated for NP and NP1EO. The levels showed remarkable consistency over the different ages, which suggested that little of the compounds had been lost. This suggests that NP in sludges disposed of to landfill are unlikely to enter the aquatic environment. However, if a landfill becomes aerobic, as it may do in the longer term, NP will be ultimately degraded (Marcomini *et al* 1991).

A2. FATE AND BEHAVIOUR IN WATER

Biodegradation and sorption to sediment are the predominant mechanisms determining the fate of nonylphenolic compounds in surface waters whereas volatisation and hydrolysis are only minor fate processes.

A2.1 Abiotic processes

There is evidence that NP can be lost from surface water by photolysis. In filtered lake water Ahel *et al* (1994b) found that the half-life of nonylphenol in the surface layer exposed to strong sunlight was 10-15 hours at 14.5-17 °C. The rate of photolysis was found to be dependent on both turbidity and dissolved organic carbon concentrations and to be significantly reduced with increasing depth. The rate was approximately 1.5 times slower at depths of 20 - 25 cm below the surface. Therefore under environmental conditions the rate of photolysis of NP in turbid river waters or reservoirs will be significantly longer than 10-15 hours.

A2.2 Biodegradation

There are very few data relating to the biodegradation of NP in the aquatic environment. It appears that NP can be fully mineralised under aerobic conditions. However, it is formed under anaerobic conditions where it is persistent. Due to the hydrophobic nature of NP, sorption to sediments is a far more important process for its removal from water than biodegradation (Williams *et al.* 1996).

Most studies (e.g. Cady *et al.* 1995) have investigated the biodegradation of the parent NPEs as most of the NP is in the environment is produced by incomplete biodegradation. An outline of this pathway is provided (see Figure A2.1).

The primary biodegradation of NPEs is well established. Giger *et al* (1984) showed that the biodegradation of NPEs under aerobic conditions occurs by progressive microbial cleavage of ethoxylate chains to short (1-3) chain nonylphenol ethoxylates and carboxylates (see Figure A2.1). The short (1-3) chain nonylphenol ethoxylates and carboxylates may be further biodegraded to NP which in turn can be degraded to full mineralisation. However, under anaerobic conditions, NP is not biodegradable.

A2.3 Sorption

NP and nonylphenol mono-and diethoxylates are hydrophobic molecules. Therefore sorption to particulate matter and sediments will be the predominant mechanism for their removal from the water column. A study of Swiss rivers by Ahel *et al* (1994b) found that the ratios of nonylphenol concentrations in river sediments to that in water ranged from 364 to 5100. This can be explained by both its affinity for sediments and their resistance to anaerobic biodegradation. In the majority of sediments nonylphenol was the predominant compound.

In a laboratory study simulating field conditions 1000 $\mu g\, l^{-1}$ NP was applied to stream and pond water. Four days after application only 5% of the original NP was detected in the water column whilst 50% of the added material was present in the sediment. Of this about 80% was aerobically degraded within 70 days (Sundaram and Szeto 1981).

A2.4 Volatility

The physico-chemical properties of NP, in particular the low Henry's law constant, suggest that volatilisation from surface waters is unlikely to be a significant loss processes for nonylphenol or its ethoxylates. However, studies with stream and pond water in open and closed flasks by Sundaram and Szeto (1981) showed that the half-life for the disappearance of 1000 $\mu g\, l^{-1}$ NP was 2.5 days in open- and 16 days in closed flasks incubated at 16 °C. The authors suggested that the disappearance of NP in the open system was due to volatilisation.

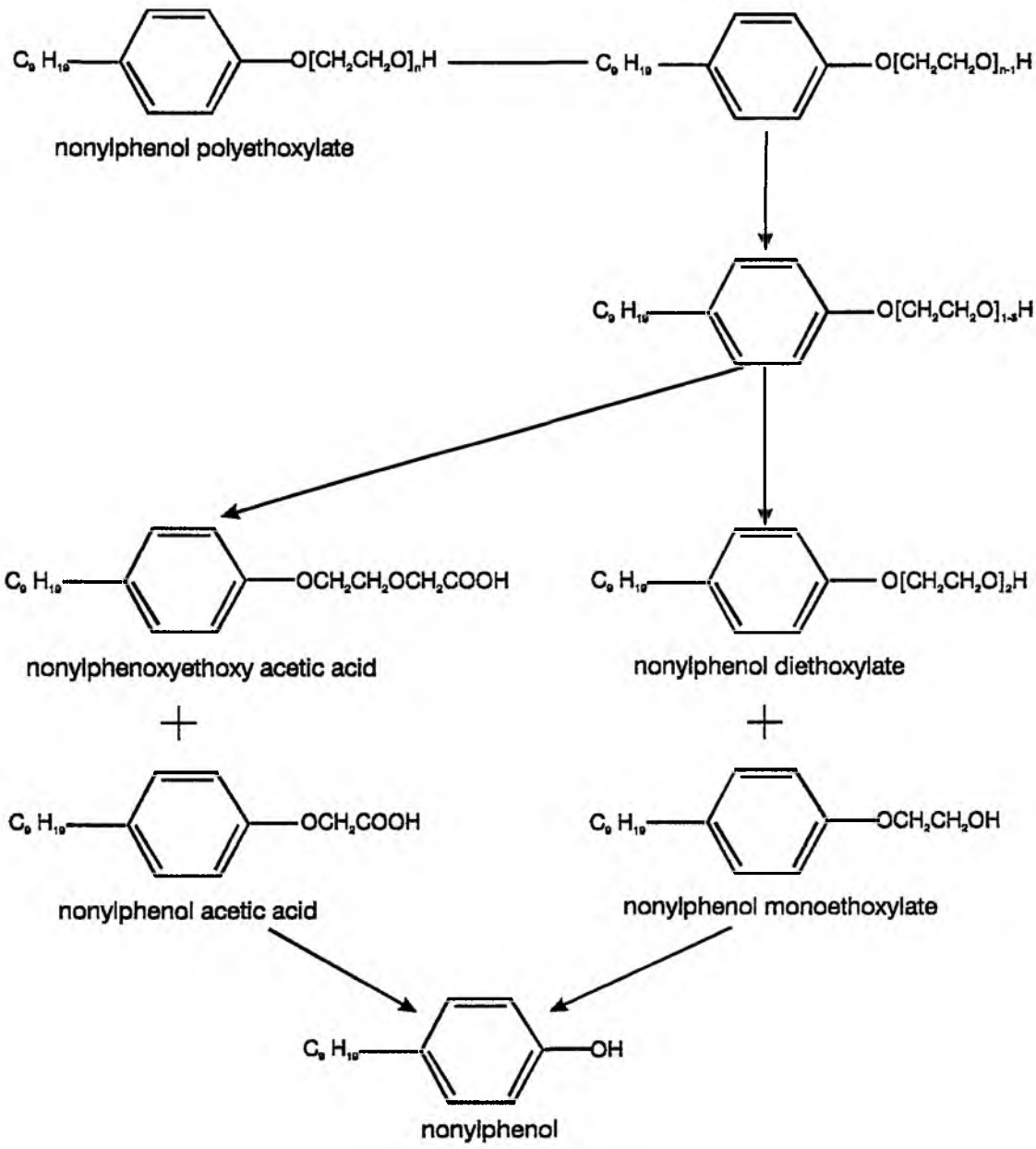

Figure A2.1 Degradation pathway of nonylphenol polythoxylates

The rate of biodegradation of alkylphenol ethoxylates decreases with:

1. increasing ethoxylate chain length;
2. increasing carbon chain length plus ethoxy chain length;
3. change of substitution site from the *para* to *meta* to *ortho* position (Giger *et al*, 1984)

A3. BEHAVIOUR IN SEWAGE TREATMENT PROCESSES

The primary source of NP in the aquatic environment is due to the incomplete biodegradation of NPE surfactants during sewage treatment. Although NPEs undergo extensive primary biodegradation, this requires acclimation of sewage treatment plants. With proper acclimation both linear and branched NPEs undergo almost complete primary biodegradation. However, full biodegradation in most sewage treatment plants is incomplete and therefore most sewage effluents contain many primary degradation by-products. Temperature is an important variable. Biodegradation and effective removal of NP and other NPE degradation by-products from effluents is less efficient at temperatures below 10-15 °C.

Ahel *et al* (1994c) studied 11 Swiss sewage treatment plants and showed that the average elimination rate for all nonylphenolic compounds was around 70% based on BOD, 90% based on weight and around 59% on a molar basis.

However, this elimination did not indicate ultimate biodegradation, but transformation into products which are more resistant to further microbial degradation. Elimination efficiency was related to number of EO groups of the NPE (see Table A3.1 below). $NPn_{3-20}EO$ were efficiently eliminated during biological treatment but $NP_{1-2}EO$ showed no net elimination (between -18 and 80%), indicating that their formation from nonylphenol polyethoxylates (NPnEO) compounds during activated sludge treatment was faster than their degradation. Furthermore, concentrations of NP1EC and NP2EC in secondary effluents were two to seven times higher than in primary effluents indicating significant formation of these compounds during aerobic biological treatment.

Table A3.1 Elimination efficiencies of different NPEs from Swiss STWs (Ahel *et al*, 1994c)

	Elimination Efficiency (loss from aqueous phase)
$NPn_{8-17}EO$	88-90%
$NPn_{>6}EO$	98%
NPn_6EO	78%
NPn_3EO	21%
$NP_{1-2}EO$	-18 - 80%
NP	9 - 94%
NPEs overall	59%

Ahel *et al* (1994c) estimated that 60-65% of all nonylphenolic compounds (on a molar basis) introduced to sewage treatment processes are discharged to the environment, and 60% of these are composed of NP, NP1EO and NP2EO. However, the relative abundance of different metabolites is highly dependent on the treatment conditions. Table A3.2 shows estimated average compositions of nonylphenolic compounds in effluents from 11 Swiss sewage treatment plants and the relative proportions of nonylphenolic compounds discharged into the environment.

Table A3.2 Relative proportions of nonylphenolic compounds during sewage treatment and discharged into the environment from sewage treatment plants Ahel *et al* (1994c)

	Primary effluents	Secondary effluents	Digested sludge	Discharged to the environment
NP	7%	8%	95%	25%
NP1EO + NP2EO	20%	26%	-	11%
NP1EC + NP2EC	5%	48%	5%	19%
NPnEO	68%	20%	-	8%

Brunner *et al* (1988) studied the behaviour of NPE through a single Swiss sewage treatment plant. There was greater than 80% removal of NP from the aqueous phase during activated sludge treatment. Most of the NP entering the activated sludge plant was determined to be bound to the sludge. Brunner *et al* (1988) also investigated the fluxes of NP and NP1EO and NP2EO through 29 similar plants. About 50% of the NPE in influents were transformed to NP and accumulated in the digested sewage sludge. The NP was not significantly degraded under anaerobic conditions but was 99% associated with sludge particulate matter.

A3.1 Temperature

Several studies have investigated the effect of temperature on the removal efficiency of alkylphenol ethoxylates by activated sludge plants. Stiff and Rootham (1973) suggested that a stable population of bacteria able to break down alkylphenol ethoxylates could not be maintained below 15 °C in their laboratory studies. This was supported by findings by Rudling and Solyom (1974) who investigated the degradation of a range of NPEs using the OECD screening test and a modification of the OECD confirmatory test. In the screening tests they reported NP2EO as a major degradation product. The NP2EO was 50% degraded after incubation at 20 °C for 28 days but not degraded at 15 °C.

Ahel *et al* (1994b) noted distinct seasonal variations in the concentrations and profiles of NPE compounds in river waters in Switzerland. In February when the water temperature was 4.8-5.6 °C, levels of NP, and (NP1EO + NP2EO) were around 2 and 20 $\mu g\, l^{-1}$, respectively. This compares to levels in August of approximately 3 $\mu g\, l^{-1}$ for NP, 10 $\mu g\, l^{-1}$ for NP1EO and NP2EO and 70 $\mu g\, l^{-1}$ for NP1EC and NP2EC when the water temperature was 18-20 °C. Although the seasonal variability was not significant for NP, the overall flow of NP, NP1EO and NP2EO showed a 30% decrease in summer, indicating a markedly higher elimination efficiency in summer (see Table A3.3)

Table A3.3 Temperature dependence of elimination efficiency of nonylphenolic compounds from a sewage treatment plant (Ahel *et al* 1994c).

	Elimination rate (%)		
Temperature (°C)	NP	NP1EO	NP2EO
10-13	65	18	-2
18	75	40	25
20	82	72	47

A4 PREDICTED FUTURE OCCURRENCE OF NP IN THE ENVIRONMENT

A model was used by CES (Consultants in Environmental Sciences Ltd) to predict the fate and distribution of NPE in the environment over the ten year period 1992 - 2001. This illustrates the likely impact of the expected changes in NPE usage and, due to the EC Urban Wastewater Directive (91/271/EC), changes in the treatment of sewage effluents and disposal of sewage sludges. The results of the ten year predictions are summarised in Table A4.1.

It is estimated that the annual consumption of NPEs in the UK will decline from 17 600 tonnes as of 1992 to 2150 tonnes by the year 2001. It was estimated that, in 1992, 14 670 tonnes of NPEs (83% of that used) entered the environment. This input is predicted to reduce to about 1500 tonnes per annum by the year 2001 (CES 1993).

Table A4.1 Ultimate fate of NPEs in the environment (CES 1993)

	NPEs Mass (Tonnes)		
	1992	**1996**	**2001**
River	2810	960	10
Estuary	1920	520	0
Sea	1770	340	0
Landfill	4680	2650	1530
Soil	3490	1240	10
Disposal Routes of NPEs from Industry 1992 -2001			
	1992	**1996**	**2001**
Discharge to sewer	10 690	3580	30
Discharge to river	10	10	10
Landfill	4310	2550	1530
Agriculture	2010	660	0
Disposal Routes of NPEs after Sewage treatment			
	1992	**1996**	**2001**
Discharge to River	2810	960	10
Discharge to estuary	1920	520	0
Discharge to sea	880	200	0
Sludge	2950	1090	10
Disposal Routes of NPEs in Sewage sludge			
	1992	**1996**	**2001**
Landfill	370	100	0
Agriculture	1490	570	10
Incineration	210	170	0
Dumping at sea	890	100	0

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APPENDIX B FRESHWATER TOXICITY AND BIOACCUMULATION

B1. FRESHWATER TOXICITY

The biodegradation of nonylphenolic ethoxylate surfactants during sewage treatment is incomplete. Consequently, sewage effluents discharged to surface waters can contain a range of nonylphenolic degradation by-products, including nonylphenol (NP). Since NP is unlikely to be present in the aquatic environment in the absence of other NPE degradation by-products, the toxicity of these (i.e. nonylphenol mono- and diethoxylates (NP1EO and NP2EO), nonylphenoxy carboxylic acids (NPEC) has also been considered.

Although there are limited toxicity data on NP1EO, NP2EO, NP1EC or NP2EC, the toxicity of NPEs decreases with increasing ethylene oxide units. This is consistent with their decreasing fat solubility. NP being the most lipophilic of the NPE metabolites appears to be the most toxic. Data on the acute and chronic toxicity of NP to freshwater organisms are presented in Table B1.1 and Table B1.2, respectively.

Table B1.1 Acute toxicity of nonylphenol to freshwater organisms

Species	Exposure	Effect	Conc. ($\mu g\ l^{-1}$)	Notes	Ref.
BACTERIA					
Pseudomonas putida		EC10 (not specified)	16000		15
Pseudomonas putida		EC10 (not specified)	10000		16
ALGAE					
Selenastrum capricornutum	96 hr	EC50 (growth rate)	410	Branched-4-NP Static, unaerated, GLP, measured	7
Scenedesmus subspicatus	72 hr	EC10 (biomass)	500		17
Scenedesmus subspicatus	72 hr	EC50 (biomass)	1300		17
Scenedesmus subspicatus	72 hr	EC90 (biomass)	3200		17
Chlamydomonas reinhardii	-	Photosynthesis inhibition	750		8
Chlamydomonas reinhardii	1 hr	Cell membrane disruption	500-700		13
Chlorella pyrenoidosa	-	Growth depression	25 - 7500		13
Chlorella pyrenoidosa	24 hr	LC50	1500		13
Chlorella pyrenoidosa	-	LC100	25000		13
MACROPHYTES					
Duckweed	96 hr	Growth inhibition	500		5
INVERTEBRATES					
Water flea (*Daphnia magna*)	24 hr	EC50 (Immobilisation)	180	4-nonylphenol	10
Daphnia magna	24 hr	EC50 (Immobilisation)	300	Measured, GLP, Semi-static	6
Daphnia magna	48 hr	EC50 (Immobilisation)	190	Measured, GLP, Semi-static	6
Daphnia magna	48 hr	NOEC	< 100	GLP	18
Daphnia magna	48 hr	EC50	140	GLP	18
Daphnia magna	48 hr	EC100	> 400	GLP	18
Daphnia magna	48 hr	LC50	440		11
Daphnia pulex	48 hr	LC50	140-190	static	4
Cladoceran (*Ceriodaphnia dubia*)	48 hr	LC50	470	nonylphenol	12
Cladoceran (*Ceriodaphnia dubia*)	48 hr	LC50	1040	NP1EO + NP2EO	12
Cladoceran (*Ceriodaphnia dubia*)	48 hr	EC50	>300	static, renewal, nominal	21
Cladoceran (*Ceriodaphnia dubia*)	48 hr	LC50	>300	static, renewal, nominal	21
Cladoceran (*Ceriodaphnia dubia*)	96 hr	EC50	>300	static, renewal, nominal	21
Cladoceran (*Ceriodaphnia dubia*)	96 hr	LC50	>300	static, renewal, nominal	21
Amphipod (*Hyalella azteca*)	96 hr	EC50 (not specified)	150	GLP, measured	19
Amphipod (*Hyalella azteca*)	96 hr	LC50	170	GLP, measured	19
Midge (*Chironomus tentans*)	96 hr	EC50 (not specified)	160	GLP, measured	22
Midge (*Chironomus tentans*)	96 hr	MATC	97	GLP, measured	22

Table B.1.1. Continued

Species	Exposure	Effect	Conc. ($\mu g\ l^{-1}$)	Notes	Ref.
FISH					
Ide (*Leuciscus idus*)	48 hr	LC0	800	Static	20
Ide (*Leuciscus idus*)	48 hr	LC50	950	Static	20
Ide (*Leuciscus idus*)	48 hr	LC100	1100	Static	20
Fathead minnow (*Pimephales promelas*)	48 hr	LC50	164	Flow through, measured	14
Fathead minnow (*Pimephales promelas*)	72 hr	LC50	137	Flow through, measured	14
Fathead minnow (*Pimephales promelas*)	96 hr	LC50	135	Flow through, measured	14
Fathead minnow (*Pimephales promelas*)	96 hr	LC50	300		3
Fingerling brook trout (*Salvelinus fontinalis*)	96 hr	LC50	145		9
Rainbow trout (*Oncorhyncus mykiss*)	24 hr	LC50	484	static	4
Rainbow trout (fingerling) (*Oncorhyncus mykiss)*	96 hr	LC50	960		4
Rainbow trout (fingerling) (*Oncorhyncus mykiss*)	96 hr	LC50	960		4
Rainbow trout (fingerling) (*Oncorhyncus mykiss*)	96 hr	LC50	230		9
Rainbow trout (embryos/ juveniles) (*Oncorhyncus mykiss*)	48 h	LC50	480		4
Rainbow trout (embryos/ juveniles) (*Oncorhyncus mykiss)*	96 h	LC50	560 - 920		4
Killifish (*Oryzias latipes*)	48 hr	LC50	1400		2.
Stickleback (*Gasterosteus aculeatus*	96 hr	LC50	400	4-NP	1

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19. CMA (1994)
20. Huels (1988)
21. CMA (1995)
22. CMA (1993)

Table B1.2 Chronic toxicity of nonylphenol to freshwater organisms

Species	Exposure	Effect	Conc. (µg l^{-1})	Notes	Ref.
INVERTEBRATES					
Midge (*Chironomus tentans*)	14 day	EC50 (various)	95 (dosed water)	GLP, Measured	5
Midge (*Chironomus tentans*)	14 day	LC50	119 (dosed water)	GLP, Measured	5
Midge (*Chironomus tentans*)	14 day	MATC	107 (dosed water)	GLP, Measured	5
Midge (*Chironomus tentans*)	14 day	LC50	75 - >252 (interstitial water)	GLP, Measured	5
Midge (*Chironomus tentans*)	14 day	MATC	56 - 190 (interstitial water)	GLP, Measured	5
Midge (*Chironomus tentans*)	14 day	LC50	> 34000 ug kg^{-1} (dosed sediment)	GLP, Measured	5
Midge (*Chironomus tentans*)	14 day	MATC	> 26100 ug kg^{-1} (dosed sediment)	GLP, Measured	5
CRUSTACEANS					
Daphnia magna	7 day	LC50	120	GLP, Measured, semi-static	1
Daphnia magna	14 day	LC50	120	GLP, Measured, semi-static	1
Daphnia magna	21 day	LC50	100	GLP, Measured, semi-static	1
Daphnia magna	21 day	NOEC (reproduction)	24	GLP, Measured, semi-static	1
Daphnia magna	21 day	NOEC (growth)	39	GLP, Measured, semi-static	1
Ceriodaphnia dubia	7 day	EC50 (mortality, size)	27	static, renewal, nominal	6
Ceriodaphnia dubia	7 day	LC50 (reproduction)	>300	static, renewal, nominal	6
Ceriodaphnia dubia	7 day	NOEC (reproduction)	100	static, renewal, nominal	6
Ceriodaphnia dubia	7 day	LOEC	300		
Ceriodaphnia dubia	7 day	MATC (Reproduction)	170	static, renewal, nominal	6
MOLLUSC					
Freshwater clam (*Anodonta cataractae*)	6 day	LC50	5000		4
Branched 4-Nonylphenol					
FISH					
Fathead minnow (*Pimephales promelas*)	33 day	NOEC (survival)	7.4	Measured, GLP Flow through, unaerated	2
Fathead minnow (*Pimephales promelas*)	33 day	LOEC (survival)	14	Measured, GLP Flow through, unaerated	2

Continued overleaf

Table B.1.2. continued

Species	Exposure	Effect	Conc. (µg l^{-1})	Notes	Ref.
Fathead minnow (*Pimephales promelas*)	33 day	MATC (survival)	10.2	Measured, GLP Flow through, unaerated	2
AMPHIBIANS					
Bullfrog Tadpole (*Rana catesbiana*)	30 days	NOEC (weight & mortality)	155 mg kg^{-1} (dosed sediment)	GLP	3
Bullfrog Tadpole (*Rana catesbiana*)	30 days	EC50 (weight & mortality)	220 mg kg^{-1} (dosed sediment)	GLP	3
Bullfrog Tadpole (*Rana catesbiana*)	30 days	LC50	260 mg kg^{-1} (dosed sediment)	GLP	3
Bullfrog Tadpole (*Rana catesbiana*)	30 days	LOEL (weight & mortality)	390 mg kg^{-1} (dosed sediment)	GLP	3
Bullfrog Tadpole (*Rana catesbiana*)	30 days	MATC	250 mg kg^{-1} (dosed sediment)	GLP	3

References:
1. Comber *et al* (1993)
2. CMA (1991)
3. CMA (1992)
4. McLeese *et al* (1980)
5. CMA (1993)
6. CMA (1995).

B1.1 Bacteria

Knie *et al* (1983) studies the effect of 4-NP on a population of *Pseudomonas putida*. They reported an EC_{50} of >10 000 µg l^{-1}. 4-Nonylphenol inhibited germination of *Bacillus megaterium* (99% inhibition within 2 hr) at 32 000 µg l^{-1} (Lewis and Jurd 1972).

A level of 50 mg carbon/l of p-nonylphenol in sludge under anaerobic conditions was considered to be inhibitory to methanogenesis (Battersby and Wilson 1989). However, Giger *et al* (1984) reported that concentrations of up to 0.14 g kg^{-1} NP, NP1EO and NP2EO in activated sludge, mixed primary and secondary sludge had no adverse effects on sewage bacteria.

At a concentration of 1000 ppm nonylphenol in soil only 60% of the nonylphenol was degraded. At this level respiration was inhibited after four days indicating an effect on the soil micro-organisms (Trocme *et al*, 1988).

B1.2 Protozoa

No data located.

B1.3 Algae

The effects of NP on the ultra-structure, photosynthetic activity and growth of several species of freshwater algae have been investigated. A concentration of 410 $\mu g\ l^{-1}$ branched *4-NP* depressed growth of *Selenastrum capricornutum* (CMA 1990), whilst most EC_{50} values for growth are greater than 1000 $\mu g\ l^{-1}$ (IUCLID 1996, Weinberger and Rea, 1981).

At concentrations of 500 to 700 $\mu g\ l^{-1}$ in the nutrient media, the flagellae and ultra-structural architecture of the cell membranes of *Chlamydomonas reinhardii* were disrupted (Weinberger and Rea 1981). Photosynthetic activity was inhibited by 55% at 500 $\mu g\ l^{-1}$ NP and by 100% at 750 $\mu g\ l^{-1}$ (Moody *et al* 1983).

Weinberger and Rea (1981) exposed cultures of *Chlorella pyrenoidosa* to NP concentrations of 25 -25 000 $\mu g\ l^{-1}$. At concentrations from 25 $\mu g\ l^{-1}$ and upwards the exponential growth was depressed, while at 25 000 $\mu g\ l^{-1}$ 100% mortality was achieved. From these values a 24 hr LC_{50} of 1500 $\mu g\ l^{-1}$ was calculated.

B1.4 Macrophytes

Because they improve wetting, penetration, absorption and water solubility characteristics, surfactants, including NP, are used in the formulation of foliar-applied agrochemicals as stabilising, emulsifying and dispersing agents.

A concentration of 20 000 $\mu g\ l^{-1}$ NP inhibited the growth of paper birch (*Betula papyrifera*), seedlings. A concentration of 100 000 $\mu g\ l^{-1}$ significantly depressed seed germination of jackpine (*Pinus banksiana*) and paper birch and depressed seedling growth of jackpine (Weinberger and Vladut 1981).

Concentrations of 500 - 5000 $\mu g\ l^{-1}$ NP had a small effect on the growth (frond multiplication) of cultures of the aquatic vascular plant *Lemna minor,* when treated once and observed for six days. Under the same conditions, concentrations of 2500-25 000 $\mu g\ l^{-1}$ were toxic to another macrophyte, *Salvinia molesta*; treated cultures were dead by Day 9. When cultures of *Lemna minor* were treated every day for four days, concentrations of 125-250 $\mu g\ l^{-1}$ produced significant effects on growth. A concentration of 500 $\mu g\ l^{-1}$ NP was lethal (Prasad, 1989)

B1.5 Invertebrates

There are limited toxicity data available on the toxicity of NP1EO, and NP2EO to aquatic invertebrates. The toxicity of NPEs decreases with increasing ethylene oxide units, which is consistent with their increasing water solubility. However, NP being the most lipophilic appears to be the most toxic metabolite (see Table B1.3). The 48 hr LC_{50} of NP to *Ceriodaphnia dubia* is between 300 and 470 $\mu g\ l^{-1}$ whereas NP1EO and NP2EO produce equivalent values of around 1040 $\mu g\ l^{-1}$ (Ankley *et al* 1990, CMA 1995, 1995a). It should be noted that although a LOEC of 10 $\mu g\ l^{-1}$ was reported for survival of *Ceriodaphnia dubia* for nonyl phenol, a clear dose response was not observed. The results are also somewhat erratic considering that the same study reported a higher LOEC of 300 $\mu g\ l^{-1}$ for effects on reproduction. Although CMA (1995) report an acute EC_{50} of 69 $\mu g\ l^{-1}$, the endpoints on which this effect concentration comprise miscellaneous abnormalities such as 'paleness', 'surfacing' and 'trailing extraneous material', whose toxicological significance is unclear.

Table B1.3 Comparison of Toxicity of NP and $NP_{1.5}EO$ to *Ceriodaphnia dubia*

Effect	Exposure	NP ($\mu g\ l^{-1}$)	$NP_{1.5}$ EO ($\mu g\ l^{-1}$)	Ref.
LC_{50}	48 hr	470	1040 ($NP_1EO + NP_2EO$)	1
EC_{50}	48 hr	>300	~ 1000	2,3
LC_{50}	48 hr	>300	~ 1000	2,3
EC_{50}	96 hr	>300	~ 1000	2,3
LC_{50}	96 hr	>300	~ 1000	2,3
Mortality				
NOEC	7 day	3	300	2,3
MATC	7 day	5.5	550	2,3
LOEC	7 day	10	1000	2,3
EC_{50} (mortality, size)	7 day	27	460	2,3
LC_{50}	7 day	>300	600	2,3
Reproduction				
NOEC	7 day	100	100	2,3
MATC	7 day	170	170	2,3
LOEC	7 day	300	300	2,3

References:

1. Ankley *et al* (1990)
2. CMA (1995)
3. CMA (1995a)

B1.6 Fish

NP is highly toxic to freshwater fish with LC_{50} values generally below 1000 $\mu g\ l^{-1}$. The most sensitive species is the fathead minnow, (*Pimephales promelas*) for which a 96 hr LC_{50} of 135 $\mu g\ l^{-1}$ is reported. It is also highly sensitive in chronic tests. In a 33 day test a NOEC (for survival) of 7.4 $\mu g\ l^{-1}$ was determined (CMA 1991). Due to the concerns regarding the oestrogenicity of NP, further chronic tests have been conducted to investigate the possible effects on fish.

B1.6.1 Oestrogenicity

Investigation of anecdotal evidence of hermaphrodite roach (*Rutilis rutilis*) in rivers receiving sewage effluents led to the discovery that sewage effluents tested contained oestrogenically active components as determined by vitellogenin induction in male rainbow trout (Harries *et al.* 1995 and 1996). More recent studies using caged male rainbow trout placed in rivers receiving effluents from sewage treatment works also exhibited induction of vitellogenin, most noticeably in a river receiving trade effluent from wool-scouring mills (Harries *et al.* 1997). Vitellogenin is an egg yolk protein that is normally only found in mature female fish but exposure to oestrogenic substances has been found to induce its production in male fish.

In an *in vitro* bioassay using trout liver hepatocytes, the ED_{50} (effective dose) for oestrogenic activity was determined to be around 3500 $\mu g\ l^{-1}$ for various nonylphenolic compounds (including nonylphenol, NP2EO, NP9EO and NP1EC) (Jobling and Sumpter 1993). The lowest concentration of NP producing a response was 10 μmol, (2200 $\mu g\ l^{-1}$). This compares with the ED_{50} value of 492 $ng\ l^{-1}$ for 17β-oestradiol. Data reported by Harries *et al* (1995) and Jobling *et al* (1996) surprisingly indicate that lower levels of nonylphenolic compounds were needed to induce vitellogenesis in fish *in vivo* (20-30 $\mu g\ l^{-1}$) compared to *in vitro* (2200-3500 $\mu g\ l^{-1}$). It was suggested that this could have been an artefact due to the different periods of exposure of the two systems. Exposure to the compounds was only two days in the *in vitro* assay but was three weeks in the *in vivo* test. Nevertheless the data indicate that the levels of nonylphenolic compounds generally present in surface waters, and in most sewage effluents (around 1 $\mu g\ l^{-1}$), are below the levels required to induce vitellogenesis in fish *in vivo*, assuming they act alone.

Whilst the rainbow trout is a very sensitive test organism for vitellogenesis, it is not an indigenous species to the UK and therefore may not be representative of British fish species. A subsequent survey, reported by Purdom *et al* (1994), used carp rather than rainbow trout to determine the oestrogenicity of sewage treatment work effluents. Of the ten sites which had originally been shown to produce vitellogenin in rainbow trout, only three induced raised vitellogenin levels in carp. Table B1.4 shows how much less sensitive carp are to oestrogenic induction of vitellogenesis than rainbow trout. In addition, significant induction of a testis-ova intersex condition have also been reported in Japanese medaka (*Oryzias latipes*) following exposure to 100 $\mu g\ l^{-1}$ nonylphenol. Although shifts in the male:female ratio were evident at 50 $\mu g\ l^{-1}$, these were not statistically significant (Gray and Metcalfe 1997).

Table B1.4 Comparison of induction of vitellogenin in Carp and Trout bioassays over 10 day exposure period (Purdom *et al* 1994)

17 α ethinyl oestradiol Dose ($ng\ l^{-1}$)	Mean plasma vitellogenin ($\mu g\ l^{-1}$)	
	Carp	Trout
Control	<0.01	1.0
1	<0.01	-
10	0.15	630
25	0.84	4970
50	216	11200

Recent studies have also investigated the oestrogenic effects on other endpoints in fish. Jobling *et al* (1996) exposed maturing male rainbow trout to up to 54 $\mu g\ l^{-1}$ of NP, NP1EC and NP2EO for three weeks. A NOEC for induction of vitellogenesis of 10 $\mu g\ l^{-1}$ was determined, which was consistent with other studies. Perhaps of greater significance was that 30-54 $\mu g\ l^{-1}$ of each of the compounds was sufficient to retard testicular growth and interfere with spermatogenesis.

Similar studies carried out at the Institute of Freshwater Ecology (IFE) by Ashfield *et al* (1995) have investigated the effects of chronic exposure to nonylphenolic compounds on the growth rate and reproductive status of rainbow trout. Juvenile fish were exposed for 35 days and their growth and reproductive status i.e. the Gonadosomatic index (GSI) observed for up to one year post treatment. Nonylphenol at 10 and 30 $\mu g\, l^{-1}$ caused significant growth suppression of juvenile trout, these effects were still apparent one year after exposure. The NOEC for growth suppression was 1 $\mu g\, l^{-1}$ and the LOEC was 10 $\mu g\, l^{-1}$. In female fish 30 $\mu g\, l^{-1}$ NP significantly increased: the GSI, vitellogenin levels, relative fecundity, progeny survival to hatch and progeny fork length. NP2EO caused a reduction of growth at both 1 and 10 $\mu g\, l^{-1}$, but not at 30 $\mu g\, l^{-1}$. In female fish, 30 $\mu g\, l^{-1}$ NP2EO increased vitellogenin levels, egg volumes and relative fecundity. NP1EC accelerated growth at 1 and 10 $\mu g\, l^{-1}$ but had no effect at 30 $\mu g\, l^{-1}$. These effects were still apparent one year after exposure. The GSI of juvenile trout was significantly decreased at both 1 and 10 $\mu g\, l^{-1}$ NP1EC. In female fish 30 $\mu g\, l^{-1}$ significantly increased vitellogenin levels, egg volume, survival of progeny to hatch and progeny fork length. Exposure of male fish to all treatments at 30 $\mu g\, l^{-1}$ increased vitellogenin levels, but no effects on sperm counts was seen at any treatment, despite the effects on the GSI. Overall it was considered that exposure to alkylphenolic compounds had a positive influence on the reproductive status of female rainbow trout.

A direct link between vitellogenesis and other oestrogenic responses in male fish and fertility, and therefore on population viability has still to be investigated. Nevertheless, such a link is a distinct possibility. Furthermore, vitellogenesis in male fish would have an impact on the energy balance of affected fish and might divert calcium from scales and skeleton. There are also indications of liver enlargement, renal dysfunction and changes to blood viscosity following NP exposure (Matthiesson, pers. comm.).

Structure Activity Relationship

Although some alkylphenols have been shown to possess weak oestrogenic activity, such activity is very sensitive to subtle changes in chemical structure. Substitution on the 4 (*para*) position on the phenol ring appears to be essential for oestrogenic activity. For alkylphenol ethoxylate compounds, oestrogenicity rapidly declines with increasing ethoxylate chain length. Jobling and Sumpter (1993) used the *in vitro* rainbow trout hepatocyte bioassay to compare the oestrogenicity of various alkylphenolic compounds to that of ethinyl oestradiol. The results are presented in Table B1.5.

Table B1.5 Oestrogenic potency of nonylphenolics in the Rainbow trout *in vitro* hepatocyte bioassay (Jobling and Sumpter 1993)

Compound	Relative potency
Ethinyl oestradiol	1.0
Nonylphenol	0.000009
NP2EO	0.000006
NP1EC	0.0000063

White *et al* (1994) investigated the validity of the trout vitellogenin assay by comparing the oestrogenicity of several alkylphenolic compounds in a variety of different *in vitro* bioassays i.e. in human, mouse and fish cells. Each of the compounds tested had the same potency in each of the different bioassays. However, it was demonstrated that the order of oestrogenic potency of a range of nonylphenolic compounds is NP1EC > NP = NP2EO. There are also now data (Arnold *et al* 1996) which indicate that environmental chemicals can act not just additively, but show strong synergistic activity. Mixtures of weak oestrogenic pesticides have been shown to be more than 1000 times more potent than when acting alone. On this basis it is prudent to consider the effects of nonylphenolic compounds together rather than considering NP alone, which in classical ecotoxicity tests appears to be the most toxic NPnEO metabolite.

B2 BIOACCUMULATION

Log K_{ow} values ranging from 3.01 for pure p-nonylphenol and 3.28 for 'technical' nonylphenol, and up to 4.2 for p-nonylphenol have been reported in the literature. These values suggest medium to high bioaccumulation potential. However the BCFs of NP which have been measured in several aquatic animal species vary quite widely. Bioconcentration factors of 13 to 1200-1300 (after 16 days) have been reported (see Table B2.1 below). All studies have found alkylphenols to depurate rapidly over a period of a few days (Ahel *et al* 1993, Ekelund *et al* 1990).

Table B2.1 Bioconcentration factors of nonylphenolic compounds

Species	BCF	Notes	Ref.
MACROPHYTIC ALGAE			
Cladophora glomerata	10,000 (NP)	Adsorption to surface ?	1
Cladophora glomerata	200 (NP1EO)	Adsorption to surface ?	1
Cladophora glomerata	500 (NP2EO)	Adsorption to surface ?	1
FISH			
Heck (*Squalius cephalus*)	246	Field study	1
Fish	13 - 408	Field studies	1
Fish	3 - 300 (NP1EO)	Field studies	1
Fish	3 - 326 (NP2EO)	Field studies	1
Stickleback	1200-1300	Flow through	3
Fathead minnow	271 NP	at 4.9 $\mu g\ l^{-1}$, measured, GLP	2
Fathead minnow	344 NP	at 22.7 $\mu g\ l^{-1}$, measured, GLP	2

References:

1. Ahel *et al* (1993) 2. CMA (1991b) 3. Ekelund *et al* (1990)

In a bioaccumulation test conforming to GLP standards the bioconcentration factor of NP was determined in fathead minnow (*Pimephales promelas*) following exposure over a 20-day period at 22 °C to two different concentrations, 4.9 and 21 $\mu g\ l^{-1}$. The NP tissue concentration increased to a steady state concentration during the first four days of exposure, and increased no further. Exposure to 4.9 $\mu g\ l^{-1}$ resulted in a BCF of 271, whilst exposure to 22.7 $\mu g\ l^{-1}$ resulted in a BCF of 344. Upon removal to clean water, depuration half-lives were 1.4 and 1.2 days, respectively (CMA 1991b). The data from this study indicate that the actual BCF is similar to estimates made using Log K_{ow} or the water solubility and suggest that the potential for bioconcentration of NP by fish is probably low to moderate.

Ahel *et al* (1993) reported the levels of NP, NP1EO and NP2EO determined in different freshwater organisms from the Glatt river in Switzerland. The Glatt river runs through a densely populated area and receives secondary effluents from ten sewage treatment works. High concentrations of NP, NP1EO and NP2EO were found in the macrophytic algae (*Cladophora glomerata*) (up to 38, 4.7 and 4.3 $mg\ kg^{-1}$ dry weight, respectively), with the bioconcentration factor (BCF) of nonylphenol reaching up to 10 000. The authors considered that much of this BCF value could have been due to adsorption of the NP to the surfaces of the algae. The concentrations of NP, NP1EO and NP2EO in various tissues of several species of fish indicated BCF values of 13-410, 3-300 and 3-330, respectively.

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APPENDIX C SALTWATER TOXICITY AND BIOACCUMULATION

C1. SALTWATER TOXICITY

The available acute and chronic toxicity data for nonylphenol to saltwater organisms is given in Tables C1.1 and C1.2, respectively.

Table C1.1 Acute toxicity of nonylphenol to saltwater organisms

Species	Exposure	Effect	Conc. ($\mu g\ l^{-1}$)	Notes	Ref.
ALGAE					
Diatom (*Skeletonema costatum*)	24 hr	EC50 (growth)	34	Branched-4-NP, GLP, Static, unaerated, measured	8
Diatom (*Skeletonema costatum*)	48 hr	EC50 (growth)	40	Branched-4-NP, GLP, Static, unaerated, measured	8
Diatom (*Skeletonema costatum*)	72 hr	EC50 (growth)	30	Branched-4-NP, GLP, Static, unaerated, measured	8
Diatom (*Skeletonema costatum*)	96 hr	EC50 (growth)	27	Branched-4-NP, GLP, Static, unaerated, measured	8
Diatom (*Skeletonema costatum*)	96 hr	EC10 (growth)	12	Branched-4-NP, GLP, Static, unaerated, measured	8
Diatom (*Skeletonema costatum*)	96 hr	MATC (growth)	14	Branched-4-NP, GLP, Static, unaerated, measured	8
CRUSTACEANS					
Mysid shrimp (*Mysidopsis bahia*)	24 hr	LC50	>0.047	branched-4-NP, GLP, FT, measured, unaerated	9
Mysid shrimp (*Mysidopsis bahia*)	48 hr	LC50	>0.047	branched-4-NP, GLP, FT, measured, unaerated	9
Mysid shrimp (*Mysidopsis bahia*)	96 hr	NOEC	18	branched-4-NP, GLP, FT, measured, unaerated	9
Mysid shrimp (*Mysidopsis bahia*)	96 hr	LOEC	30	branched-4-NP, GLP, FT, measured, unaerated	9
Mysid shrimp (*Mysidopsis bahia*)	96 hr	MATC	23	branched-4-NP, GLP, FT, measured, unaerated	9
Mysid shrimp (*Mysidopsis bahia*)	96 hr	LC50	43	branched-4-NP, GLP, FT, measured, unaerated	9
Copepod (*Nitocra spinipes*)	-	LC50	118 - 139	NP	4
Brown shrimp (*Crangon crangon*)	96 hr	LC50	600	4-nonylphenol	5
Brown shrimp (*Crangon crangon*)	96 hr	LC50	420	nonylphenol	1
Sand shrimp (*Crangon septemspinosa*)	96 hr	LC50	300	p-nonylphenol, measured	3
Sand shrimp (*Crangon septemspinosa*)	96 hr	LC50	400	nonylphenol	6
Lobster (*Homarus americanus*)	96 hr	LC50	200	nonylphenol	6

Continued overleaf

Table C.1.1 continued

Species	Exposure	Effect	Conc. ($\mu g\ l^{-1}$)	Notes	Ref.
MOLLUSCS					
Mussel (*Mytilus edulis*)	96 hr	LC50	3000	nonylphenol	7, 5
FISH					
Sheepshead minnow (*Cypridon variegatus*)	96 h	LC50	310	Branched NP, Flow through, unaerated	10
Sheepshead minnow (*Cypridon variegatus*)	96 h	NOEC	240	Branched NP, Flow through, unaerated	10
Hook nose (*Agonus cataphractus*)	96 h	LC50	510		1
Cod (*Gadus morhua*)	96 hr	LC50	3000		2
Atlantic salmon (*Salmo salar*)	96 hr	LC50	130-190	p-nonylphenol, Measured	3

References

1. Waldock and Thain (1991)
2. Swedmark (1968)
3. McLeese *et al* (1981)
4. Bergstrom (1984)
5. Granmo *et al* (1991)
6. McLeese *et al* (1980)
7. Granmo *et al* (1989)
8. CMA (1990a)
9. CMA (1990)
10. CMA (1990b)

Table C1.2 Chronic toxicity to saltwater organisms

Nonylphenol	Exposure	Effect	Concn ($\mu g\ l^{-1}$)	Notes	Ref.
CRUSTACEAN					
Brown shrimp (*Crangon crangon*)	7 day	LC50	340		3
MOLLUSC					
Soft-shelled clam (*Mya arenaria*)	15 day	LC50	>1000		4
FISH					
Hook nose (*Agonus cataphractus*)	7 day	LC50	360		3
Branched-4-nonylphenol					
INVERTEBRATES					
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	NOEC (body length of F1)	3.9	GLP, Measured, FT, unaerated	1
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	LOEC (body length of F1)	6.7	GLP, Measured, FT, unaerated	1
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	MATC (body length of F1)	5.1	GLP, Measured, FT, unaerated	1
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	NOEC (No. & survival of F1)	6.7	GLP, Measured, FT, unaerated	1
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	LOEC (No. & survival of F1)	9.1	GLP, Measured, FT, unaerated	1
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	MATC (No. & survival of F1)	7.8	GLP, Measured, FT, unaerated	1
Juvenile mysid shrimp (*Mysidopsis bahia*)	28 day	LC50	> 21	GLP, Measured, FT, unaerated	1
MOLLUSC					
Mussel (*Mytilus edulis*)	15 day	LC50	500		2
Mussel (*Mytilus edulis*)	35 day	LC50	140		2
Mussel (*Mytilus edulis*)	15-30 day	LOEC (byssus strength)	56		2
Mussel (*Mytilus edulis*)	35 day	NOEC (fertilisation)	200		2

MATC: Calculated as the geometric mean of the NOEC and LOEC.

Reference:

1. CMA (1991a)
2. Granmo *et al* (1989, 1991)
3. Waldock and Thain (1991)
4. McLeese *et al* (1980)

C1.1 Algae

The only data available for marine algae are from a single acute study on the diatom, *Skeletonema costatum* (CMA 1990a). In this study a 96 hr EC_{50} (for growth) of 27 $\mu g\, l^{-1}$ was determined. This indicates that *Skeletonema* is a particularly sensitive species of algae in contrast to the freshwater algae species tested which appear to be relatively tolerant.

C1.2 Macrophytes

No data available.

C1.3 Invertebrates

The most sensitive species tested appears to be the mysid shrimp with a reported chronic LOEC of 6.7 $\mu g\, l^{-1}$ for the body length of the F1 generation after 28 days. A maximum acceptable toxicant concentration (MATC) of 5.1 $\mu g\, l^{-1}$ was calculated (CMA 1991a). In acute tests with this species a 96 hr LC_{50} and NOEC of 43 and 18 $\mu g\, l^{-1}$ have been reported (CMA 1990), which are lower than data from other comparable acute tests for other invertebrates. Each of these tests complied with GLP. In chronic tests on the common mussel (*Mytilus edulis)* a concentration of 56 $\mu g\, l^{-1}$ NP caused sub-lethal effects including decreased byssal strength and change of scope for growth. At a concentration of 100 $\mu g\, l^{-1}$, no byssus thread formation occurred. Fertilisation and early development were not affected by 200 $\mu g\, l^{-1}$, the highest concentration tested (Granmo *et al* 1989, 1991). The acute LC_{50} for this species is 3000 $\mu g\, l^{-1}$.

C1.4 Fish

For all species tested, the 48 hr and 96 hr LC_{50} values for NP ranged from 130 $\mu g\, l^{-1}$ (average measured concentration for juvenile Atlantic salmon (*Salmo salar*) in flow through tests (McLeese *et al* 1980) to 3000 $\mu g\, l^{-1}$ for the cod (*Gadus morhua*) (test conditions not given) (Swedmark, 1968). There are no chronic data.

C2. BIOACCUMULATION

Table C2.1 Bioconcentration factors of nonylphenolic compounds in marine organisms

Species	BCF	Notes	Ref.
MOLLUSC			
Mussel (*Mytilus edulis*)	1.4 - 13	Not constant water concentration	2
Mussel (*Mytilus edulis*)	3430	Constant flow through concentration	3
Mussel (*Mytilus edulis*)	320	Field study	4
Mussel (*Mytilus edulis*)	170	NP1EO Field study	4
Mussel (*Mytilus edulis*)	100	NP2EO Field study	4
CRUSTACEAN			
Shrimp (*Crangon crangon*)	100		
FISH			
Juvenile Atlantic salmon (*Salmo salar*)	280	p-nonylphenol	1

References

1. McLeese *et al* (1981)
2. McLeese *et al* (1980)
3. Ekelund *et al* (1990)
4. Granmo *et al* (1991).

McLeese *et al* (1980) examined uptake and excretion of nonylphenol by mussels (*Mytilus edulis*) exposed over a four-day period to concentrations of 100 and 1130 $\mu g\, l^{-1}$. Maximum tissue concentrations occurred after one or two days. Estimated bioconcentration factors ranged between 1.4 and 13. Calculated rate constants indicated relatively low uptake and high excretion rates. The depuration half-life was 0.3 days.

Higher bioconcentration factors have been found in other studies and other organisms. McLeese *et al* (1981) examined bioaccumulation of p-nonylphenol in juvenile Atlantic salmon (*Salmo salar*) exposed to an original concentration of 310 $\mu g\, l^{-1}$ over a 96 hr period. A BCF of 280 was determined. Following transfer to clean water depuration was rapid, with a calculated half-life of four days.

Ekelund *et al* (1990) investigated bioaccumulation of nonylphenol in mussels, and shrimp (*Crangon crangon*) over a 16-day exposure period and 32 days post-exposure. For shrimp, accumulation reached a steady state within about ten days and post-exposure elimination was rapid. In contrast, mussels were still accumulating nonylphenol at Day 16 and after transfer to clean water a residue still remained after 30 days. Bioconcentration factors recorded (mean of two experiments) were 100 for shrimp and 3430 for mussels. This is in marked contrast to the figure of 13 for mussels reported by McLeese *et al* (1980). Ekelund *et al* speculated that the large discrepancy could have been due to differences in dosing (the flow-through system of Ekelund *et al* maintained more constant concentrations than the McLeese studies).

In a field test in which mussels were placed in cages at several sites close to an area receiving wastewaters containing a known concentration of NP a BCF of 320 was determined (Granmo *et al* 1991).

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APPENDIX D MAMMALIAN TOXICITY

D1 MAMMALIAN METABOLISM OF NONYLPHENOL

Pharmacokinetic studies have shown that NP is metabolised identically when administered either orally or by intra-peritoneal injection. Over a period of four days nonylphenol was excreted predominantly in the faeces (70%) with less (19%) being excreted in the urine. Hence, over a period of four days around 90% of administered nonylphenol was excreted (Knaak *et al* 1966). There are no details regarding the final proportion of nonylphenol that is excreted, how much remains in the body, or its location.

D1.1 Acute Toxicity

NP appears to be of low acute oral toxicity in laboratory animals. Several acute oral toxicity tests have been conducted using rats. LD_{50} values are consistently reported to be in the range 1000 - 2000 mg kg^{-1} bodyweight, with two exceptions. One reported an LD_{50} value of 580 mg kg^{-1} and another reported a range up to 5000 mg kg^{-1} bodyweight. The average LD_{50} is around 1500 mg kg^{-1} (BUA 1988).

D1.2 Sub-Chronic toxicity

In a 28-day rat feeding study, animals were given daily doses of either 25, 100 or 400 mg kg^{-1} body weight. There were no mortalities or any clinical signs to suggest any adverse effects of the treatments. The only significant effect was observed in males in the 400 mg kg^{-1} day^{-1} group. There were increases in mean relative kidney, liver and testes weights. The No Observed Adverse Effect Level (NOAEL) for both males and females was determined to be 100 mg kg^{-1} bodyweight.

A 90 day sub-chronic feeding study in rats, using dose levels of 200, 650 and 2000 ppm was started in December 1995. The results are not yet available (IUCLID 1996). A multi-generation reproduction study in rats is also currently underway in the USA. It is sponsored by the National Toxicology Program (NTP) and started in January 1996. Dose levels in the diet are 200, 650 and 2000 ppm. (IUCLID 1996).

D1.3 Reproductive Toxicity

In a developmental toxicity/teratogenicity test, rats were given NP by gavage at doses of 75, 150 and 300 mg kg^{-1} during Days 6 to 15 of gestation. At a dose level of 150 mg kg^{-1} body weight, three of the 21 females showed effects (not specified) on kidneys or spleens. A dose of 300 mg kg^{-1} caused clear maternal toxicity, including increased mortality, reduced body weight gain and food consumption. This maternal dose was found not to produce any significant effects in foetuses. The NOAEL for maternal effects was determined to be 75 mg kg^{-1} bodyweight, whilst the NOAEL for teratogenic effects was determined to be 300 mg kg^{-1} bodyweight. (IBR 1992).

No other data were available on the chronic toxicity, carcinogenicity, or reproductive toxicity of nonylphenol.

D1.4 Mutagenicity

p-Nonylphenol was not found to be mutagenic in bacterial Ames tests with or without metabolic activation when tested in strains TA97, TA98, TA100, TA102, TA104 at concentrations of up to 5 mg/plate (Huels 1984).

NP was not mutagenic, either with or without metabolic activation, in an HGPRT assay using mammalian, Chinese Hamster cells (IUCLID 1996). Neither was NP found to be mutagenic in an *in vivo* mouse micronucleus test at doses of up to 500 mg kg^{-1} bodyweight per day, the maximum tolerated dose (Huels 1988).

D1.5 Taste and Odour

A taste threshold of 1 µg l^{-1} for NP has been reported by Dawson *et al* (1970) and an odour threshold of 1000 µg l^{-1} for p-NP has been listed by Dietz and Traud (1978). The taste threshold appears to be very low and since it has not been possible to obtain the original paper from which this value is cited, there is low confidence, in this value.

D1.6 Oestrogenicity

Soto *et al* (1991) measured endometrial cell proliferation in ovariectomised rats, the classic test for oestrogenicity, to determine the activity of nonylphenol. Some cell proliferation was observed at an intra-peritoneal (i.p.) dose of 20 mg per animal, but a dose of 50 mg per animal was required to produce significant proliferation. This dose, however, was still three times weaker than a dose of 1.25 µg ethinyl oestradiol per animal. The polystyrene derived nonylphenol was therefore about four orders of magnitude (40 000 times) weaker in this assay than ethinyl oestradiol.

Endometrial and uterotrophic assays use the measurement of increased endometrial proliferation and increased uterine weight of ovariectomised rats or mice to measure oestrogenic potency. In these assays the relative potency of NP compared to oestradiol was found to be 1:0.00051 (Connor *et al* 1995).

The oestrogenicity of various alkylphenols (p-octyl and p-nonylphenols and the ethoxylated and carboxylated derivatives of p-nonylphenol) was investigated by White *et al* (1994) in *in vitro* tests, using two different human breast cancer cell cultures. Each alkylphenol was around three orders of magnitude (around 1000 times) less potent than ethinyl oestradiol. Ethinyl oestradiol was generally active in the tens of nanograms range, whereas the alkylphenols and derivatives were not significantly active below concentrations of tens of micrograms. In general the order of oestrogenicity of the compounds tested were: p-octylphenol > p-nonylphenoxy-carboxylic acid > p-nonylphenol = p-nonylphenol diethoxylate.

The oestrogenicity of NP was studied in immature female rats by giving daily oral doses of either 9.5, 47.5 or 285 mg kg^{-1}. The increase in absolute uterine weight ranged from 1.29-fold at 47.5 mg kg^{-1} to 1.82-fold at 285 mg kg^{-1}, the Maximum Tolerated Dose (MTD). The uterine: bodyweight ratio increased from 1.35-fold to 2.2-fold at the same dose levels. The NOAEL for these effects was 9.5 mg kg^{-1} with a LOAEL of 47.5 mg kg^{-1} (CTL, 1996).

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