Environmental Impacts of Alkylphenol Ethoxylates and Carboxylates. Part 1: Proposals for the Development of Environmental Quality Standards.

R&D Technical Report P2-115/TR3

Environmental Impacts of Alkylphenol Ethoxylates and Carboxylates

Part 1: Proposals for the Development of Environmental Quality Standards

P. Whitehouse

Research Contractor:
WRc plc

R&D Technical Report P2-115/TR3

Publishing Organisation:
Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol BS32 4UD

Tel: 01454 624400 Fax: 01454 624409

ISBN: 1 85705 383 4

Dissemination status
Internal: Release to Regions
External: Public Domain

Statement of use
This report reviews data on the occurrence, environmental fate and aquatic toxicity of alkylphenol ethoxylate surfactants and their breakdown products. It sets out options for the development of EQSs for this class of compounds and details an approach based on 'Toxic Equivalence Factors'. Proposals for the control of point source releases of APEOs based on these EQSs are being considered.

Research contractor
This document was produced under R&D Project P2-115 by:

WRc plc
Henley Road
Medmenham
Marlow
Buckinghamshire
SL7 2HD

Tel: 01491 636500 Fax: 01491 636501

WRc Report No.: EA 4903

Environment Agency's Project Manager
The Environment Agency's Project Manager for R&D Project P2-115 was Helen Wilkinson, National Centre for Ecotoxicology and Hazardous Substances, Wallingford, Thames Region.

Further copies of this report are available from:
Environment Agency R&D Dissemination Centre, c/o
WRc, Frankland Road, Swindon, Wilts SN5 8YF

tel: 01793-865000 fax: 01793-514562 e-mail: publications@wrcplc.co.uk

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Page

EXECUTIVE SUMMARY

This report reviews the available data on the occurrence, environmental fate and toxicity of alkylphenol ethoxylate (APnEO) surfactants and their breakdown products, resulting from degradation during sewage treatment and in surface waters. The report goes on to prioritise the breakdown products arising from alkylphenol ethoxylates with respect to the risk they pose to aquatic life. Finally, options for developing water quality standards for these substances are explored and proposals for the development of Environmental Quality Standards for the protection of aquatic life in surface waters are described in detail.

Standards for the protection of aquatic life and potable water supplies have recently been developed for the alkylphenols, nonylphenol (NP) and octylphenol (OP). These substances are produced as a result of biodegradation of APnEOs during sewage treatment and also in the environment, along with APnEOs with varying numbers of ethylene oxide units, alkylphenol-mono and di-carboxylates (APECs) and possibly metabolites which are oxidised in both the alkyl and ethylene oxide chains. Thus, APnEOs and APECs represent a source of alkylphenols in the environment but they are not subject to any form of regulatory control currently.

NP and OP are persistent in the environment and are of much higher toxicity than their ethoxylated counterparts. However, APnEOs and APECs with small numbers (1-3) of ethylene oxide (EO) units may also be rather resistant to degradation and, under certain circumstances, appear to accumulate in surface waters. Whilst they are less toxic than either NP or OP, there is good evidence to show that these 'lower' APnEOs are appreciably more toxic to aquatic life than the parent surfactants or 'higher' APnEOs. An assessment of the risk posed by alkylphenolic compounds to aquatic life indicates that the alkylphenols NP and OP probably pose the greatest risks by virtue of their toxicity and environmental persistence. Based on the limited evidence available, the APnEOs appear to be of greater toxicological concern than the carboxylate (APEC) derivatives. The 'lower' APnEOs with between 1 and 4 EO units are of greater concern than the 'higher' APnEOs because they are also more toxic and, probably, more persistent. However, with the exception of NP and OP, standards for other alkylphenolic compounds do not yet exist.

Several possible approaches to the development of Environmental Quality Standards can be recognised. The preferred approach is one in which standards for individual APnEOs are derived by interpolation from the relationship between acute toxicity and EO chain length for these substances, coupled with a safety factor to extrapolate to a 'no effects' concentration. Standards relating to both short-term (e.g. intermittent discharge) and long-term (e.g. continuous discharge) have been derived in this way. Significantly, standards for mixtures of APnEOs have also been developed. This is achieved by expressing the toxicity of ApnEO oligomers detected in an effluent or water sample in terms of the equivalent concentration of NP. These 'Toxic Equivalent Factors' (TEFs) are then combined and the sum of the TEFs compared with the EQS for NP.

There is evidence to indicate that the toxicities of nonylphenolic and octylphenolic compounds are equivalent and that the relationship between EO chain length applies equally to NPnEOs and OPnEOs. Furthermore, based on our understanding of the toxic mode of action of these alkylphenolic compounds, it is reasonable to suppose that additive toxicity will result when both nonylphenolic and octylphenolic compounds are present at the same time. Therefore,

standards may be expressed in terms of total alkylphenolic compounds, irrespective of whether they are derivatives of NPnEOs or OPnEOs.

Reliance on surface water standards for alkylphenolic compounds alone is flawed because both NP and OP will tend to sorb to sediments. As a result, there is a risk that concentrations in samples of overlying water will under-estimate the true environmental burden. A better approach may be to rely on sediment standards for NP and OP but currently, such standards are unavailable. Furthermore, APECs represent a potentially persistent form of alkylphenolics in the environment and may occur at higher concentrations than the 'lower' APnEOs, although it is not clear whether they are formed predominantly during sewage treatment or subsequently, in the environment. They may also be more water-soluble, raising questions about possible different exposure routes for APnEOs and APECs. The relative environmental risks posed by APnEOs and APECs remains unclear and a lack of information prevents the derivation of standards for APECs using the approach outlined in this report. However, it is possible that the approach to regulating APnEOs could be extended to APECs if a better understanding of their environmental fate and toxicity develops.

1. INTRODUCTION

The alkylphenols, nonylphenol (NP) and octylphenol (OP), are among the substances for which EQSs have been developed recently (Whitehouse *et al*, 1998a; 1998b). This reflects concerns about the occurrence of these substances in surface waters and the possible risks their presence may pose to aquatic life and to consumers of drinking water. It is clear that the most important source of these substances in water is through degradation of alkylphenol (nonylphenol and octylphenol) ethoxylate surfactants (APnEOs) in biological sewage treatment. However, a number of intermediates and metabolites are also formed during biological treatment, notably alkylphenol ethoxylates with small ethylene oxide (EO) chains and alkylphenol carboxylates. In order to be sure that water quality is adequately protected, it is necessary to review the need for standards for these substances as well as the alkylphenols for which EQSs have recently been developed.

The aim of the review is:

1. to identify those alkylphenol ethoxylates and carboxylates for which EQSs are warranted;

2. to identify a suitable approach for deriving standards for alkylphenol ethoxylates and carboxylates in surface waters, bearing in mind the availability of credible data;

3. to describe, in detail, proposed standards for priority APnEOs.

Sections 2-4 review the sources, occurrence and fate of alkylphenol ethoxylates and carboxylates in the environment whilst Section 5 considers their toxicities to aquatic life, possible oestrogenic effects and bioaccumulation. Priorities for the development of EQSs are addressed in Section 6 and Section 7 sets out options for different technical approaches that may be employed. Section 8 goes on to develop the preferred option in more detail, including proposed standards for individual APnEDs and also mixtures of APnEDs.

2. USES AND PRODUCTION OF APnEO SURFACTANTS

2.1 Structure and nomenclature

Alkylphenol ethoxylates are non-ionic surfactants based on ethoxylated derivatives of nonylphenol and octylphenol. Nonylphenol ethoxylates are the more important class because they are used more extensively and, consequently, occur more frequently in wastewaters and in the environment. A wide range of oligomers is available, varying in the length of the hydrophilic EO chain (typically between 4 and 50 EO units) which confers different properties to the molecule. Most alkylphenol ethoxylates are based on alkyl substitution of the phenyl ring at the 4-position (as shown in Fig. 2.1), although small amounts of 2-substituted isomers may also be present in commercial products. This alkyl chain may be linear or branched and in commercial products, a mixture of both is usually present.

Throughout this review, the following conventions are used as abbreviations for the substances under discussion.

[1]APnEO — Alkylphenol ethoxylate with n ethylene oxide units. In commercial products, it is normal for a range of EO chain lengths to be represented and the value of n indicates the average number of EO units on the molecule. The most common length of the EO chain is around 9 and, in the case of nonylphenol, this is denoted as NP9EO.

[1]AP1EO — alkylphenol monoethoxylate

[1]AP2EO — alkylphenol diethoxylate

[1]AP1EC — the carboxylic acid of AP1EO formed by oxidation of the terminal OH group

[1]AP2EC — the carboxylic acid of AP2EO formed by oxidation of the terminal OH group

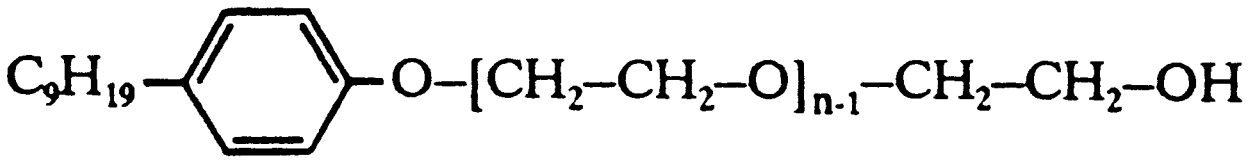

Figure 2.1 Structure of alkylphenol ethoxylates

[1] The prefix ‘alkylphenol’ is a generic term including nonylphenol and octylphenol compounds. When these are specifically described, the AP notation is replaced with the more specific NP or OP notation e.g. NP1EC or OP9EO.

2.2 Uses and production

The surface active properties of APnEOs have been exploited in a wide variety of industrial and domestic applications since the 1940s. Details of quantities of NPnEOs used in different applications are to be found in a review by CES for DETR (CES, 1993) and are summarised in Table 2.1.

Table 2.1 Estimated uses of nonylphenol ethoxylate surfactants (CES, 1993)

Sector	Usage (tonnes p.a.)
Industrial cleaning products	7500-8500
Paint	2000-3000
Agrochemical formulations, for aiding product stability and aids to wetting and penetration	2000
Emulsion polymers e.g. coatings, adhesives	1500
Textiles (scouring*, fibre lubrication, dyeing)	1000
Metal finishing, especially cleaning	1000
Lubricating oils (as NPnEO phosphate esters)	600
Other applications e.g. photographic applications, metal working fluids, spermicidal lubricants, fuel additive, hand cleaning gels, dust suppression	100-1000
Total	14500-18500

* a process designed to remove the natural oils present in wool

In 1993, a significant proportion of European production took place in the UK. Then, independent figures for UK production were estimated to be between 17000 - 20000 tonnes p.a. (CES, 1993), which are supported by the figures shown in Table 2.1. Of this, CES (1993) estimated that around 37% of the NPnEOs produced was released to the aquatic environment (ca. 6500 tonnes p.a.). As described below, there has been a decline in usage and production in recent years although significant usage continues (Section 2.2.1).

2.2.1 Constraints on usage and production

EC Directives on the properties exhibited by detergents apply to APnEOs. These are particularly concerned with primary biodegradability (EC Directive 73/404/EEC) determined as specified in another Directive, 82/242/EEC. APnEOs have recently been subject to a variety of voluntary and statutory constraints on their production and use, arising out of concerns about environmental safety of some of the breakdown products, especially NP and OP. Recent concerns about possible oestrogenic effects of some of these breakdown products (e.g. Jobling and Sumpter, 1993; Routledge and Sumpter, 1996) have raised the profile of these substances and seem likely to accelerate further restrictions on production and use. APnEOs are often replaced by alcohol ethoxylate surfactants in such circumstances.

Since 1976 NPnEOs have not been used in domestic applications (domestic cleaning products, detergents etc.) following their voluntary withdrawal from such products in the UK (ENDS,

1998), and a ban on their use in these products in Switzerland and Germany. A ban on their use in Denmark, to take place by 2000, is also reported (ENDS, 1996). As a result of pressure from the Environment Agency, the use of APnEOs for wool scouring is being reduced (ENDS, 1997), previously regarded a major source of alkylphenolic compounds in certain regions of the UK where textile industries are prominent.

In 1992, the Paris Commission issued a Recommendation (PARCOM 92/8) to phase out the use of NPnEOs in domestic cleaning agents by 1995 and to extend this to industrial cleaning products by 2000. Significantly, this recommendation is confined to NPnEOs and does not extend to OPnEOs. However, Sweden is calling for a ban on all alkylphenol ethoxylates (both NPnEOs and OPnEOs) as well as NP and OP. Consumption of APnEOs appears to be declining: The Nordic Council of Ministers estimates that, since 1992-3, NPnEO consumption in Scandinavian countries has declined by about 40% to a current level of about 5300 tonnes p.a. (Renner, 1997). Also relevant is an EU-ESR Risk Assessment for nonylphenol which is currently being carried out; it has undergone technical review within the EU and is due to be issued later in 2000.

Despite the measures outlined above, APnEOs continue to remain in widespread use in cleaning products, leading to wide occurrence in wastewater discharges and, in some cases, to locally high concentrations. Thus, APnEOs represent a highly hazardous and persistent group of chemicals which are prevalent in UK wastewaters and surface waters. Therefore an understanding of the occurrence, fate and aquatic toxicity of APnEOs and their breakdown products is warranted. This review and the EQSs proposed is the first step in this process.

3. SOURCES OF APnEOs AND APECs IN SURFACE WATERS

3.1 Introduction

Biodegradation plays a key role in an environmental assessment of APnEOs and APnECs because, with the possible exception of certain short (*ca.* 5) EO chain APnEOs, these substances occur in the environment entirely as a result of biodegradation of APnEOs during sewage treatment. There is a substantial research literature on the biodegradation of APnEO surfactants. The pathways and products of degradation are reviewed in detail in Appendix A.

3.2 Primary biodegradation

There is compelling evidence demonstrating a high level of primary biodegradation of APnEO surfactants, meeting the requirements set out in the EEC Directive on biodegradability of non-ionic surfactants (EEC, 1982). Details of studies into the ready biodegradability of APnEOs are to be found in Appendix A but a number of general points can be drawn from laboratory and field studies:

- Rates of APnEO removal are generally high in both standard laboratory tests and in field (sewage treatment plant) studies, typically of the order of around 90% (Gerike, 1987; Rudling and Solyom, 1974; Ahel *et al*, 1994a; Naylor, 1992; Naylor *et al*, 1992).

- The primary biodegradability of APnEOs may drop under conditions of stress and in particular in trickling filter sewage works at winter temperatures (Mann and Reid, 1971; Ahel *et al*, 1994a) or in plants with high sludge loading rates (Ahel *et al*, 1994a).

3.3 Ultimate biodegradation

The ultimate biodegradation of APnEOs is of greater environmental significance than their primary biodegradation. This is because degradation results in metabolites which are progressively more persistent, liable to bioaccumulate and toxic than the parent APnEO. Ahel *et al* (1994a) predict that around 60-65% of the NPEOs introduced to sewage treatment works with primary and secondary treatment are discharged to the environment as nonylphenolic compounds.

Numerous authors (e.g. Rudling and Solyom, 1974; Bruschweiler *et al*, 1983; Giger *et al*, 1984; 1987; Reinhard *et al*, 1982; Ahel *et al*, 1987; Holt *et al*, 1992) have shown that, in laboratory tests and during biological treatment in sewage works, the first step in the breakdown of APnEOs is the rapid removal of ethylene oxide (EO) groups, eventually giving rise to 'lower' APnEOs with less than four EO units, and usually one or two EO units (AP1EO and AP2EO). Under aerobic conditions, these may be further oxidised to the corresponding carboxylic acids (predominantly AP1EC and AP2EC; Frazee *et al*, 1964; Field and Reed, 1996; Ball *et al*, 1989; Ahel *et al*, 1987; Lee and Peart, 1998) or they may be

converted to the alkylphenols, NP or OP, especially where anaerobic conditions prevail (Bennie, 1999).

The generally accepted view of APnEO biotic degradation is shown in Figure 3.1. Very recent studies (DiCorcia *et al*, 1998) show that a significant proportion of the metabolites produced during biological treatment are actually oxidised in both the EO and alkyl chains (not shown in Figure 3.1). The indications are that these metabolites are resistant to further biodegradation.

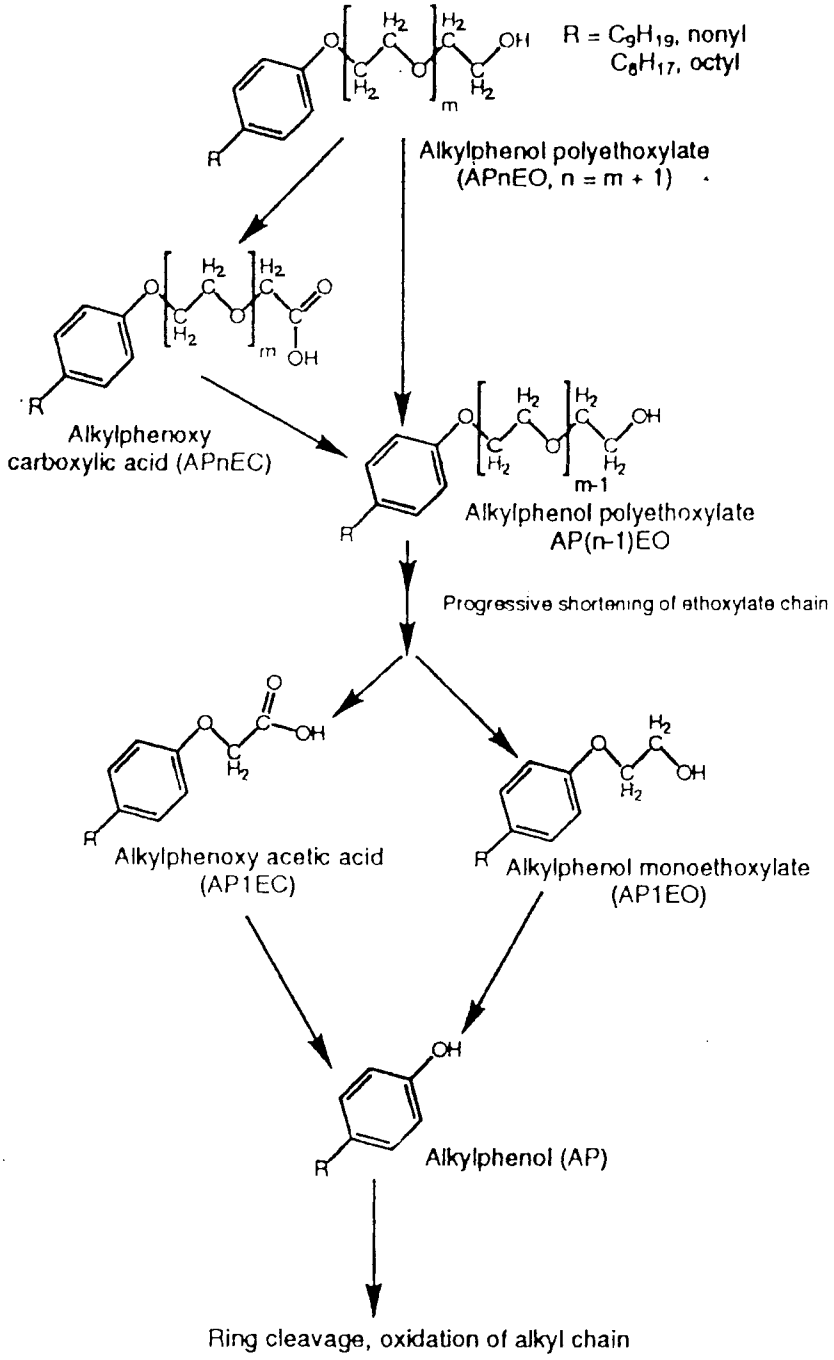

Figure 3.1 Biodegradation of APnEO surfactants (from Ahel *et al*, 1994a)

Because they tend to accumulate in sewage effluents and in the environment, it is thought that the carboxylic acids are more resistant to biodegradation than APnEOs. A recent report indicated that NP1EC and NP2EC exceeded 60% theoretical $C0_2$ generation in 28 days during an OECD 301B (Modified Sturm) ready biodegradation test, but degradation of NP1EC did not fulfil the 10 day window for ready biodegradability (Williams *et al*, 1996; Staples *et al*, 1998). Similar results were obtained for the corresponding octylphenolic compounds, OP1EC and OP2EC, although in this case, the 10-day window criterion was satisfied (Staples *et al*, 1998). Ball *et al*, (1989) previously showed that OP1-3EC were slower to degrade under aerobic conditions than their EO counterparts and OPECs with more than 2EO units were not transformed under anaerobic conditions. Together, these results indicate that the carboxylic acids are less readily degraded than the 'higher' NPnEO s.

Being more hydrophilic, the carboxylic acids tend to be associated with the aqueous phase and so are mainly discharged in the treated sewage effluent whilst the remainder of the more lipophilic 'lower' APnEOs tend to be adsorbed onto the sewage works sludge. If this sludge is treated by anaerobic digestion, the APnEOs are liable to conversion to NP and OP, which are also lipophilic and so will mostly be retained in the sludge. Accordingly the sludge-bound fraction is a less important contributor to mass flow of 'lower' APnEOs in sewage treatment plants than it is for NP and OP. Losses via sludge are estimated to be 14-15% for NP1EO and only 6-7% for NP2EO compared with around 50% for NP (Ahel *et al*, 1994a). A similar distribution would be expected for OPnEOs and their breakdown products. Table 3.1 summarises the results of the key studies into APnEO biodegradation.

It follows that the distribution of APnEO oligomers in wastewaters which have undergone biological treatment may be quite distinct from that found in either commercial APnEO formulations or in the influents to such works. Based on a detailed study of eleven Swiss biological treatment plants, Ahel *et al* (1994a) estimated that 19% of the nonylphenolic compounds discharged are released in the form of NP1EC and NP2EC, 11% in the form of NP1EO and NP2EO, 25% as NP and 8% as unconverted NPnEOs (i.e. 92% removal of NPnEOs). These are expressed on a molar basis and so take account of differences in molecular weight. Again, a similar distribution would be expected for OPnEOs and their breakdown products.

In contrast to these experiences, studies in the US (Naylor, 1992 and Naylor *et al*, 1992) provide cases in which the oligomer distribution is not obviously skewed toward the more lipophilic APnEOs. The relatively high proportion of NP1EC and NP2EC in effluents from biological treatment plants in the Swiss studies appears to be a feature of secondary treatment because they were only very minor components in the effluent arising from primary treatment in the US studies (Figure 3.2). In a survey of treated wastewaters from the Swiss plants discharging into the River Glatt (Stephanou and Giger, 1982) in which treated wastewater accounted for up to 15-20% of the flow, the greatest skews toward the 'lower' ethoxylated and carboxylated derivatives were associated with discharges from plants providing only partial or no nitrification and those plants receiving a lower proportion of industrial waste.

Table 3.1 Summary of biodegradation of APnEO surfactants

Substance tested	Type of test	Results	Reference
NP9EO	Coupled Units test	48.6% DOC removal; 97% primary degradation seen in OECD screening test	Gerike, 1987
NP9EO	Semi-continuous activated sludge test	Overall 93% removal of the NPnEO; 20.8% was mineralised to CO_2, 23.1% converted to highly degraded metabolites, 26% in effluent as NPnEC. Conversion to nonylphenol could be around 4.5% of the NPnEO (by weight), of which around 1/4 was found in effluent.	Varineau *et al*, 1996
NPnEO (n=8;10;14; 16; and 30)	Lab-scale activated sludge system	82-96% removal of the original surfactant was seen	Rudling and Solyom, 1974
NP9EO	Semi-continuous activated sludge system	93-97% removal of parent surfactant	Varineau *et al* (*pers. comm.*)
NP9EO	Lab-scale bioreactor attached to sewage treatment plant, United States	>95% removal of the NPnEO; 35-50% of the hydrophobe was discharged in effluent from the system, probably as NPnEO/NPnEC, with n=0-3	Kravetz *et al*, 1982
NPnEO	Sewage treatment plants, Switzerland	50% on a molar basis and 17% on a mass basis of the NPnEO entering the plant was estimated to form nonylphenol ethoxylate during anaerobic sludge digestion	Brunner *et al*, 1988
NPnEO	Sewage treatment plants, Switzerland	Overall removal on NPnEO (n>2) is 92%. Of the total entering the plant: 19% release via effluent as NPnEC, 11% release via effluent as NP1EO + NP2EO, 25% released as nonylphenol (of which 90% is adsorbed onto digested sludge → <2.5% released as nonylphenol in effluent) 8% released as un-transformed	Ahel *et al*, 1994a
NPnEO	Sewage treatment plants in the United States	>92% removal of the original surfactant	Naylor *et al*, 1992 Naylor, 1992 Kubeck and Naylor, 1990
NPnEO	Sewage treatment plant, Canada	Elimination rate of NPnEOs of 53%. Major products in effluent were NP1-2ECs.	Lee and Peart, 1998

The treated effluent concentrations of the NP1EO and NP2EO metabolites reported by Giger *et al* (1987) from works with primary, secondary and tertiary (sludge digestion) treatment are generally in the range 20-40 μg l^{-1} (one figure of 160 μg l^{-1}) and of NP in the range 1-14 μg l^{-1}. The concentrations of the NP1-2ECs in the treated effluents were in the range 71-330 μg l^{-1}. In a survey of wastewater effluents carried out in the US, between 1 and 14 μg l^{-1} NP was detected in the treated effluents from municipal wastewater treatment works. By comparison, concentrations of NP1EC-NP4EC in a selection of the same discharges ranged between 144-273 μg l^{-1} (Field and Reed, 1996) but lower concentrations of these carboxylates (1.5-3.9 and 5.1-9.4 μg l^{-1}, respectively) were reported by Di Corcia *et al*, (1994) following analysis of wastewaters around Rome, Italy. UK experiences are limited but

concentrations of NP2EO of approximately 150 μg l^{-1} were detected in effluent from a sewage treatment works in Yorkshire (Blackburn and Waldock, 1995).

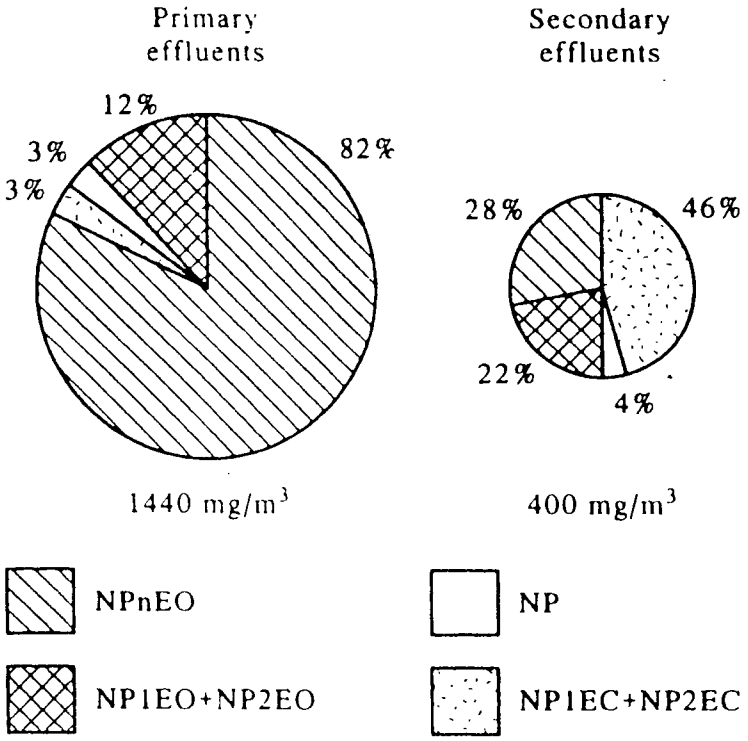

Figure 3.2 Relative abundance of APnEOs, APnECs and alkylphenols in primary and secondary effluents from eleven sewage treatment plants in Switzerland (from Ahel *et al*, 1994a)

3.4 Concluding remarks

In summary, primary biodegradation of APnEO surfactants is rapid but may be regarded as a process by which a range of substances, particularly 'lower' APnEOs and APECs are generated. There is some debate about the abundance of APECs as degradation products from sewage treatment works but there is evidence to show they can be the dominant breakdown products in the effluent stream. Whilst some 'lower' APnEOs and APs will tend to associate with sludges and sediments, the corresponding carboxylic acids appear to be discharged to the environment predominantly in the aqueous phase. Metabolites which are oxidised in both the EO and alkyl chains may also be generated.

It is known that the breakdown products of APnEO surfactants are more toxic, persistent, oestrogenic and lipophilic that the 'parent' surfactant. Clearly, any measures to regulate these substances in effluent discharges and surface waters needs to address the combined effects of a mixture of compounds and also their potential to generate further hazardous metabolites in the environment.

4. OCCURRENCE AND FATE OF APnEOs AND APnECs IN SURFACE WATERS

4.1 Introduction

'Lower' ethoxylated alkylphenols (i.e. those with fewer than 3 or 4 EO units), their carboxylated derivatives and the alkylphenols NP and OP have no commercial applications. Consequently, their presence in water occurs as a result of degradation of APnEO surfactants, much of it arising from biological treatment of domestic and industrial wastes, as described in Section 3. Ahel *et al* (1994b) undertook a mass balance of alkylphenolic compounds in the Glatt catchment in Switzerland and concluded that over 95% of the alkylphenolic compounds entered the river by direct discharge of secondary effluents. It follows that any differences in distribution of metabolites between that found in sewage effluent discharges and that found in surface and groundwaters can be attributed to chemical and biological transformations occurring in the river.

A recent review by Bennie (1999) summarises reported information on the occurrence of APs, APnEOs and APECs in effluent discharges, sludges resulting from sewage treatment, drinking water, surface waters, sediments and biota.

4.2 Freshwaters

4.2.1 Concentrations and identity of APnEO breakdown products

In response to growing regulatory pressure on the use of APnEOs in the US, several US manufacturers of APnEO surfactants embarked on a major survey of concentrations of APnEOs and their metabolites in rivers across the US in the early 1990s to provide exposure data which could be used for risk assessment purposes. Combined with similar studies in Europe, particularly in the Glatt catchment in Switzerland, this has resulted in a substantial body of data on concentrations of alkylphenolic compounds in surface waters. UK data are restricted but are included in Table 4.1 along with the results of US and Swiss studies in which a range of alkylphenolic compounds have been looked for. In addition, recent surveys indicate total APnEO concentrations in the River Aire downstream of Marley STW (which treats waste from a heavily industrialised catchment) generally lie between 0 and 20 $\mu g\ l^{-1}$ although peaks of up to 190 $\mu g\ l^{-1}$ total APnEOs are evident (Kennedy, EA North-East Region, *pers. comm.*).

Table 4.1 Measured concentrations of APnEOs in surface freshwaters

Substance	Range (µg l^{-1})	Mean (µg l^{-1})	Location(s)	Number of sites/rivers where measurable concentrations found	Ref
NP1EO	<0.06 - 1.2	0.09	Thirty rivers throughout US	13/30	1
	<3 - *69	ca. 7.0!	Glatt River, Switzerland	-	2
	<0.5 - 17.0	4.8	Glatt River, Switzerland	5/6	3
	0.04 - 0.37	0.13	Fox River, Wisconsin	3/5	5
	<0.5 - 18.0	-	Glatt River, Switzerland	-	6
	<0.1 - 0.6	-	R. Chelmer, Sandford Mill	5/18	8
	<0.02– 7.8	1.3	Lake Ontario, Canada	22/38	9
NP2EO	<0.07 - 1.2	0.10	Thirty rivers throughout US	18/30	1
	<0.3 - 30.0	ca. 6.0!	Glatt River, Switzerland	-	2
	<0.5 - 10.0	3.7	Glatt River, Switzerland	5/6	3
	<0.5 - 16.0	-	Glatt River, Switzerland	-	6
	-	25.0#	R. Aire, d/s of Marley STW	-	7
	<0.1 - 2.3	-	R. Chelmer, Sandford Mill	6/18	8
	<0.02-10.0	1.4	Lake Ontario, Canada	12/38	9
NP3-17EO	<1.6- 14.9	2.0	Thirty rivers throughout US	11/30	1
	<1 - 7.1	-	Glatt River, Switzerland	-	2
	0.8 - 3.4$	1.8	Fox River, Wisconsin	4/5	5
	<0.1 - 1.5	-	R. Chelmer, Sandford Mill	8/18	8
NP	<0.11-0.64	0.12	Thirty rivers throughout US	13/30	1
	<0.3 - *45	ca. 2.0!	Glatt River, Switzerland	-	2
	0.12 - 0.29	0.23	Fox River, Wisconsin	4/5	5
	<0.5 - 1.5	-	Glatt River, Switzerland	-	6
	<0.1 - 2.0	-	R. Chelmer, Sandford Mill	17/18	8
	<0.01-0.92	0.21	Lake Ontario, Canada	9/38	9
NP1EC	<0.04 - 2.0	-	Fox River + 8 other US rivers	4/10	4
			Glatt River, Switzerland		
	<1.0 - 45	ca. 17.5!		4/6	2,3
NP2EC	<0.2 - 11.8	-	Fox River + 8 other US rivers	5/10	4
			Glatt River, Switzerland		
	2.0 - 71	ca. 37.5!		6/6	2,3
NP3EC	not detected	-	Fox River + 8 other US rivers	0/10	4
NP4EC	not detected	-	Fox River + 8 other US rivers	0/10	4

* The upper end of the range was strongly influenced by an isolated large value
$ Actually NP2-17EO
! Median rather than mean
No further details given

References:

1 Naylor *et al*, (1992)
2 Ahel *et al*, (1994b)
3 Ahel *et al*, (1987)
4 Field and Reed (1998)
5 Naylor *et al*, (1998)
6 Ahel and Giger (1985a)
7 Blackburn and Waldcock (1995)
8 Janbakhsh (1996)
9 Bennie (1997)

Broadly speaking, the alkylphenolic compounds detected in freshwaters reflect those discharged from sewage treatment works i.e. a predominance of 'lower' ethoxylated derivatives (NP1EO and NP2EO) and their carboxylated derivatives, NP1EC and NP2EC, and, compared to the surfactant products from which these substances originate, only low concentrations of 'higher' NPnEOs. Although not verified experimentally, it seems likely that the proportion of 'higher' APnEOs will be greater in winter when conditions are less favourable for degradation in sewage works (CES, 1993).

There are clearly differences in the concentrations of these 'lower' ethoxylated derivatives and their carboxylated derivatives in a survey of US rivers reported by Naylor *et al,* (1992) and studies in the Glatt catchment in Switzerland (Ahel *et al*; 1987, 1994b), but this is largely a reflection of differences in dilution afforded by the two study sites.

Of greater significance are differences in the distributions of alkylphenolic compounds. For example, substantial concentrations of un-degraded NPnEOs were found in the US study but these were very minor components in the Swiss studies. Indeed, in the latter study, no oligomers with more than 6EO units were detected. Some sites in the US study were associated with a skew in the distribution of metabolites toward the 'lower' ethoxylated derivatives (NP1EO and NP2EO) but in others, no such skew was reported. In the Swiss studies (Ahel *et al*, 1987, 1994b) there was always a marked skew in the distribution of alkylphenolic compounds in the river samples analysed toward the 'lower' ethoxylated derivatives (NP1EO and NP2EO) and, especially their carboxylated derivatives, NP1EC and NP2EC. Indeed, NP1EC and NP2EC were the most abundant metabolites found in the River Glatt. Concentrating on the carboxylated derivatives, Field and Reed (1998) showed that concentrations of NP1EC and NP2EC varied between undetectable and 13.5 $\mu g\ l^{-1}$. In their survey of US rivers, Naylor *et al,* (1992) detected NPnECs in 5 out of the 8 rivers for which these substances were analysed in the range 1.4-6.3 $\mu g\ l^{-1}$. As with sewage effluents, NP2EC was the dominant oligomer among the carboxylic acids detected.

4.2.2 Transformations in rivers

Studies by Ahel and co-workers demonstrate that shifts in the composition of alkylphenolic compounds can occur in rivers. By comparing loads discharged from the Glatt River in Switzerland with the inputs to the river from eleven sewage treatment works discharging into the catchment, Ahel *et al,* (1994b) concluded that 24% of the surfactant-derived nonylphenolic compounds were eliminated over a 35km stretch of river (residence time of 10-15h). Most of the degradation taking place was of NPnEO, NP1EO and NP2EO but this was balanced by a net increase in concentrations of NP1EC and NP2EC (mass flow at the output from the river was 27% higher than the total input). This is consistent with aerobic degradation of ethoxylated alkylphenols to their carboxylated counterparts at a faster rate then their subsequent breakdown. These compositional changes are illustrated in Fig. 4.1.

Reports of the degradation of APnEO surfactants under realistic e.g. 'die-away' conditions have only become available very recently. In a study of the fate of NP18EO under sub-tropical estuarine conditions, primary degradation was complete within 4 - 24 days with the main degradation products identified as NP2EO and NP2EC, which appeared to be relatively resistant to further degradation (Potter *et al*, 1999). A river water die-away study (Varineau *et*

al, *pers. comm*.) demonstrated extensive degradation of NP9EO after 128 days but, in this case, ring-cleaved metabolites of the carboxylates were formed, leading the authors to conclude that the carboxylates were not persistent products of APnEO degradation.

The only example of a study into the biodegradation of the 'lower' ethoxylated or carboxylated alkylphenols under realistic conditions was also provided by Potter *et al*, (1999). Die-away of NP2EC in estuarine water declined to only 50% of the starting concentration after 32 days, leading the authors to describe it as 'relatively resistant' to degradation. This conclusion is in marked contrast with the assertion by Staples *et al*, (1998) that NP1EC, NP2EC, OP1EC and OP2EC would not persist in the environment. However, it is important to note that their conclusion was based on the results of ready biodegradability testing using conditions which are highly favourable to degradation. Further circumstantial evidence for slower biodegradation of NP1EC and NP2EC compared to the corresponding lower ethoxylates also comes from an earlier study of the infiltration into groundwater of river water contaminated with alkylphenolic compounds (Ahel *et al*, 1996). The slow biodegradation of the carboxylates in laboratory tests (Ball *et al*, 1989) would also suggest they are likely to be resistant to degradation in surface waters.

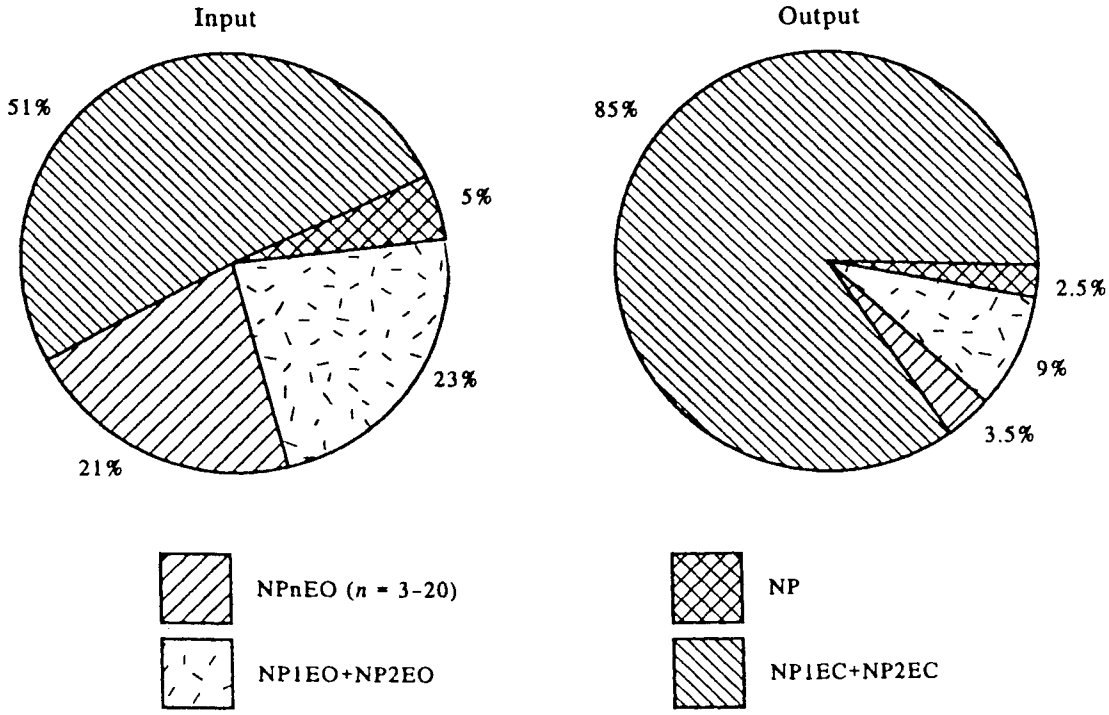

Figure 4.1 Compositional change of nonylphenolic compounds in the Glatt River, Switzerland (after Ahel *et al*, 1994b)

4.3 Sediments

Measured concentrations of NP, NP1EO and NP2EO in river sediments tend to be higher than those determined in the overlying water (Ahel *et al*, 1994b; Bennie, 1999). A summary of reported concentrations of alkylphenolic compounds in sediments is given in Table 4.2. In marked contrast to the water column, NP was the most abundant alkylphenolic compound associated with the sediment with concentrations 364-5100 times higher than in the water. This is probably a reflection of the greater resistance to degradation (Garrison and Hill, 1972) of NP compared with other metabolites.

Removal of ethylene oxide units during biodegradation leads to the formation of progressively more lipophilic compounds. Predicted log P values for NP, the 'lower' ethoxylates and carboxylates are all very similar (5.3 - 5.9), indicating that all these metabolites will tend to partition from the aqueous phase onto bed and suspended sediments. Recent research (Johnson *et al*, 1998) suggests that bed and suspended sediments play a key role in the fate of OP in rivers although their relative importances in sequestering OP appears to differ between river catchments. Although the carboxylates have not been reported in sediment, it is unclear whether they have actually been analysed for in this medium.

Table 4.2 Reported concentrations of alkylphenolic compounds in sediments

Substance	Location	Number of samples	Mean (mg kg^{-1})	Range (mg kg^{-1})	Ref
NP1EO	Rhine River, Germany	-	800	-	1
	Glatt River catchment, Switzerland (7 sites)	-	-	100-8850	2
	Besos River, Spain	2	-	2400, <100[a]	3
	Marine lagoon, Venice, Italy	20	-	9-82[ab]	4
	Thirty US rivers	81	18.1	<2.3-175	5
	Fox River, US (12 sites)	-	121.4	4-215	5
	Tees Estuary, UK	8	-	0.13-3.9	6
	Tyne Estuary, UK	9	-	0.16-1.4	6
	Lake Ontario, Canada	9	7.1	<0.015-38	7
NP2EO	Rhine River, Germany	-	700	-	1
	Glatt River catchment, Switzerland (7 sites)	-	-	LOD-2720	2
	Besos River, Spain	1	1200	-	3
	Marine lagoon, Venice, Italy	20	-	3-20[ab]	4
	Thirty US rivers	-	-	-	5
	Lake Ontario, Canada	9	1.2	0.015-6.0	7
NP3-17EO	Thirty US rivers	-	-	not detected	5

[a] coastal sediment [b] easily resuspendible sediment from the sediment surface

References:

1 Marcomini and Giger, 1987
2 Ahel *et al*, 1991
3 Grifoll *et al*, 1990
4 Marcomini *et al*, 1990
5 Naylor 1998
6 Lye *et al*, 1999
7 Bennie *et al*, 1997

4.4 Estuarine waters

Studies into the occurrence of APnEOs and their breakdown products in marine and estuarine waters have again revealed the presence of 'lower' ApnEOs, although at low concentrations (Marcomini *et al*, 1990; Kvestak and Ahel, 1994; Lye *et al*, 1999). The results of die-away tests using brackish and saline water also point to a similar degradation pathway for APnEO surfactants to that seen in sewage treatment and in freshwaters (Kvestak and Ahel, 1995).

4.5 Biota

Data are limited to analyses of 'lower' NPnEOs in duck, several fish species and aquatic plants (Ahel *et al*, 1993). These revealed concentrations of NP1EO of between <0.03 and 80 ug l^{-1} and lower concentrations <0.03-3.0 ug l^{-1} of NP2EO in biota.

4.6 Current regulatory monitoring

The carboxylates are not routinely analysed for by the Environment Agency. APnEOs are analysed for in fresh and marine waters in the Environment Agency's North-East Region (Daniels, EA, *pers. comm*.) but the method routinely used (extraction followed by HPLC with fluorescence detection) will only determine APnEOs with >3 EO units. Methods are available which will also detect AP1EO and AP2EO (see Appendix D) but these are employed only as part of specific studies.

4.7 Concluding remarks

The distribution of alkylphenolic compounds in surface waters tends to reflect that found in effluents discharged from sewage treatment works. Particularly where wastes have been subjected to secondary treatment, the carboxylates AP1EC and AP2EC appear to predominate in the watercourse and concentrations of these substances can be augmented by further degradation and oxidation of APnEOs in the water. There is evidence to suggest that the carboxylates may be more persistent in the environment than the APnEOs but this remains an area of debate.

Following release of treated effluents containing degradation products of APnEO surfactants to a watercourse, further degradation is probably slow. However, based on predicted log P values and a small amount of data describing measured concentrations of alkylphenolic compounds in water and sediments, the 'lower' ethoxylates, carboxylates and alkylphenols would all be expected to be lost from solution as a result of sorption onto suspended and bed sediments. In this compartment, it appears that NP (and presumably OP) are more resistant to further degradation than the other alkylphenolic compounds. The consequences of this loss mechanism would be to reduce bioavailability to biota in the water column but at the same time, to raise the risk of exposure for benthic organisms, and then possibly the aquatic food chain.

5. TOXICITY TO AQUATIC LIFE

5.1 Introduction

There is a substantial amount of toxicity data relating to the effects of APnEO surfactants and also the alkylphenols NP and OP on both freshwater and marine organisms. Recent reviews by Talmage (1994) and Staples *et al,* (1999) provide a comprehensive summary of available ecotoxicity data although the majority of these data relate to the alkylphenols NP and OP rather than the ethoxylates or carboxylates. Appendix C summarises the available aquatic toxicity data for APnEO surfactants and their breakdown products. Data for the alkylphenols have been reviewed by Whitehouse *et al,* (1998a; 1998b) and are not covered in any detail here, although they are referred to for comparative purposes.

It is important to note that the majority of studies on APnEO surfactants and their breakdown products have been performed on commercial products, which typically contain a range of oligomers. Thus, although toxicity summaries are expressed in terms of an APnEO with a particular EO chain length, in fact the toxicity observed will have been due to the combined effects of all the oligomers present in the sample tested. It appears that most commercial products contain a distribution of oligomers which is approximately symmetrical (Fig 5.1) and so the contribution of less toxic oligomers (those with longer EO chains) probably cancels out the contribution made by the more toxic oligomers (those with shorter EO chains). Therefore, the toxicity stated for a particular EO chain length may be taken as a reasonable reflection of the toxicity of that oligomer if it had been tested in its pure form. A similar assumption was made by Roberts (1991) when deriving structure-activity relationships for non-ionic surfactants. This was developed further by Roberts and Marshall (1994) who were able to generate accurate predictions of the acute toxicity to *Daphnia* of mixtures of alcohol ethoxylates based on an additive contribution to toxicity by each oligomer according to its mole fraction in the commercial product. It is reasonable to suppose the same relationship also holds for alkylphenol ethoxylates.

5.2 APnEOs

5.2.1 Acute toxicity

Most data on the acute toxicity of APnEO surfactants relate to studies with chain lengths of between 9 and 11 EO units, reflecting the prevalence of these substances in commercial products. Acute toxicities of these substances are summarised in Appendix C. Effect concentrations for the undegraded surfactants with between 9 and 11 EO units range between 1.6 and 44.2 mg l^{-1} and so these substances are somewhat less toxic than the corresponding alkylphenols, where effect concentrations in acute tests are usually below 1 mg l^{-1} (Whitehouse *et al*, 1998a, 1998b).

Sections 3 and 4 show that, unless untreated wastes containing APnEO surfactants are released to surface waters, these substances are less likely to be found as environmental contaminants than the ‘lower’ ethoxylated and carboxylated alkylphenolic compounds. It is also clear that the ethoxylated compounds become more toxic with progressive loss of EO

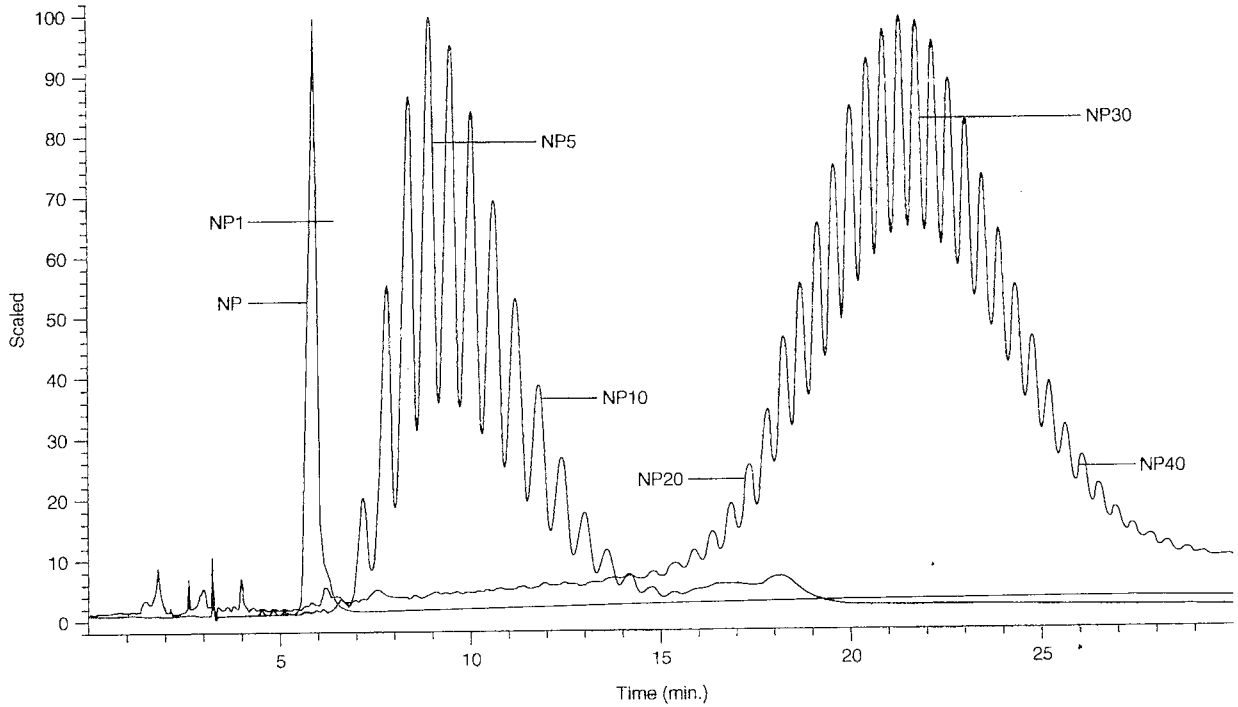

Figure 5.1 Distributions of oligomers in NP, NP1EO and two commercial surfactant products, NP5EO and NP30EO (reproduced with the permission of ICI Surfactants)

units: the toxicities of APnEOs with a range of EO units have been tested against several species of aquatic organisms (*Daphnia* sp., the mysid crustacean *Mysidopsis bahia,* bluegill sunfish *Lepomis macrochirus,* the Japanese killifish *Oryzias latipes,* the freshwater alga, *Scenedesmus sp.* and the marine bacterium, *Vibrio fischeri*) (Janicke *et al*, 1969; Hall *et al*, 1989; Macek and Krzeminski, 1975; Yoshimura, 1986; Ribosa *et al*, 1993) and all show a clear inverse relationship between EO chain length and toxicity. The data of Yoshimura (1986), who tested a series of nine NPnEOs, including synthesised biodegradation intermediates on Japanese killifish show this trend most clearly with 48h EC50 values increasing from 1.4 mg l^{-1} for NP9EO to 110 mg l^{-1} for NP16.6EO.

Figure 5.2 summarises data from experiments in which the effect of EO chain length of NPnEOs on toxicity to the freshwater species mentioned above was investigated. The inverse relationship between EO chain length and acute toxicity is evident for all these species and when all the data from these studies are combined, a correlation coefficient (r^2) of 0.93 results (regression parameters: $y=1.18e^{0.270x}$) between EO chain length and log LC/EC50. A very similar relationship between structure and acute toxicity is evident when the toxicity data are expressed in terms of molar concentrations rather than absolute concentrations. As a result, we can be confident that the relationship shown in Fig. 5.2 is not merely a function of the molecular weight of the APnEOs.

Although the intercepts vary between species (reflecting differences in sensitivity), the slope of the regression is very similar for the different species (Table 5.1), suggesting that this relationship is a general one which can be applied to a range of species. Support for this is provided by Figure 5.3 which includes data for other freshwater species where only one or two oligomers have been tested (all NP7EO-NP10EO). A useful correlation between the number of EO units and acute toxicity is maintained ($r^2 = 0.86$) with similar parameters for the slope and intercept ($y=0.82e^{0.28x}$), despite data having been amalgamated from around 25 different studies using a wide range of species, test methodologies and exposure duration.

Table 5.1 Regression parameters for different species

Species	**Intercept (mg l^{-1})**	**Slope**	**Correlation coefficient (r^2)**
Bluegill sunfish (*Lepomis macrochirus*)	0.67	0.246	0.99
Killifish (*Oryzias latipes*)	1.33	0.259	0.96
Water flea (*Daphnia magna*)	0.93	0.316	0.97
Unicellular alga (*Scenedesmus subspicatus*)	2.35	0.241	0.97

The corresponding data for saltwater mysid (*Mysidopsis bahia*) exposed to a series of highly branched NPnEOs (Figure 5.3) also exhibit a similar relationship to that seen in Figure 5.2 and with a high correlation coefficient ($r^2 = 0.98$). However, mysids appear to be more sensitive than the freshwater species, as indicated by a lower intercept with the y axis ($y=0.08e^{0.282x}$).

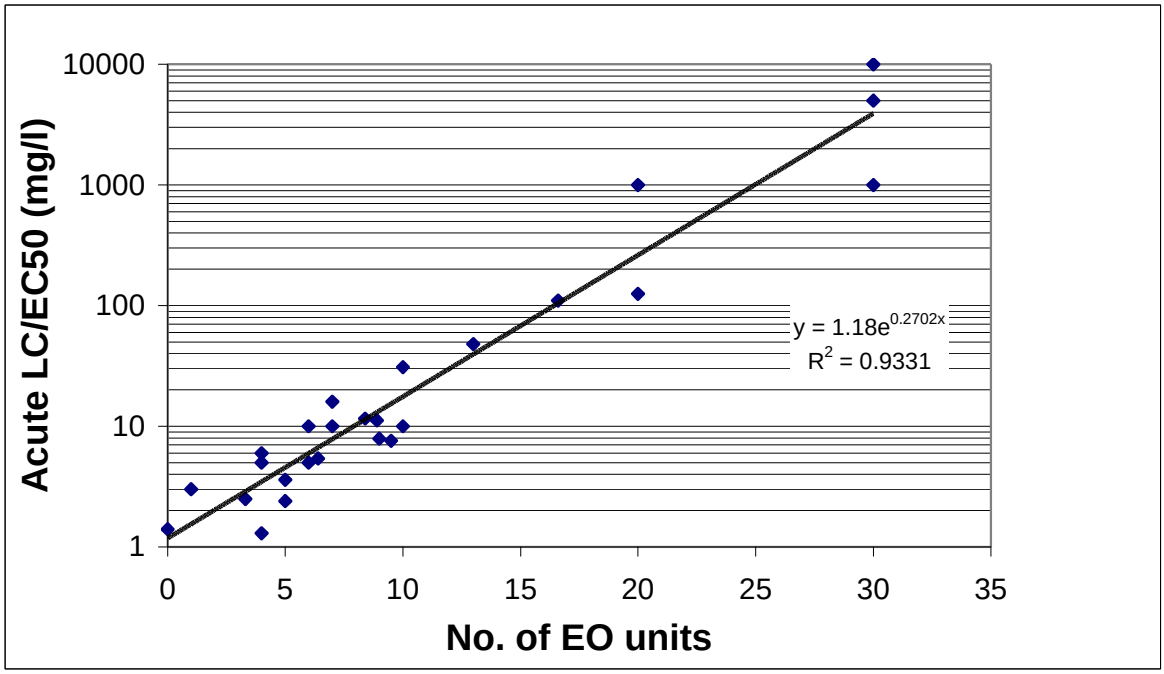

Figure 5.2 Relationship between EO chain length and acute toxicity of NPnEOs to selected species of freshwater fish, invertebrates and algae

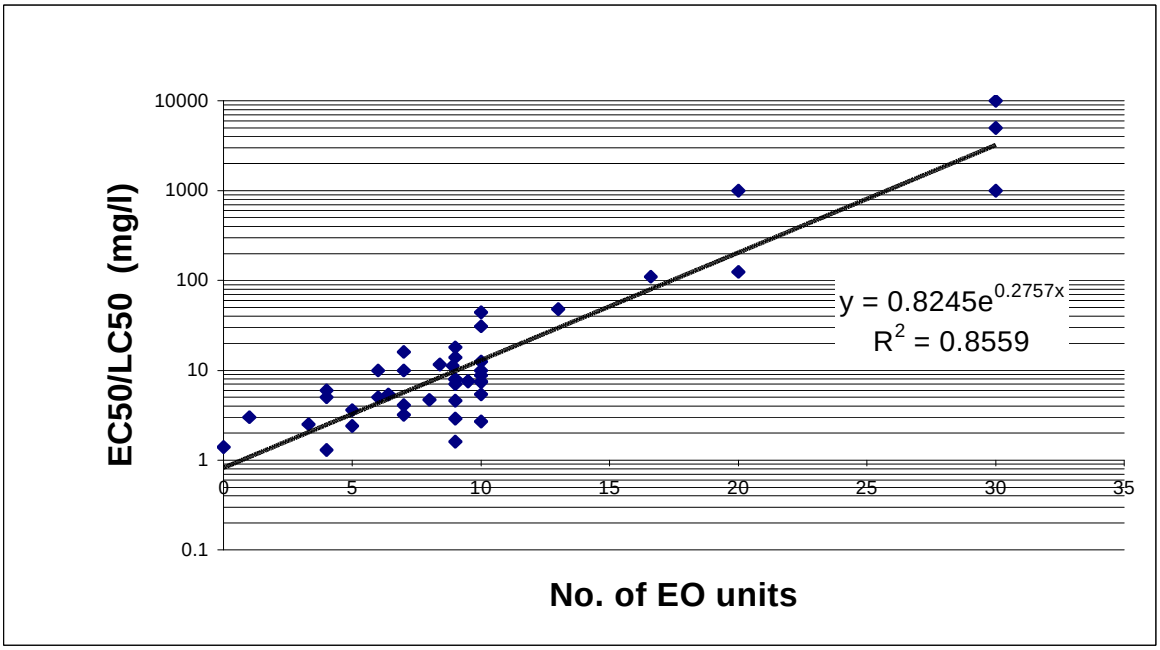

Figure 5.3 Relationship between EO chain length and acute toxicity of NPnEOs (all species)

Fewer data are available for OPnEOs but these indicate a similar relationship to that seen with NPnEOs (Table 5.2).

Roberts (1991) developed Quantitative Structure-Activity Relationships (QSARs) for non-ionic surfactants, including APnEOs based on their octanol-water partition coefficients. Using the fish toxicity data reported by Yoshimura (1986), he showed that toxicity conformed to a general narcosis QSAR. Moreover, differences in toxicity of APnEOs with different EO chain lengths could be explained in terms of the change in lipophilicity (each EO unit changed the log P increment by -0.1) when a certain branching pattern was assumed in the alkyl chain. This work by Roberts (1991) provides a mechanistic basis to the observation that toxicity is inversely related to EO chain length. Therefore we conclude that this relationship is sufficiently robust to predict acute toxicity from information on EO chain length, at least for nonylphenolic compounds. This will form an important part of the recommendations for standard-setting suggested in Section 7.

5.2.2 Chronic toxicity

Data for chronic toxicity of APnEOs are sparse by comparison and most of the data refer to studies with NP. In chronic studies with freshwater organisms, MATCs of 10 and 14 mg l^{-1} were reported for 7-day growth and 7-day mortality, respectively, of *Daphnia magna* when exposed to a branched alkyl NP9EO (Kravetz *et al*, 1991; Dorn *et al*, 1993). Studies by the same authors using fathead minnow (*Pimephales promelas*) gave rise to MATCs of 1.4 mgl^{-1} for the same substance/endpoints.

There are few studies which investigate the toxicity of low or intermediate EO chain lengths or the relationship between EO chain length and chronic toxicity. Nevertheless, in studies with the water flea *Daphnia magna* (Kravetz *et al*, 1991; Dorn *et al*, 1993; Comber *et al*, 1993) and fathead minnow, *Pimephales promelas* (Kravetz *et al*, 1991; Dorn *et al*, 1993; CMA, 1991) it is again clear that NP is much more toxic than NP9EO. OPnEO (Triton X) surfactants were also tested on the aquatic vascular plant, duckweed *(Lemna minor)* (Caux *et al*, 1988): at 50 mg l^{-1}, OP1EO and OP3EO depressed frond development by 25 to 50%, about twice as much as OP9-10EO suggesting that the toxicity of the surfactants to *L. minor* is inversely related to EO chain length.

Clearly, the chronic dataset for APnEOs and carboxylates is very small and standards for long-term protection (e.g. an Annual Average) would therefore have to rely on extrapolation from acute toxicity data with the uncertainty associated with acute to chronic extrapolation.

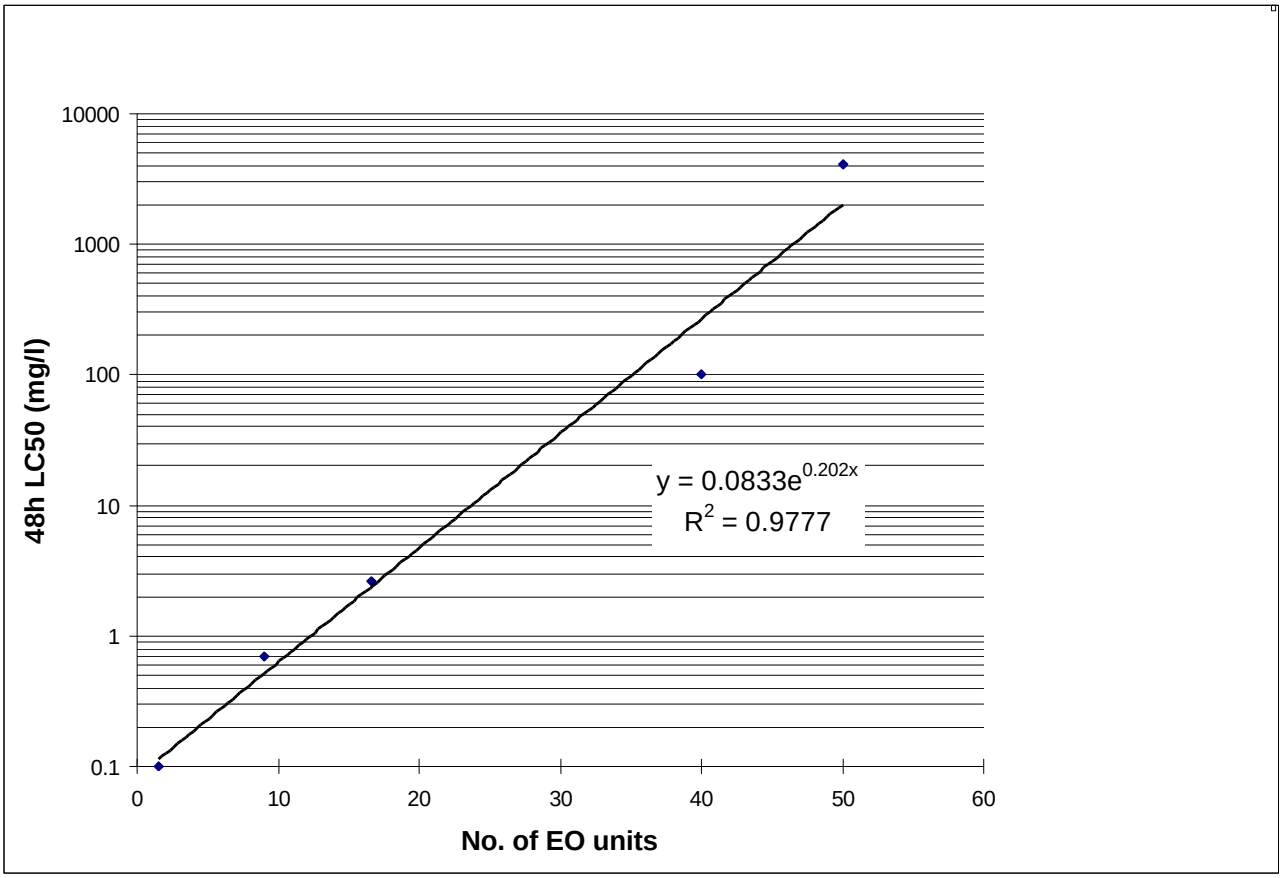

Figure 5.4 Relationship between EO chain length and acute toxicity of NPnEOs to mysid shrimps (*Mysidopsis bahia*) (after Hall *et al*, 1989)

Table 5.2 Relationship between EO chain length and acute toxicity of OPnEOs

Species/endpoint	No. of EO units	Acute LC50 (mg l^{-1})	Reference
Bluegill sunfish (*Lepomis macrochirus*)/96h survival	5	2.8 - 3.2	
	10	12.0	1
	30	531	
Mysid shrimp (*Mysidopsis bahia*)/48h survival	1.5	6.5 - 7.1	2
	5	1.8	

References:

1 Macek and Krzeminski (1975)
2 Hall *et al*, (1989)

5.3 APECs

Few aquatic toxicity data have been reported for the carboxylates, AP1EC and AP2EC. Yoshimura (1986) included both NP1EC and NP2EC in his study of the effects of a range of NPnEOs on Japanese killifish. This led to 48h LC50 values of 9.6 and 8.9 mg l^{-1} being estimated for NP1EC and NP2EC, respectively. These toxicities correspond to that exhibited by nonylphenol ethoxylates with between 6.4 and 8.4 EO units. Thus, on the basis of these experiments, NP1EC and NP2EC may be regarded as intermediate in toxicity between the 'lower' NPnEOs (NP1-3EO) and the undegraded alkylphenol ethoxylate surfactants with around 9-11 EO units.

In recent studies with the water flea, *Ceriodaphnia dubia*, a 96h LC_{50} of 110 mg l^{-1} was estimated for NP1-2EC along with 7-day NOECs of 8.4 and 2.2 mgl^{-1} for survival and fecundity, respectively (Staples, APE Research Council, *pers. comm*.). Since no other toxicity data have been reported for this species it is difficult to suggest what an equivalent EO chain length might be in the NPnEO series.

NP1EC was included as a test substance in an experiment to examine the effects of a NPnEO surfactant, NP and NP1EC on mitochondrial respiration in sub-mitochondrial particles isolated from beef heart (Argese *et al*, 1994). The relative toxicities were different to those seen in aquatic organisms, with EC50s of 1.3, 1.8 and 8.2 mg l^{-1} for the surfactant, NP and NP1EC, respectively. In this assay, the un-degraded NPnEO surfactant appears to be more toxic than would be expected, based on 'conventional' aquatic toxicity tests.

5.4 Relationship between acute and chronic toxicity

Although chronic effects occur at concentrations lower than those giving rise to acute toxicity, the ratio between acute effects concentrations and chronic no-effects concentrations is small. Studies in which acute and chronic toxicity has been estimated on the same species and by the same author reveals ratios of between 1.4 and 6.8.

5.5 Oestrogenic activity

The oestrogenic properties of certain alkylphenolic compounds have led to considerable interest in this aspect of their toxicology. Indeed, the restrictions on use of APnEO surfactants described in Section 2 have largely been driven in recent years by concerns about the generation of oestrogenic substances in the environment following biodegradation.

Several workers have demonstrated oestrogenic effects of certain of the lower APnEOs and APnECs in a variety of *in vitro* and *in vivo* assays. NP2EO and NP1EC have been shown to exhibit potencies similar to that of NP, although they are all considerably less oestrogenic than the naturally-occurring oestrogen, β17-oestradiol. Oestrogenic effects of NP, NP1EC and NP1EO *in vivo* (vitellogenesis and testicular growth in rainbow trout) occurred at much lower concentrations than those required to elicit responses *in vitro* (vitellogenesis in trout hepatocytes), although this may reflect the very different exposure times used in these studies and so they may not be directly comparable. The 'higher' ethoxylates (NP9EO, NP12EO and NP40 EO have been tested) are either only very weakly oestrogenic or have no demonstrable

activity, reflecting the structure-activity pattern seen between EO chain length and acute toxicity. Further details of these studies are given in Appendix C.

5.6 Bioaccumulation

Laboratory and field studies into the bioaccumulation of APnEO surfactants indicate a moderate tendency to bioaccumulate although metabolism and depuration rates are often rapid. Appendix C summarises the available bioaccumulation data for degradation products of APnEO surfactants and indicates BCF values for NP1EO and NP2EO of between 3 and 330 in fish and values of 200 and 500 for NP1EO and NP2EO respectively in a macroalga. These are broadly consistent with those found with NP (Whitehouse *et al*, 1998a).

5.7 Mammalian toxicity

Appendix D summarises the available mammalian toxicity data for APnEOs. Of the numerous APnEOs in existence, one of the more studied is nonoxynol-9 (NP9EO), which is largely due to its use as a spermicide. No mammalian toxicity data for APnECs were located other than some data on their oestrogenic activity.

In general, the data indicate that for acute exposures, APnEOs exhibit a low order of mammalian toxicity by the oral, dermal or inhalation route of exposure and for APnEOs with greater than 30 EO units, they can be considered essentially non-toxic. Although APnEOs with an average ethoxylate chain length of 9-10 units exhibited the greatest toxicity in some studies, the data are too scattered to make conclusions concerning toxicity and chemical structure. This is unlike the data for aquatic life in which there is a clear inverse relationship between acute toxicity and ethoxylate chain length (Section 5.2). The lower chain APnEO surfactants with chain lengths of up to 20 ethoxylate units are of similar acute toxicity to NP and OP.

APnEOs are also of low subchronic and chronic oral toxicity although, again, the data are too limited and scattered to make robust conclusions concerning toxicity and chemical structure. Any assessment of the relationship between toxicity and chemical structure is further complicated by the fact that there can be significant species differences in toxicity for a particular APnEO. For example, cardiotoxicity has been demonstrated in dogs and guinea pigs when exposed to AP15-20EO but not in rats, rabbits or cats. The lowest NOAEL of 10 mg kg^{-1} body weight day^{-1} reported in a 90 day subchronic feeding study in dogs and rats is for NP9EO.

In recent years, there has been some concern about the potential oestrogenic properties of APnEOs, in particular NP1EO and NP2EO based on a variety of *in vitro* assays and an *in vivo* study in rainbow trout (see Appendix C and section 5.5). However, it would appear that longer-chain NPnEOs with greater than 9 EO units either have only very weak or no demonstrable oestrogenic activities in such tests. Furthermore, NP4EO and NP9EO showed no evidence of oestrogenic activity when tested in the uterine weight assay in immature female rats.

6. PRIORITIES FOR DEVELOPMENT OF EQSS

6.1 Prioritisation of compounds

It is clear from Section 4 that the 'lower' APnEOs, containing fewer than 6 EO units, and the alkylphenols NP and OP, are more likely to be found in surface waters receiving inputs from sewage works where there is some biological treatment than the 'higher' oligomers. The carboxylates, AP1EC and AP2EC may also be important environmental contaminants, especially where wastes have been subjected to aerobic degradation. Undegraded surfactants with larger EO chain lengths are only likely to be found in the vicinity of discharges from industrial sites where these surfactants are being manufactured or used, or sewage works where there is no biological treatment or where this is of impaired efficiency.

With the limited monitoring data available, it is not possible to estimate how widespread these substances are as significant contaminants in surface waters. However, it may be possible to prioritise degradation products of APnEOs by the following methods:

1. The prioritisation scheme developed by Hedgecott and Cooper (1991) can be applied to surfactants and to those products of APnEO breakdown for which suitable data (tonnage, toxicity, bioaccumulation and persistence) are available. This approach has previously been used to rank substances with respect to priorities for EQS development.

2. The risks to aquatic life posed by particular breakdown products may be examined by comparing concentrations which have been determined in surface waters with their toxicities to aquatic life. Such an approach has been used by Weeks *et al,* (1998), based on a survey of US rivers, to assess the risks posed to aquatic life by NP.

Approach 1: Prioritisation scheme

Table 6.1 summarises the threshold values conventionally employed for prioritising substances with respect to EQS development. Using these criteria, undegraded APnEOs, NP1EO, NP2EO and NP attract the classifications shown in Table 6.2. Applying the decision scheme proposed by Hedgecott and Cooper (1991) to these data (Fig. 6.1) would result in a 'moderate' priority rating for APnEO surfactants NP1EO, NP2EO and NP (Table 6.2) although NP is expected to pose the greatest risk by virtue of its greater toxicity, persistence and possibly bioaccumulation potential. It is also more oestrogenic than any of the other alkylphenolic compounds.

Table 6.1 Threshold values for prioritising substances (after Hedgecott and Cooper, 1991)

Property	Classification		
	High	**Medium**	**Low**
Amount (tonnes p.a.)	>10000	1000-10000	<1000
Acute toxicity (LC/EC50, mg l^{-1})	<1	1-100	>100
Bioaccumulation (BCF)	>1000	100-1000	<100
Persistence (half-life, days)	>100	10-100	<10

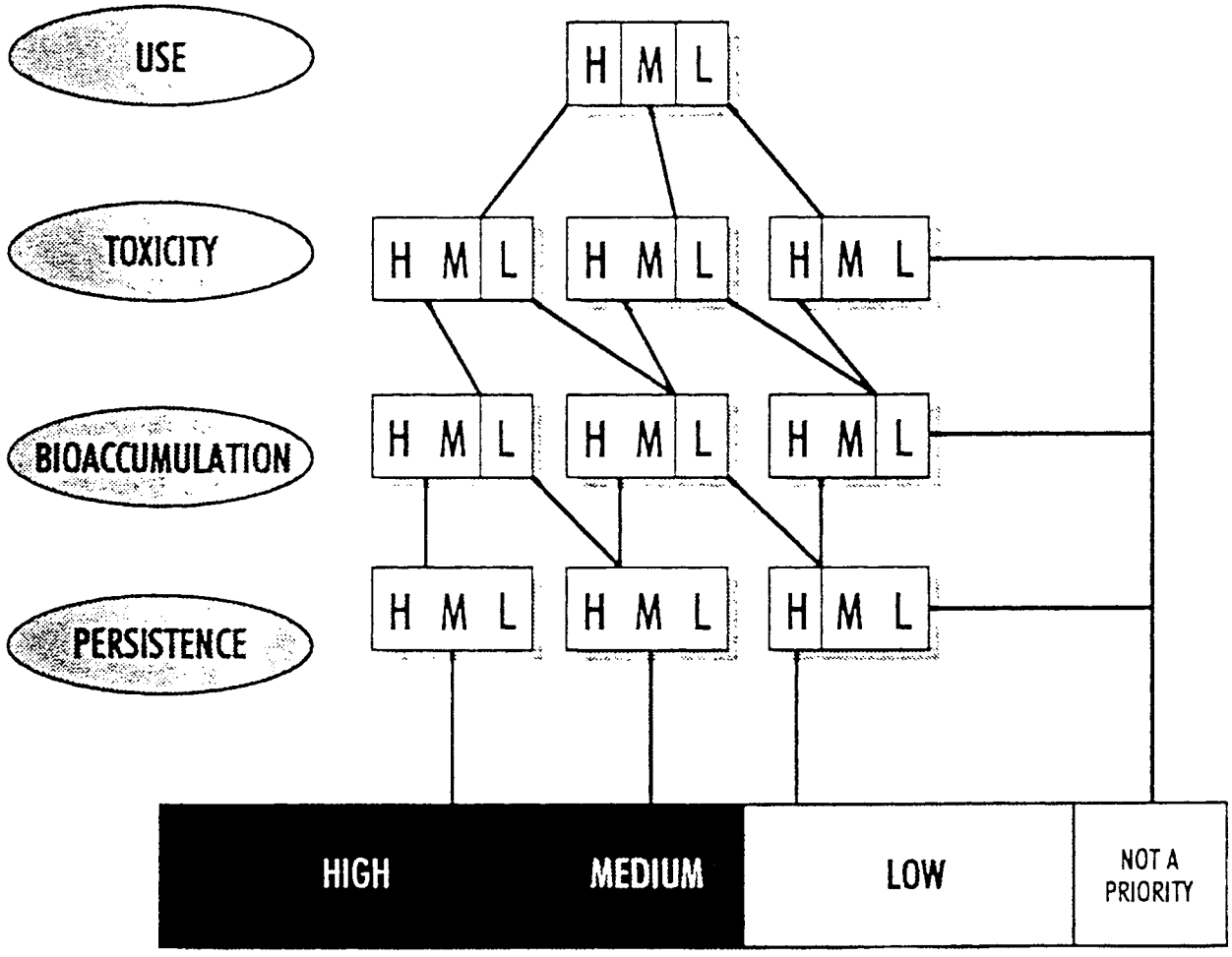

Figure 6.1 Decision criteria for prioritisation of substances (after Hedgecott and Cooper, 1991)

Table 6.2 Prioritisation classifications for alkylphenolic compounds (based on nonylphenolic compounds)

	Substance		
Property	**NP>9EO**	**NP1-2EO / NP2EC**	**NP**
Amount (tonnes p.a.)	5059 (M)[1]	5280 (M)[2]	3840 (M)[3]
Acute toxicity (LC/EC50, mg l^{-1})[6]	2.7-44.2 (M)[4]	1.0-9.6 (M)[4]	0.012-1.4 (H)[4,5]
Bioaccumulation (BCF)	M[6]	M[7]	200-1300 (M)[5]
Persistence (half-life, days)	L	L (NP1-2EO)[8]	H
Overall Classification	**'Moderate'**	**'Moderate'**	**'Moderate'**

Notes:

1. Estimated annual UK production of NPnEO (17600 tonnes p.a., CES, 1993) where the assumed 'average' product is NP9EO and after allowing for NP contribution (6261 tonnes produced and 3840 tonnes released into the environment p.a.)
2. Based on combined release of NP1-2EO and NP1-2EC from Swiss sewage treatment works estimated by Ahel *et al*, (1994): 30% of total nonylphenolic load to works released asNP1-2EO and NP2EC, multiplied by annual UK production of NPnEOs (CES, 1993)
3. Total quantity of NP discharged to sewer and river by industry (CES, 1993)
4. Toxicity data taken from Appendix C; 'worst-case' data used in risk classification
5. There are no reliable data for the 'higher' ethoxylates; a worst-case' assumption is that bioaccumulation is as high as for the 'lower' ethoxylates.
6. Toxicity and bioaccumulation data for NP extracted from Whitehouse *et al*, (1998a)
7. Assumed bioaccumulation classification, based on classifications of more polar NP10EO and less polar NP
8. In laboratory die-away tests, NP1-2EC were estimated to persist for at least 30 days (Yoshimura, 1986; Potter *et al*, 1999)

Approach 2: Comparison of Toxicity Data with Environmental Concentrations

Using the data on measured environmental concentrations of alkylphenolic compounds generated by Ahel and co-workers from their work on the River Glatt in Switzerland (Section 4) and comparing these with reported effect concentrations for the same substances (Section 5), margins of safety for these compounds may be estimated (Table 6.3).

It should be remembered that this assessment is really only applicable to the circumstances of the Glatt River at that time and in some cases, particularly the NP1EC and NP2EC, aquatic toxicity data are very sparse, adding further uncertainty to the margins of safety for these compounds in particular. There has been no attempt to extrapolate to a 'no effects' concentration or to take account of other species which may be more sensitive than those for which toxicity data are available. Consequently, the margins of safety shown in Table 6.3 may be regarded as over-estimates, possibly by around two orders of magnitude if conventional safety factors are applied to these acute effects concentrations to extrapolate to an acceptable concentration.

On the other hand, it is likely that a significant proportion of the measured substances could actually be sorbed onto bed and suspended sediments and thereby may not be bioavailable. Indeed, level 1 fugacity modelling indicates that losses of NP2EO from water onto bed sediments and suspended sediments may be very significant, although the extent of this loss is strongly influenced by the amount of suspended sediment in particular (Table 6.4). Because log P values for the other nonylphenolic compounds are very similar, we would expect a similar pattern to emerge for these. Consequently, the environmental concentrations of bioavailable NP, NP1-2EO and NP1-2EC may be appreciably lower than those shown in Table 6.3 and the safety margins would be underestimated accordingly. The relative contributions of these two opposing factors will depend on the particular physical characteristics (suspended and bed sediment and organic mater content of the sediment). However, it is reasonable to conclude that any potential risk to water column organisms will be reduced when high concentrations of suspended sediments are present.

Based on this preliminary assessment of risk to aquatic organisms, the priorities for development of standards should follow the order:

NP >> NP1EO = NP2EO > NP2EC > NP1EC

As noted above, this ranking is based on a very limited dataset for NP1EC and NP2EC in particular (only two toxicity studies are available) and so the position of these substances in the ranking is subject to considerable uncertainty.

A very similar rank order was concluded in a recent risk assessment described by Staples *et al* (1998b) in which exposure concentrations were taken from studies of freshwater locations in the US and no effect concentrations were predicted from laboratory studies using *Ceriodaphnia*. Again, a narrower margin of safety was evident for NP1EO and NP2EO than for NP1EC and NP2EC although in no case did the PEC:PNEC ratio exceed 1.0.

Table 6.3 Assessment of risks posed by alkylphenolic compounds (based on Ahel *et al*, 1994b)

Substance	Estimated 95%-ile for concentration in the River Glatt[1] (μg l^{-1})	Acute LC/EC50 (μg l^{-1})	Margin of safety (based on lowest LC/EC50)
NP1EO	15	1040[3], 3000[2]	69
NP2EO	16	1040[3]	65
NP1EC	27	9600[4]	356
NP2EC	57	8900[4]	156
NP	6	12.7-1400[5]	2

Notes:

1 Extracted from Ahel *et al*, (1994b)
2 48h LC50 to killifish (*Oryzias latipes*) (Yoshimura, 1986)
3 48h LC50 of mixture of NP1EO and NP2EO to *Ceriodaphnia dubia* (Ankley *et al*, 1990)
4 48h LC50 to killifish (*Oryzias latipes*) (Yoshimura, 1986)
5 Acute toxicity data from Whitehouse *et al*, (1998a); lowest value (12.7 μg l^{-1}) is 96h EC50 to *Gammarus pulex* (Sims *et al*, 1997)

Table 6.4 Predicted losses of NP2EO from water column by sorption onto suspended and bed sediments

% suspended solids[1](w/v)	% associated with water column[2]	% associated with bed sediment[2,3]	% associated with suspended sediment[1,2]
0.001	94.0	4.7	1.2
0.01	85.0	4.3	10.6
0.1	43.5	2.2	54.3
1.0	7.4	0.4	92.9

Notes:

1 Organic carbon content of 20%
2 Assumes equilibrium
3 Organic carbon content of 4%

6.2 Concluding remarks

An assessment of priority for EQS development based on tonnage in use, toxicity, persistence and bioaccumulation indicates that NPnEO surfactants, their ethoxylated (NP1EO and NP2EO) and carboxylate (NP1EC and NP2EC) breakdown products and also NP may be regarded as 'moderate' priorities for regulation. This assessment discounts possible oestrogenic effects which may further raise their priorities with respect to the development of standards.

Data are too sparse to permit further refinement of priorities with any confidence. However, of the breakdown products which are known to occur, NP poses a potentially greater risk to aquatic life than the 'lower' NPnEOs (NP1EO and NP2EO) and these in turn may pose a greater risk than the carboxylates, NP1EC and NP2EC.

All the products of alkylphenol ethoxylate surfactant breakdown are considerably more lipophilic than the parent surfactants and, as a consequence, are expected to preferentially sorb onto sediments. This will have the effect of reducing their bioavailability in the water column, although it might increase the risks posed to benthic organisms. It appears that this partitioning is strongly influenced by the level of suspended and bed sediments and, presumably, the organic matter contents of those sediments. Thus, there may be a strong site-specific influence on the level of risk posed by these substances to aquatic organisms. Although we would expect the APs, the 'lower' APnEOs and carboxylates to partition between water and sediment in a similar manner, this has yet to be confirmed experimentally. Moreover, there remains considerable uncertainty about their relative persistence in the environment.

The ranking suggested above is based on the relative toxicities of the compounds under review. However, this is subject to very considerable uncertainties caused by a lack of data in some areas, particularly with respect to data on environmental concentrations and data for the carboxylates, NP1EC and NP2EC. Acquisition of monitoring data for 'lower' APnEOs and APECs in particular, is highlighted as a priority for further investigation.

7. POSSIBLE APPROACHES TO THE DERIVATION OF EQSS FOR ALKYLPHENOLIC COMPOUNDS

7.1 Introduction

The assessments shown in Section 6 indicate that, in addition to the alkylphenols, the 'lower' APnEOs are also a priority for development of standards. The importance of the carboxylates remains uncertain but, based on the limited data available, they would seem to assume lower priorities. It is doubtful whether there are sufficient data to derive standards for the APECs although control of APEOs would be expected also to reduce the environmental burden of APECs and any adverse effects resulting from their presence.

It is clear from the preceding sections that a mixture of APnEOs will almost invariably occur together, usually with APECs and APs. Consequently, standards based on single substances are unlikely to be adequate. A different approach is needed, which will also address the toxicity of mixtures of alkylphenolic compounds.

In Section 7.2.1, two possible approaches to the derivation of EQSs for APnEOs are outlined. Emphasis is placed on protection against short-term exposure to APnEOs because most of the available data describe effects following acute exposure. However, standards to protect against long-term exposure may also be obtained by applying a safety factor to derived short-term standards. Section 7.2.2 suggests approaches for developing standards for the carboxylates.

7.2 Possible approaches to standard-setting

7.2.1 APnEOs

Option 1: Standards based on the assumption that APnEOs will give rise to alkylphenols

It is clear that all the alkylphenolic compounds described in this review may ultimately give rise to their respective alkylphenols (NP and OP) which, as shown earlier are the most toxic of the alkylphenolics. Reducing environmental concentrations of NP and OP would be a key objective of deriving standards for APnEOs. If we regard all these alkylphenolic compounds as intermediates to the formation of the more toxic alkylphenols, then it may be possible to encompass APnEOs within the existing standards for NP and OP. Whilst there is evidence of accumulation of alkylphenols (NP and OP) in sediments, these may not be the predominant metabolite in the overlying water. There is evidence that the carboxylates and 'lower' APnEOs may actually be more abundant in the water column. Because these are less toxic than NP and OP, this approach is inherently over-precautionary, at least as far as the water column is concerned.

Nevertheless, this approach can be achieved in principle because standards for NP and OP are already available (Whitehouse *et al*, 1998).

Monitoring for this option would simply require measured concentrations of total alkylphenolic compounds (APnEOs, APnECs and APs), possibly determined from the area under the peak resulting from a non-selective method such as HPLC with fluorescence detection (Appendix D) and comparing this estimated concentration with existing standards for NP and OP. The standards for NP and OP are identical and so it would not even be necessary to identify the alkyl moiety in the molecule.

However, there are some disadvantages with this approach and these are set out below alongside those features which favour such an approach.

	Advantages		**Disadvantages**
1.	The potential for generating more toxic and persistent breakdown products is explicitly taken into account.	1.	Likely to be over-precautionary because: (a) NP and OP are the most toxic alkylphenolic compounds. Increasing EO chain length reduces toxicity and so extent of 'over-precaution' could be large if sample contains high proportion of 'higher' ApnEOs (b) Molecular weights of oligomers are higher than those of NP and OP and increase with increasing EO chain length. So, if monitoring is based on concentration rather than molarity, the concentration of 'alkylphenol equivalents' will be over-estimated (c) The generation of NP and OP may not actually be realised because the residence time in a river of a discharge containing 'higher' APnEOs may be less than the time taken to convert the APnEOs to the alkylphenols. This would apply particularly in short rivers and discharges close to the sea where extensive dilution can be expected.
2.	Simple to implement; standards (for NP and OP) have already been derived.	2.	HPLC/fluorescence method prone to overestimating concentrations of alkylphenolic compounds because interferents cannot be identified without recourse to MS. This compromises analytical simplicity of this approach.
3.	Requires only estimation of total alkylphenolics, so it is not necessary to resolve individual oligomers and relatively simple HPLC/fluorescence method could suffice.	3.	Assumes that any interactions (synergism) between oligomers does not result in toxicity which is greater than that seen with NP or OP.
4.	There is a much more substantial database on the toxicity of NP and OP than the other alkylphenolic compounds and so standards derived for NP and OP are likely to be more robust than any derived for individual oligomers.	4.	Provides no information on the chemicals actually present and so provides no pointers about e.g. efficiency of biodegradation of inputs to water body

Option 2: Standards based on 'Toxic Equivalent Factors'

Conventionally, separate standards would be derived for each substance based on their respective toxicity datasets. However, in the case of APnEOs, a lack of toxicity data for many oligomers prevents derivation of robust standards. However, use can be made of the relationship between acute toxicity of APnEOs (or at least NPnEOs) and EO chain length, as described in Section 5.2.1. This relationship is sufficiently well defined to permit the toxicity of an APnEO with any number of EO units to be estimated even though toxicity data for that particular oligomer are sparse or lacking.

Essentially, standards may be derived from the relationship between EO chain length and acute toxicity, as illustrated in Figure 5.1. A parallel regression line which describes the relationship between EO chain length and a predicted 'no effects' concentration could be developed simply by applying a safety factor, to the regression for acute EC/LC50 data. There is an extensive dataset for NP and OP, and standards for these substances have been developed (Whitehouse *et al*, 1998a; 1998b). This standard could be used to determine the intercept of the 'no effects' line (i.e. APOEO) and so define the parameters of the 'no effects' regression. Because short-term standards (MAC) and long-term standards (AA) exist for NP and OP, it follows that corresponding standards could be derived for APnEO oligomers.

For compliance monitoring, samples are extracted and the constituent oligomers separated. Measured concentrations may then be compared with standards for individual APnEO oligomers. In addition to establishing standards for individual oligomers, standards for 'total' APnEOs may also be derived. This is important because oligomers will usually occur in combination and this allows for the possibility that no individual oligomer is present in sufficient concentrations to cause adverse effects but, in combination, the threshold for toxic effects is exceeded. The steps involved in assessing compliance would then be as follows:

1. Using the predicted differences in toxicity between NP or OP and each APnEO oligomer, the concentration of each oligomer detected (e.g. in an effluent discharge) is converted into the concentration of NP or OP which is predicted to result in a similar toxicity as the APnEO oligomer (i.e 'Toxic Equivalent Factors', TEFs).

2. The TEFs for all the oligomers present to toxicity is then combined in terms of the equivalent concentration of NP/OP ('Toxic Equivalents').

3. The sum of the TEFs for all the oligomers present is then compared with the appropriate standard for NP/OP.

Consider the situation in which just two oligomers, NP6EO and NP8EO, are detected: because NP6EO and NP8EO are predicted to be less toxic than NP, higher concentrations of these oligomer are deemed to be acceptable. The measured concentration of these oligomers are converted into concentrations of NP which would result in an equivalent level of toxicity (TEFs). These TEFs are then summed and compared with the standards for NP.

It is recommended that concentrations are normalised to those of NP (or OP) because EQSs for these substances have already been proposed and there is a much more substantial amount of ecotoxicity data for these compounds than for any of the ethoxylates or carboxylates.

Again, there are advantages and disadvantages of this approach and these are detailed below.

	Advantages		Disadvantages
1.	Utilises all available data relating toxicity to EO chain length and therefore resulting standards are based on the largest possible dataset	1.	Only addresses the hazards posed by the alkylphenolic compounds present at the time; does not account for the possibility of generating more toxic and persistent breakdown products as a result of biodegradation
2.	Allows standards to be derived for individual APnEOs and also for 'total' APnEOs	2.	Analysis of samples needs to resolve all oligomers/isomers
3.	Changes in oligomer distribution over time can be monitored e.g. as a result of management action	3.	Assumes toxicity of different APnEOs is additive
4.	May be possible to incorporate control of APECs (see Section 7.2.2)		

Conclusions

On balance, Option 1 would be highly precautionary because it makes assumptions about the ultimate fate of ApnEOs. Moreover, standards should be developed on a *prima facie* case of toxicity of the substance to be controlled, at the point of discharge and when monitored for in the receiving environment. Separate standards need to be derived for any ultimate degradation products if they also pose a toxic threat to aquatic life. EQSs developed for APnEOs must be able to reflect the incipient toxicity of individual APnEO oligomers but also account for the combined effects of all the oligomers present. These considerations are more closely satisfied by Option 2.

7.2.2 APnECs

The available toxicity data for APnECs are restricted to just two acute LC50 values for killifish (*Oryzias latipes*) from a study reported by Yoshimura (1986). This is insufficient to derive standards and so this remains an important gap in the available data, particularly since these degradation products have been found to be the most abundant alkylphenolic compounds in sewage effluents and surface waters in several studies (Section 5).

One possible way in which EQSs for these substances might be developed is by incorporating them into a variant of Option 2, described above. We have already seen that, within the APnEOs, there is a clear relationship between EO chain length and acute toxicity. There is also evidence to suggest that this arises through changes in lipophilicity as the number of EO units in the molecule changes and that the length of the EO chain may simply be a surrogate for log K_{ow} (Roberts, 1991). If so, Figure 5.2 could be simplified so that it relates acute

EC/LC50 to log K_{ow} instead of EO chain length. If it can be demonstrated that the toxicities of NP1EC and NP2EC are also a function of their lipophilicities (log K_{ow}) then measured concentrations of peaks corresponding to NP1EC and NP2EC could simply be compared with the standards for the log K_{ow} appropriate to these substances. Indeed, the HPLC conditions used to separate alkylphenolic compounds could effectively become a way of determining the log K_{ow} of any substances present including NPnECs (in the same way that OECD Test Guideline 117 uses reverse-phase HPLC to estimate log K_{ow}).

This approach has the advantage that it makes use of a much larger dataset to derive standards which are appropriate to the APnECs. However it is only plausible if the following assumptions can be supported:

- the toxicities of the APnEOs and also the APnECs are explicable in terms of their lipophilicity;
- the only substances present in the sample undergoing chromatography are alkylphenolics or the sample can be treated in such a way (e.g. using HPLC-MS) that only alkylphenolic compounds are considered;
- the retention characteristics of the carboxylates are known or can be identified.

8. PROPOSED APPROACH TO THE DEVELOPMENT OF STANDARDS FOR ALKYLPHENOLIC COMPOUNDS IN SURFACE WATERS

8.1 Introduction

The environmental risks posed by alkylphenolic compounds to aquatic life are unusual in that the substances normally present in wastes ('higher' and possibly partially degraded APnEOs) are degraded to substances which are more persistent, toxic and oestrogenic than the original APnEOs. The alkylphenols, NP and OP, give rise to particular concern because these are the most toxic and persistent of the breakdown products. Environmental contamination by alkylphenolic compounds arises predominantly as a result of point source discharges of APnEOs and their breakdown products to surface waters but, currently, the lack of standards for this class of substances prevents effective control. Below, standards for APnEOs are described based on Option 2 outlined in Section 7.

8.2 Derivation of standards

8.2.1 Standards for individual APnEO oligomers:

Standards for individual APnEO oligomers may be derived as described in Option 2 (Section 7). Essentially, the regression of acute LC_{50} vs EO chain length is taken and a safety factor applied to extrapolate to a standard which is intended to protect against toxicity in the receiving water. The safety factor used is equivalent to the ratio between the predicted LC_{50} for NP (i.e. NPOEO) and the EQSs derived for NP and OP (Whitehouse *et al*, 1998a; 1998b). In the case of the 'short-term' standards, this corresponds to a safety factor of 472[2] whilst for the 'long-term' standards, a safety factor of 1180[3] is necessary. In Figure 8.1 these safety factors are the intervals between the regression line for acute toxicity and the regression lines for the corresponding standards.

It follows that the standards for APnEOs with increasing numbers of EO units will become progressively higher, reflecting the predictable decline in toxicity. To derive the standard for a particular EO chain length, the corresponding acute LC_{50} is obtained from the regression and the safety factor applied to this value. For example, for NP5EO, the predicted log LC_{50} is 0.662 mg l^{-1} which corresponds to an LC_{50} of 4.59 mg l^{-1}. To extrapolate to a 'short-term' standard for NP5EO, the safety factor of 472[2] is applied, resulting in a concentration of 9.7 μg l^{-1}. To extrapolate to a 'long-term' standard, a safety factor of 1180[3] is applied, resulting in a standard of 3.9 μg l^{-1} for NP5EO. NP8EO is predicted to be less toxic to aquatic

[2] This factor is obtained from the ratio between the predicted LC_{50} for NP (1.18 mg l^{-1}) and the MAC that has been derived for this substance in freshwater (2.5 μg l^{-1}; Whitehouse et al, 1998)

[3] This factor is obtained from the ratio between the predicted LC_{50} for NP (1.18 mg l^{-1}) and the AA that has been derived for this substance in freshwater (1.0 μg l^{-1}; Whitehouse et al, 1998a;1998b)

life. In this case, the predicted acute LC_{50} is 10.31 mg l^{-1}. To extrapolate to a 'short-term' standard for NP8EO, the safety factor of 472 is again applied, resulting in a concentration of 21.8 μg l^{-1}. In this case, the corresponding 'long-term' standard is 8.7 μg l^{-1}.

The resulting standards for individual NPnEOs up to NP15EO are presented as a 'look up' table in Table 8.1 and are illustrated in Figure 8.2.

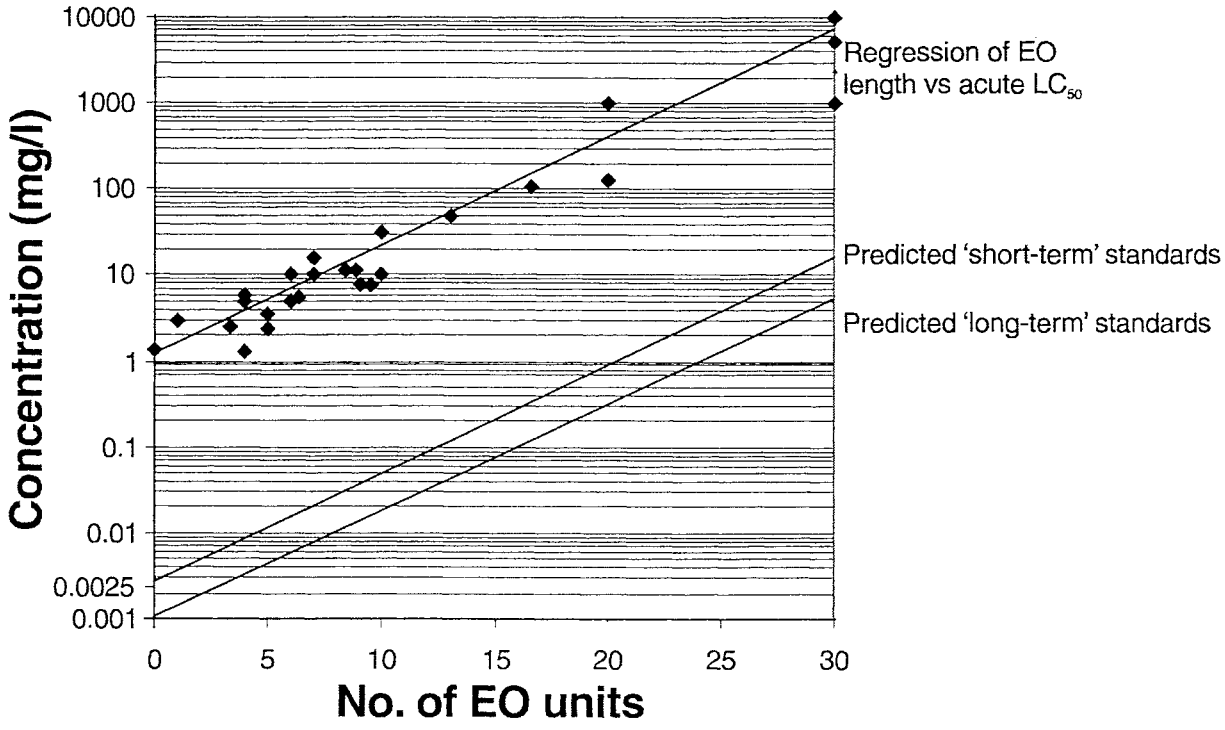

Figure 8.1 Derivation of standards for individual APnEOs, based on the relationship between EO chain length and acute toxicity

The values in brackets in Table 8.1 represent the ratio between the standard for each oligomer and that for NP. These effectively indicate the 'Toxic Equivalent Factor' for each oligomer. For example, NP5EO is predicted to be 3.9 times less toxic than NP and therefore may occur at a concentration 3.9 times higher than that of NP before the standard is considered to have been exceeded (assuming that no other oligomers are present).

Table 8.1 Derivation of standards for individual NPnEOs

APnEO oligomer	Acute LC_{50} (mg l^{-1})[1]	Standard (short-term exposure) (mg l^{-1})[2]	Standard (long-term exposure) (mg l^{-1})[3]
NP	1.18	2.5[4]	1.0[4]
NP1EO	1.56	3.3 (1.3)	1.3
NP2EO	2.05	4.3 (1.7)	1.7
NP3EO	2.68	5.7 (2.3)	2.3
NP4EO	3.50	7.4 (3.0)	3.0
NP5EO	4.59	9.7 (3.9)	3.9
NP6EO	6.01	12.7 (5.1)	5.1
NP7EO	7.87	16.7 (6.7)	6.7
NP8EO	10.31	21.8 (8.7)	8.7
NP9EO	13.12	27.8 (11.1)	11.1
NP10EO	17.66	37.4 (15.0)	15.0
NP11EO	23.12	49.0 (19.6)	19.6
NP12EO	30.20	64.0 (25.6)	25.6
NP13EO	39.63	84.0 (33.6)	33.6
NP14EO	51.88	109.9 (44.0)	44.0
NP15EO	67.99	144.0 (57.6)	57.6

1. From regression of acute LC_{50} vs EO chain length (Figure 5.1)
2. Application of safety factor of 472, based on the ratio between the predicted LC_{50} for NP (1.18 mg l^{-1}) and the MAC that has been derived for this substance in freshwater (2.5 µg l^{-1}; Whitehouse *et al*, 1998a; 1998b)
3. Application of safety factor of 1180, based on the ratio between the predicted LC_{50} for NP (1.18 mg l^{-1}) and the AA that has been derived for this substance in freshwater (1.0 µg l^{-1}; Whitehouse *et al*, 1998a;1998b)
4. These are the EQSs (MAC and AA, respectively) derived for NP and OP for the protection of freshwater life

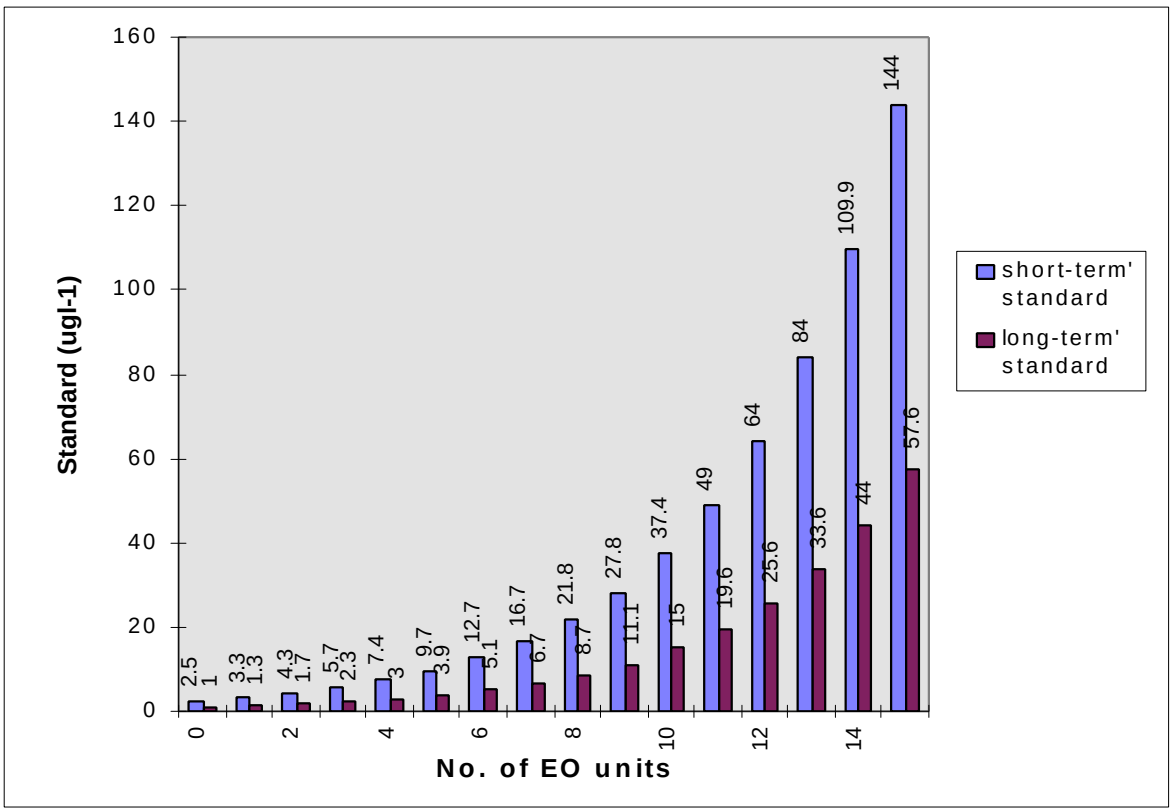

Figure 8.2 Proposed standards for individual APnEO oligomers

8.2.2 Standards for 'total' APnEO oligomers

Clearly, APnEO oligomers will rarely occur in isolation but together in a complex mixture, perhaps containing ten or more oligomers and possibly in combination with the alkylphenols.

Standards for 'total' APnEO oligomers are required to take account of the combined toxicity arising from exposure to several APnEOs simultaneously but which differ in their toxicities to aquatic life. For example, it is possible that several oligomers fall just below the standards for individual oligomers yet, in combination, their effects are additive[4] and together they exceed the threshold for toxicity. This may be addressed by combining the 'Toxic Equivalent Factors' for all the oligomers present into a single group parameter, as described below. In this approach, NP and OP may be regarded as alkylphenol ethoxylates with no EO units (i.e. 'AP0EO') so that the combined effects of the alkylphenols, NP and OP, and the ethoxylated alkylphenols are taken into account.

[4] The assumption of additivity is reasonable because the toxicity data for the oligomers is log-linear and it is very probable that the oligomers share the same toxic mode of action, differing in toxicity only by virtue of differences in uptake or transfer to the site of toxic action.

The concentration of each oligomer detected (e.g. in an effluent discharge) is converted into 'Toxic Equivalent Factors' i.e. the concentration of NP that is predicted to result in a similar toxicity as the APnEO oligomer. This is achieved based on the predicted differences in toxicity between NP and each APnEO oligomer, as shown in Table 8.1. Essentially, this process results in an equivalent concentration of NP and the sum of 'Toxic Equivalent Factors' for all the oligomers present is then simply compared with the appropriate standard for NP. Compliance with short-term and long-term standards for 'total' APnEOs may be then be assessed as described below.

In the following formulations, the denominator is the ratio between the standard for the oligomer of concern (shown in the numerator) and that for NP. So, for NP4EO, the short-term standard is 7.4 μg l^{-1}, 3.0 times higher than the corresponding standard for NP (Table 8.1), reflecting the difference in toxicity between NP4EO and NP. NP8EO is predicted to be even less toxic and so the numerator is correspondingly larger i.e. NP8EO is predicted to be 8.7 times less toxic than NP.

Maximum Acceptable Concentration for total APnEOs to protect against ***intermittent*** releases of APnEOs:

$$\frac{[NP]}{1.3} + \frac{[NP1EO]}{1.7} + \frac{[NP2EO]}{2.3} + \frac{[NP3EO]}{3.0} + \frac{[NP4EO]}{3.9} + \frac{[NP5EO]}{5.1} + \frac{[NP6EO]}{6.7} + \frac{[NP7EO]}{8.7} + \frac{[NP8EO]}{11.1} + \frac{[NP9EO]}{15.0} + \frac{[NP10EO]}{19.6} + \frac{[NP11EO]}{25.6} + \frac{[NP12EO]}{33.6} + \frac{[NP13EO]}{44.0} + \frac{[NP14EO]}{57.6} + \frac{[NP15EO]}{57.6} < \mathbf{2.5\ mg\ l^{-1}}$$

Maximum Acceptable Concentration for total APnEOs to protect against ***continuous*** release of APnEOs:

$$\frac{[NP]}{1.3} + \frac{[NP1EO]}{1.7} + \frac{[NP2EO]}{2.3} + \frac{[NP3EO]}{3.0} + \frac{[NP4EO]}{3.9} + \frac{[NP5EO]}{5.1} + \frac{[NP6EO]}{6.7} + \frac{[NP7EO]}{8.7} + \frac{[NP8EO]}{11.1} + \frac{[NP9EO]}{15.0} + \frac{[NP10EO]}{19.6} + \frac{[NP11EO]}{25.6} + \frac{[NP12EO]}{33.6} + \frac{[NP13EO]}{44.0} + \frac{[NP14EO]}{57.6} + \frac{[NP15EO]}{57.6} < \mathbf{1.0\ mg\ l^{-1}}$$

One important consequence of this 'Toxic Equivalent Factors' approach is that compliance with the standards will be determined by the distribution of oligomers present. Unlike the situation with conventional chemical-specific standards, it is not possible to say at the outset what the standard for a particular watercourse or discharge will be without first gaining an understanding of its alkylphenolics composition.

8.2.3 Combining standards for octyl - and nonylphenolic compounds

It is worth pointing out that the current EQSs for NP and OP are identical (a Maximum Allowable Concentration of 2.5 μg l^{-1} and an Annual Average of 1.0 μg l^{-1}), reflecting the view that these substances exhibit identical toxicities to aquatic life. Based on the available evidence, it is reasonable to suppose this also extends to the ethoxylates (i.e. OP4EO exhibits the same toxicity as NP4EO) and that additive toxicity will result when both nonylphenolic and octylphenolic compounds are present at the same time.

Therefore, standards may be expressed in terms of total alkylphenolic compounds, irrespective of whether they are derivatives of NPnEOs or OPnEOs, as follows:

'Short-term' standards:

Σ ([NP] + [Σ'toxic equivalents' for NPnEOs] + [OP] + [Σ'toxic equivalents' for OPnEOs]) < 2.5 ug l^{-1}.

'Long-term' standards:

Σ ([NP] + [Σ'toxic equivalents' for NPnEOs] + [OP] + [Σ'toxic equivalents' for OPnEOs]) < 1.0 ug l^{-1}.

8.3 Monitoring for compliance

8.3.1 Analytical requirements

As noted earlier, determination of concentrations of the alkylphenols, NP and OP, and individual APnEOs is required. The analytical techniques used should be capable of resolving different oligomers and a combination of extraction procedures employed to ensure that both polar (high EO chain length) and non-polar (small EO chain length) APnEOs are recovered. Guidance on suitable methods is given in Appendix E.

Assessment of compliance with 'short term' standards may be simplified where the distribution of oligomers is symmetrical and sufficiently well-defined so that the mean EO chain length can be estimated with confidence. In this situation, the total concentration of all oligomers (effectively the area under the trace describing the concentration of all oligomers - see Fig. 5.1) is compared with the standard for the oligomer corresponding to the mean EO chain length. This assumes that the distribution of oligomers in the effluent sample matches the distribution in the samples that were originally used to define the standards. We are unable to validate this assumption and so we caution against the routine application of this approach. Furthermore, it is only really applicable to 'short-term' standards where compliance assessment is based on the results of individual analyses rather than a summary of several analyses as would be the case when assessing compliance with 'long term' standards.

8.4 Constraints on the effectiveness of standards for APnEOs

The approach outlined above provides a means of deriving standards for a wide range of APnEO oligomers. It addresses the immediate hazards posed by alkylphenolic compounds but fails to account for the effects of their breakdown products, which will usually be the more persistent and hazardous alkylphenols, NP and OP.

An effective approach is one which addresses both the immediate hazards posed by alkylphenolics discharged to surface water and the possibility that other, more toxic, compounds will be formed in the environment over time. This could be achieved by a combination of point source controls coupled with a programme of environmental monitoring for the most hazardous and persistent substances, at least NP and OP. The monitoring could provide an assessment of the effectiveness of the effluent control measures. If there was evidence that EQSs for either NP or OP were being exceeded, then this would act as a trigger

to identify the major discharges of alkylphenolic compounds and, where necessary, to refine the discharge limits. Details of the ways in which these standards may be implemented for point source control are covered in detail in a separate report (Whitehouse, 2000).

It is important to recognise that reliance on EQSs for NP and OP as a means of judging the extent of environmental contamination by these substances may be subject to error. This stems from the tendency of these substances to sorb to sediments (see Section 4.3) and thereby be removed from the water column. As a result, analysis of water samples could lead to a environmental contamination by NP and OP being under-estimated. This situation would be aided by the development of sediment standards for NP and OP and this is identified as a key research need.

Environmental monitoring for NP and OP is also subject to uncertainty arising from the rate at which APnEOs are converted to NP and OP in the receiving water (and associated sediments). For example, if substantial quantities of 'lower' ethoxylates are released, they may be degraded to NP and OP only some considerable distance downstream of the discharge. The rate at which NP and OP appear in the receiving water at any particular point depends on the level of inoculum present, physical factors e.g. temperature and, of course, the rate of flow. This will be offset by losses due to sorption of NP and OP onto bed and suspended sediments and, to a lesser extent, on the ultimate biodegradation of NP and OP. Clearly, the location of monitoring stations could have a major bearing on our assessment of environmental contamination by alkylphenols. Johnson *et al* (1998) have described the sorption of OP onto bed and suspended sediments in different UK rivers but highlight differences between sites in the extent to which OP will remain in solution or suspension.

Currently, our understanding of the fate of APnEOs and alkylphenols in rivers is inadequate to guide the selection of suitable sampling points for environmental monitoring of NP and OP. This problem highlights a second area of research - to gain a better understanding of the fate and redistribution of alkylphenolic compounds under realistic conditions - which is needed to improve the quality and accuracy of environmental monitoring of alkylphenols.

9. CONCLUSIONS AND RECOMMENDATIONS

9.1 The need for standards

The environmental risks posed by alkylphenolic compounds to aquatic life are unusual in that the substances normally present in wastes ('higher' and possibly partially degraded APnEOs) give rise to other substances which are more persistent, toxic and oestrogenic than the original APnEOs. The alkylphenols, NP and OP give rise to particular concern because these are without doubt the most hazardous and persistent of the breakdown products. Environmental contamination by alkylphenolic compounds arises predominantly as a result of point source discharges of APnEOs and their breakdown products but the lack of standards for these substances means there is currently no mechanism for regulating discharges of any alkylphenolic compounds (other than NP and OP) to surface waters.

An assessment of the risks posed by alkylphenolic compounds to aquatic life (Section 6) indicates that the alkylphenols NP and OP probably pose the greatest risks by virtue of their toxicity and environmental persistence. Their ethoxylated counterparts (APnEOs) are also acutely toxic to aquatic life but the extent of the hazard posed is a function of their EO chain length. The 'lower' ethoxylates (APnEOs with between 1 and 4 EO units) probably pose greater immediate hazards to aquatic life than the carboxylate (APEC) derivatives. Of course, they may also break down to more toxic substances in the receiving water, which adds to the case for developing standards for these substances.

9.2 Proposals for the development of standards

Possible measures to identify and, if necessary, control the possible impacts caused by APnEOs have been considered. The favoured approach is one based on standards for individual and total APnEOs (including the alkylphenols with no EO units) derived by interpolation from the relationship between acute toxicity and EO chain length for these substances, coupled with a safety factor to extrapolate to a 'no effects' concentration. This allows 'Toxic Equivalent Factors' for all the oligomers to be developed in which concentrations of individual oligomers are expressed in terms of an equivalent concentration of NP. The resulting standards for the more toxic oligomers are correspondingly more stringent and allowance is made for the effects of combinations of APnEO oligomers when they occur together. Standards relating to both short-term (e.g. intermittent discharge) and long-term (e.g. continuous discharge) have been derived in this way.

There is evidence to indicate that the toxicities of nonylphenolic and octylphenolic compounds are equivalent and that the relationship between EO chain length applies equally to NPnEOs and OPnEOs. Furthermore, based on our understanding of the toxic mode of action of these alkylphenolic compounds, it is reasonable to suppose that additive toxicity will result when both nonylphenolic and octylphenolic compounds are present at the same time. Therefore, standards may be expressed in terms of total alkylphenolic compounds, irrespective of whether they are derivatives of NPnEOs or OPnEOs.

The application of these standards as a means of regulating point source discharges are described in detail in a separate report (Whitehouse, 2000).

Currently, there are no sediment standards available for any of the alkylphenolic compounds discussed here and this remains a significant shortcoming in any measures to control emissions of this class of substances. Furthermore, APECs represent a potentially persistent form of alkylphenolics in the environment. Although they may not pose such a serious environmental risk as the lower APnEOs, NP or OP it has not been possible to derive standards for APECs using the approach described here.

9.3 Research needs

As suggested above, there are a number of uncertainties associated with the standards proposed here. However, these may be addressed through the following areas of research:

1. EQSs for NP and OP are only available for the water column although sediments are known to be an important environmental sink for these compounds, where they typically occur at much higher concentrations. This may result in failure to detect unacceptable contamination of water courses by NP and OP and therefore inadequate environmental protection.

   The derivation of sediment standards for NP and OP should be considered. Unpublished data for Chironomids and benthic and epibenthic amphipods (*Hyalella*, *Gammarus*) are available but the range of species would need to be extended. Alternatively, 'interim' standards may be inferred from water column toxicity data in conjunction with an equilibrium partitioning approach.

2. APECs represent a potentially important source of alkylphenols in the environment. Although they may not pose such a serious environmental risk as the lower APnEOs, NP or OP (Section 6.1), they are not covered at all by the approach outlined above. A better understanding of their occurrence in effluent discharges and surface waters would help to indicate the extent of environmental contamination by APECs and, if significant, consideration of chemical-specific standards may be warranted. In this case, new ecotoxicity data would have to be generated because the dataset for APECs is currently restricted to only two studies.

10. REFERENCES

Ahel, M. and Giger, W. (1985a) Determination of alkylphenols and alkylphenol mono- and diethoxylates in environmental samples by high-performance liquid chromatography. *Anal. Chem.*, **57,** 1577-1583.

Ahel, M. and Giger, W. (1985b) Determination of non-ionic surfactants of the alkylphenol polyethoxylate type by high-performance liquid chromatography. *Anal. Chem.*, **57,** 2584-2590.

Ahel, M., Conrad, T. and Giger, W. (1987) Persistent organic chemicals in sewage effluents. 3. Determination of nonylphenoxy carboxylic acids by high-resolution gas chromatography/mass spectrometry and high-performance liquid chromatography. *Environs. Sci. Technol.*, **21,** 67-703.

Ahel, M., Giger, W. and Schaffner, C. (1991) Environmental occurrence and behaviour of alkylphenol polyethoxylates and their degradation products in rivers and groundwaters. In: Swedish EPA Seminar on Nonylphenol Ethoxylate/Nonylphenol held in Saltsjobaden, Sweden, 6-8 February, pp. 105-151.

Ahel, M., Giger W. and Koch, M. (1994Aa) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - I. Occurrence and transformation in sewage treatment. Water Res., **28,** 1131-1142.

Ahel, M., Giger, W. and Schaffher, C. (1994b) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - II. Occurrence and transformation in rivers. Water Res., **28,** 1143-1152.

Ahel, M., Schaffner, C. and Giger, W. (1996) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - III. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. *Wat. Res.*, **30,** 37-46.

Ankley, G.T., Peterson, G.S., Lukasewycz, M.T. and Jenner, D.A. (1990) Characteristics of surfactants in toxicity identification evaluations. *Chemosphere,* **21,** 3-12.

Argese, E., Marcomini, A., Miana, P., Bettiol, C. and Perin, G. (1994) Sub-mitochondrial particle response to linear alkylbenzene sultanates, nonylphenol polyethoxylates and their biodegradation derivatives. *Environs. Toxicol. Chem.*, **13,** 737-742.

Ball, H.A., Reinhard, M. and McCarty, P. (1989). Bio-transformation of halogenated and non-halogenated octylphenol polyethoxylate residues under aerobic and anaerobic conditions. Environs. Sci. Technol., **23,** 951-961.

Bennie, D.T. (1999) Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates. *Water Qual. Res. J. Canada*. **34**, 79-122.

Brunner, P.H., Capri, S., Marconiini, A. and Giger, W. (1988). Occurrence and behaviour of linear alkylbenzene sulphonates, nonylphenol, nonylphenol mono- and nonylphenol diethoxylates in sewage and sewage sludge treatment. Water Res., **22,** 1465-1472.

Blackburn, M. and Waldock, M.J. (1995) Concentrations of alkylphenols in rivers and estuaries in England and Wales. Report from the Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Bunnham-on-Crouch, Essex, UK.

Brown, D., de Henau, H., Garrigan, J.T., Gerike, P., Holt, M., Kunkel, E., Matthijs, E., Waters, J. and Watkinson, R.J. (1987) Removal of non-ionics in sewage treatment plants. II. Removal of domestic detergent non-ionic surfactants in a trickling filter sewage treatment plant. *Tenside,* **24,** 14-19.

Brüschweiler, H. and Gämperle, H. (1982) Primary and ultimate biodegradation of alkylphenol ethoxylates. *J. Com. Esp. Deterg.*, pp. 55-71.

Caux, P.Y., Weinberger, P. and Carlisle, D.B. (1988) A physiological study of the effects of Triton surfactants on *Lemna minor* L. *Environ. Toxicol. Chem.*, **7,** 671-676.

CES (1993) *Uses, Fate and Entry to the Environment of Nonylphenol Ethoxylates*. Consultants in Environmental Sciences Ltd (for the Department of the Environment), Beckenham, Kent.

CMA (Chemical Manufacturers Association) (1991b) Early life stage toxicity of nonylphenol to the fathead minnow, *Pimephales promelas*. Unpublished report, Alkylphenol & Ethoxylates Panel, CMA, Washington, DC.

Comber, M.H.I., Williams, T.D. and Stewart, K.M. (1993) The effects of nonylphenol on *Daphnia magna*. *Water Res.,* **27,** 273-276.

Di Corcia, A., Samperi, R. and Marcomini, A. (1994). Monitoring aromatic surfactants and their biodegradation intern-iediates in raw and treated sewage by solid-phase extraction and liquid chromatography. *Environ. Sci. Technol.*, **28,** 850-858.

Dorn, P.B., Salanitro, J.P., Evans, S.H. and Kravetz, L. (1993) Assessing the aquatic hazard of some linear and branched non-ionic surfactants by biodegradation and toxicity. *Environ. Toxicol. Chem.,* **12,** 1751-1762.

EEC (1982) Directive 82/242/EEC: Methods of testing the biodegradability of non-ionic surfactants. Official Journal L109/1, 22/4/82.

ENDS (1996) Denmark takes lead in curbs on oestrogenic chemicals. ENDS Report 252, January 1996, 35-36.

ENDS (1997) Wool scourers phase out APEs. ENDS Report 266, March 1997, 9.

ENDS (1998) Phase out for APEs draws closer. ENDS Report 280, May 1998, 3-4.

Field, J.A. and Reed, R. (1996) Nonylphenol polyethoxycarboxlate metabolites of non-ionic surfactants in US paper mill effluents, municipal sewage treatment plant effluents, and river waters. *Environ. Sci. Technol.,* **30,** 3544-3550.

Frazee, C.D., Osburn, Q.W. and Crisler, R.O. (1964) Application of infrared spectroscopy to surfactant degradation studies. *J. Am. Oil Chem. Soc.*, **41,** 808-812.

Garrison, A.W. and Hill, D.W. (1972) Organic pollutants from mill persist in downstream waters. *Am. Dyestuff. Reporter*, **61,** 21-25.

Gerike, P. and Jasiak, W. (1986). How completely are surfactants biodegraded?. *Tenside Detergents*, **23,** 300-304.

Giger, W., Brunner, P.H. and Schaffner, C. (1984). 4-Nonylphenol in sewage sludge: Accumulation of toxic metabolises from non-ionic surfactants. Science, **225,** 623-625.

Giger, W., Ahel, M., Koch, M., Laubscher, H.U., Schaffner, C. and Schneider, J. (1987). Behaviour of alkylphenol polyethoxylate surfactants and of nitrilotriacetate in sewage treatment. Water Sci. Technol., **19,** 449-460.

Grifoll, M., Solanas, A.M. and Bayona, J.M. (1990) Characterisation of genotoxic components in sediments by mass spectrometric techniques combined with *Salmonella*/microsome test. *Arch. Environ. Contam. Toxicol.*, **19,** 175-184.

Hall, W.S., Patoczka, J.B., Mirenda, R.J., Porter, B.A. and Miller, E. (1989) Acute toxicity of industrial surfactants to *Mysidopsis bahia*. *Arch. Environ. Contam. Toxicol.*, **18,** 765-772.

Hedgecott, S. and Cooper, M. (1991) Priority setting scheme for potentially hazardous substances in the aquatic environment in the UK. WRc Report N. DoE 2605 -M/1.

Holt, M.S., Mitchell, G.C. and Watkinson, R.J. (1992) The environmental chemistry, fate and effects of non-ionic surfactants. In: *The Handbook of Environmental Chemistry, Volume 3 Part F: Anthropogenic Compounds: Detergents*, pp. 89-144. Hutzinger, O. and de Oude, N.T. (Eds), Springer-Verlag, Berlin.

Janbakhsh, A. (1996) Oestrogenic substances in water: the analytical determination of alkylphenols and alkylphenol ethoxylates in rivers and sewage effluents. M.Sc. Thesis, Dept. of Biological and Chemical Science, University of Essex.

Janicke, W., Bringmann, G. and Kühn, R. (1969) Aquatic toxicological studies of the harmful action of ethoxylate non-ionic surfactants. *Gesund. Ing.*, **90,** 133-138.

Johnson, A.C., White, C., Besien, T.J. and Jurgens, M.D. (1998) The sorption potential of octylphenol, a xenobiotic oestrogen to suspended and bed sediments collected from industrial and rural reaches of three English rivers. *Sci. Tot. Env*. **210/211**, 271-282.

Kravetz, L., Guin, K.F., Shebs, W.T. and Smith, L.S. (1982). Ultimate biodegradation of an alcohol ethoxylate and a nonylphenol ethoxylate under realistic conditions. Soap Cosmetics Chem. Specialities, **58,** 34-42.

Kravetz, L., Chung, H., Guin, K.F., Shebs, W.T. and Smith, L.S. (1984) Primary and ultimate biodegradation of an alcohol ethoxylate and an alkylphenol ethoxylate under average winter conditions in the USA. *Shell Chemical Company Technical Bulletin*, **SC,** 779-83, *Tenside*, **21,** 1-6.

Kravetz, L., Salanitro, J.P., Dorn, P.B. and Guin, K.F. (1991) Influence of hydrophobe type and extent of branching on environmental response factors of non-ionic surfactants. *J. AM. Oil Chem. Soc.*, **68,** 610-618.

Kubeck, E. and Naylor, C.G. (1990). Trace analysis of alkylphenol ethoxylates. J. Am. Oil Chem. Soc., **67**, 400-405.

Kvestak, R. and Ahel, M. (1994) Occurrence of Toxic Metabolites from Non-ionic Surfactants in Krka River Estuary. *Ecotoxicology and Environmental Safety*, **28,** 25-34.

Lee, H-B. and Peart, T.E. (1998) Occurrence and elimination of nonylphenol ethoxylates and metabolites on municipal wastewater and effluents. *Wat. Qual. Res. J. Can.* **33**, 389-402.

Lye, C.M., Frid, C.L.J., Gill, M.E., Cooper, D.W. and Jones, D.M. (1999) Estrogenic alkylphenols in fish tissues, sediments and waters from the UK Tyne and Tees estuaries. *Env. Sci. Technol*. **33**, 1009-1014.

Macek, K.J. and Krzeminski (1975) Susceptibility of bluegill sunfish (*Lepomis macrochirus*) to non-ionic surfactants. *Bull. Environ. Contam. Toxicol.,* **13,** 377-384.

Mann, A.H. and Reid, V.W. (1971) Biodegradation of synthetic detergents: evaluation by community trials. Part 2. Alcohol and alkylphenol ethoxylates. *J. Am. Oil Chem. Soc.*, **48,** 794-797.

Marcomini, A. and Giger, W. (1987) Simultaneous determination of linear alkylbenzene-sulfonates, alkylphenol polyethoxylates and nonylphenol by high-performance liquid chromatography. *Anal. Chem.*, **59,** 1709-1715.

Marcomini, A., Pavoni, B., Sfriso, A. and Orio, A.A. (1990) Persistent metabolites of alkylphenol polyethoxylates in the marine environment. *Marine Chem.,* **29,** 307-323.

Naylor, C.G. (1992). Environmental fate of alkylphenol ethoxylates. Soap Cosmetics Chemical Specialities, August 1992, pp27-31 and 72

Naylor, C.G., Mieure J. P., Morici 1. and Romano R. R. (1992) Alkylphenol ethoxylates in the environment. Proc. 3rd CESIO International Surfactants Congress, 111-124.

Naylor, C.G. Environmental Fate and Safety of Nonylphenol Ethoxylates. pp 23-31 in: *The Alkylphenols and Alkylphenol Ethoxylates Review*, **1**, CMA 1998.

Potter, T.L., Simmons, K., Wu, J., Sanchez-Olvera, M., Kostecki, P. and Calabrese, E. (1999) Static die-away of a nonylphenol ethoxylate surfactant in estuarine water samples. *Env. Sci. Technol.* **33**, 113-118.

Reinhard, M., Goodman, W. and Mortelmans, K.E. (1982) Occurrence of brominated alkylphenol polyethoxy carboxylates in mutagenic wastewater concentrates. *Environ. Sci. Technol.,* **16,** 351-363.

Renner, R. (1997) European bans on surfactant trigger Transatlantic debate. Internet article: Environmental Science and Technology, American Chemical Society publications, July 1997 (http://pubs.acs.org/hotartcl/est/97/jul/euro.html)

Roberts, D.W. (1991) QSAR issues in aquatic toxicity of surfactants. *Sci. of the Tot. Env.* **109/110**, 557-568.

Roberts, D.W. and Marshall S. J. (1995) Application of hydrophobicity parameters to prediction of the acute aquatic toxicity of commercial surfactant mixtures. *SAR and QSAR in Env. Res*., **4**, 167-176.

Ribosa, I., Garcia, M.T., Sanchez, Leal, J. and Gonzalez, J.J. (1993) *Photobacterium phosphoreum* test data of non-ionic surfactants. *Tox. and Env. Chem*. **39**, 237-241.

Rudling, L. and Solyom, P. (1974). The investigation of biodegradability of branched nonyl phenol ethoxylates. Water Research, **8,** 115-119.

Sims, I.R., Whitehouse, P., Wilkinson, H. and McEvoy J. (1997) The acute toxicity of 4-nonylphenol to nymphs of the freshwater shrimp, *Gammarus pulex* and the damselfly, *Ischnura elegans*. WRc/EA Technical Report.

Staples, C.A., Weeks, J., and Naylor, C.G. (1998a) An environmental risk assessment of nonylphenol ehoxylates and their biodegradation intermediates in water and sediments. Presentation to SETAC Conference, November 15-18, Charlotte NC, USA.

Staples, C.A., Weeks, J., Hall, J.F. and Naylor, C.G. (1998b) Evaluation of aquatic toxicity and bioaccumulation of C8- and C9- alkylphenol ethoxylates. *Env. Toxicol. Chem.* **17**, 2470-2480.

Stephanou, E. and Giger, W. (1982) Persistent organic chemicals in sewage effluents. 2. Quantitative determinations of nonylphenols and nonylphenol ethoxylates by glass capillary gas chromatography. *Environ. Sci. Technol.*, **16,** 800-805.

Talmage, S.S. *Environmental and Human Safety of Major Surfactants: A Report to the Soap and Detergent Association*. Lewis Publishers, 1994.

Varineau, P.T., Williams, J.B., Naylor, C.G., Yunick, R.P. and Cady, C. (1996a). The biodegradation of ^{14}C ring-labelled nonylphenol ethoxylate in a semi-continuous activated sludge system. Unpublished report.

Varineau, P.T., Williams, J.B., Naylor, C.G., Serak, K. and Cady, C. (1996b). The biodegradation of a ^{14}C ring-labelled nonylphenol ethoxylate in river water. Unpublished report.

Weeks, J., Adams, W.J., Guiney, P.D., Hall, J.F. and Naylor, C.G. Risk assessment of nonylphenol and its ethoxylates in US river water and sediment. pp 64-74 in: *The Alkylphenols and Alkylphenol Ethoxylates Review*, **1**, CMA 1998.

Whitehouse, P., Wilkinson, M., Fawell, J.K. and Sutton, A. (1998a) Proposed Environmental Quality Standards for nonylphenol in water. R&D Technical Report P42. WRc Report No. EA 4217/3.

Whitehouse, P., Wilkinson, M., Fawell, J.K. and Sutton, A. (1998b) Proposed Environmental Quality Standards for octylphenol in water. R&D Technical Report P59/i688. WRc Report No. EA 4288.

Whitehouse, P. (2000) Environmental Impacts of Alkylphenol Ethoxylates and Carboxylates. Part 2: Proposals for Point Source Controls. WRc R&D Technical Report in preparation.

Williams, J.B., Varineau, P.T. and Blessing, R.L. (1996) Aerobic Degradation of Nonylphenol Ether Carboxylates and Octylphenol Ether Carboxylates. Abstract of submission to Society of Environmental Toxicology and Chemistry Annual Meeting.

Yoshimura, K. (1986) Biodegradation and fish toxicity of non-ionic surfactants. *J. Am. Oil Chem. Soc.*, **63,** 1590-1596.

APPENDIX A BIODEGRADATION OF APnEO SURFACTANTS

A1 Introduction

There is a large body of work concerning the degradation of APnEO surfactants and a comprehensive summary of the published data is to be found in the Appendix to Talmage's authoritative review of alkylphenol ethoxylate surfactants (Talmage, 1994). The examples described below are regarded as being 'key' with respect to the elucidation of breakdown pathways, likely fate in sewage treatment works and studies on the 'lower' APnEOs and APnECs. Those reviewed here include laboratory and bench-scale studies with 'higher' NPnEOs and OPnEOs (Section A2), , degradation of the 'lower' NPnEOs (NP1-2EO) and APnECs (Section A3) and field studies describing the fate of NPnEOs in sewage treatment works (Section A4).

A2 Laboratory studies into biodegradation of 'higher' APnEOs (>3EO units)

A2.1 Standard tests

Lashen and Booman (1967) concluded that OP10EO was degraded in excess of 90% in an activated sludge sewage treatment plant and that this degradation was not adversely affected by an increased plant loading or reduced detention time. They also showed the effect of acclimation in both laboratory scale activated sludge tests and in river water samples, concluding that rivers which regularly receive inputs of surfactants contain acclimated micro-flora which result in their rapid biodegradation.

Later, Stiff *et al*, (1973) showed that the biodegradability of 20 mg l^{-1} of an OP8EO surfactant in a laboratory scale activated sludge unit fell from 95% at 15^{o}C to approximately 70% at 11^{o}C and to 50% at 8^{o}C. This effect appeared to be influenced by the concentration of OP8EO present because at a test concentration of 5 mg 1^{-1} the biodegradability of the OP8EO was also hardly affected by temperature with mean removals of 93% and 90% in duplicate experiments at 8^{o}C.

The results from standard biodegradation tests on APnEOs reported by Gerike (1987) confirmed that primary biodegradation is occurring. In addition, Rudling and Solyom (1974) showed that degradation of several NPnEOs (n=8, 10 and 14 EO units) using the OECD Screening Test exceeded 90% within 12 days (primary degradation). Analysis of the test media after 4 days at 20^{o}C indicated that NP2E0 was the major degradation product and around 50% of this had itself degraded after 28 days. However, when incubated at 15^{o}C, no further degradation of NP2E0 was seen.

A2.2 Other laboratory biodegradation tests

Kravetz *et al*, (1982) examined the biodegradation of radio labelled NP9EO during waste water treatment in a bench-scale bioreactor. The compound was ^{14}C-labelled in the ethoxylate chain and ^{3}H-labelled in the phenolic ring. During a 14-day acclimation period during which time the reactor was supplied with unlabelled NPnEO, >95-98% removal of NPnEO was seen,

again indicating substantial primary biodegradation. When the radio labelled NPnEO was used, about 40-60% of the ^{14}C was converted to $^{14}C0_2$ and around 10-40% of the ^{3}H converted to $^{3}H_20$ indicating that some mineralisation of both the ethoxylate chain and phenolic ring was occurring. The authors estimated that around 35-50% of the hydrophobe of NPnEO was discharged in the effluent from the system, probably as short EO chain NPnEO or NPnEC. In later studies (Kravetz *et al*, 1984) the influence of temperature was clearly demonstrated, with formation of $^{3}H_2O$ declining from 29% at 25°C to 10% at 12°C and just 2% at 8°C. A similar effect on $^{14}CO_2$ evolution was also evident, with formation of 58%, 50% and 10% $^{14}CO_2$ at these temperatures.

In a variant of the OECD Screening Test, primary degradability of NP11EO and NP23EO was approximately 98% after 30 days and ultimate degradation, as determined by DOC removal, was 82% and 70%, respectively (Bruschweiler and Gamperle, 1982). Degradation of the phenyl ring, as determined by changes in UV absorption, was approximately 63% after 30 days incubation. Several other authors refer to changes in UV absorbance during biodegradation, indicating loss of the phenyl ring (e.g. Sato *et al*, 1963).

The biodegradation of ^{14}C ring-labelled NP9EO has also been studied more recently in a semi-continuous activated sludge (SCAS) treatment system using activated sludge acclimated to the primary effluent (Varineau *et al*, 1996). The authors report that most of the radioactivity was recovered as NP, NPnEO and NPnEC (see below) and there was a 93% removal of NPnEO from the influent, again confirming its ready biodegradability.

Compound(s)/location	**Proportion of influent radioactivity (%)**
CO_2	20.8%
NP, NPnEO, NPnEC, 'highly degraded' metabolites in effluent	55.9%
NP/NPnEO associated with sludge	6%
NP, NPnEO, NPnEC, other metabolites remaining within aqueous phase of test system	8.4%
Unaccounted for	8.2%

The first indication in the literature that the primary biodegradation of APnEO surfactants may lead to relatively stable metabolites appears to have been given in 1974 by Rudling and Solyom. They used a lab-scale activated sludge system to study the behaviour of several NPnEO (8, 10, 14, 16 and 30 EO units) surfactants. Activated sludge from a municipal waste water treatment plant was used as seed for the system and, after a week of operation, 5 mg 1^{-1} of NP8EO was added to the influent. The other NPnEOs were added to the influent over the next 7-24 days depending on the degradation seen. Degradation of the original NPnEO was determined by monitoring the effluent using methods that detected NPnEOs containing more than 2EO units. Removals of around 90% were determined for all the NPnEOs regardless of EO chain length.

Reports of the degradation of APnEO surfactants under realistic e.g. 'die-away' conditions have only become available very recently. In a study of the fate of NP18EO under sub-tropical

estuarine conditions, primary degradation was complete within 4 - 24 days with the main degradation products identified as NP2EO and NP2EC, which appeared to relatively resistant to further degradation (Potter *et al*, 1999). A river water die-away study (Varineau *et al, pers. comm*.) demonstrated extensive degradation of NP9EO after 128 days but, in this case, ring-cleaved metabolites of the carboxylates were formed, leading the authors to conclude that the carboxylates were not persistent products of APnEO degradation. However, half-lives of the carboxylates and partially oxidised carboxylates were greater than those of the more extensively oxidised, ring-cleaved metabolites which are formed as a result of the degradation of the carboxylates.

Finally, Maki *et al*, (1994) showed that a Pseudomonad bacterium from activated sludge degraded the ethylene oxide chain of NP9EO and NP2E0 was identified as a major metabolic product.

A3 Degradation of 'lower' APnEOs and APnECs

A3.1 NP1-4EOs

The earliest study of biodegradation of 'lower' APnEOs was a die-away study of NP4EO (Frazee *et al*, 1964). After 34 days, the found that all surfactant activity had been lost and IR spectroscopy showed that the predominant product was a carboxylated derivative. A later experiment into the aerobic biodegradation of a commercial product (Imbetin N/7A: 75% NPIEO, 20% NP2E0 and 5% NP3E0) was conducted using a shake culture test with acclimated inoculum (Ahel *et al*, 1994). Three bacterial cultures were derived from (a) the waste water of a detergent manufacturing plant, (b) a chronically polluted river water and (c) a pristine forest soil and grown in a medium containing NP9EO prior to use. The removal of NP1EO and NP2E0 was monitored (primary biodegradation) and it was clear that similar rates of removal for both compounds was observed in all media. Furthermore, removal was extensive (ca. 90%) after 6-23 days with the carboxylates, NP1EC and NP2EC predominating as reaction products. Of these, NP1EC was clearly the most abundant metabolite. Ahel *et al,* (1994) concluded that the half-life for NP1-3EO under these conditions was between 1 and 2 days.

These findings compare with those of Rudling and Solyom (1974) who reported that 50% of the NP2EO produced in an OECD Screening Test had degraded after 28 days when incubated at 20^{o}C but degradation was halted at 15^{o}C (Section A2).

A3.2 OPnEOs and OPnECs

Lashan *et al* (1966) carried out tests using radio labelled p-tert-OP10EO using bench-scale activated sludge units. The compound used was ^{14}C-labelled in the ethoxylate chain and ^{3}H-labelled on the phenol ring to distinguish between degradation of the ethoxylate chain and the alkylphenol parts of the molecule. In shake-flask cultures inoculated with acclimated activated sludge, >90% primary biodegradation of the octylphenol ethoxylate was seen in 7 days, reflecting the ready biodegradation seen with NPnEOs. Extensive (90-95%) removal was also evident in bench-scale activated sludge units. Experiments using the radio labelled compound showed that degradation of the OP10EO occurred almost entirely by degradation of the ethoxylate chain, with little or no degradation of the phenolic ring being seen. In a further experiment carried out under anaerobic conditions, 84-93% biodegradation of OPnEO

occurred, with around half the ^{14}C being lost from the system. Again, no loss of ^{3}H was observed, indicating that little or no degradation of the phenolic ring was occurring but rather degradation of the ethoxylate chain only.

Ball *et al*, (1989) carried out an extensive study of the biodegradation of 'lower' OPnEOs and the corresponding carboxylic acids (OPnECs) under a variety of aerobic and anaerobic conditions. The 0PnEO material used was a mixture of 13% OPIEO, 40% OP2E0, 29% OP3E0, 14% OP4E0 and 4% OP5E0. In addition, the corresponding 0PnEC (same relative composition of oligomers) was used in some tests. The test substances were incubated with either (a) BOD dilution water seeded with solids from an activated sludge plant, (b) settled effluent from the same plant or (c) anaerobic bacteria maintained under anaerobic conditions.

The experiments using activated sludge inoculation (a) clearly show that the OPnEOs degrade to OPnEC (mainly OP2EC) with little or no mineralisation. Results from the primary sludge inoculated tests (b) show that the longer chain OPnEO (>3EO units) degraded rapidly (within 2 days) with a concurrent increase in OP2E0. Degradation of OPIEO and OP2E0 appeared to require an adaptation period of approximately 5 and 17 days respectively before degrading to unidentified products. Some oxidation of the OPnEO to OPnEC did occur but this was only a very minor route of degradation since little or no OPnEC with >3 EO units were seen in the test. OP1-2EC was degraded to some extent under the conditions used, with the possible formation of small amounts of OP.

Under anaerobic conditions (c), OPnEO was degraded predominantly to OP1EO within 10 days and this was subsequently converted to OP which appeared to be stable under the conditions of the test. The oxidative pathway (to form the carboxylates) did not occur under these conditions. The OP2-4ECs were not degraded under anaerobic conditions although OPlEC was rapidly degraded with OP again being produced.

It is clear from these experiments that, under both aerobic and anaerobic conditions, fairly recalcitrant substances were formed after 10 days. Under aerobic conditions, OPnEOs are transformed by ether cleavage to relatively stable OP2E0 and 0PnEC (with 2-3 EO units) which tend to accumulate but after a period of acclimation are then transformed further to unidentified products. Oxidation of the 'lower' OPnEOs is favoured over oxidation of 'higher' OPnEOs with the result that no 'higher' OPnECs were found. Under anaerobic conditions, removal of EO units again takes place but there is no oxidation to the carboxylates; instead transformation proceeds via progressive removal of EO units, via OP1EO, and eventually formation of OP, which persists for at least 190 days. Under these conditions and when OPnECs are used as starting materials, the only OPnEC to be significantly degraded was OP1EC but further degradation to OP was not complete even after 120 days.

Degradation experiments on 'lower' NPnEOs reported by Ahel *et al*, (1994) and described in Section A3.1 were also carried out on p-tert OP2EO. These led to very similar conclusions as those drawn based on the fate of NPnEOs, i.e. that OP1-3EOs are rapidly removed (>95%) in the presence of a variety of inocula although it appears that the extent of degradation adopted the following pattern: OP3EO>OP2EO>OP1EO. There was also near-quantitative formation of the corresponding OPnECs.

Recent experiments with the carboxylates (Staples *et al*, 1998) suggest they may not be as recalcitrant as implied by some of the previous studies. When OP1EC, OP2EC, NP1EC and NP2EC were used as starting materials in ready biodegradability tests (modified Sturm test: OECD 301B) more than 60% of the theoretical CO_2 production was evolved after 28 days, although the nonylphenolic carboxylates did not attain the 10-day window for 'ready' biodegradability. These results led the authors to suggest that these carboxylates are unlikely to persist or accumulate in the environment, although such conclusions drawn from 'ready' biodegradation tests of this type need to be treated with caution. The only example of a study into the biodegradation of the 'lower' ethoxylated or carboxylated alkylphenols under realistic conditions is provided by Potter *et al* (1999). Die-away of NP2EC in estuarine water declined to only 50% of the starting concentration after 32 days, leading the authors to describe it as 'relatively resistant' to degradation.

A4 Field studies

Mann and Reid (1971) monitored the biodegradation of two alcohol ethoxylates and an OPnEO surfactant at a small trickling filter plant in the UK. These trials showed that the alcohol ethoxylates degraded in excess of 80% under both summer and winter temperature conditions but that degradation of the OPnEO was adversely affected by winter temperatures with the biodegradability being approximately 80% in the summer and 20% in the winter. From more recent monitoring work by Brown *et al* (1986, 1987) it appears that the removal of APnEOs in activated sludge sewage treatment plants is rather better than that found in trickling filter plants but with less difference in removal rates between the trickling filter results under summer (75%) and winter (70%) conditions.

Several detailed studies of the behaviour of nonylphenol ethoxylates and their degradation products in sewage works have been reported. Many of these refer to wastewater treatment plants in Switzerland carried out in the 1970s, before controls were introduced to limit the use of nonylphenol ethoxylates in domestic products. Although the data are relevant in the context of the overall behaviour of APnEOs during waste water treatment, the actual concentrations measured may not reflect the current situation in Europe. Relevant data from studies carried out in the US, Italy, Germany and the UK have also been located.

Studies into the fate of APnEO surfactants in sewage treatment works in the UK and Italy have confirmed the extensive removal (typically greater than 90%) of these substances seen in laboratory tests (Brown *et al.*, 1986; 1987; Holt *et al*, 1986; Di Corcia *et al*, 1994). Brunner *et al*, (1988) studied the fluxes of NP, NP1EO and NP2E0 through sewage treatment plants in Switzerland, focusing on the digestion/stabilisation of the sewage sludge at the plants. Both NPIEO and NP2E0 were present in the sewage treatment works and were thought to be precursors to the formation of NP which accumulated in sewage sludge during anaerobic treatment of sludge. Based on detailed measurements at one plant with anaerobic digestion of sludge it was estimated that 50% on a molar basis or 17% on a weight/weight basis of the NPnEO entering into the plant was converted to NP in the final sewage sludge.

Ahel and co-workers (Stephanou and Giger, 1982; Ahel and Giger, 1985a; 1985b; Ahel *et al*, 1986; Giger *et al*, 1986; 1987; Marcomini *et al*, 1988) have published an important series of papers on the fate of APnEO surfactants in sewage treatment works in Switzerland (detailed below) and also in the environment receiving discharges from those works (Appendix B). The

results of detailed studies of four activated sludge treatment plants discharging into the Glatt River in Switzerland (Ahel *et al*, 1994c; Giger *et al*, 1986; 1987) are described below:

Concentrations of alkylphenolic compounds were determined in primary and secondary effluents and it was seen that between 81-99% of NPnEOs (3-20 EO units) were eliminated in all plants. The concentrations of NP1EO and NP2E0 were only slightly lower in secondary effluent as compared to primary effluent, and at one plant their concentration was higher in secondary effluent. The concentration of NP was always found to be lowered by activated sludge (secondary treatment), while the concentration of NP1EC and NP2EC increased appreciably in the effluent after secondary treatment. Tertiary treatment (anaerobic sludge digestion) further reduced the concentration of NP, NP1EO and NP2E0 in the effluent, but had little or no effect on the concentration of NPIEC and NP2EC. Samples taken during sludge digestion indicated that NP was accumulating in the sludge (concentration in sludge increased by a factor of 15), while the concentration of NPIEC and NP2EC in sludge declined slightly (Giger *et al*, 1987). Analysis of NP, NP1EO and NP2EO in the effluents from the same wastewater treatment plants by GC/MS (Stephanou and Giger, 1982) found none at one plant and the following ranges of concentrations at the other three:

Compound	**Concentration range (mg l^{-1})**
NP	<10 - 35 μg l^{-1}
NP1EO	24 - 133 μg l^{-1}
NP2EO	nd - 70 μg l^{-1}

Ahel *et al*, (1994) subsequently reported results from surveys of 11 mechanical-biological waste water treatment plants in the Glatt Valley, Switzerland. The waste water treatment plants typically consisted of a primary clarifier for mechanical treatment, aeration tank and secondary clarifier for biological treatment and an anaerobic digester for sewage sludge treatment. Samples were analysed for the presence of NP, 'NPnEOs (with between 1 and 20 EO units), and the carboxylates NPIEC and NP2EC.

In untreated sewage and primary effluent, the main components found were generally undegraded 'higher' NPnEOs which accounted for 82.4% of the total nonylphenol derivatives present, followed by NPIEO and NP2E0 (11.5% of the total), and NP (3% of the total) and NP1EC and NP2EC as minor components (3.1% of the total). In secondary effluent the composition of the alkylphenolic compounds had changed markedly, with 'higher' NPnEO only present in trace amounts. NP1EC and NP2EC were now the most abundant substances found (46.1% of the total), followed by the 'lower' NPnEOs (21.8% of the total) and NP (3.9% of the total). Based on analysis of the various effluents and sludges in the plants, an overall budget for the nonylphenolic compounds (mainly NPnEO) entering the plant was given as:

- 19% released to the environment as NPnEC
- 11% released as NP1EO and NP2E0
- 25% released as NP (>90% of which is adsorbed onto digested sewage sludge)
- 8% released as un-transformed NPnEO

Thus the overall removal of 'higher' NPnEOs is around 92% but approximately 60-65% of NPnEO compounds are released into the environment via secondary effluents. There is also clearly a marked shift in the distribution away from the 'higher' NPnEOs entering the works in untreated wastewater toward the 'lower' NPnEOs and particularly the carboxylates, NP1EC and NP2EC after secondary treatment (Figure A1).

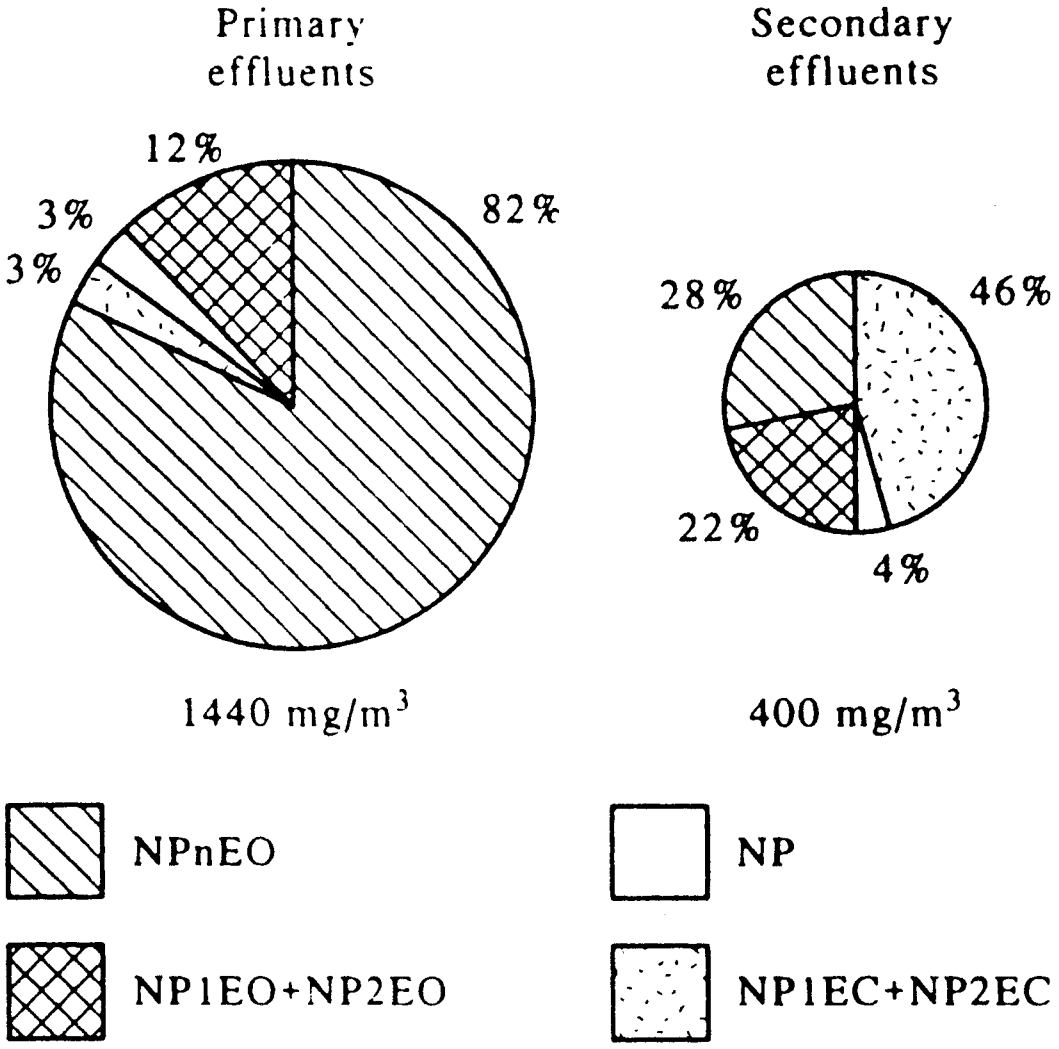

Figure A1 Change in distribution of oligomers following primary and secondary treatment of APnEO surfactants (Ahel *et al*, 1994)

Discharge of predominantly 'lower' APnEOs and APnECs from sewage treatment works was borne out in subsequent studies of the behaviour of NPnEO surfactants in a study of the discharge from a sewage treatment works in Italy (Di Corcia *et al*, 1994) and two sewage treatment plants in the UK (Holt *et al*, 1986). A survey of UK sewage treatment works effluents carried out in 1988 (James *et al*, 1989) also found significant concentrations of NP1-2EO and OP1-2EO but 'higher' ethoxylates were not detected, although the analytical methods used would not have been suitable for the 'higher' NPnEOs (James, WRc, *pers. comm*.). A more recent study (Janbakhsh, 1996) includes an extensive survey of concentrations and the composition of alkylphenolics entering Chelmsford STW and also

those present in the final effluent from the same works. Although alkylphenols and the 'lower' APnEOs dominated, significant concentrations of AP4-7EOs were also detected in the final effluent. Concentrations of alkylphenols in final effluent from Chelmsford STW ranged between 0.6 and 7.0 μg l^{-1} whilst concentrations of APnEOs ranged between 1.4 and 52.1 μg l^{-1}. These compare with concentrations of 0-1400 μg l^{-1} and 0-1900 μg l^{-1} APnEOs in the final effluents of Huddersfield and Marley STW in Yorkshire (Kennedy, EA North-East Region, *pers. comm.*), both of which serve large industrial catchments.

A very recent development is the discovery of metabolites from laboratory biodegradation test systems which are oxidised in both the EO chain and the alkyl chain (Di Corcia *et al*, 1998), designated here as CAPnECs. These species appear to be generated from the APnECs with relatively little branching in the alkyl chain but in addition, small quantities of compounds which were oxidised only in the alkyl chain (i.e. CAPnEOs) were also detected. These CAPnECs and CAPnEOs were highly resistant to further degradation, persisting for at least 5 months. Subsequent analysis of effluent from a sewage treatment plant in Rome yielded concentrations of 58 μg l^{-1}, accounting for 63% of the alkylphenolic compounds leaving the plant.

Sorption of NP and OP to solids represents significant removal mechanisms for these compounds but APnEOs and especially APnECs sorb less strongly (Table A1). Accordingly the sludge-bound fraction is a less important contributor to mass flow of these substances in sewage treatment plants: losses via sludge are estimated to be 14-15% for NP1EO and only 6-7% for NP2EO compared with around 50% for NP (Ahel *et al*, 1994). Whilst most of the NP released to the environment will be via digested sewage sludge, most of the other metabolites and any residual parent NPnEO will be discharged in the final effluent. A similar distribution would be expected for OPnEOs and their breakdown products.

Table A1 Distribution coefficients (concentration in activated sludge: secondary effluent) for 'lower' NPnEOs and NP2EC (from Ahel *et al,* 1994)

Substance	Distribution coefficient (l kg^{-1})
NP1EO	1800
NP2EO	900
NP2EC	500

In the late 1980s, largely in response to increasing regulatory concerns about APnEOs and their breakdown products in the environment, the Chemical Manufacturers Association (CMA) in the US established the 'Alkylphenol and Ethoxylates Panel'. This comprised several US manufacturers of APnEOs to initiate research into the fate and effects of these substances in sewage treatment works and in the receiving environment. A major component of this research programme was the so-called 'Thirty Rivers Study', which set out to determine concentrations of APnEOs and their breakdown products in US rivers subjected to effluent discharges from wastewater treatment plants. A similar study was also initiated on the Fox River in Wisconsin and a study into the fate of APnEOs in wastewater treatment plants was also initiated. It is this latter area that is discussed here but data on concentrations in receiving waters are dealt with in Appendix B.

As was seen in the Swiss studies, described above, removal of NPnEO in the US plants studied plants was generally >92% (Naylor, 1992; Naylor *et al*, 1992). However, unlike the pattern seen in the Swiss studies, the oligomer distribution in effluent showed only a slight increase in the proportion of 'lower' NPnEO oligomers when compared with the influent but nothing like to the same extent as that seen in the Swiss studies. NP was detected in the effluent at concentrations of 0.5-4.0 μg l^{-1}, but no influent concentrations were measured so it is not possible to say anything about the possible formation and/or removal during wastewater treatment. The only case where a significant skew in oligomer distribution was seen was in the effluent from a works treating waste from a pulp mill, which was subject to particularly high NPnEO loadings. A typical example of the results from one of the works investigated is shown in Figure A2 and for comparison, an equivalent Figure from the Swiss studies is shown in Figure A3.

In the 'Thirty US rivers' study, Kubek and Naylor (1990) used a simplified extraction technique to look at the behaviour of NPnEO in a US wastewater treatment plant. They reported that the presence of oxygen in the extraction and work-up procedure could lead to a skewing of the NPnEO oligomer distribution to those with a low number of EO units and this could, in part, explain the accumulation of these compounds seen in other studies (particularly those from the Swiss studies). On the basis of this type of data, Naylor and co-workers take issue with a number of the conclusions drawn by Ahel and co-workers, particularly that recalcitrant metabolites are produced when APnEO surfactants are broken down in sewage treatment works.

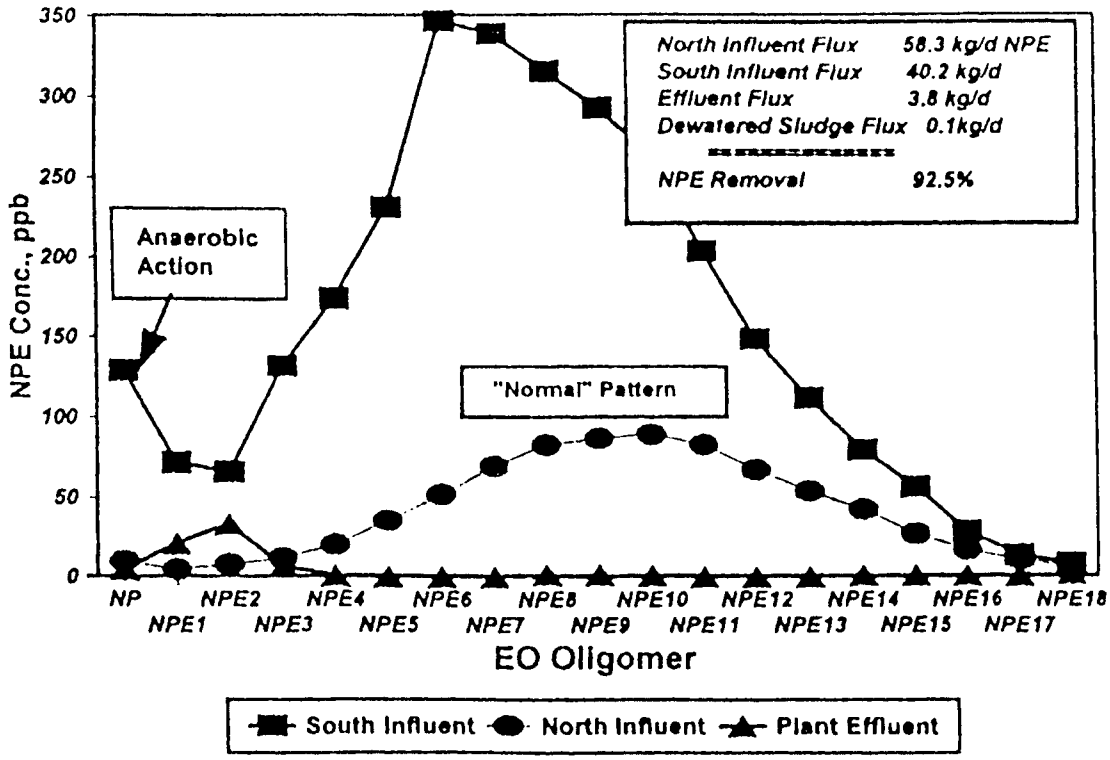

Figure A2 Oligomer distributions of influent and effluent streams from a mid-western city's wastewater facility (from Naylor, 1995)

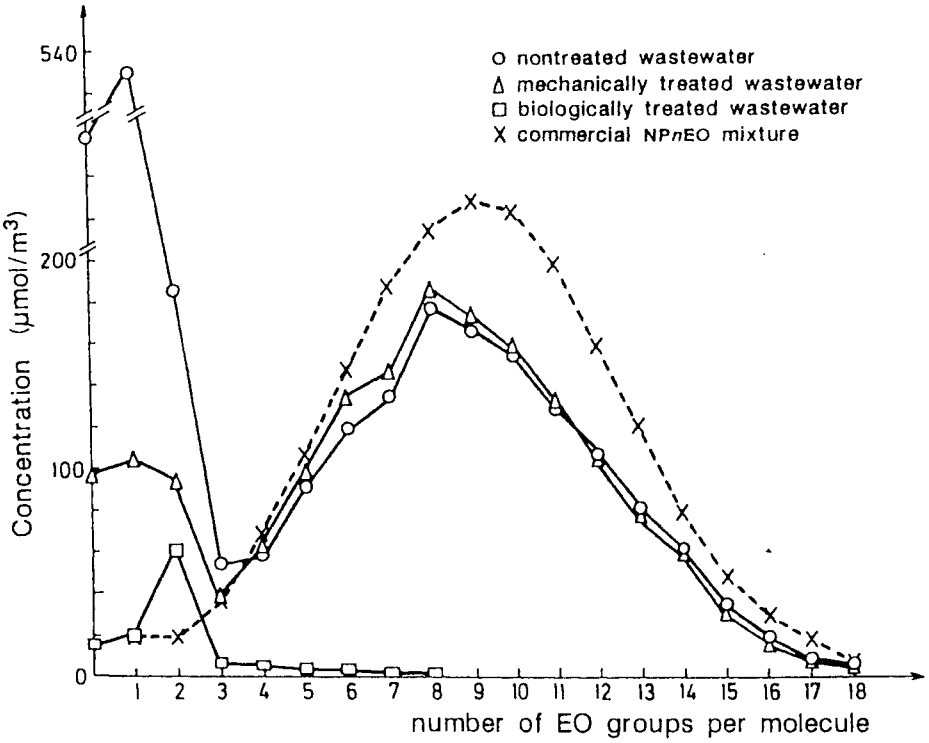

Figure A3 Distribution of NPnEO oligomers in raw sewage, primary effluent and secondary effluent from Uster sewage treatment works, Switzerland (Ahel *et al*, 1994)

Although Naylor and co-workers did not analyse for the carboxylates described by Ahel and co-workers, recent studies in the US (Field and Reed, 1996) have demonstrated the presence of NPnECs in the effluents discharged from paper mills and sewage treatment works. All but one of the 21 discharges examined contained detectable concentrations of NPnECs. In paper mill effluents concentrations were generally between 100-1300 µg l^{-1} whilst in the discharges from sewage treatment works, concentrations of NPnECs ranged between 140 and 270 µg l^{-1}, which are similar to those reported in the Swiss studies. As in the Swiss studies, NP2EC was the dominant carboxylate found in sewage treatment works effluents.

It is likely that the relatively poorer performance of the Swiss treatment plants may contribute to these differences and seasonal effects do seem to be important. We have seen, for example, that primary degradation of OPnEOs was poorer in winter than in summer (Mann and Reid, 1971). Smaller differences were found in a study at a German site (Brown *et al*, 1987) but at a works in Switzerland, a marked seasonal effect on removal of NP1EO, NP2EO and to a lesser extent, NP, was evident (Ahel *et al*, 1994), as shown in Table A2.

Table A2 Temperature dependence of NP1EO, NP2EO and NP removal in Zurich-Glatt sewage treatment works (after Ahel *et al*, 1994)

Date	Temperature (^{o}C)	Elimination (%)		
		NP	NP1EO	NP2EO
31-01-84	10-13	65	18	-2
20-06-83	18	75	40	25
27-8-84	20	82	72	47

In addition, the relatively high proportion of NP1EC and NP2EC in effluents from biological treatment plants appears to be a feature of secondary treatment because they were only very minor components in the effluent arising from primary treatment, even in the studies of Ahel and co-workers. This may also go some way to reconciling the apparently anomalous results of Naylor *et al*, (1996) who found levels of NP1EC and NP2EC in the discharges from activated sludge plants which were only comparable to those of the corresponding ethoxylates and NP.

APPENDIX B OCCURRENCE AND FATE OF ALKYLPHENOLIC COMPOUNDS IN SURFACE WATERS

B1 Freshwaters

In response to growing regulatory pressure on the use of APnEOs in the US, several US manufacturers of APnEO surfactants embarked on a major survey of concentrations of APnEOs and their metabolites in rivers across the US in the early 1990s to provide exposure data which could be used for risk assessment purposes. Combined with similar studies in Europe, particularly in the River Glatt in Switzerland, this has resulted in a substantial body of data on concentrations of alkylphenolic compounds in surface waters. The only UK data we have been able to locate are from a survey carried out by WRc in 1991 in which alkylphenol ethoxylate surfactants were detected in a number of water samples but degradation products were not analysed for.

B1.1 Transformation in rivers

An early study by Osburn and Benedict (1966) is revealing because it demonstrated removal of EO units from NP10EO in a river die-away test over 34 days using IR spectroscopy. Their work also clearly demonstrated more rapid degradation of the straight alkyl chain analogue compared to a branched alkyl chain analogue. In a later study, Yoshimura (1986) studied the degradation of a NP9EO in sediment and river water. Around 98% primary degradation of the NP9EO was seen within 5 days with stirring and within 10 days without stirring. New peaks were observed to be formed in the HPLC trace which still remained 30 days after inoculation, indicating the formation of less biodegradable metabolites, which were subsequently identified as NP1EC and NP2EC. NP1-3EO were formed only in small amounts after 5-10 days incubation. The concentration of surfactant associated with the sediment also decreased after 10 days, indicating that primary biodegradation was also occurring in the sediment-bound fraction.

In the Glatt River, Switzerland (Ahel *et al*, 1994c), the main input of nonylphenolic compounds into the river was thought to be from secondary effluents from municipal waste water treatment plants. The study was undertaken in 1983-1986 using sampling campaigns that simultaneously collected 1-day composite samples from several parts of the river and secondary effluent samples from waste water treatment plants along the river. This was carried out in such a way that the same "package" of water was sampled at each point. The most abundant compounds detected were NPIEC and NP2EC, followed by NP1EO and NP2E0, then NP and finally NPnEO (n>3 EO units), which made up only a very small fraction of the total. The hydraulic residence time of the river was 10-15 hours and it was estimated that 85% of the ‘higher’ NPnEOs, 70% of the NP1EO and NP2E0 and 62% of the NP were eliminated in the river (by biodegradation and/or adsorption to sediment) during this period. However, there was a corresponding increase in NP1EC and NP2EC concentrations in the river of around 27% so that, overall, a net loss of alkylphenolic compounds from the river of 24% was estimated. This is consistent with aerobic degradation of ethoxylated alkylphenols to their carboxylated counterparts at a faster rate then their subsequent breakdown. These compositional changes are illustrated in Figure B1.

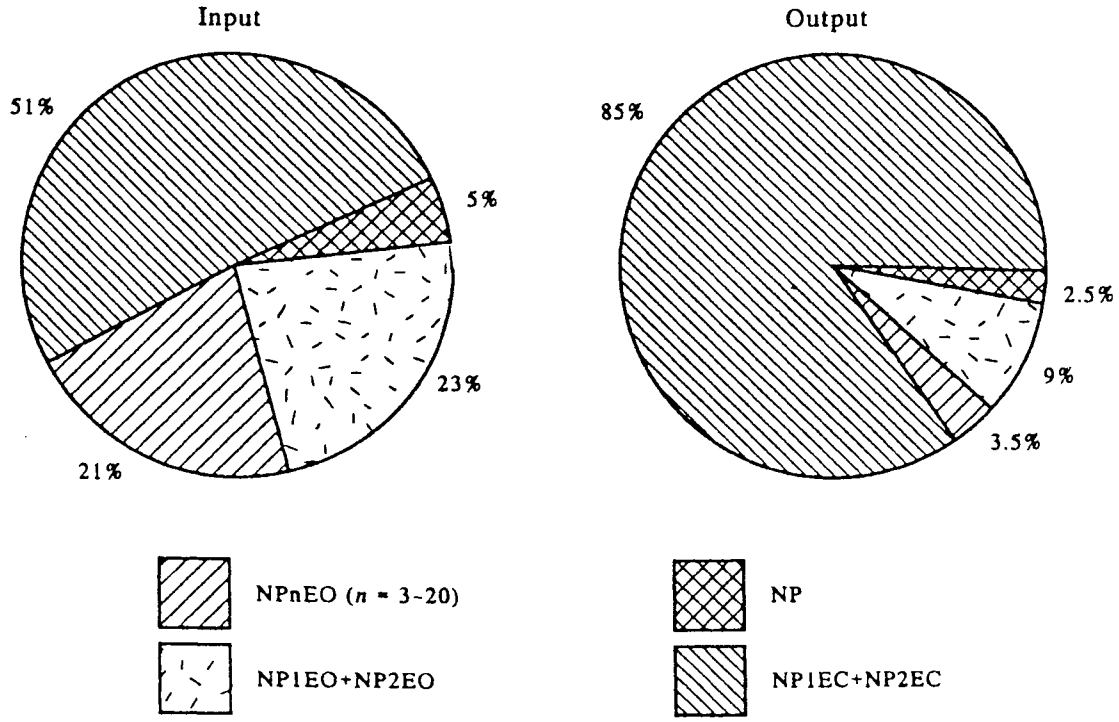

Figure B1 Compositional changes in nonylphenolic compounds in the Glatt River, Switzerland (after Ahel *et al*, 1994)

The degradation of ^{14}C ring-labelled NP9EO has also been studied in river die-away tests (Varineau *et al*, 1996). The river water for the tests was from the Missouri River, several miles downstream from a waste water treatment plant. The water was spiked with secondary effluent from a publicly owned waste water treatment plant to ensure that the bacteria present in the water had been previously exposed to NPnEOs. In the die-away tests, 89% primary degradation (defined as degradation into species not identifiable as NP and NPnEO) of radio labelled NP9EO occurred after 28 days and 96% degradation after 128 days.

We are unaware of any studies into the rates of biodegradation of the 'lower' ethoxylated or APnECs under natural conditions e.g. in river die-away tests. However, the relatively slow biodegradation of these compounds in laboratory tests (Ball *et al*, 1989) and their accumulation in die-away tests (e.g. Yoshimura, 1986) would suggest they are also likely to be more resistant to degradation in surface waters than APnEOs. There is also circumstantial evidence for this from field surveys (Ahel *et al*, 1994b; 1996) which show accumulation of NP1EC and NP2EC in samples of river water, suggesting a greater resistance to degradation than the 'lower' ethoxylates.

B1.2 Measured concentrations of APnEOs and degradation products

It should be remembered that most of the studies described here were undertaken at a time when restrictions on APnEO surfactants had either not been put in place or had not taken full effect. Therefore, the reported measured concentrations are likely to overestimate current environmental concentrations of APnEOs and their breakdown products.

Broadly speaking, the alkylphenolic compounds detected in freshwaters reflect those discharged from sewage treatment works i.e. a predominance of 'lower' ethoxylated derivatives (NP1EO and NP2EO) and their carboxylated derivatives, NP1EC and NP2EC, and, compared to the surfactant products from which these substances originate, only low concentrations of 'higher' NPnEOs. Although not verified experimentally, it seems likely that the proportion of 'higher' APnEOs will be greater in winter when conditions are less favourable for degradation in sewage works (CES, 1993).

Measured concentrations of APnEO surfactants in surface waters vary widely. Talmage (1994) summarises the available data and states that up to 1000 $\mu g\ l^{-1}$ (as 'total non-ionics') was detected in the Rivers Aire and Calder in the UK between 1965 and 1974, which was probably influenced by the wool scouring activities in this region at the time. Data on environmental concentrations of APnEO degradation products in surface waters are summarised in Table B1. These show a wide range but the highest measured concentration was a concentration of 71 $\mu g\ l^{-1}$ NP2EC in the Glatt River in Switzerland (Ahel *et al*, 1994), though typical concentrations from studies carried out during the 1980s and 1990s are usually much lower. In the extensive CMA-sponsored study of US rivers described earlier, the highest concentration of an NPnEO and associated metabolites found was 17.3 $\mu g\ l^{-1}$ in the Grand Calumet River, Indiana.

There are clearly differences in the concentrations of the 'lower' ethoxylated derivatives and their carboxylated derivatives reported by Naylor *et al*, (1992) and in the River Glatt by Ahel *et al*, (1987, 1994), but this is largely a reflection of differences in dilution afforded by the two study sites.

Of greater significance are differences in the distributions of alkylphenolic compounds. For example, substantial concentrations of undegraded NPnEOs were found in the US study but these were very minor components in the study carried out by Ahel and co-workers. Indeed, in the latter study, no oligomers with more than 6EO units were detected. Some sites in the US study were associated with a skew in the distribution of metabolites toward the 'lower' ethoxylated derivatives (NP1EO and NP2EO) but in others, no such skew was reported. In the Swiss studies (Ahel *et al*, 1987, 1994b) there was always a marked skew in the distribution of alkylphenolic compounds in the river samples analysed toward the 'lower' ethoxylated derivatives (NP1EO and NP2EO) and, especially their carboxylated derivatives, NP1EC and NP2EC. Indeed, NP1EC and NP2EC were the most abundant metabolites found in the River Glatt. Seasonal effects may also be important: Ahel *et al*, (1994b) show a marked decline in concentrations of NP1EO, NP2EO and NP as water temperature rises and it is possible that differences in the times at which samples were taken could have given rise to differences between the US and Swiss studies.

Table B1 Measured concentrations of APnEOs and APnEO degradation products in surface freshwaters

Substance	Range ($\mu g\ l^{-1}$)	Mean ($\mu g\ l^{-1}$)	Location(s)	Number of sites/rivers where measurable concentrations found	Ref
NP1EO	<0.06 - 1.2	0.09	Thirty rivers throughout US	13/30	1
	<3 - *69	ca. 7.0!	Glatt River, Switzerland	-	2
	<0.5 - 17.0	4.8	Glatt River, Switzerland	5/6	3
	0.04 - 0.37	0.13	Fox River, Wisconsin	3/5	5
	<0.5 - 18.0	-	Glatt River, Switzerland	-	6
	<0.1 - 0.6	-	R. Chelmer, Sandford Mill	5/18	8
	<0.02– 7.8	1.3	Lake Ontario, Canada	22/38	9
NP2EO	<0.07 - 1.2	0.10	Thirty rivers throughout US	18/30	1
	<0.3 - 30.0	ca. 6.0!	Glatt River, Switzerland	-	2
	<0.5 - 10.0	3.7	Glatt River, Switzerland	5/6	3
	<0.5 - 16.0	-	Glatt River, Switzerland	-	6
	-	25.0[#]	R. Aire, d/s of Marley STW	-	7
	<0.1 - 2.3	-	R. Chelmer, Sandford Mill	6/18	8
	<0.02-10.0	1.4	Lake Ontario, Canada	12/38	9
NP3-17EO	<1.6- 14.9	2.0	Thirty rivers throughout US	11/30	1
	<1 - 7.1	-	Glatt River, Switzerland	-	2
	0.8 - 3.4[$]	1.8	Fox River, Wisconsin	4/5	5
	<0.1 - 1.5	-	R. Chelmer, Sandford Mill	8/18	8
NP	<0.11-0.64	0.12	Thirty rivers throughout US	13/30	1
	<0.3 - *45	ca. 2.0!	Glatt River, Switzerland	-	2
	0.12 - 0.29	0.23	Fox River, Wisconsin	4/5	5
	<0.5 - 1.5	-	Glatt River, Switzerland	-	6
	<0.1 - 2.0	-	R. Chelmer, Sandford Mill	17/18	8
	<0.01-0.92	0.21	Lake Ontario, Canada	9/38	9
NP1EC	<0.04 - 2.0	-	Fox River + 8 other US rivers	4/10	4
	<1.0 - 45	ca. 17.5!	Glatt River, Switzerland	4/6	2,3
NP2EC	<0.2 - 11.8	-	Fox River + 8 other US rivers	5/10	4
	2.0 - 71	ca. 37.5!	Glatt River, Switzerland	6/6	2,3
NP3EC	not detected	-	Fox River + 8 other US rivers	0/10	4
NP4EC	not detected	-	Fox River + 8 other US rivers	0/10	4

* The upper end of the range was strongly influenced by an isolated large value
[$] Actually NP2-17EO
! Median rather than mean
No further details given

References:

1 Naylor *et al*, (1992)
2 Ahel *et al*, (1994b)
3 Ahel *et al*, (1987)
4 Field and Reed (1998)
5 Naylor *et al*, (1998)
6 Ahel and Giger (1985)
7 Blackburn and Waldock (1995)
8 Janbakhsh (1996)
9 Bennie (1997)

Surveys of contamination of surface waters by alkylphenolics carried out by the Environment Agency (Kennedy, EA North-East Region, *pers. comm.*) between 1996 and 1998 have shown that concentrations of APnEOs in the River Aire at Crossflatts, downstream of a large STW which receives surfactant waste, are usually less than 10 μg l^{-1} although peaks of up to 190 μg l^{-1} have occurred over a two-year period. Although some of these peaks appear to coincide with peaks in measured NP concentrations, this is not always the case. A recent study of Chelmsford STW and the River Chelmer into which it discharges (Janbakhsh, 1996) has revealed concentrations of 0-7.3 μg l^{-1} APnEOs in the river (predominantly the 'lower' ethoxylates) just downstream of the STW discharge, along with concentrations of up to 2.0 μg l^{-1} alkylphenols. This study represents the most up-to-date work describing contamination of river water by APnEOs in the UK. For this reason, a summary of Janbakhsh's data for the River Chelmer at Sandford Mill is shown in full in Table B2.

B2 Sediments

Measured concentrations of NP, NP1EO and NP2EO in river sediments are higher than those determined in the overlying water (Ahel *et al*, 1994b). In the Glatt River catchment, concentrations of NP ranged between 0.19 and 13.1 mg kg^{-1} whilst for NP1EO and NP2EO the corresponding ranges were 0.1 - 8.85 mg kg^{-1} and 0.08 - 2.72 mg kg^{-1}, respectively. It was clear that, in marked contrast to the water samples, NP was the most abundant alkylphenolic compound associated with the sediment with concentrations being 364-5100 times higher than in the water. This is almost certainly a reflection of the lipophilic nature of NP (log P = 4.5) and also its relative resistance to degradation (Garrison and Hill, 1972).

As part of the thirty rivers study conducted by the Radian Corporation in the US (Naylor *et al*, 1998) described earlier, residues of NP, NP1EO and NP2EO were also detected in the sediments of rivers receiving treated wastewaters but 'higher' NPnEOs (with >3 EO units) were not found. In a survey of marine sediments from Venice lagoon, Marcomini *et al*, (1990) were able to show that the superficial layers of readily suspended sediment contained significantly higher concentrations of NP, NP1EO and NP2EO than the underlying sediment. Preliminary data for analyses of sediments taken from the Rivers Aire and Calder in West Yorkshire during 1997 and 1998 (Daniels, EA North-East Region, *pers. comm.*) indicate concentrations of NP1EO, NP2EO and NP of between 0.5-30.0 mg l^{-1}, 0.6-9.5 mg l^{-1} and 1.3-227.0 mg l^{-1}, respectively.

Table B2 APs and APnEOs detected in the River Chelmer at Sandford Mill between February and November 1995 (from Janbakhsh, 1996)

sampling month	AP	AP1-EO	AP2-EO	AP3-EO	AP4-EO	AP5-EO	AP6-EO	AP7-EO	AP8-EO	AP9-EO	AP10-EO	AP11-EO	AP12-EO	AP13-EO	TOTAL APEO
8/2/95	<0.1	<0.1	<0.1	< 0.1	<0.1	<0.1	< 0.1	<0.1	<0.1	< 0.1	<0.1	<0.1	< 0.1	<0.1	<0.2
15/2/95	2.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.0
22/2/95	1.1	0.2	0.35	0.7	0.6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.95
1/3/95	0.77	<0.1	<0.1	<0.1	<0.1	0.75	0.82	0.6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.94
8/3/95	0.18	<0.1	0.3	<0.1	0.12	0.24	0.5	0.7	0.6	0.35	<0.1	<0.1	<0.1	<0.1	2.99
15/3/95	1.0	0.6	1.2	0.8	0.9	0.8	0.9	0.6	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	7.3
5/4/95	1.8	0.5	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.5
12/4/94	1.6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.6
3/5/95	0.62	<0.1	0.3	<0.1	0.4	<0.1	0.4	0.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.1
6/6/95	0.39	<0.1	<0.1	<0.1	<0.1	0.52	0.63	0.59	0.46	0.45	<0.1	<0.1	<0.1	<0.1	3.0
19/7/95	0:51	<0.1	1.85	0.1	0.2	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	2.86
9/8/95	0.47	0.3	0.54	0.35	0.29	0.5	0.53	0.49	0.55	0.49	0.3	0.28	<0.1	<0.1	4.4
16/8/95	0.6	<0.1	0.24	0.13	0.15	0.17	0.25	0.2	0.2	0.12	<0.1	<0.1	<0.1	<0.1	2.1
7/9/95	0.55	0.6	0.9	0.78	0.76	0.91	0.82	0.74	0.55	0.52	0.47	<0.1	<0.1	<0.1	6.4
13/9/95	0.71	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
19/9/95	1.1	<0.1	<0.1	<0.1	1.26	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.2
26/9/95	0.4	<0.1	2.3	1.48	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	3.78
4/10/95	0.28	0.1	0.2	0.46	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.66
26/10/95	0.21	<0.1	0.3	0.55	<0.1	0.25	0.75	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.88
8/11/95	0.27	0.1	0.22	0.28	0.28	0.34	0.31	0.38	0.30	0.34	0.3	0.15	0.22	0.11	3.3
22/11/95	0.28	0.25	0.17	0.21	0.37	0.24	0.18	0.33	0.31	0.27	<0.1	<0.1	<0.1	<0.1	2.3

Table B3 summarises published data on measured concentrations of NPnEOs in freshwater sediments and two marine sediments. No equivalent data are available for the carboxylates.

Table B3 Reported concentrations of NPnEOs in sediments

Substance	Location	Number of samples	Mean (mg kg^{-1})	Range (mg kg^{-1})	Ref
NP1EO	Rhine River, Germany	-	800	-	1
	Glatt River catchment, Switzerland (7 sites)	-	-	100-8850	2
	Besos River, Spain	2	-	2400, <100[a]	3
	Marine lagoon, Venice, Italy	20	-	9-82[ab]	4
	Thirty US rivers	81	18.1	<2.3-175	5
	Fox River, US (12 sites)	-	121.4	4-215	5
	Tees Estuary, UK	8	-	0.13-3.9	6
	Tyne Estuary, UK	9	-	0.16-1.4	6
	Lake Ontario, Canada	9	7.1	<0.015-38	7
NP2EO	Rhine River, Germany	-	700	-	1
	Glatt River catchment, Switzerland (7 sites)	-	-	LOD-2720	2
	Besos River, Spain	1	1200	-	3
	Marine lagoon, Venice, Italy	20	-	3-20[ab]	4
	Thirty US rivers	-	-	-	5
	Lake Ontario, Canada	9	1.2	0.015-6.0	7
NP3-17EO	Thirty US rivers	-	-	not detected	5

[a] coastal sediment [b] easily re-suspendible sediment from the sediment surface

References:

1 Marcomini and Giger, 1987
2 Ahel *et al*, 1991
3 Grifoll *et al*, 1990
4 Marcomini *et al*, 1990
5 Naylor, 1998
6 Lye *et al*, 1999
7 Bennie *et al*, 1997

B3 Estuarine waters

Studies on breakdown products of NPnEO surfactants in Venice lagoon, Italy (Marcomini *et al*, 1990) revealed between 0.6 and 4.5 μg l^{-1} total NPnEOs. Later studies on the Krka River estuary in Croatia which receives untreated sewage from the town of Sibenik (Kvestak and Ahel, 1994) showed the range of concentrations of NP, NP1EO and NP2EO to lie between <20-1.2, <20-0.4 and <20-1.3 μg l^{-1}, respectively. Because there is no treatment of the sewage prior to discharge, these compounds must have arisen as a result of degradation in the sewer and in the receiving water. No studies on the presence of breakdown products of APnEO surfactants (other than NP) appear to have been carried out in the UK.

The degradation of a NP10EO (range: 1-18 EO units) was studied in brackish and saline water using a static die-away method (Kvestak and Ahel, 1995). The disappearance of the total nonylphenol ethoxylates present in the sample was found to occur faster in the brackish water than the saline water, possibly due to an increased amount of pre-exposure of the brackish water to NP10EO compared to the saline layer. The half-life for disappearance of the NP10EO was found to be longer at low temperatures (half-life > 1 month at 13^{o}C) than at temperatures simulating summer conditions (half-life of 2,5-4 and 14-35 days in brackish and saline water respectively). The changes in oligomer distribution of the parent NPnEO was also

investigated: the added NP10EO was found to be relatively unchanged during the first 3 days incubation but after 8 days incubation, there was a shift from the higher oligomers (all NPnEO with n>5 had disappeared) to lower oligomers (an increase in the amounts of NPnEO with n<4, with the biggest increase in NP2E0). This then degraded at a slower rate than seen for the higher oligomers, with residual amounts of NP2E0 being seen after 30 days.

A recent survey of alkylphenolic concentrations in sediments of the Tees Estuary has indicated concentrations (subject to confirmation) of NP1EO, NP2EO and NP of between <0.3-3.1 mg l^{-1}, <0.3-1.2 mg l^{-1} and <0.3-170.0 mg l^{-1}, respectively (Daniels, EA North-East Region, *pers. comm.*). In samples of water from the Tyne estuary, adjacent to a sewage outfall, NP1EO concentrations of 0.94 μg l^{-1} were reported (Lye *et al,* 1999).

APPENDIX C AQUATIC TOXICITY OF APnEOS / APECS

Introduction

The aquatic toxicity APnEOs and their breakdown products has received some interest in recent years and comprehensive reviews have been published by Talmage (1994) and Staples *et al* (1998). Some commercial reports were included in the review by Staples *et al,* which have not been available for review by us.

The majority of studies on APnEO surfactants and their breakdown products have been performed on commercial products, which typically contain a range of oligomers. Thus, although toxicity summaries are expressed in terms of an APnEO with a particular EO chain length, in fact the toxicity observed will have been due to the combined effects of all the oligomers present. Since commercial products contain a distribution of oligomers which is approximately symmetrical the contribution of less toxic oligomers (those with longer EO chains) probably cancels out the contribution made by the more toxic oligomers (those with shorter EO chains). Therefore, the toxicity stated for a particular EO chain length may be taken as a reasonable reflection of the toxicity of that oligomer if it had been tested in its pure form. A similar assumption was made by Roberts (1991) when deriving structure-activity relationships for non-ionic surfactants. When estimating octanol-water coefficients (P), a value was estimated for each component of the mixture and multiplied by the mole fraction for that component. The products were summed and the log of the sum used as the estimate of log P for the product.

Acute toxicity to fish

Acute toxicity data for freshwater and saltwater fish exposed to APnEOs are summarised in Tables C1 and C2. For APnEOs, acute LC50 values range between 1 and 1000 mg l^{-1} although, as explained below, toxicity is highly dependent on chemical structure and especially the length of the EO chain. For the most important commercially relevant APnEOs (those with 9-11 EO units), acute LC50 values range between 1.0 and 64 mg l^{-1}.

The toxicity of the alkylphenols, NP and OP have been reviewed extensively by Whitehouse *et al,* (1998a, 1998b) and are not discussed in more detail here except for comparative purposes. Only two studies are reported for the carboxylates (Yoshimura, 1986) and these gave rise to 48h LC_{50} values to killifish (*Oryzias latipes*) of 8.9 mg l^{-1} for NP2EC and 9.6 mg l^{-1} for NP1EC, similar to those of NPnEOs with between 6 and 8 EO units.

Differences between 'non-toxic' and acutely toxic concentrations appear to be very small i.e. concentration-response curves are steep, though data are limited. For NPnEOs, Fischer (1973) cited 48-hr LC0, LC50 and LC100 values of 5, 7, and 10 mg l^{-1}, respectively, for golden orfe *(Leuciscus idus).*

Table C1 Acute toxicity of APnEOs to freshwater fish

Species/Common Name	Surfactant/Trade Name	LC_{50} (mg l^{-1})	Test Duration (h)	Reference
Carassius auratus goldfish	NP9EO	18	48	Tomiyama, 1974
Carassius auratus goldfish	NP10EO	5.4	48	Kurata et al, 1977, cited in Talmage, 1994
Carassius auratus goldfish	NP9-10EO	6.9	6	Reiff et al, 1979
Lebistes reticulatus guppy	NP11EO	52-64[c]	24	Van Emden et al, 1974
Lepomis macrochirus bluegill sunfish	NP4EO (Surfonic N-40)	1.3	96	Macek and Krzeminski, 1975
	NP9.5EO (Surfonic N-95)	7.6	96	
	NP5EO (Igepal CO-520)	2.4-2.8	96	
	NP9EO (Igepal CO-630)	7.9	96	
	NP30EO (Igepal CO-880)	>1000	96	
	OP5EO (Triton X-45)	2.8-3.2	96	
	OP10EO (Triton X-100)	12.0	96	
	OP30EO (Triton X-305)	531	96	
Leuciscus idus golden orfe	NP9EO	7	48	Fischer, 1973
Leuciscus idus golden orfe	NPnEO	3.7>10	48	Hamburger et al, 1977
Leuciscus idus golden orfe	NP9-10EO	4.9, 7.0, 11.2	48	Reiff et al, 1979
Oryzias latipes killifish	NP	1.4	48	Yoshimura, 1986
	NP1EO	3.0	48	
	NP3.3EO	2.5	48	
	NP5EO	3.6	48	
	NP6.4EO	5.4	48	
	NP8.4EO	11.6	48	
	NP8.9EO	11.2-14	48	
	NP13EO	48	48	
	NP16.6EO	110	48	
Pimephales promelas fathead minnow	NP9EO	1.6	96	Salanitro et al, 1988

Species/Common Name	Surfactant/Trade Name	LC_{50} (mg l^{-1})	Test Duration (h)	Reference
Pimephales promelas fathead minnow	NP7EO	3.2	96	Markarian et al, 1989
Pimephales promelas fathead minnow	NP9EO	4.6[a]	96	Kravetz et al, 1991 Dorn et al, 1993
Oncorhynchus mykiss rainbow trout	NP10EO	2.5-6.2[i]	3	Marchetti, 1965
Oncorhynchus mykiss rainbow trout	NP8EO	4.7	96	Calamari and Marchetti, 1973
Salmo trutta brown trout	NP9-10EO	1.0	96	Reiff et al, 1979

Table C2 Acute toxicity of APnEOs to marine fish

Species/Common Name	Surfactant/ Trade Name	LC_{50} (mg l^{-1})	Test Duration (h)	Reference
Gadus morhua cod	NP10EO	2.5-6.8	96	Swedmark et al, 1971; 1976
Pleuronectes flesus flounder	NP10EO	3.0	96	Swedmark et al, 1971
Rasbora heteromorpha harlequin fish	NP9-10EO	8.6	96	Reiff et al, 1979

Studies of toxicity coupled to biodegradation tests

To determine whether toxic effects persist after primary biodegradation, several investigators have coupled biodegradation tests to aquatic toxicity tests. The bioassays were carried out by exposing organisms to the biodegrading medium or effluent and comparing survival times to those in known concentrations of the starting materials. In a laboratory river water die away test, rainbow trout were exposed to biodegrading OP9EO, at an initial concentration of 20 mg l^{-1} (Reiff, 1976). Ten to eleven weeks were required for the surfactant to be rendered non-toxic to the trout, at which time the concentration was less than the LC50.

Yoshimura (1986) tested the toxicity to Japanese killifish *(Oryzias latipes) of* biodegrading NP9EO (initial concentration 20 mg l^{-1}) in a river-water die away system. Although no peaks of the parent material or its biodegradation intermediates were present on HPLC chromatograms after 10 days, 50% of the tested fish died at this time (96-hr test). The author

speculates that carboxylate metabolites may be responsible for the toxicity remaining after the NP9EO disappeared. All fish survived when tested after 14 days of biodegradation although the chemical composition of the test medium at this time is difficult to judge.

More recently, fathead minnows *(Pimephales promelas)* were subjected to effluent from activated sludge bio treated units degrading 50 mg l^{-1} br-NP9EO at 25^{o}C. Acute toxicity was still present (20-40% of the LC50 for NP9EO), indicating the presence of the intact surfactant or its metabolites (Kravetz *et al*., 1991; Dorn *et al*., 1993). However, following biodegradation of NP9EO in an activated sludge reactor, the effluent toxicity to the mysid shrimp *(Mysidopsis bahia)* was 17% that of the 48-hr LC50 of the intact surfactant (Patoczka and Pulliam, 1990).

Toxicity to aquatic invertebrates

Toxicity data on freshwater and marine invertebrates exposed to APnEOs are included in Tables C3 and C4. Again, LC50 values for APnEOs vary widely among species but NP and OP are more toxic to all tested species (Whitehouse *et al*, 1998a; 1998b). Recently, data for NP1-2EC have become available (Staples, APE Research Council, *pers. comm*.) from experiments with the water flea, *Ceriodaphnia dubia*: following exposure for 96h, an LC_{50} of 110 mg l^{-1} was estimated and, following a 7 day exposure, NOECs for mortality and fecundity were 8.4 and 2.2 mg l^{-1}, respectively.

Table C3 Acute toxicity of APnEOs to freshwater invertebrates and algae

Species/common name	Substance	LC/EC_{50} mg l^{-1}	Test duration (h)	Reference
ARTHROPODS				
Daphnia magna water flea	NP7EO	4.1	96	Markarian *et al*, 1989
Daphnia magna water flea	NP9EO	14.0[b]	48	Kravetz *et al*, Dorn *et al*, 1993
Daphnia pulex water flea	NP10EO	12.5	48	Moore *et al*,1987
Daphnia pulex water flea	NP9EO	2.9	48	Salanitro *et al*, 1988
Daphnia sp. water flea	NP4EO	5	-	Janicke *et al*, 1969
	NP6EO	5	-	
	NP7EO	10	-	
	NP10EO	10	-	
	NP20EO	1000	-	
	NP30EO	10000	-	
Ceriodaphnia dubia cladoceran	NP1-2EO	1.04	48	Ankley *et al*, 1990
	nonlyphenol	0.47	48	
Gammarus pulex amphipod	C_9APE_n NP_nEO (Lissapol NXA)	>1-2	-	Madai and An der Lan, 1964
Aedes aegypti Mosquito larva	NP11EO	500	24	Van Emden *et al*, 1974

Species/common name	Substance	LC/EC_{50} mg l^{-1}	Test duration (h)	Reference
MOLLUSCS				
Biomphalaria glabrata	NP11EO	23 (LC_{100})	24	Van Emden *et al*, 1974
ALGAE				
Chlamydomonas reinhardii	nonylphenol	cell membrane disruption	0.5-0.7	Weinberger and Rea, 1981
Chlamydomonas reinhardii	nonylphenol	inhibition of photosynthesis	0.75	Moody *et al*, 1983
Chlorella pyrenoidosa	nonylphenol	growth depression 24-hr LC_{50} LC_{100}	0.025-7.5 1.5 25	Weinberger and Rea, 1981;1982
Chlorella fusca	OP10EO (Triton X-100)	no effect, 14-day growth	131, 263, 525	Wong, 1985
Microcystis aeruginosa	C8APE10 OP10EO	growth (96-hr EC_{50})	7.4	Lewis and Hamm, 1986
Nitzschia actinastroides	OP9.5EO (Triton X-100)	5-day growth inhibition	10-15	Nyberg, 1985
Nitzchia holsatica	OP9.5EO (Triton X-100)	no effect 5-day growth (EC_{45}) 5-day growth (EC_{100}) 5-day growth (EC_{57}) 5-day growth (EC_{100})	10 (25°C) 15 (25°C) 25 (25°C) 5 (15°C) 15 (15°C)	Nyberg, 1976
Phorphyridium purpuretum	OP9.5EO (Triton X-100)	5-day growth inhibition	5-10	Nyberg, 1985
Poterioochromonas malhamensis	OP10EO OP40EO	"lethality" "lethality"	124 17,784	Röderer, 1987
Scendesmus sp.	NP4EO NP6EO NP7EO NP10EO NP20EO NP30EO	"lethal threshold" "lethal threshold" "lethal threshold" "lethal threshold" "lethal threshold" "lethal threshold"	6 10 16 31 125 5000	Janicke *et al*, 1969
Selenastrum capriocornutum	NP8EO (Emulgen 910) NP9EO (Emulgen 909)	growth (48-hr EC_{50}) growth (48-hr EC_{50})	20 50	Yamane *et al*, 1984
Selenastrum caprocornutum	OP10EO	growth (96-hr EC_{50})	0.21	Lewis and Hamm, 1986
Selenastrum capricornutum	NP6EO	3-week growth, slight decrease	100	Nyberg, 1988

Species/common name	Substance	LC/EC_{50} mg l^{-1}	Test duration (h)	Reference
Selenastrum capricornutum	(Synperionic NP6)	3-week growth rate increase	200, 300, 400, 500	
	NP9EO (Synperionic NP9)	3-week growth rate decrease (~50%)	100-500 100-500	
	NP30EO (Symperonic NP30)	3-week growth rate increase	100 300-500	
	NP9.5EO (Triton X-100)	3-week growth, slight decrease 3-week growth rate increase		
Selenastrum capricornutum	NP7EO	growth (96-hr EC_{50})	>1000	Markarian *et al*, 1989, 1990
Selenastrum capricornutum	br-4-nonylphenol	growth (96-hr EC_{50})	0.41	CMA, 1990c
Selenastrum capricornutum	NP9EO	growth (96-hr EC_{50}) 96-hr NOEC 96-hr LOEC MATC[b]	12 8 16 11.3	Dorn *et al*, 1993

Table C4 Acute toxicity of APnEOs to marine invertebrates and algae

Species/Common Name	Surfactant/ Trade Name	LC_{50}/EC_{50} (mg l^{-1})	Test duration (h)	Reference
INVERTEBRATES				
Leander adspersus decapod	NPIOEO	>100 (6-8[C)]) 10-50 (15-17°C)	96 96	Swedmark *et al*., 1971
Leander squilla decapod	NPIOEO	> 100	96	Swedmark *et al*, 1971
Mysidopsis bahia mysid shrimp	NP9EO	1.23	48	Patoczka and Pulliam, 1990
Mysidopsis bahia mysid shrimp	NP1.5EO NP9EO NP50EO tp-NP1.5EO tp-NP9EO tp-NP15EO tp-NP40EO tp-NP50EO OP1.5EO OP5EO	1.66-3.34 1.23-1.89 4148 0.11 0.71-2.2 2.57 100 4110 6.51-7.07 1.83	48 48 48 48 48 48 48 48 48 48	Hall *et al*., 1989

Species/Common Name	Surfactant/ Trade Name	LC_{50}/EC_{50} (mg l^{-1})	Test duration (h)	Reference
Crangon crangon brown shrimp	NP12EO	89.5	48	Portmann and Wilson, 1971
Pandalus montagui pink shrimp	NP12EO	19.3	48	Portmann and Wilson, 1971
Balanus balanoides barnacle (adults: nauplii)	NP10EO	<25; 1.5	96	Swedmark *et al*., 1971
Carcinus meanas shore crab	NP10 EO	<100	96	Swedmark *et al*., 1971
Carcinus maenas shore crab	NP12EO	<100	48	Portmann and Wilson, 1971
Eupagurus bernhardus hermit crab	NP10EO	>100	96	Swedmark *et al*., 1971
Hyas araneus spider crab (adults: larvae)	NP10EO	>1070; 10	96	Swedmark *et al*., 1971
MOLLUSCS				
Cardium edule cockle (adult; juvenile)	NP10 EO	5(6-8°C) <<10 (15-17°C)	96 96	Swedmark *et al*., 1971
Cardium edule cockle	NP12EO	92.5	48	Swedmark *et al*.,1971
Mya arenaria clam	NP10EO	18(6-8°C) <10(15-17°C)	96	Portmann and Wilson, 1971
Mytilus edulis mussel	NP10EO	12(6-8°C) <10(15-17°C)	96	Swedmark *et al*., 1971; 1976
Pecten maximus scallop	NP10EO	<<5.0	96	Swedmark *et al*., 1971
Pecten opercularis Scallop	NP10EO	<<10	96	Swedmark *et al*., 1971
ALGAE				
Skelatonema costatum	br-4-nonylphenol	growth (6-hr EC_{50})	0.027	CMA, 1990d
BACTERIA				
Vibrio fischeri	NP2EO NP4EO NP6EO NP8EO NP10EO	1.3 4.3 14.3 32.5 208.6	exposure duration not given	Ribosa *et al*, 1993

As noted in the previous section on fish, toxicity is related to chemical structure, particularly the length of the EO chain. Using *Daphnia* sp., Janicke *et al*. (1969) tested a series of technical products containing mixtures with different EO chain lengths. The "lethal threshold' decreased with increasing EO chain length from 4 to 30 units. In addition, Hall *et al*. (1989)

found that EO chain length was a good predictor of toxicity to the marine mysid shrimp, *Mysidopsis bahia.*

For a series of alkylphenols with the alkyl chain ranging in carbon number from 4 to 12, toxicity to shrimp *(Crangon septemspinosa)* increased with increasing molecular weight which also correlated with increasing log P (McLeese *et al*., 1981). This demonstrates that variations in both the EO and alkyl chains may influence acute toxicity.

Toxicity to algae and bacteria

Numerous algal toxicity studies have been conducted using surfactants, particularly those based on OP. Toxicity data for freshwater and saltwater algae are included in Tables C3 and C4. They suggest that growth of most species of algae, is not inhibited by APnEO concentrations of $\leq$ 10 mg l^{-1} although a few species of green algae appear to be sensitive to concentrations as low as 0.21 mg l^{-1}.

Changes in the community structure of natural assemblages of algae, based on common indices of species diversity and similarity, were affected at 8.4 mg l^{-1} OP11EO but not at 3.2 mg l^{-1} (Lewis, 1986). In a continuation of this study, the effects of the same surfactant on lake community photosynthesis were determined using a ^{14}C technique (Lewis and Hamm, 1986). The mean 3-hr EC50 for photosynthesis was 28.7 mg l^{-1}, with a range of 2.5 to 101.5 mg l^{-1}. When compared to predictions from laboratory single-species tests for toxicity, the concentrations that produced effects in the laboratory were lower than or similar to concentrations that produced effects in the field. Lewis (1990) reviewed studies on the chronic toxicities of surfactants to algae. He concluded that the toxicity of most surfactants to natural assemblages of algae under natural conditions is less than that predicted from laboratory tests, although this is a recurring theme in lab/field comparisons for many chemicals.

There is only one example where the effect of APnEO structure on toxicity to algae has been addressed (Janicke *et al*, 1969). In this study, the freshwater alga, *Scenedesmus* sp. was employed and, as noted earlier, it showed that the 'lethal threshold' concentration of NPnEOs with varying numbers of EO units increased (i.e. became less toxic) with increasing length of the EO chain.

Recently, Ribosa *et al*, (1993) reported the effects of different NPnEO oligomers on light output from the bioluminescent bacterium, Vibrio fischeri. Exposure times are not given but IC_{50} values ranged between 1.3 and 209 mg l^{-1}, depending on the EO chain length.

Higher plants

OPnEO ('Triton X') surfactants were also tested on the aquatic vascular plant, duckweed *(Lemna minor)* (Caux *et al*, 1988). The authors monitored growth, frond florescence and chlorophyll content, conductivity of the test media, and specific ion leakage. Solutions of 1 mg l^{-1} of each surfactant and a 10 mg l^{-1} solution of OP7-8EO (Triton X-114) were without effects but a 10 mg l^{-1} solution of the other Triton surfactants (OP1EO, OP3EO and OP9-10EO*)* depressed frond development by 25 to 50%. At 50 mg l^{-1}, the two lower homologues were about twice as phytotoxic as the higher ethoxylates so again, the toxicity of the surfactants to *L. minor* appears to be inversely related to EO chain length and directly related to log P.

Other test systems

NPECs were included as test substances in an experiment to examine the effects of a NPnEO surfactant, NP and NP1EC on mitochondrial respiration in sub-mitochondrial particles isolated from beef heart (Argese *et al*, 1994). The relative toxicities were different to those seen in aquatic organisms, with EC50s of 1.3, 1.8 and 8.2 mg l^{-1} for NPnEO, NP and NP1EC, respectively. In this assay, the undegraded NPnEO surfactant appears to be more toxic than would be expected, based on 'conventional' aquatic toxicity tests. Although the authors suggest the assay provides good prediction of toxicity to aquatic organisms, the examples used to support this claim are highly selective and these predictions are not borne out when all the available data are considered, particularly toxicity data for undegraded NPnEOs.

Chronic toxicity

Chronic toxicity tests using NPnEO surfactants have been conducted with *Daphnia magna*, cod (*Gadus morhua*) and fathead minnow (*Pimephales promelas*) and the results are summarised in Table C5. Chronic toxicity data for 'lower' APnEOs are lacking, however.

Neufahrt *et al*, (1987) undertook a study of the chronic (six months exposure) toxicity of effluents from biotreater units to several aquatic organisms. The effluent, which contained 0.46 mg l^{-1} NPnEOs, 2 ug l^{-1} NP, 1 ug l^{-1} NP1EO and 3 ug l^{-1} NP2EO was diluted 1:5 with water to simulate the concentrations likely to occur in the receiving water. The authors found no long-term reproductive effects on *Daphnia magna* or the snail *Planorbis comeus* or growth effects on the aquatic vascular plant *Lemna minor*. The number of young produced by the guppy *(Lebistes reticulatus)* was smaller than that of controls, but the difference was not evaluated. It should be noted that the design of the study was not one which would be capable of resolving small differences between treatment groups.

As expected, chronic effects are evident at lower concentrations than those eliciting toxicity in acute studies. Where the same species has been used to investigate both acute and chronic toxicity, ratios between acute LC/EC50 and chronic NOECs appear to be small:

- 1.4 (*Daphnia magna* exposed to br-NP9EO (Dorn *et al*, 1993))
- 2.5 - 6.8 (Cod exposed to NP10EO (Swedmark *et al*, 1971))
- 2.6-4.6 (fathead minnows exposed to br-NP9EO (Dorn *et al*, 1993))

Factors affecting toxicity of APnEOs

EO chain length

Several studies describe the acute toxicity of 'lower' APnEOs, usually arising from experiments which have examined the effect of EO chain length on toxicity. It is clear from these studies that the ethoxylated compounds become more toxic with progressive loss of EO units: the toxicities of APnEOs with a range of EO units have been tested against several species of aquatic organisms (*Daphnia* sp., the mysid crustacean *Mysidopsis bahia*, bluegill sunfish *Lepomis macrochirus*, the Japanese killifish *Oryzias latipes* and the freshwater alga, *Scenedesmus sp.*) (Janicke *et al*., 1969; Hall *et al*., 1989; Macek and Krzeminski, 1975; Yoshimura, 1986) and all show a clear inverse relationship between EO chain length and

toxicity. The data of Yoshimura (1986), who tested a series of nine NPnEOs, including synthesised biodegradation intermediates, on Japanese killifish most clearly show this trend (Table C1) in which toxicity declined from a 48h LC50 of 1.4 mg l^{-1} for NP1EO to a value of 110 mg l^{-1} for NP16.6EO.

It is important to note that the majority of studies have been performed on commercial products which typically contain a range of oligomers. Thus, although toxicity summaries are given for a particular EO chain length, in fact the toxicity observed will have been due to the combined effects of all the oligomers present. It appears that most commercial products contain a distribution of oligomers which is approximately symmetrical and so the contribution of less toxic oligomers (those with longer EO chains) probably cancels out the contribution made by the more toxic oligomers (those with shorter EO chains). Therefore, the toxicity stated for a particular EO chain length is probably a reasonable reflection of the toxicity of that oligomer if it had been tested in its pure form. Nevertheless, this assumption remains untested.

Age/developmental stage

Age of the test species and experimental conditions appears to affect sensitivity to APnEOs: tolerance to NP10EO varied among alevin, fry, and fingerling rainbow trout *(Oncorhynchus mykiss)* (Marchetti, 1965). Alevins with completely absorbed yolk sacs were more sensitive than younger alevins whilst tolerance increased in the fry and fingerlings. Swedmark *et al,* (1971) have also shown that early developmental stages of aquatic invertebrates are less tolerant than adults: mortality of adult mussels (*Mytilus edulis*) held at 5 mg l^{-1} for 16 days was less than 50%, but at 2 mg l^{-1} embryos did not develop beyond the blastula stage and at 1 mg l^{-1} they did not develop beyond the veliger larval stage. Likewise, larvae of both the spider crab *(Hyas araneus)* and the barnacle *(Balanus balanoides)* were also more sensitive than adults.

Temperature

Uptake of surfactants may be more rapid and reach higher tissue concentrations at higher temperatures than at low temperatures, thus affecting toxicity. For example, the surfactant NP10EO was taken up by cod *(Gadus morhua)* more rapidly and reached higher concentrations in several tissues at 18^{o}C than at 11^{o}C (Granmo and Kollberg, 1976). This appears to have been translated into effects on toxicity: for cod, toxicity was greater at 15 to 17^{o}C than at 6 to 8^{o}C and toxicity of NP10EO increased with an increase in temperature for several other marine species (Swedmark *et al,* 1971). Golden orfe *(Leuciscus idus)* were also less sensitive at the lower temperature of 15^{o}C ('critical concentration' range of 12-20 mg l^{-1}) than at 20^{o}C ('critical concentration'' range of 6-11 mg l^{-1}) (Fischer and Gode, 1978).

Changes in temperature have been shown to affect the response of individual species of algae and phytoplankton communities to surfactants. In the absence of surfactant, growth of the diatom *Nitzschia holsatica* was slower at 15^{o}C than at 25^{o}C and the inhibitory effect of OP9.5EO was more marked at the lower temperature (Nyberg, 1976). However, the inhibitory effect of OPnEO and other surfactants on the photosynthetic activity of lake phytoplankton increased with increasing water temperature (Lewis and Hamm, 1986).

Table C5 Chronic toxicity of APnEOs to aquatic life

Species/Common Name	Chemical	Parameter Measured	NOEC (mg l^{-1})	LOEC (mg l^{-1})	MATC (mg l^{-1})	LC_{50} (mg l^{-1})	Reference
Daphnia magna	NP9EO	7-day growth	10	>10	>10		Kravetz *et al*, 1991
water flea		7-day mortality (LC_{50})	10	20	14	9.0	Dorn *et al*, 1993
	NP	21-day survival, growth, reproduction	0.024				Comber *et al*, 1993
Pimephales promelas	NP9EO	7-day growth	1.0	2.0	1.4		Kravetz *et al*, 1991
		7-day mortality	1.8	2.0	1.4	2.9	Dorn *et al*, 1993
fathead minnow							
	br-4-NP	length at 28 days	0.023				CMA, 1991b
		survival at 33 days	0.0074	0.014	0.010		
Gadus morhua	NP10EO	several months	<1				Swedmark *et al*, 1971
cod							

Oestrogenic effects

Several workers have examined the oestrogenic effects of APnEOs and APnECs *in vitro* and *in vivo*, often alongside the alkylphenols NP and OP, whose oestrogenic properties are well documented.

Jobling and Sumpter (1993) used an *in vitro* assay based on the ability of cultures of rainbow trout hepatocytes to synthesise vitellogenin when stimulated by oestrogenic substances. They tested two NPnEOs with 2 and 9 EO units alongside the carboxylate NP1EC and the natural oestrogen, 17β-oestradiol, and their results are summarised in Table C6.

TableC6 Oestrogenic potencies of nonylphenolic compounds in a trout hepatocyte assay (Jobling and Sumpter, 1993)

Compound	Mean ED50 (mM)	Standard error	Relative potency
17β-oestradiol	1.8	0.81 (n=8)	1.0
NP	16.2	0.79 (n=4)	0.000009
NP2EO	17.3	0.77 (n=2)	0.000006
NP9EO	82.3	7.79 (n=2)	0.0000002
NP1EC	15.3	2.76 (n=2)	0.0000063

Similar potencies were exhibited by NP, NP2EO, NP9EO and NP1EC although greater quantities of vitellogenin were produced following exposure to NP2EO and NP1EC than NP or NP9EO. The authors report that NP40EO had no effect in the assay and concluded that oestrogenicity declined with increasing length of the EO chain. They also discovered that, as the exposure time was extended from 48h to 96h, NP2EO and NP1EC were actually more potent than NP in the trout hepatocyte assay. The authors suggested this maybe due to activation following metabolism (presumably to NP).

Using a different assay (a recombinant yeast screen developed for the detection of oestrogenic compounds), Routledge and Sumpter (1996) found significant responses when NP and OP were introduced at concentrations of between 0.1 and 10 mg l^{-1}. The carboxylates, NP1EC and NP2EC, gave rise to smaller responses over this concentration range whilst NP12EO was completely without effect, bearing out the findings of the earlier trout hepatocyte study reported by Jobling and Sumpter (1993). Relative potencies of these alkylphenolic compounds with respect to 17β-oestradiol are shown in Table C7.

In another study into the effects of alkylphenolic compounds on the stimulation of vitellogenin gene expression in trout hepatocytes (White *et al*, 1994) NP, NP2EO and NP1EC again gave rise to similar levels of effect but, as seen in other *in vitro* studies, OP appears to be a more potent oestrogen than NP.

These effects are reflected *in vivo*. Based on assessments of testicular growth and vitellogenesis in rainbow trout, Jobling *et al*, (1996) also concluded that NP, NP1EC and NP1EO exhibited similar oestrogenic potencies following a three week exposure period. However, the concentrations required to elicit these responses *in vivo* were around two orders

of magnitude lower than those causing vitellogenesis *in vitro* (in trout hepatocytes, Jobling and Sumpter, 1993). Oestrogenic responses *in vivo* by alkylphenolic compounds appear to be influenced by the period over which animals are exposed and the stage of sexual development (Jobling *et al*, 1996) and this may explain the varied results obtained when juvenile rainbow trout were exposed various alkylphenolic compounds (including NP2EO and NP1EC) in a study reported by Ashfield *et al,* (1995).

TableC7 Relative potencies of alkylphenolic compounds in a recombinant yeast screen (Routledge and Sumpter, 1996)

Substance	Relative potency
17β-oestradiol	1.0
NP	0.00014
OP	0.00067
NP1EC	0.00004
NP2EC	0.00004
NP2EO	0.000002
NP12EO	-

Bioaccumulation

Talmage (1994) points out that, whilst NP and the 'lower' APnEOs are relatively lipophilic with log P values greater than 3.0, they are rapidly excreted from aquatic organisms. Exposure of cod *(Gadus morhua) to* 5 mg l^{-1} of NP10EO, labelled in the EO chain with ^{14}C resulted in rapid uptake through the gills and distribution to the tissues, reaching an equilibrium after eight hours (Granmo and Kollberg, 1976). Highest concentrations of radioactivity were to be found in the gall bladder (4000 ppm) which corresponds to a bioconcentration factor of *ca.* 800-fold although corresponding factors for the gills and blood were only 20-fold. Apart from the gall bladder, 60% of the radioactivity had been eliminated from other tissues after 24h.

Other studies addressed have uptake and bioaccumulation under field conditions:

Mussels (*Mytilus edulis*) placed in cages near the wastewater outlet of a facility that manufactured NPnEO surfactants contained concentrations of NP of 0.20 to 0.40 ug g^{-1} wet weight (Wahlberg *et al*., 1990). Concentrations of NP1EO ranged from 0.08 to 0.28 ug g^{-1} but unfortunately, concentrations in the water and sediment were not measured and so a BCF cannot be estimated.

NP, NP1EO and NP2EO were determined in several freshwater organisms from surface waters of the Glatt Valley, Switzerland (Ahel *et al*., 1993). High concentrations occurred in the macroalga, *Cladophora glomerata,* 38.0, 4.7, and 4.3 mg kg^{-1} dry weight for NP, and NPE, respectively, where respective water concentrations were 3.9, 24, and 9.4 ug l^{-1}. Based on the dry weight concentrations, BCF were 10,000, 200, and 500, respectively. Dry weight concentrations for the respective compounds in two species of aquatic plants were lower: 4.2, 0.9, and 0.6 mg kg^{-1} in *Fontinalis antipyretica* and 2.5, 1.1, and 1.9 mg kg^{-1} in *Potamogeton*

crispus. Concentrations of NP, NP1EO,, and NP2EO in various tissues of several species of fish were <0.03-1.6, 0.06-7.0, and 0.03-3.1 mg kg^{-1} dry weight, respectively, resulting in BCF of 13-410, 3-300, and 3-330, respectively. Concentrations in a single mallard duck captured on the bank of the river were similar to those of fish.

Marcomini *et al*, (1990) analysed NPnEO metabolites (NP, NP1EO, and NP2EO) in macroalga *(Ulva rigida)*, water, and marine sediments of the Venice lagoon, Italy. The mean concentration of total alkylphenols in the water was 1.8 ug l^{-1} of which NP, NP1EO and NP2EO constituted < 10%. The total concentration of these three metabolites in algal samples was 0.049 μg g^{-1} dry weight, suggesting they were not bioaccumulated.

APPENDIX D MAMMALIAN TOXICITY

D1 Introduction

The mammalian toxicity of APnEO surfactants has been reviewed by Nimrod and Benson (1996) and in-depth by Talmage (1994). Of the numerous APnEOs in existence, one of the more studied is nonoxynol-9 (NP9EO), which is largely due to its use as a spermicide. No mammalian toxicity data for APnECs were located other than some data on their potential oestrogenicity (see Appendix C, section 5.5).

D2 Acute oral toxicity

Studies of various APnEO surfactants to rodents indicate that they are of low acute oral toxicity (Talmage 1994). The acute oral LD50 for the rat ranges from 1300 mg kg^{-1} (NP10EO) to >28,000 mg kg^{-1} body weight (OP40EO). Toxic symptoms include lethargy, depression, diarrhoea, tremors and coma. Autopsies showed congestion and discoloration of the liver, congestion and haemorrhage of the lungs, kidney effects and inflammation and congestion of the gastric mucosa. Although APnEOs with an average ethoxylate chain length of 9-10 units exhibited the greatest toxicity in some of the studies, the data from other studies are too scattered to make conclusions concerning toxicity and chemical structure. However, at ethoxylate chains of $\geq$30, the materials were essentially non-toxic. The length and degree of branching of the alkyl chain does not appear to influence toxicity.

D3 Subchronic toxicity

The data available indicate that APnEOs are also of low subchronic chronic oral toxicity, although cardiotoxicity has been demonstrated in dogs when exposed to AP15-20EO. Table E1 lists reported no-effect levels on growth and liver weight in the 90 day feeding studies with rats and dogs.

Larson *et al,* (1963) fed male and female albino rats on diets containing 2500 mg kg^{-1} day^{-1} of OP40EO for 90 days. No effects on weight gain, haematological indices, organ to body weight ratios or tissues were noted. Beagle dogs treated with 88 or 50 mg kg^{-1} body weight day^{-1} in the diet were similarly unaffected.

Smyth and Calandra (1969) conducted 90-day subchronic feeding studies with several species at dose levels of 40 to 5000 mg kg^{-1} body weight day^{-1} and found that ingestion of APnEOs with 20 or more ethoxylate units resulted in little toxicity to rats. Ingestion of APnEOs with less than 20 ethoxylate units caused growth retardation (attributed to the un-palatability of the food) and increased absolute and relative liver weights for the lower homologues at 20 or 100 mg kg^{-1} body weight day^{-1}. In several additional feeding studies with rats using NP9EO the following effects were reported: retardation of weight gain $\geq$ 320 mg kg^{-1} day^{-1} and reversible cellular changes in the liver and kidney at 125 and 625 mg kg^{-1} day^{-1}. In one dietary study, no effects were observed at 50 mg kg^{-1} body weight day^{-1}, whereas in another study there was a reduced weight gain at a dose of 5 mg kg^{-1} body weight day^{-1}.

Table D1 No observed effect levels in 90-day rat and dog feeding studies

Chemical	Rat NOAEL (mg kg^{-1} body weight day^{-1})	Dog NOAEL (mg kg^{-1} body weight day^{-1})
OP9EO	40	-[a]
OP40EO	2500	1250
NP4EO	40	40
NP6EO	-[a]	40
NP9EO	10	10
NP15EO	40	40
NP20EO	1000	-[a]
NP30EO	5000	1000
NP40EO	150	-
Dodecylphenol6EO	-[a]	-
Dodecylphenol9EO	-[a]	-
Dodecylphenol40EO	5000	-

[a] Retarded growth and/or liver weight changes were present at 40 mg kg^{-1} day^{-1}, the lowest dose tested.

Smyth and Calandra (1969) also addressed the effect of chemical structure on toxicity in a similar subchronic feeding study with dogs. Feeding of 40, 200, 1000 or 5000 mg kg^{-1} day^{-1} of several APnEO surfactants with 4 to 40 ethoxylate units resulted in toxic effects for only NP20EO. At 40 mg kg^{-1} day^{-1} there was microscopic evidence of cardiotoxicity, whereas at 1000 mg kg^{-1} day^{-1} six of the eight dogs died and cardiotoxicity could be grossly observed. By comparison, a dose of 5000 mg kg^{-1} of the same surfactant had no effect on rats. Both dogs and guinea pigs showed evidence of cardiac lesions, but rabbits, rats and cats did not. The cardiotoxic action appears to be a direct pharmacologic effect on the heart muscle and regardless of the alkyl group (octyl, nonyl or dodecyl) focal myocardial lesions were present for APEs with an average of 15, 17.5 or 20 EO units.

D4 Chronic toxicity/carcinogenicity

In 2-year feeding studies, a dose of 200 mg kg^{-1} day^{-1} of NP4EO produced no significant toxicological effects in rats whereas a dose of 1000 mg kg^{-1} day^{-1} resulted in reduced body weights and enlarged livers. No evidence of carcinogenic effects was observed. In another study involving the same compound but in dogs, no significant effects were seen in dogs at 40 mg kg^{-1} day^{-1} whereas 1000 mg kg^{-1} day^{-1} caused reduced weight, emesis, and increased serum alkaline phosphatase in dogs.

For NP9EO doses of 140 and 30 mg kg^{-1} day^{-1} in the diet for 2 years caused no significant toxicological effects in rats and dogs, respectively. Parameters considered were body and organ weights and histopathology of 28 tissues. A dose of 88 mg kg^{-1} day^{-1} produced increased liver to body weight ratios in dogs which were attributed to reduced food intake. No evidence of carcinogenicity was observed for either species.

Male and female Wistar rats fed OP40EO at 0, 17.5, 175 or 700 mg kg^{-1} day^{-1} for two years showed no adverse effects on growth and survival, food consumption, haematological values,

urinary concentrations of reducing substances and protein, organ to body weight ratios, or incidence of pathologic lesions (Larson *et al*, 1963).

D5 Genotoxicity

Talmage (1994) reviewed the genotoxicity data for APnEO surfactants. Overall, the data indicated that APnEOs are non-genotoxic when tested for mutations or genetic damage in a variety of *in vitro* and *in vivo* test systems. These short-term tests include forward and reverse mutations in bacterial and mammalian cell systems; clastogenic effects such as aneupoidy in fungi and chromosome aberrations and breaks both *in vivo* and *in vitro* in bacterial and mammalian cell transformations. All of the bacterial and mammalian cell mutational assay results reviewed were negative as were the *in vivo* tests for genetic damage in somatic and germ cells. A few of the tests, such as the cell transformation and unscheduled DNA synthesis assays produced conflicting results.

D6 Developmental/reproductive toxicity

Some APnEO surfactants such as NP9EO have been used in contraceptive preparations. A number of studies have focused on mammalian reproductive toxicity data following NP9EO exposure when administered intravaginally, although limited oral studies are available. There was no evidence of teratogenic effects in offspring of rats treated orally or vaginally with doses that resulted in maternal or foetal toxicity (Talmage 1994).

Rats were administered NP9EO according to several dosing regimes: 50, 250 or 500 mg kg^{-1} by gavage on days 6-15 of pregnancy, 500 mg kg^{-1} by gavage on days 1-20 of pregnancy or 50 or 500 mg kg^{-1} dermally on days 6-15 of pregnancy (Meyer *et al.,* 1988). The dams administered the two highest oral doses exhibited a statistically significant decrease in weight gain. A slight, but statistically significant small litter size and pre-implantation loss, not dose related, was observed in rats treated orally. There was also a statistically significant dose-related increase in foetuses with both extra ribs and slightly dilated pelvic cavities which was thought to be attributable to the high toxic doses of NP9EO. No effects were present in rats treated dermally. No toxic, reproductive or teratogenic effects were present in dams treated in a similar manner with NP30EO.

Pregnant CD-1 mice were administered OP9EO and NP10EO at dose levels of 800 and 600 mg kg^{-1} by gavage on days 6-13 of pregnancy. No teratogenic effects were seen as measured by litter size, birth weight and neonatal growth and survival to postnatal day 3 (Hardin *et al.,* 1987).

D7 Oestrogenicity

The oestrogenic properties of APnEOs and APnECs have been studied utilising a variety of *in vitro* and *in vivo* assays (White *et al.,* 1994, Jobling and Sumpter 1993, Routledge and Sumpter 1996, Jobling *et al.,* 1996) (see section 5.5 and Appendix C). These assays have utilised systems such as isolated rainbow trout hepatocytes and recombinant yeast screen as well as effects on testicular growth and vitellogenin in rainbow trout *in vivo*.

Only limited data were located on oestrogenic effects seen in vivo mammalian test assays. NP4EO and NP9EO were examined for their ability to exert oestrogenic activity using the

uterine weight assay in immature female rats (Williams *et al* 1998). The animals were given single doses of 0, 20, 100, 200, 500 or 1000 mg kg^{-1} day^{-1} for three consecutive days. As a positive control, animals were given 0.5 μg estradiol benzoate per day sub-cutaneously. The oral administration of either compound had no effects on absolute or relative uterine weight.

APPENDIX E CHEMICAL ANALYSIS FOR APnEOS AND APnECS

E1 Possible methods

Because of their polymeric nature, APnEOs and APnECs cover a wide range of volatilities. Also, compared to APnEOs, APnECs are more polar compounds. Because of these differences, methods based on gas chromatography (GC) can be used to analyse the lower APnEOs (n = 1-4) but for the higher APnEOs (n ≥ 5) and APnECs, methods based on liquid chromatography (LC) are needed. Derivatisation can be used to convert APnECs to e.g. their methyl esters which are less polar than the APnECs themselves, allowing the lower APnECs (n = 1-3) to be analysed using GC-based methods.

As with almost all organic compounds in aqueous matrices, the initial step in analysis involves extracting the determinands of interest from water. For APnEOs, several analytical methods have been reported which used Wickbold sublation as an extraction technique. This is effective for all types of surfactants, and often some type of clean-up step is used to remove other co-extracted surface active compounds such as ionic surfactants (e.g. "Blue Book" method, 1993). Other extraction techniques include combined steam distillation/solvent extraction (Ahel *et al*, 1996), solvent extraction with dichloromethane (Rudel *et al*, 1998), solid phase extraction using graphitised carbon black cartridges (Crescenzi *et al*, 1995) or octadecyl-silica cartridges (Scullion *et al*, 1996). For APnECs, extraction techniques include the use of ion-exchange (SAX) disks (Field and Reed, 1996), solvent extraction with dichloromethane (Rudel *et al*, 1998) and solvent extraction with chloroform (after pH adjustment of the samples to pH2) (Ahel *et al*, 1996)

As noted above, both GC- and LC-based methods have been used to detect and quantify APnEOs and APnECs in extracts from environmental samples. Although GC with flame ionisation detection (FID) can be used, generally GC with mass spectrometry (MS) has been used because MS provides more specific detection than FID. LC-based methods have used either ultraviolet (UV) or fluorescence spectrophotometry or MS detection but again, the latter technique provides better specificity.

Usually, the GC-based techniques used separate mixtures of APnEOs and APnECs into individual oligomers. Because APnEOs can be present in water samples as fairly complex mixtures (due to the various isomers of octyl- and nonylphenols that are present in the technical alkylphenol mixes used in the synthesis of the APnEOs) it is possible to detect, say, three or four different, varying in the structure of the alkyl chain. This does cause some analytical problems as standards are rarely available, and inevitably assumptions have to be made e.g. that the signals for all octyl or nonyl phenol diethoxylates are the same (which is probably untrue) and consequently quantitative data may be inaccurate. The same is true for data produced using LC-based techniques although individual isomers of oligomers do not appear to be resolved when normal-phase LC is used (i.e. only one peak is obtained for NP2EO). Quantification is usually based on using a particular surfactant (e.g. a Marlophen) as a reference. Use of reversed-phase LC resolves the various alkyl analogues (Kiewiet & Devoogt, 1996) although there is still the problem of referring the results to e.g. Marlophen. If required, it is possible to reduce the resolution of a reversed-phase LC column so that all isomers of oligomers elute as a single peak (Crescenzi *et al*, 1995).

The main problem preventing quantification with both GC and LC is the absence of individual oligomers (or the various isomers of each oligomer) as reference standards. This difficulty could potentially be resolved by using preparative LC to separate (and collect) individual isomers of oligomers, which could be used to make up standards for reference purposes. However, this requires large-scale HPLC to provide sufficient quantities of material and is therefore rarely done. To our knowledge, no reference standards of APnEOs and APnECs are commercially available.

E2 Compliance monitoring

From the point of view of monitoring for compliance with standards, there are various factors that might need to be taken into account:

1. Lack of commercially available standards for individual oligomers makes quantification difficult. This is worse for APnECs than APnEOs because, at least for APnEOs, it is theoretically possible to obtain mixtures commercially and separate them; APnECs mixtures are not commercially available.

2. Even if standards for individual oligomers were available, there may be problems with quantification of "total" APnEOs or APnECs (as proposed as one of the options in Section 7) because of errors introduced by summation of individual oligomers (e.g. if individual oligomers are quantified to ± 20%, the sum of all four will be ± 80%).

3. Any analytical technique which does not use MS for detection/identification may give falsely high results because interferences cannot be recognised as such and there is no way of checking. Thus, in compliance monitoring, there is a risk of false positives, especially where compounds which extract and co-chromatograph with APnEOs and APnECs are present in the water samples.

4. GC-MS is much more widely available than LC-MS. Because GC-MS resolves APnEOs where n = 1-4 and APnECs where n = 1-3, which in both cases is the most toxic end of the range of oligomers, GCMS is probably the preferred approach. Costs for GC-MS are approximately £100-150 per sample.

5. None of the methods described above are validated (i.e. fully performance tested) If validated methods are needed, this would be very expensive, perhaps running into several tens of thousands of pounds. However, without validation it is difficult to be confident about the validity of the analytical results.

REFERENCE: FOR APPENDICES A-F

Ahel, M. and Giger, W. (1985a) Determination of alkylphenols and alkylphenol mono- and diethoxylates in environmental samples by high-performance liquid chromatography. *Anal. Chem.,* **57,** 1577-1583.

Ahel, M. and Giger, W. (1985b) Determination of non-ionic surfactants of the alkylphenol polyethoxylate type by high-performance liquid chromatography. *Anal. Chem.*, **57,** 2584-2590.

Ahel, M., Conrad, T. and Giger, W. (1987) Persistent organic chemicals in sewage effluents. 3. Determination of nonylphenoxy carboxylic acids by high-resolution gas chromatography/mass spectrometry and high-performance liquid chromatography. *Environ. Sci. Technol.,* **21,** 67-703.

Ahel, M., Giger, W. and Schaffner, C. (1991) Environmental occurrence and behaviour of alkylphenol polyethoxylates and their degradation products in rivers and groundwaters. In: Swedish EPA Seminar on Nonylphenol Ethoxylate/Nonylphenol held in Saltsjobaden, Sweden, 6-8 February, pp. 105-151.

Ahel, M., J. McEvoy and W. Giger. (1993). Bioaccumulation of the lipophilic metabolites of non-ionic surfactants in freshwater organisms. *Environ. Pollut.* **79**, 243-248.

Ahel, M., Hrsak, D. and Giger, W. (1994a). Aerobic transformation of short-chain alkylphenol polyethoxylates by fixed bacterial cultures. *Arch. Environ. Contam. Toxicol.,* **26,** 540-548.

Ahel, M., Giger, W. and Koch, M. (1994b). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - 1. Occurrence and transformation in sewage treatment. *Wat. Res.*, **28,** 1131-1142.

Ahel, M., Giger, W. and Schaffher, C. (1994c). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-11. Occurrence and transformation in rivers. *Wat. Res.*, **28,** 1143-1152.

Ahel, M., Schaffner, C. and Giger, W. (1996) Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - III. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. *Wat. Res.*, **30,** 37-46.

Ankley, G.T., Peterson, G.S., Lukasewycz, M.T. and Jenner, D.A. (1990) Characteristics of surfactants in toxicity identification evaluations. *Chemosphere,* **21,** 3-12.

Argese, E., Marcomini, A., Miana, P., Bettiol, C. and Perin, G. (1994) Submitochondrial particle response to linear alkylbenzene sulfonates, nonylphenol ethoxylates and their biodegradation intermediates. *Environ. Toxicol. Chem.,* **13,** 737-742.

Ashfield, L.A., Pottinger, T.G. and Sumpter, J.P. (1995) Exposure of trout to alkylphenolic compounds results in modifications to growth rate and reproductive status. Abstract of paper presented at SETAC conference, John Moores University, November 1995.

Ball, H.A., Reinhard, M. and McCarty, P. (1989). Biotransformation of halogenated and nonhalogenated octylphenol polyethoxylate residues under aerobic and anaerobic conditions. *Environ. Sci. Technol.*, **23,** 951-961.

Blackburn, M. and Waldock, M.J. (1995) Concentrations of alkylphenols in rivers and estuaries in England and Wales. Report from the Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Bunnham-on-Crouch, Essex, UK.

Bennie, D.T. (1999) Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates. *Water Qual. Res. J. Canada*. **34**, 79-122.

Brunner, P.H., Capri, S., Marcomini, A. and Giger, W. (1988). Occurrence and behaviour of linear alkylbenzenesulphonates, nonylphenol, nonylphenol mono- and nonylphenol diethoxylates in sewage and sewage sludge treatment. *Wat. Res*. **22**, 1465-1472.

Brown, D., de Henau, H., Garrigan, J.T., Gerike, P., Holt, M., Keck, E., Kunkel, E., Matthijs, E., Waters, J. and Watkinson, R.J. (1986) Removal of non-ionics in a sewage treatment plant. Removal of domestic detergent non-ionic surfactants in an activated sludge sewage treatment plant. *Tenside,* **23,** 190-195.

Brown, D., de Henau, H., Garrigan, J.T., Gerike, P., Holt, M., Kunkel, E., Matthijs, E., Waters, J. and Watkinson, R.J. (1987) Removal of non-ionics in sewage treatment plants. II. Removal of domestic detergent non-ionic surfactants in a trickling filter sewage treatment plant. *Tenside*, **24,** 14-19.

Brüschweiler, H. and Gämperle, H. (1982) Primary and ultimate biodegradation of alkylphenol ethoxylates. *J. Com. Esp. Deterg.*, pp. 55-71.

Calamari, D. and Marchetti, R. (1973) The toxicity of metals and surfactants to rainbow trout (*Salmo gairdneri* Rich.). *Water Res.,* **7,** 1453-1464.

Caux, P.Y., Weinberger, P. and Carlisle, D.B. (1988) A physiological study of the effects of Triton surfactants on *Lemna minor* L. *Environ. Toxicol. Chem.*, **7,** 671-676.

CMA (Chemical Manufacturers Association) (1990c) Acute static toxicity of nonylphenol to the freshwater alga, *Selenastrum capricornutum*. Unpublished report, Alkylphenol & Ethoxylates Panel, CMA, Washington, DC.

CMA (Chemical Manufacturers Association) (1990d) Acute static toxicity of nonylphenol to the marine alga, *Skeletonema costatum*. Unpublished report, Alkylphenol & Ethoxylates Panel, CMA, Washington, DC.

CMA (Chemical Manufacturers Association) (1991b) Early life stage toxicity of nonylphenol to the fathead minnow, *Pimephales promelas*. Unpublished report, Alkylphenol & Ethoxylates Panel, CMA, Washington, DC.

Comber, M.H.I., Williams, T.D. and Stewart, K.M. (1993) The effects of nonylphenol on *Daphnia magna*. *Water Res.,* **27,** 273-276.

Crescenzi, C., Di Corcia, A., Sampari, R. and Marcomini, A. (1995) Determination of Non-ionic Polyethoxylate Surfactants in Environmental Waters by Liquid Chromatography/Electrospray Mass Spectrometry. *Anal. Chem.*, **67**(11), 1797-1804.

Di Corcia, A., Samperi, R. and Marcomini, A. (1994). Monitoring aromatic surfactants and their biodegradation intern-iediates in raw and treated sewage by solid-phase extraction and liquid chromatography. Environ. Sci. Technol., **28,** 850-858.

Di Corcia, A., Constantino, A., Crescenzi, C., Marinoni, E. and Samperi, R. (1998) Characterisation of recalcitrant intermediates from biotransformation of the branched alkyl side chain of nonylphenol ethoxylate surfactants. *Environ. Sci. Technol*. **32**, 2401-2409.

Dorn, P.B., Salanitro, J.P., Evans, S.H. and Kravetz, L. (1993) Assessing the aquatic hazard of some linear and branched non-ionic surfactants by biodegradation and toxicity. *Environ. Toxicol. Chem.*, **12,** 1751-1762.

Field, J.A. and Reed, R. (1996) Nonylphenol polyethoxycarboxlate metabolites of non-ionic surfactants in US paper mill effluents, municipal sewage treatment plant effluents, and river waters. *Environ. Sci. Technol.*, **30,** 3544-3550.

Fischer, W.K. 1973 (Personal communication cited in Gloxhuber, 1974)

Fischer, W.K. and Gode, P. (1978) Comparative investigation of various methods of examining fish toxicity, with special consideration of the German golden orfe test and the ISO zebra fish test. *A.F. Wasser Abwasser Forsch.*, **11,** 99-105. (Cited in Goyer *et al.* 1981).

Frazee, C.D., Osburn, Q.W. and Crisler, R.O. (1964) Application of infrared spectroscopy to surfactant degradation studies. *J. Am. Oil Chem. Soc.*, **41,** 808-812.

Garrison, A.W. and Hill, D.W. (1972) Organic pollutants from mill persist in downstream waters. *Am. Dyestuff. Reporter,* **61,** 21-25.

Gerike, P. (1987). Environmental impact of surfactants. *Surfactants Consum. Prod.* pp450-474.

Giger, W., Ahel, M., Koch, M., Laubscher, H. U., Schaffner, C. and Schneider, J. (1987). Behaviour of alkylphenol polyethoxylate surfactants and of nitrilotriacetate in sewage treatment. *Wat. Sci. Technol.*, **19,** 449-460.

Giger, W., Ahel, M. and Koch, M. (1986) Behaviour of alkylphenol polyethoxylate surfactants in mechanical-biological sewage treatment. *Vom Wasser***, 67**, 69-81.

Granmo, A. and Kollberg, S. (1976) Uptake pathways and elimination of a non-ionic surfactant in cod (*Gadus morhua* L.). *Water Res.*, **10,** 189-194.

Grifoll, M., Solanas, A.M. and Bayona, J.M. (1990) Characterisation of genotoxic components in sediments by mass spectrometric techniques combined with *Salmonella*/microsome test. *Arch. Environ. Contam. Toxicol.* **19,** 175-184.

Hall, W.S., Patoczka, J.B., Mirenda, R.J., Porter, B.A. and Miller, E. (1989) Acute toxicity of industrial surfactants to *Mysidopsis bahia*. *Arch. Environ. Contam. Toxicol.* **18,** 765-772.

Hamburger, B., Haberling, H. and Hitz, H.R. (1977) Comparative tests on toxicity to fish using minnows, trout and golden orfe. *Arch. Fischerewiss.*, **28,** 45-55. (English Abstract) (Cited in Goyer *et al.* 1981).

Hardin, B.D., Schuler, R.L., Berg, J.R., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J. and Smith, K.N. (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratogenesis, Carcinogenesis and Mutagenesis* **7**,29-48.

Holt, M.S., McKerrell, E.H., Perry, J. and Watkinson, R.J. (1986) Determination of alkylphenol ethoxylates in environmental samples by high-performance liquid chromatography coupled to fluorescence detection. *J. Chromatog.*, **362,** 419-424.

James, H. A., Taylor, L., Gibson, T. and Hammond, I. (1989) The effects of organics on fish (EC 9408 SLD). WRc Progress Report DoE 2103-M to DoE, September 1988 to February 1989.

Janbakhsh, A. (1996) Oestrogenic substances in water: the analytical determination of alkylphenols and alkylphenol ethoxylates in rivers and sewage effluents. M.Sc. Thesis, Dept. of Biological and Chemical Science, University of Essex.

Janicke, W., Bringmann, G. and Kühn, R. (1969) Aquatic toxicological studies of the harmful action of ethoxylate non-ionic surfactants. *Gesund. Ing.*, **90,** 133-138.

Jobling, S and Sumpter, J.P. (1993) Detergent components in sewage effluent are weakly oestrogenic to fish: an *in vitro* study using rainbow trout (*Oncorhynchus mykiss* (hepatocytes). *Aquatic Toxicology* **27**, 361.

Jobling, S., Sheahan, D., Osborne, J.A., Matthiessen, P. and Sumpter, J.P. (1996) Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry* **15**, 194.

Kiewiet, A.T. and Devoogt, P. (1996) Chromatographic tools for analysing and tracking non-ionic surfactants in the aquatic environment. *Journal of Chromatography* **733**(1,2) 185-192.

Kravetz, L., Guin, K.F., Shebs, W.T. and Smith, L.S. (1982). Ultimate biodegradation of an alcohol ethoxylate and a nonylphenol ethoxylate under realistic conditions. Soap Cosmetics Chem. Specialities, **58,** 34-42.

Kravetz, L., Chung, H., Guin, K.F., Shebs, W.T. and Smith, L.S. (1984) Primary and ultimate biodegradation of an alcohol ethoxylate and an alkylphenol ethoxylate under average winter conditions in the USA. *Shell Chemical Company Technical Bulletin*, SC 779-83,*Tenside*, **21,** 1-6.

Kravetz, L., Salanitro, J.P., Dorn, P.B. and Guin, K.F. (1991) Influence of hydrophobe type and extent of branching on environmental response factors of non-ionic surfactants. *J. Am. Oil Chem. Soc.*, **68,** 610-618.

Kubeck, E. and Naylor, C.G. (1990). Trace analysis of alkylphenol ethoxylates. *J. Am. Oil Chem. Soc.*, **67**, 400-405.

Kvestak, R. and Ahel, M. (1994) Occurrence of Toxic Metabolites from Non-ionic Surfactants in Krka River Estuary. *Ecotoxicology and Environmental Safety,* **28,** 25-34.

Kvegtak, R. and Ahel, M. (1995). Biotransforniation of nonylphenol polyethoxylate surfactants by estuarine mixed bacterial cultures. *Arch. Environ. Contam. Toxicol*., **29,** 551-556.

Larson, P.S., Borzelleca, J.R., Bowman, E.R., Crawford, E.M., Smith, R.B. and Hennigar, G.R. (1963) Toxicologic studies on a preparation of p-tertiary octylphenoxy-polyethoxyethanols (Triton X-405). *Toxicol. Appl. Pharmacol.*, **5**, 782-798.

Lashen, E.S., Blankenship, F.A., Booman, K.A. and Dupro, J. (1966). Biodegradation studies on a *p* tert- octylphenoxypolyethoxyethanol. *J. Am. Oil Chem. Soc.* **43,** 371-376.

Lashen, E.S. and Booman, K.A. (1967) Biodegradability and treatability of alkylphenol ethoxylates: A class of non-ionic surfactants. *Water Sew. Works*, **114,** R155-R163.

Lewis, M.A. and Hamm, B.G. (1986) Environmental modification of the photosynthetic response of lake plankton to surfactants and significance to a laboratory-field comparison. *Water Res.*, **20,** 1575-1582.

Lewis, M.A. (1986) Comparison of the effects of surfactants on freshwater phytoplankton communities in experimental enclosures and on algal population growth in the laboratory. *Environ. Toxicol. Chem.*, **5,** 319-332.

Lewis, M.A. (1990) Chronic toxicities of surfactants and detergent builders to algae: A review and risk assessment. *Ecotox. Environ. Safety,* **20,** 123-140.

Lye C.M., Frid C.L.J., Gill M.E., Cooper D.W. and Jones D.M. (1999) Estrogenic alkylphenols in fish tissues, sediments and waters from the UK Tyne and Tees estuaries. *Env. Sci. Technol*. **33**, 1009-1014.

Macek, K.J. and Krzeminski (1975) Susceptibility of bluegill sunfish (*Lepomis macrochirus*) to non-ionic surfactants. *Bull. Env. Contam. Toxicol.*, **13,** 377-384.

Madai, J. and H. An der Lan. (1964). Zur wirkung einiger detergentien auf sübwasserorganismen. *Wasser Abwasser. Forsch.* **4**, 168-183.

Maki, H., Masuda, N., Fujiwara, Y., lke, M. and Fujita, M. (1994). Degradation of alkylphenol ethoxylates by *Pseudomonas* sp. Strain TR01. A*ppl. Environ. Microbiol.*, **60,** 2265-2271.

Mann, A.H. and Reid, V.W. (1971) Biodegradation of synthetic detergents: evaluation by community trials. Part 2. Alcohol and alkylphenol ethoxylates. *J. Am. Oil Chem. Soc.*, **48,** 794-797.

Marchetti, R. (1965) The toxicity of nonyl phenol ethoxylate to the development stages of rainbow trout, *Salmo gairdneri* Richardson. *Ann. Appl. Biol.*, **55,** 425-430.

Marcomini, A., Capri, S., Brunner, P.H. and Giger, W. (1988) Mass fluxes of linear alkylbenzenesulphonates, nonylphenol, nonylphenol mono- and diethoxylate through a sewage treatment plant. *Commun. Eur. Comm. Rep.*, **11350,** 266-277.

Marcomini, A. and Giger, W. (1987) Simultaneous determination of linear alkylbenzene-sulfonates, alkylphenol polyethoxylates and nonylphenol by high-performance liquid chromatography. *Anal. Chem.*, **59,** 1709-1715.

Marcomini, A., Pavoni, B., Sfriso, A. and Orio, A.A. (1990) Persistent metabolites of alkylphenol polyethoxylates in the marine environment. *Marine Chem.*, **29,** 307-323.

Markarian, R.K., Pontasch, K.W., Peterson, D.R. and Hughes, A.I. (1989) Review and analysis of environmental data on Exxon surfactants and related compounds. Technical Report. Exxon Biomedical Sciences, Inc.

Markarian, R.K., Hinman, M.L. and Targia, M.E. (1990) Acute and chronic toxicity of selected non-ionic surfactants. Presented at the Eleventh Annual Meeting of the Society of Environmental Toxicology and Chemistry, Arlington, VA. (Poster abstract)

McLeese, D.W., Zitko, V., Sergeant, D.B., Burridge, L. and Metcalfe, C.D. (1981) Lethality and accumulation of alkylphenols in aquatic fauna. *Chemosphere*, **10,** 723-730.

Meyer, O., Haubro Anderson, P., Hansen, E.V. and Larson, J.C. (1988) Teratogenicity and *in vitro* mutagenicity studies on nonoxynol-9 and -30. *Pharmacology and Toxicology* **62**, 235-238.

Moore, S.B., Diehl, R.A., Barnhardt, J.M. and Avery, G.B. (1987) Aquatic toxicity of textile surfactants. *Text. Chem. Col.*, **19,** 29-32.

Naylor C. G. (1992). Environmental fate of alkylphenol ethoxylates. *Soap Cosmetics Chemical Specialities, August 1992*, pp27-31 and 72

Naylor C. G., Mieure J. P., Morici 1. and Romano R. R. (1992). Alkylphenol ethoxylates in the environment. Proc. 3rd CESIO International Surfactants Congress, 111-124.

Naylor C.G., Williams J.B., Varineau P.T. and Webb D.A. Nonylphenol ethoxylates in an industrial river. pp. 44-53 in: *The Alkylphenols and Alkylphenol Ethoxylates,* **1**(1), CMA, 1998.

Neufahrt, A., Hofmann, K., Täuber, G. and Damo, Z. (1987) Biodegradation of nonylphenol-ethoxylate (NPE) and certain environmental effects of its catabolites. Hoechst Aktiengesellschaft, unpublished paper.

Nimrod, A.C. and Benson, W.H. (1996) Environmental estrogenic effects of alkylphenol ethoxylates. *Crit. Rev. in Toxicol.,* **26**, 35-364.

Nyberg, H. (1976) The effects of some detergents on the growth of *Nitzschia holsatica* Hust. (Diatomeae). *Ann. Bot. Fennici*, **13,** 65-68.

Nyberg, H. (1985) Physiological effects of four detergents on the algae *Nitzschia actinastroides* and *Porphyridium purpureum*. Publication 12, Department of Botany, University of Helsinki. (Cited in Lewis 1990).

Nyberg, H. (1988) Growth of *Selenastrum capricornutum* in the presence of synthetic surfactants. *Water Res.*, **22,** 217-223.

Osburn, Q.W. and Benedict J.H. (1966) Polyethoxylated alkyl phenols: Relationship of structure to biodegradation mechanism. *J. Am. Oil Chem. Soc*. **43**, 141-146.

Patoczka, J. and Pulliam, G.W. (1990) Biodegradation and secondary effluent toxicity of ethoxylated surfactants. *Water Res.*, **24,** 965-972.

Portmann, J.E. and Wilson, K.W. (1971) Shellfish Information Leaflet. Ministry of Agriculture, Fisheries and Food (UK). (Cited in McLeese *et al.* 1980b).

Potter, T.L., Simmons, K., Wu, J., Sanchez-Olvera, M., Kostecki, P. and Calabrese, E. (1999) Static die-away of a nonylphenol ethoxylate surfactant in estuarine water samples. *Env. Sci. Technol.* **33**, 113-118.

Reiff, B. (1976) Proceedings of the Seventh International Congress on Surface-Active Substances, Moscow, USSR, September 1976. (Cited in Scharer *et al.* 1979).

Reiff, B., Lloyd, R., How, M.J., Brown, D. and Alabaster, A.S. (1979) The acute toxicity of eleven detergents to fish: Results of an interlaboratory exercise. *Water Res.*, **13,** 207-210.

Ribosa, I., Garcia, M.T., Sanchez, Leal, J. and Gonzalez, J.J. (1993) *Photobacterium phosphoreum* test data of non-ionic surfactants. *Tox. and Env. Chem*. **39**, 237-241.

Roberts, D.W. (1991) QSAR issues in aquatic toxicity of surfactants. *Sci. Tot. Env.* **109/110**, 557-568.

Röderer, G. (1987) Toxic effects of tetraethyl lead and its derivatives on the chrysophyte *Poterioochromonas malhamensis*. VIII. Comparative studies with surfactants. *Arch. Environ. Contam. Toxicol.*, **16,** 291-301.

Routledge, E.J. and Sumpter, J.P. (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Envir. Tox. Chem.*, **15,** 241-284.

Rudel, R.A., Melly, S.J., Geno, P.W., Sun, G. and Brody, J.G. (1998) Identification of alkylphenols and other oestrogenic phenolic compounds in waste water, sewage and groundwater on Cape Cod, Massachusetts. *Env. Sci. Technol.*, **32**(7), 861-869.

Rudling, L. and Solyom, P. (1974). The investigation of biodegradability of branched nonyl phenol ethoxylates. *Wat. Res.*, **8,** 115-119.

Salanitro, J.P., Langston, G.C., Dorn, P.B. and Kravetz, L. (1988) Activated sludge treatment of ethoxylate surfactants at high industrial use concentrations. Presented at the International Conference on Water and Wastewater Microbiology, Newport Beach, CA, 8-11 February.

Sato, M., Hashimoto, K. and Kobayashi, M. (1963) Microbial degradation of non-ionic synthetic detergent, poly EO nonylphenol ethers. *Water Treat. Eng.*, **4,** 31-36.

Scullion *et al.* (1996) Determination of surfactants in surface water by solid phase extraction, liquid chromatography and liquid chromatographic and mass spectroscopy. *Journal of Chromatography* **733**(1,2) 207-216.

Smyth, H.F. and Calandra, J.C. (1969) Toxicologic studies of alkylphenol polyoxyethylene surfactants. *Toxicol. Appl. Pharmacol.* **14**, 315-334.

Staples, C.A., Weeks, J., and Naylor, C.G. (1998a) An environmental risk assessment of nonylphenol ehoxylates and their biodegradation intermediates in water and sediments. Presentation to SETAC Conference, November 15-18, Charlotte NC, USA.

Staples, C.A., Weeks, J., Hall, J.F. and Naylor, C.G. (1998b) Evaluation of aquatic toxicity and bioaccumulation of C8- and C9- alkylphenol ethoxylates. *Env. Toxicol. Chem.* **17**, 2470-2480.

Stephanou, E. and Giger, W. (1982) Persistent organic chemicals in sewage effluents. 2. Quantitative determinations of nonylphenols and nonylphenol ethoxylates by glass capillary gas chromatography. *Environ. Sci. Technol.*, **16,** 800-805.

Stiff, M.J., Rootham, R.C. and Culley, G.E. (1973a) The effect of temperature on the removal of non-ionic surfactants during small scale activated sludge sewage treatment. I. Comparison of alcohol ethoxylates with a branched chain alkylphenol ethoxylate. *Water Res.*, **7,** 1003-1010.

Stiff, M.J., Rootham, R.C. and Culley, G.E. (1973b) II. Comparison of a linear alkylphenol ethoxylate with branched chain alkylphenol ethoxylates. *Water Res.*, **7,** 1407-1415.

Swedmark, M., Braaten, B., Emanuelsson, E. and Granmo, A. (1971) Biological effects of surface active agents on marine animals. *Marine Biol.*, **9,** 183-201.

Swedmark, M., Granmo, A. and Kollberg, S. (1976) Toxicity testing at Kristinberg Marine Biology Station. In: *Pollutants in the Aquatic Environment*. FAO/SIDA/TF 108, Suppl. 1. (Cited in Goyer *et al.* 1981)

Talmage, S.S. *Environmental and Human Safety of Major Surfactants: A Report to the Soap and Detergent Association*. Lewis Publishers, 1994.

Tomiyama, S. (1974) Effects of surfactants on fish. *Bull. Jpn. Soc. Sci. Fish*, **40,** 1291-1296.

Van Emden, H.M., Kroon, C.C.M., Schoeman, E.N. and Van Seventer, H.A. (1974) The toxicity of some detergents tested on *Aedes aegypti* L., *Lebistes reticulatus* Peters, and *Biomphalaria glabrata* Say. *Environ. Pollut.*, **6,** 297-308.

Varineau, P. T., Williams, J. B., Naylor, C. G., Yunick, R. P. and Cady, C. (1996a). The biodegradation of ^{14}C ring-labelled nonylphenol ethoxylate in a semi-continuous activated sludge system. Unpublished report.

Varineau, P. T., Williams, J. B., Naylor, C. G., Serak, K. and Cady, C. (1996b). The biodegradation of a ^{14}C ring-labelled nonylphenol ethoxylate in river water. Unpublished report.

Wahlberg, C., Renberg, L. and Wideqvist, U. (1990). Determination of nonylphenol and nonylphenol ethoxylates as their pentafluorobenzoates in water, sewage sludge and biota. *Chemosphere*, **20**, 179-195.

Weinberger, R. and Rea, M. (1981) Nonylphenol: A perturbant additive to an aquatic ecosystem. In: Bermington, N. *et al.* (Eds), Proc. 7th Annual Aquatic Toxicity Workshop, 5-7 November, Montreal, Quebec. *Can. Techn. Rep. Fish Aquatic Sci.* No. 990, 371-380.

Weinberger, R. and Rea, M. (1982) Effects of aminocarb and its formulation adjuncts on the growth of *Chlorella pyrenoidosa* Chick. *Environ. Exper. Bot.*, **22,** 491-496.

White, R., Jobling, S., Hoare, S.A., Sumpter, J.P. and Parker, M.G. (1994) Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 135, 175.

Whitehouse, P., Wilkinson, M., Fawell, J.K. and Sutton, A. (1998a) Proposed Environmental Quality Standards for nonylphenol in water. R&D Technical Report P42. WRc Report No. EA 4217/3.

Whitehouse, P., Wilkinson, M., Fawell, J.K. and Sutton, A. (1998b) Proposed Environmental Quality Standards for octylphenol in water. R&D Technical Report P59/i688. WRc Report No. EA 4288.

Williams, J., Brady, A.M., Lewis, R.W. and Hughes, L. Assessment of alkylphenol derivatives for estrogenic activity in a rat uterotrophic model. pp. 102-106 in: *The Alkylphenols and Alkylphenol Ethoxylates Review*. **1**(1), CMA, 1998.

Wong, S.L. (1985) Algal assay evaluation of trace contaminants in surface water using the non-ionic surfactant, Triton X-100. *Aquatic Toxicol.*, **6,** 115-131.

Yamane, A.N., Okada, M. and Sudo, R. (1984) The growth inhibition of planktonic algae due to surfactants used in washing agents. *Water Res.*, **18,** 1101-1105.

Yoshimura, K. (1986) Biodegradation and fish toxicity of non-ionic surfactants. *J. Am. Oil Chem. Soc.*, **63,** 1590-1596.