

An Environmental Assessment of Alkylphenol Ethoxylates and Alkylphenols

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Summary

1. Alkylphenol ethoxylate surfactants are usually made from a branched-chain nonylphenol or octylphenol, reacted with ethylene oxide. They are used in a variety of industrial processes, including wool washing, but are no longer in domestic detergents in the UK. Alkylphenols are also used by some industries.
2. Alkylphenol ethoxylates are biodegraded by removal of ethoxy groups, producing less biodegradable products: alkylphenol mono- and di-ethoxylates, alkylphenoxy acetic and alkylphenoxy polyethoxy acetic acids, and alkylphenols. These metabolites frequently persist through sewage treatment and in rivers. Anaerobic conditions generally lead to the accumulation of alkylphenols.
3. Rivers in Switzerland have been found to contain concentrations of tens of $\mu\text{g l}^{-1}$ of a wide range of alkylphenolic metabolites. Low levels have also been found in drinking water in the USA, with a total concentration of alkylphenolic compounds of almost $1 \mu\text{g l}^{-1}$. There is little UK data on concentrations in the environment, but a recent modelling study concluded that 83 % of UK nonylphenol ethoxylate production enters the environment, with 37 % entering the aquatic environment.
4. Metabolites of alkylphenol ethoxylates accumulate in organisms, with bioconcentration factors varying from ten to several thousand, depending on species, metabolite and organ.
5. The metabolites of alkylphenol ethoxylates are generally more toxic than the original compounds. Alkylphenol ethoxylates have LC_{50} s above about 1.5 mg l^{-1} , whereas nonylphenol LC_{50} s are generally around 0.1 mg l^{-1} .
6. Alkylphenols were first found to be oestrogenic (oestrogen-mimicking) in the 1930s, but more recent research has highlighted the implications of these effects. The growth of cultured human breast cancer cells is affected by nonylphenol at concentrations as low as $1 \mu\text{M}$ ($220 \mu\text{g l}^{-1}$), or concentrations of octylphenol as low as $0.1 \mu\text{M}$ ($20 \mu\text{g l}^{-1}$). Oestrogenic effects have also been shown on rainbow trout hepatocytes, chicken embryo fibroblasts and a mouse oestrogen receptor.
7. Studies on UK rivers receiving sewage effluent have shown that an oestrogenic pollutant is present, as male rainbow trout placed in these rivers produce a female egg yolk protein.
8. By comparing environmental concentrations, bioconcentration factors and *in vitro* oestrogenic effect levels, this report concludes that current environmental levels of alkylphenolic compounds are probably high enough to be affecting the hormonal control systems of some organisms. It is also possible that human health could be being affected.
9. Some progress has been made in phasing out alkylphenols and alkylphenol ethoxylates, but many industries are still using them.

Recommendations

1. The production of all alkylphenols and their derivatives should be stopped as soon as possible. An immediate ban should not present a problem to most industries. Any industries which require time to modify their processes could be given a couple of years to comply.
2. Research should continue into evaluating the human health and environmental significance of the oestrogenic toxicity of alkylphenolic compounds. Safety levels for the sum of all alkylphenolic compounds in drinking water, rivers and estuaries must be rapidly established. The safety level for drinking water is likely to be below $1 \mu\text{g l}^{-1}$ (1 ppb).
3. The National Rivers Authority should establish standardised analytical methods for all alkylphenolic compounds. Any analysis must measure alkylphenol ethoxylates (of all chain lengths), alkylphenols and alkylphenoxy carboxylic acids.
4. A thorough survey of UK rivers, and the organisms within them, should be undertaken to establish the levels of all alkylphenolic compounds. Levels in the human body must also be established. All data obtained must be available to the public.
5. Drinking water in the UK should be analysed for all alkylphenolic compounds, particularly if the drinking water is extracted from a river. Extra treatment should be introduced if the total alkylphenolic compound content is above the safety level.
6. When the safety of chemicals is being assessed, it is important to keep an open mind on possible mechanisms of toxicity, and the literature should be searched for possible biochemical effects of the compound under assessment, its metabolites and similar compounds. The oestrogenic effects of *para*-alkylphenols were established in 1938 - 6 years before the introduction of alkylphenol ethoxylates in the UK. These effects were not connected with alkylphenol ethoxylates until 1991 - 53 years later.
7. A wide-ranging investigation is needed into potential oestrogenic, immune system and nervous system effects of man-made chemicals. This should include analysis of past literature, and development of new toxicological assessment procedures, using cell culture where possible. The study should look first at any chemicals which can accumulate in fat, and which are not easily metabolised. Chemicals that are only used in small volumes, such as perfumes and colourants, may be able to accumulate in fat, and should be included in any investigation.

1. Introduction

There have been many concerns raised recently regarding the environmental safety of alkylphenol ethoxylate surfactants, particularly whether their metabolites are able to mimic hormones. This review examines the published material regarding the fate and toxicity of these compounds, and draws conclusions on the prudence of continuing to use these chemicals.

2. Structure

Alkylphenol ethoxylates are non-ionic surfactants, consisting of a branched-chain alkylphenol which has been reacted with ethylene oxide, producing an ethoxylate chain (Figure 1). Commercial formulations are usually a complex mixture of homologues, oligomers and isomers. The main alkylphenols used are nonylphenol (NP) and octylphenol (OP), with nonylphenol ethoxylates (NPnEO) taking approximately 80% of the world market, and octylphenol ethoxylates (OPnEO) taking the remaining 20% (White *et al.*, 1994). The length of the ethoxylate chain varies between 1 to 50 ethoxy units, depending on the application. Cleaning products usually have an approximately Poisson distribution of ethoxylate chain length, centred around 9 (NP9EO) or 10 (NP10EO) units long (Swisher, 1987). The nonyl group is usually synthesised from a propylene trimer, producing a branched chain (Porter, 1991; CES, 1993). The two groups are usually (>90%) *para* (Porter, 1991).

3. Uses

Alkylphenol ethoxylates were first introduced into the UK in 1944, and were used in a wide range of domestic and industrial applications (CES, 1993). The use of nonylphenol ethoxylates in domestic detergents in the UK was phased out in 1976 (DoE, 1992). They are now used mainly as industrial surfactants, though they still have some domestic uses, for example the spermicide nonoxynol-9 (Soto *et al.*, 1991). About 17, 600 t of nonylphenol ethoxylates were used in the UK in 1992, distributed as shown in Table 1 (CES, 1993). Alkylphenols are also used by some

Industry	Consumption (tonnes p.a.)
Industrial and institutional cleaning	7500 - 8500
Paint	2000 - 3000
Agrochemical	2000
Emulsion polymers	1500
Textiles	1000 (including 400 for wool scouring)
Metal finishing	1000
NPE phosphate esters	600
Miscellaneous	100 - 1000
Total	14500 - 18500

Table 1: The uses of nonylphenol ethoxylates in the UK in 1992 (CES, 1993)

industries, for example in Germany in 1986, 4950 t of the 13500 t of nonylphenol used was not ethoxylated, including 1200 t as trisnonylphenol phosphite, an antioxidant in plastics, and 250 t as a Ba or Cd salt to stabilise PVC (BUA, 1991).

4. Environmental fate

4.1 Microbial metabolism

The full breakdown pathway for alkylphenol ethoxylates has not yet been determined, and all studies have so far focused on identification of intermediates in bacterial culture media, rather than studying cell-free systems or purified enzymes. It is, however, likely that microbial metabolism usually starts by an attack on the ethoxylate chain, rather than on the ring or the hydrophobic chain (Ball *et al.*, 1989; Ahel *et al.*, 1994d). The ethoxylate groups are progressively removed, either by ether cleavage, or by terminal alcohol oxidation followed by cleavage of the resulting carboxylic acid (APnEC; Figure 1). Ball *et al.* (1989) have studied the biotransformation of a mixture of octylphenol polyethoxylates by sewage-related bacterial cultures, under both anaerobic and aerobic conditions. With an activated sludge inoculum, under aerobic conditions, the longer OPnEO (n = 4 - 5) were rapidly (<12 h) cleaved to shorter OPnEO (n = 1 - 3). At the same time, OPnEC were produced, which were degraded more slowly, with traces present after 127 days. Little OP was detected. It was also found that a mixture of OPnEC could be transformed to OPnEO, though long adaptation times were required, with >36 days for OP3EC. Under anaerobic conditions, conversion of OPnEO to OP1EO occurred within 10 days, and this was then transformed slowly to OP. Those OPnEC with n = 2 - 4 appeared to be totally recalcitrant, however OP1EC, OP5EC and OP6EC were transformed, mainly to OP and OP1EO. Anaerobic cultures seemed to accumulate OP. The eventual fate of the short chain OPnEO and OPnEC under aerobic conditions was not established, although attempts were made to locate intermediates with modified octyl

Abbreviations

APCs	Alkylphenolic compounds (see also Figure 1):
NP, OP or AP	Nonylphenol, octylphenol or alkylphenol
APEO	Alkylphenol ethoxylate (also NPEO, OPEO)
APEC	Alkylphenoxy carboxylic acid (also NPEC, OPEC)
APnEO	Alkylphenol polyethoxylate, n = number of ethoxy groups in the hydrophilic chain
APnEC	Alkylphenoxypolyethoxy acetic acid, n = number of ethoxy groups in the chain plus one acetate
EC _{xx}	Effect concentration for xx% of test organism
EO	Ethoxylate
LOEC/LOEL	Lowest observed effect concentration/level
NOEC	No observed effect concentration
NRA	National Rivers Authority

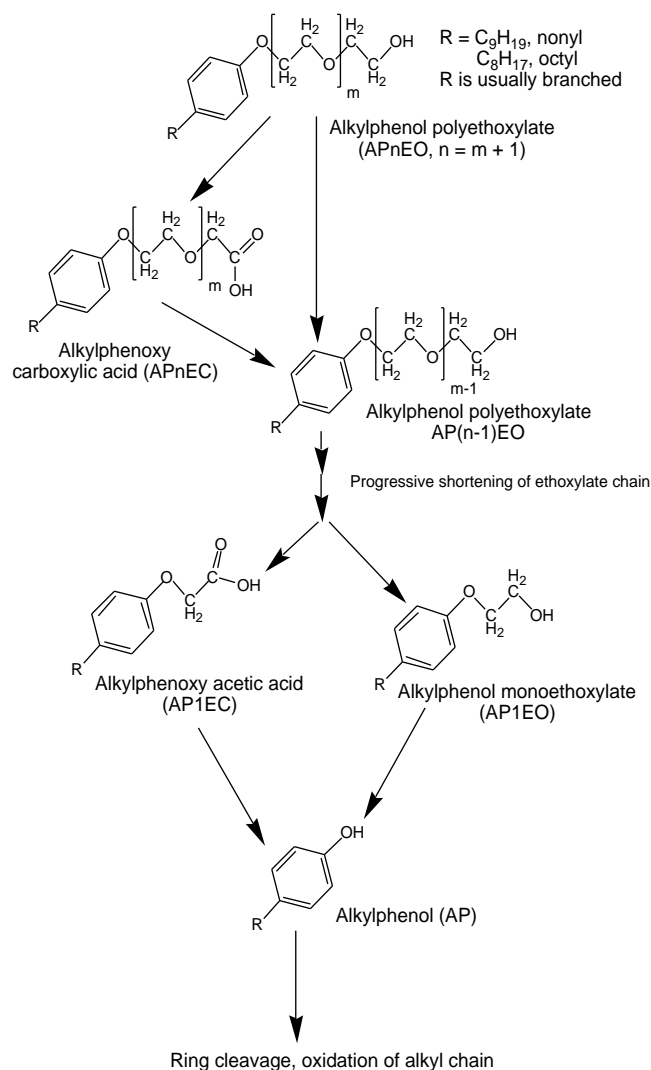


Figure 1: Microbial metabolism of alkylphenol ethoxylates (Ball *et al.*, 1989, Ahel *et al.*, 1994d) groups. These compounds are clearly fairly recalcitrant under aerobic conditions.

Similar results have been obtained by Ahel *et al.* (1994d), who examined breakdown of both octyl- and nonylphenol ethoxylates by bacterial cultures from wastewater, river water and forest soil. They found that all the cultures could transform 0.5 - 2.5 mg l⁻¹ of NPnEO and OPnEO (n=1-3) in synthetic sewage within 6-23 days. Cultures were also able to transform the APEOs as the sole carbon source in a minimal medium, though after 23 days up to 90% of the substrates were present as APnEC. Although extracts of these cultures were thoroughly examined for other metabolites, none were found. It is possible, therefore, that all of the APEO was transformed to APEC, with no further metabolism occurring under these conditions.

Maki *et al.* (1994) have isolated a bacterium, *Pseudomonas* sp. strain TR01, capable of growth on NP(9.5)EO as a sole carbon and energy source. However, this bacterium was unable to mineralise the NPEO, producing mainly NP2EO, with some NP2EC.

Some evidence for further metabolism of the aromatic ring of APEO has been obtained by using NPEO ring-

labelled with tritium, which showed 20% conversion to ³H₂O in bench-scale activated sludge tests at 25°C (Holt *et al.*, 1992). Monitoring of the u.v. absorbance bands of the phenolic ring during biodegradation tests has indicated possible breakdown of the ring, but this is not a very reliable analytical technique (Swisher, 1987).

These studies clearly show that bacteria are able to transform APEO into short-chain APEO, APEC and AP. It is likely that further metabolism of these products does occur, but very slowly. The APEOs are an example of the inadequacy of tests of primary biodegradation, which in this case would test for loss of surfactant capability, which occurs as the ethoxylate chain is shortened, but gives no indication of the ultimate fate of the chemical.

4.2 Photochemical breakdown

In addition to microbial breakdown, Ahel *et al.* (1994c) have established that NP is susceptible to photochemical degradation. Using natural, filtered, lake water, it was found that NP had a half life of approximately 10 - 15 h under continuous, noon, summer sun in the surface water layer, with a rate approximately 1.5 times slower at depths of 20-25 cm. No attempt was made to identify the products. Photolysis was found to be much slower with NPnEO, and so unlikely to be significant in the environment.

4.3 Breakdown during sewage treatment

There have been several studies which have followed APEO transformation in sewage treatment, however early studies did not measure APECs, so they are of limited use. Ahel *et al.* (1994a) have undertaken a detailed study of the fate of NPnEOs and their metabolites in 11 sewage works in Switzerland. An average mass-balance was calculated for the NPnEO and metabolites in the 11 works, which concluded that less than 40 % (calculated on a molar basis) of the influent was subject to ultimate biodegradation, with 20 % ending up absorbed to the sludge, and 40-45 % in the secondary effluent.

The distribution of nonylphenolic compounds in the digested sludge was 95 % NP and 5% NP1EO + NP2EO, partly due to the hydrophobic nature of NP, and partly because the anaerobic digestion of the sludge resulted in other nonylphenolic compounds being transformed into NP. Other work by Giger *et al.* (1984) has shown that NP concentrations in anaerobically digested sludge range from 0.45 to 2.53 g kg dry weight⁻¹, whilst in aerobically stabilised sludge levels were lower, at 0.08 to 0.5 g kg dry weight⁻¹, in addition, Giger *et al.* (1987) found that the concentration of NP in sewage sludge increased 15 fold during anaerobic digestion.

The more hydrophilic compounds were present in the secondary effluent of the Swiss sewage works, with the nonylphenolic compounds distributed as follows (on a molar basis): 48 % NP1EC+NP2EC, 26 % NP1EO+NP2EO, 20 % NPnEO (n > 2) and 8 % NP (Ahel *et al.*, 1994a). Total concentration of nonylphenolic compounds in the secondary effluents was up to 2.15 μM, with a maximum NP concentration of 0.2 μM (43 μg l⁻¹). The

most effective treatment was observed in those plants with low sludge loading rates and nitrifying conditions. Tests on one plant showed that elimination was far more effective in summer, at temperatures of 18 - 20 °C, than in winter, at temperatures of 10 - 13 °C, with NP2EO elimination being lower than its formation under the latter conditions (Ahel *et al.*, 1994a). Studies on a sewage works in Italy have found secondary effluent concentrations of up to 4 µg l⁻¹ for NP, 27 µg l⁻¹ for NPEO and 145 µg l⁻¹ for NPEC (Di Corcia *et al.*, 1994).

There is little published UK data on levels of APEO during sewage treatment, but one study has compared influent and effluent concentrations of APEO through two sewage works. The first works, an activated sludge plant, showed APEO removal of 89% in warm weather, compared with 68% in cold weather, resulting in effluent concentrations of 0.21 mg l⁻¹ and 0.49 mg l⁻¹ respectively (CES, 1993). The second works studied was a trickling filter system, which showed summer removal efficiencies of 80%, and a much lower winter efficiency of 30% (CES, 1993). This study examined only APEO levels, rather than any metabolites, but it gives an indication of the ineffectiveness of UK sewage works in treating APEO, particularly in cold weather.

The south west region of the NRA has analysed levels of NP and OP at an outfall from a sewage works into the river Dart in Devon. This sewage works, which has secondary treatment, receives trade effluent from a wool processing factory which uses APnEO surfactants. Levels in the sewage outfall were found to be up to 2.4 µg l⁻¹ of NP and 0.36 µg l⁻¹ of OP, but no other alkylphenolic compounds were analysed for (Southwest NRA, personal communication).

Another source of NPEO contamination to the environment in the UK is the ICI plant at Wilton, Teeside, according to the most recent UK Government report (CES, 1993). The NPEOs are manufactured in two 40 tonne reactors, and efforts are made to ensure that the next batch of surfactant is compatible with the previous one. However, when this does not occur, the reactors are washed out. The washout water contains, on average, 0.5 g l⁻¹ of the product, and is discharged *untreated* into the River Tees, about 5 km from the estuary head. If necessary, antifoam is added to avoid visible foaming. These discharges are now being reduced by means of process and equipment modification, but when the CES report was written '*ICI were unable to quantify the amount of NPEs discharged by this route*' (CES, 1993).

In some wastewater treatments the wastewaters are treated by chlorination, to sterilise the water, but this has been found to increase the mutagenicity of the wastewater (Reinhard *et al.*, 1982). Analysis of the mutagenic fraction of wastewater by Reinhard *et al.* (1982) showed that it contained primary carboxylic acids, including brominated and non-brominated APnEC, the bromination having occurred during chlorination. Chlorinated and brominated OPEO and OPEC have been found in other chlorinated wastewaters, with halogenated OPEO concentrations of

up to 51 µg l⁻¹ (Ball *et al.*, 1989).

Clearly, substantial amounts of nonylphenolic compounds enter the environment, both from secondary wastewater discharge to rivers and the sea and from sewage sludge, which may be spread on agricultural land, or (at present), dumped in the sea. The production of halogenated metabolites by chlorination is also of concern.

4.4 Fate and levels in rivers

The 11 Swiss sewage works described in the previous section discharge into the River Glatt, and the distribution of nonylphenolic compounds in this river has been determined by Ahel *et al.* (1994b). This study has established that approximately 108.2 mol d⁻¹, or 35 kg d⁻¹ of nonylphenolic compounds flow into this river, 95 % coming from the treated sewage discharges. At the end of the river (where it joins the Rhine), 82 mol d⁻¹ of nonylphenolic compounds flowed, an overall elimination of only 24 %. The distribution of compounds through the river was similar to that in the sewage effluent, though with higher percentage of NP1EC and NP2EC in the output (85 %) than the input (51 %). Maximum concentrations measured in the river were: 45 µg l⁻¹ NP1EC, 71 µg l⁻¹ NP2EC, 69 µg l⁻¹ NP1EO, 30 µg l⁻¹ NP2EO and 45 µg l⁻¹ NP, with both the NPEC and NPEO groups often exceeding 10 µg l⁻¹. Only one NP result was above 10 µg l⁻¹, however 84 % were above 1 µg l⁻¹. Little NPnEO (n=3-5) was detected, and concentrations of all compounds were lower in summer than in winter. In the most polluted part of the river, nonylphenolic compounds were up to 1 % of the dissolved organic carbon concentration.

The 24 % elimination observed in the river included adsorption to sediments, and mud sediments were found to contain up to 13.1 mg kg dry weight⁻¹ of NP, 8.85 mg kg dry weight⁻¹ of NP1EO and 2.72 mg kg dry weight⁻¹ of NP2EO. Lower levels were found in sand, which contained less organic matter.

Other investigations of NP levels in rivers have found values varying between 2 µg l⁻¹ in Delaware River, Philadelphia to 10 µg l⁻¹ in the Rhine and 1000 µg l⁻¹ in a tributary of the Savannah river, USA, below a wool laundry (BUA, 1991). Levels of NP and NP1EO in the sediments of USA rivers were up to 3 mg kg⁻¹ and 0.17 mg kg⁻¹ respectively (Naylor *et al.*, 1992).

As mentioned above, the south west region of the NRA have analysed levels of NP and OP in the river Dart in Devon. The maximum concentrations found in the mixing zone of the river downstream from the contaminated sewage outfall were 0.055 µg l⁻¹ of NP and 0.12 µg l⁻¹ of OP (Southwest NRA, personal communication). No other alkylphenolic compounds were measured, so the full extent of contamination cannot be determined. The Dart is, however, a fairly rural river, so would be unlikely to have high levels of contamination. The Water Research Centre have also done some analysis of APnEO surfactants in rivers as part of a project investigating foaming incidents in rivers (WRc, 1991). Many samples contained APnEO

surfactants at concentrations of 1 - 80 $\mu\text{g l}^{-1}$, but no other alkylphenolic compounds were measured.

Foaming has been a problem for many years on the River Aire, which flows through the West Yorkshire conurbation, receiving discharges from many sewage works. Yorkshire/Northumbrian NRA have a regular monitoring programme for APnEO in this river, detecting those with n greater than 3. During 1994 this sampling has shown levels of AP(n>3)EO of up to 490 $\mu\text{g l}^{-1}$ at Salt's Weir, below Dowley Gap sewage works, up to 310 $\mu\text{g l}^{-1}$ at Crossflats, below Marley sewage works and up to 210 $\mu\text{g l}^{-1}$ at Leeds Bridge, some miles downstream from Horsforth sewage works (NRA Leeds Laboratory, personal communication). Although the levels detected seem to reduce downstream from the sewage works discharges, it is likely that this is partly due to biodegradation to AP(n=1-3)EO, AP and APnEC.

4.5 Fate and levels in estuaries and the ocean

Estuaries and oceans are exposed to APEOs and their metabolites from both polluted rivers and direct sewage discharge. Marcomini *et al.* (1990) have investigated levels of NP, NP1EO and NP2EO in the shallow marine bay of the Venice lagoon, which is contaminated by both urban and industrial wastes. NPEC were not determined. A resuspension device was used in order to analyse easily resuspendable sediment, approximately the first 0.01 - 0.15 mm. The sum of NP, NP1EO and NP2EO in this layer varied between 0.15 - 13.7 mg kg dry weight⁻¹, including up to 5.6 mg kg dry weight⁻¹ of NP. Underlying sediment contained concentrations of at least 5 times lower, but by comparison with the known behaviour of recalcitrant polychlorinated biphenyls, it was concluded that this reduction was probably due to dilution, not breakdown. There was seasonal variation in the levels in sediment, with particularly high levels in February. This may have been due to decomposing macroalgae in this sediment;

Table 2: An estimate of the fate of NPEO in the UK in 1992 (CES, 1993)

Disposal Route	Mass (t)		% of total
	as NPEO	as NP	
River	2810	999	16
Estuary	1920	683	11
Sea	1770	629	10
<i>Total to aquatic environment.</i>	<i>6500</i>	<i>2311</i>	<i>37</i>
Landfill	4680	1664	26
Soil	3490	1241	20
<i>Total to land</i>	<i>8170</i>	<i>2905</i>	<i>46</i>
Total to environment	14670	5216	83
Mineralised	2120	754	12
Incinerated	800	284	5
Total Destroyed	2920	1038	17

these macroalgae were found to contain 0.25 mg kg dry weight⁻¹ of NP + NP1EO + NP2EO. The maximum concentration of total NPEO in water was found to be 4.5 $\mu\text{g l}^{-1}$, with the most common oligomers being those with 5 - 10 units, showing some degradation from the normal oligomer distribution of commercial products (Marcomini *et al.*, 1990).

Kvestak *et al.* (1994) have examined the NPnEO distribution in the Krka river estuary, a highly stratified estuary in the mid-Adriatic region, into which untreated municipal wastewaters are discharged. Levels of NPnEO in the wastewaters varied between 70 and 2960 $\mu\text{g l}^{-1}$, whilst those in the estuary varied between 1.1 - 6 $\mu\text{g l}^{-1}$ in the brackish water and 0.1 - 0.7 $\mu\text{g l}^{-1}$ in the saline layer. Phase boundaries displayed higher NPnEO concentrations, with up to 17 $\mu\text{g l}^{-1}$ in a 0.2 - 0.4 μm deep sample of the brackish water - air boundary, and 9 $\mu\text{g l}^{-1}$ in a 2 cm diameter sample at the brackish water - saline water interface. There was evidence of limited biodegradation, indicated by a change in ethoxylate chain length between the wastewater discharge and the estuary.

Ekelund *et al.* (1993) have investigated the biodegradation of 4-nonylphenol, labelled with ¹⁴C on the phenol carbons, in seawater, with and without sediment. No degradation occurred (no ¹⁴CO₂ was produced) when formalin was added to the seawater, showing that microbial activity was necessary for NP metabolism. Without sediment the NP was initially degraded very slowly, only 0.06% degraded per day, but after 28 days the rate increased to 1% per day, presumably because of adaptation of the microbial culture. When sediment was present, 1.2% of the nonylphenol degraded per day, with no increase in the rate with longer incubation. If N₂ was bubbled through the seawater before the start of the sediment-containing experiment, reducing the O₂ present, NP was degraded at about half this rate. Approximately 45% of the NP was released as ¹⁴CO₂ after 58 days in both seawater-only and sediment-seawater experiments. This experiment indicates that NP can be degraded in seawater, though breakdown is slow.

The studies of estuaries show that significant amounts of APEO remain in both sediment and the water column. Biodegradation of APEO and NP is clearly possible, but is slow, particularly if conditions are anoxic or anaerobic. More research, including APEC determination, is needed before firm conclusions can be reached regarding the behaviour of APEOs in estuaries and the ocean.

4.6 Fate and levels in soil

Sewage sludge is often disposed of to land, or utilised as a fertiliser on land, frequently after anaerobic digestion. Since sewage sludge contains high levels of alkylphenols (see above), it is important to establish the fate of these compounds in soil. Marcomini *et al.* (1989) have examined the fate of NP, NP1EO and NP2EO in soil which had been treated with sludge. The initial concentrations in the soil were 4.7, 1.1 and 0.1 mg kg dry weight⁻¹ respectively. The concentration of each of the contaminants reduced by more than 80 % in the first month, eventually reducing to

residual concentrations of 0.5, 0.12 and 0.01 mg kg dry weight⁻¹ for NP, NP1EO and NP2EO respectively. These residual concentrations were reached by the 100th day, and were still present after 320 days. Presumably most of the loss of the contaminants was due to biodegradation, and maybe photolysis, though some could have leached away in groundwater. The residual concentrations could be due to strong binding to soil material, reducing availability for biodegradation.

Kirchmann and Tensved (1991) have examined the level of NP in barley grains cropped from soil fertilised by sewage sludge, but found no sign of any uptake.

It seems that NP, NP1EO and NP2EO are degraded in soil, and that NP is not taken up by barley. However, research aimed at finding the full pathway of APEO breakdown in soil should continue, to ensure that there is no environmental risk.

4.7 Levels in drinking water

Drinking water is frequently abstracted from rivers, and so can become contaminated with compounds from these rivers, if not adequately treated. Clark *et al.* (1992) have detected alkylphenolethoxylates and their metabolites in drinking water in the USA. NP3EC was found at 164 ng l⁻¹, OP2EO at 32 ng l⁻¹, and the total NPnEO (n=1-7) concentration was approximately 757 ng l⁻¹; therefore the total concentration of alkylphenolic compounds in this drinking water sample was almost 1 µg l⁻¹. It has also been found that chlorination of river water during the treatment of drinking water can produce brominated NP, NPEO and NPEC products (Ventura *et al.*, 1988; Ball *et al.*, 1989).

Table 3: Concentrations of nonylphenol and nonylphenol ethoxylates found in wild biota

Organism	[Contaminant] in water µg l ⁻¹	[Contaminant] in organ mg kg dry weight ⁻¹		Bioconcentration factor (dry weight)
			µM* (wet)	
<i>Cladophora glomerata</i> Macrophytic alga (Ahel <i>et al.</i> , 1993)	NP: 3.9 NP1EO: 23 NP2EO: 9.4	25 80 29	23 61 19	6600-7700 3500-5000 1000-1800
<i>Squalius cephalus</i> Fish, liver (Ahel <i>et al.</i> , 1993)	NP: 3.9 NP1EO: 23 NP2EO: 9.4	1.4 1.8 1.4	1.9 2.1 1.4	358 78 149
<i>Anas boscas</i> Mallard duck, muscle (Ahel <i>et al.</i> , 1993)	NP: 3.9 NP1EO: 23 NP2EO: 9.4	1.2 2.1 0.35	1.1 1.6 0.2	308 91 37
<i>Anas boscas</i> Mallard duck, liver (Ahel <i>et al.</i> , 1993)	NP: 3.9 NP1EO: 23 NP2EO: 9.4	0.1 0.16 <0.03	0.1 0.2 -	26 7 -
Marine macroalgae (mainly <i>Ulva rigida</i>) (Marcomini <i>et al.</i> , 1990)	Total NPEO: 1.6	NP+NP1EO+ NP2EO: 0.25	-	
<i>Mytilus edulis</i> Mussel (Wahlberg <i>et al.</i> , 1990)		NP: 0.4 mg kg wet weight ⁻¹	1.8	

* For calculation of the approximate concentration of the contaminant in the organ the following percentages of water were assumed: liver, 70%; muscle, 80%; algae, 80%.

Organism	Example of data [NP] in water, [NP] in organism	Calculated bioconcentration factor (wet weight)
<i>Salmo salar</i> Atlantic salmon (McLeese <i>et al.</i> , 1981)	310 µg l ⁻¹ in water 54, 91 mg kg wet weight ⁻¹ in two fish	280
<i>Crangon crangon</i> Shrimp (Ekelund <i>et al.</i> , 1990)	6.4 µg l ⁻¹ in water 0.680 mg kg wet weight ⁻¹ in fish	100
<i>Mytilus edulis</i> Mussel (Ekelund <i>et al.</i> , 1990)	6.2 µg l ⁻¹ in water 20 mg kg wet weight ⁻¹ in mussel (still rising after 16 days)	3400
<i>Gasterosteus aculeatus</i> Sticklebacks (Ekelund <i>et al.</i> , 1990)	4.8 µg l ⁻¹ in water 5.730 mg kg wet weight ⁻¹ in fish	1300

Table 4: Experimentally determined bioconcentration factors for nonylphenol

4.8 An estimation of NPEO entry into the UK environment

A recent report, commissioned by the UK Department of the Environment, has combined information on NPEO use and biodegradation in sewage works to provide an estimate of discharges of NPEO into the UK environment in 1992 (CES, 1993). The results, shown in Table 2, indicate that the vast majority, 83%, of UK NPEO production ends up the environment, with 37% in the aquatic environment. Although biodegradation will continue slowly after discharge into the environment, these results clearly indicate an unacceptable level of environmental contamination. Unfortunately, this report includes no actual measurements of NP or NPnEO levels in the UK environment.

4.9 Concentrations found in biota and levels of bioconcentration

Nonylphenol is a lipophilic compound, with a log K_{ow} of 4.48, whilst NP1EO, NP2EO and NP3EO are slightly less lipophilic, with log K_{ows} of around 4.2 (Ahel and Giger, 1993). It would be expected that this lipophilicity would lead to bioconcentration, and this has been shown to be the case. Table 3 shows the levels of NP and NPnEO that have been found in various biota, along with approximate bioconcentration factors calculated by measuring the concentration of the relevant substance in the environment the organism was in. Particularly notable is the bioconcentration of approximately 7000 times (dry weight) in the macrophytic algae, leading to levels of NP of up to 38 mg kg⁻¹ (Ahel *et al.*, 1993). In the higher organisms the bioconcentration levels may include biomagnification through the food chain, and also uptake of sediment containing higher levels than in

Test substance	Species	Test	Value (mg l ⁻¹)
NPEO	<i>Salmo trutta</i> (Argese <i>et al.</i> , 1994)	48h LC ₅₀	2.7
NPEO	<i>Daphnia magna</i> (Argese <i>et al.</i> , 1994)	48h EC ₅₀	1.5
APEO	<i>Mytilus edulis</i> Mussel (Lewis, 1991)	21 d LOEC larval growth and development	2.4
NP10EO	<i>Mytilus edulis</i> Mussel (Lewis, 1991)	LOEC Byssal thread formation, Adductor muscle closing	5.0
NP10EO	<i>Astarte montagui</i> Cockle (Lewis, 1991)	LOEC Burrowing	2.0
NP10EO	<i>Gadus morrhua</i> Cod (Lewis, 1991)	LOEC Swimming activity	2.0
NP10EO	<i>Gadus morrhua</i> Cod (Lewis, 1991)	LOEC Avoidance	0.002
NP10EO	<i>Balanus balanoides</i> Barnacle (Lewis, 1991)	LOEC Cirral activity	5.0
NPEO	<i>Selenastrum capricornutum</i> Green alga (Argese <i>et al.</i> , 1994)	96h EC ₅₀	0.09
APEO	<i>Selenastrum capricornutum</i> Green alga (Lewis, 1990)	LOEC 3d growth	20-50

Table 5: Toxicity of alkylphenol ethoxylates

the water (Ahel *et al.*, 1993). In addition, there may also be metabolism within the organism, for example of NP2EO to NP1EO and NP1EO to NP.

Levels of bioconcentration of NP have been determined in laboratory experiments for salmon, shrimp, sticklebacks and mussels (Table 4), with values of 100 - 3400 times (wet tissue weight) found. The accumulation test by McLeese *et al.* (1981) was of 4d duration, whereas those by Ekelund *et al.* (1990) were of 16d duration, and all were done in a flow-through system.

5. Toxicity

The toxicity of alkylphenols usually increases as the length of the hydrophobic chain increases (McLeese *et al.*, 1981). Toxicity may occur by partition into lipid membranes in the organism, for example the mitochondrial membrane, leading to the uncoupling of energy production (Argese *et al.*, 1994). In many organisms NP is more toxic than NPEO. Table 5 lists the toxicity of APEO to various aquatic species; effects normally start at concentrations of more than 1.5 mg l⁻¹. Nonylphenol, however, is often toxic at levels well below this (Table 6), down to concentrations of 0.118 mg l⁻¹ for *Nitocra spinipes*.

Species	Test	Value (mg l ⁻¹)
<i>Salvelinus fontinalis</i> Salmonid fish (Wahlberg <i>et al.</i> , 1990)	96hr LC ₅₀	0.145
<i>Salmo gairdneri</i> Salmonid fish (Wahlberg <i>et al.</i> , 1990)	96hr LC ₅₀	0.230
<i>Salmo salar</i> Atlantic salmon (juvenile) (McLeese <i>et al.</i> , 1981)	96hr LC ₅₀	0.13 - 0.19
Fathead minnow (Holcombe <i>et al.</i> , 1984)	96hr LC ₅₀ 96hr LOEL (some loss of equilibrium)	0.135 0.098
<i>Gadus morrhua</i> Cod (Argese <i>et al.</i> , 1994)	96h LC ₅₀	3.0
<i>Nitocra spinipes</i> Small crustacean (Wahlberg <i>et al.</i> , 1990)	96hr LC ₅₀	0.118 0.139 (2 techniques)
<i>Crangon septemspinosa</i> Shrimp (McLeese <i>et al.</i> , 1981)	96hr LC ₅₀	0.30
<i>Mytilus edulis</i> L. Common mussel (Granmo <i>et al.</i> , 1989)	96hr LC ₅₀ 850hr LC ₅₀	3.0 0.14
<i>Daphnia magna</i> (Comber <i>et al.</i> , 1993)	48hr EC ₅₀ 504hr NOEC	0.18 0.024
<i>Selenastrum capricornutum</i> Green alga (Argese <i>et al.</i> , 1994)	96hr EC ₅₀	0.41

Table 6: Toxicity of nonylphenol

No whole-organism toxicity studies have been done on NPEC, but Argese *et al.* (1994) have studied the effects of NP, NPEO and NP1EC on energy-coupled reverse electron transfer in beef heart submitochondrial particles. This test is sensitive both to chemicals that slow down the electron flow in the respiratory chain, and to those that uncouple ATP production. EC₅₀ levels for NP and NPEO of 2.0 and 1.3 mg l⁻¹ were found, which are similar to those in real organisms. The EC₅₀ for NP1EC was found to be 8.2 mg l⁻¹, slightly but not significantly higher. This suggests that carboxylated metabolites of NPEO may also be toxicity risks.

6. Oestrogenic effects

Oestradiol (Figure 2) is a hormone that influences the development and maintenance of female sex characteristics, and the maturation and function of accessory sex organs (Alberts *et al.*, 1983). It is now becoming clear that a wide variety of both man-made and natural chemicals, including phenol, bisphenol A and some polychlorinated biphenyls, are capable of mimicking the activity of this hormone (Korach, 1993)

The first evidence that *para*-alkylphenols could be oestrogenic was published in 1938, when Dodds and Lawson (1938) reported the results of feeding 100 mg of

4-propylphenol to ovariectomized rats. The chemical caused vaginal cornification in the rats, as occurs during a normal oestrus cycle, thus mimicking the activity of oestradiol. Cornification also occurred with 4-*tert*-pentylphenol, but not with 2-*n*-pentylphenol, indicating the importance of the two groups being *para*-on the ring.

The next evidence for oestrogenic effects of alkylphenols was published in 1978, by Mueller and Kim (1978). Their research found that various alkylphenols were able to displace oestradiol from its receptor, and also to prevent oestradiol binding the receptor. This effect was most marked at low temperatures (0 - 4 °C), and about 30, 000 - 100, 000 times as many molecules of the competitor were required to show measurable effects on oestradiol binding. The order of effectiveness at inhibiting binding was: 4-*sec*-pentylphenol > 4-*isopentyl* phenol > 4-*tert*-pentylphenol > 2-*sec*-pentyl phenol = 4-*sec*-butyl phenol. No activity was found with 4-ethoxyphenol or 4-(-OH-isopentyl)phenol, suggesting that the carbon chain must be hydrophobic (Mueller and Kim, 1978).

Unfortunately the health and environmental implications of these studies were not realised at the time, and it was not until 1991, by chance, that concerns were raised about 4-nonylphenol (Soto *et al.*, 1991). Soto *et al.* were working on oestrogen-sensitive MCF₇ human breast tumour cells when they discovered that a component leaching out of a new batch of centrifuge tubes was causing cell proliferation, the normal response to oestrogens. This component was purified and found to be nonylphenol, which had been added to the tubes to improve their resistance to breakage. Similar proliferation results were found with commercial 4-NP, with significant proliferation at a NP concentration of 1 μM (220 μg l⁻¹), and the proliferation produced by 10 μM 4-NP similar to that produced by 30 pM oestradiol, a concentration about 300,000 times lower. Further work showed that 4-NP could also induce expression of the progesterone receptor (as oestradiol does), and caused cell proliferation in ovariectomized rats (Soto *et al.*, 1991).

This study encouraged Jobling and Sumpter (1993) to test the effect of alkylphenols and some of the metabolites of alkylphenol ethoxylates for oestrogenic effect in Rainbow Trout, *Oncorhynchus mykiss*. An *in vitro* assay of

Table 7: Oestrogenic potencies of various compounds on rainbow trout hepatocytes (Jobling and Sumpter, 1993)

Compound	Mean ED ₅₀	Relative potency
17 -Oestradiol	1.81 nm	1
4-Nonylphenol	16.15 μM	0.0000090
4- <i>t</i> -Butylphenol	2.06 μM	0.0001600
4- <i>t</i> -Octylphenol	2.11 μM	0.0000370
NP2EO	17.27 μM	0.0000060
NP9EO	82.31 μM	0.0000002
NP1EC	15.25 μM	0.0000063

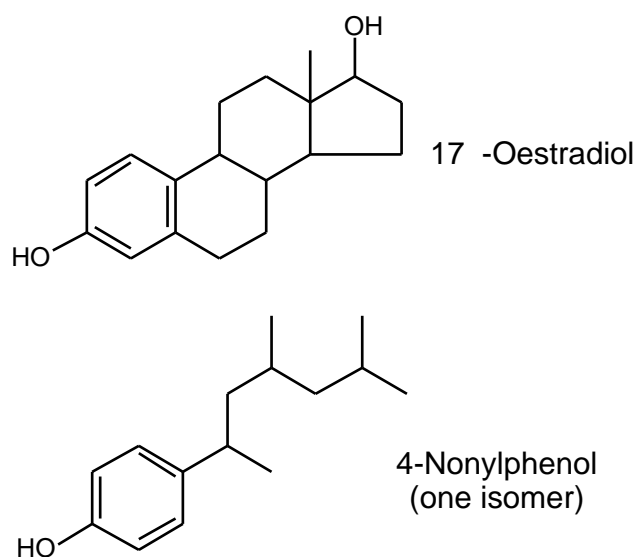


Figure 2: A comparison of the structures of oestradiol and 4-nonylphenol

liver cells was used, which detected the production of the egg-yolk protein vitellogenin, a large lipoglycophosphoprotein which is secreted by the liver of female fish and then circulates to their ovaries. This protein is produced in response to endogenous oestrogens. Both the ED₅₀ and the relative potency were determined, the latter by determining the concentrations of the test compound required to give an excretion of vitellogenin equal to that resulting from various concentrations of 17 - oestradiol. The results are shown in Table 7.

The most detailed study so far of the oestrogenic effects of APCs has found that 4-OP, 4-NP, 4-NP1EC and 4-NP2EO are able to stimulate vitellogenin gene expression in trout hepatocytes, gene transcription in transfected cells and the growth of breast cancer cell lines (White *et al.*, 1994). This study shows that these effects were due to binding to the oestrogen receptor, since they required the presence of the receptor, and were blocked by oestrogen agonists such as tamoxifen. OP, NP and NP1EC were all able to compete with oestrogen for binding of the oestrogen receptor. OP was found not to bind a mutant receptor, which also did not bind oestradiol. The oestrogen receptor has two different transcriptional activation functions; OP was found to activate both.

This study used a wide variety of cell lines and receptors: human breast cancer cell lines MCF₇ and ZR-75-1, chicken embryo fibroblasts, rainbow trout hepatocytes and a mouse oestrogen receptor. All of them showed oestrogenic responses to APCs, strongly suggesting that all oestrogen receptors will respond to APCs. The most potent APC was found to be OP, which was active in many assays at 0.1 μM, and was able to stimulate responses similar to those produced by oestradiol at concentrations only 1000 times higher than those of oestradiol. Oestrogenic effects occurred with NP, NP1EC and NP2EO concentrations of 1-10 μM, with NP1EC usually the most potent, and NP2EO the least. NP2EO did not bind cell-free receptors, suggesting that it may be metabolised within the cell to give an oestrogenic product. OPnEO were also

Organism, organ	Expected organ concentration of NP when exposed to 10 $\mu\text{g l}^{-1}$ NP in water		Water concentration of NP which would give a concentration in organ of $1\mu\text{M}$ $\mu\text{g l}^{-1}$
	mg kg wet weight ⁻¹	μM	
<i>Salmo salar</i> Atlantic salmon	2.8	12.9	0.78
<i>Gasterosteus aculeatus</i> Sticklebacks	13	60	0.17
<i>Squalius cephalus</i> Fish, liver	Dry Weight: 3.6	5 *	2
<i>Anas boscas</i> Mallard duck, liver	Dry Weight: 0.3	0.4 *	25
<i>Anas boscas</i> Mallard duck, muscle	Dry Weight: 3.1	2.9 *	3.4
<i>Crangon grangon</i> Shrimp	1.0	4.6	2.2
<i>Mytilus edulis</i> Mussel	34	156	0.06
<i>Cladophora glomerata</i> Macrophytic alga	Dry Weight: 71	65 *	0.15

* For calculation of the approximate concentration of the contaminant in the organ the following percentages of water were assumed: liver, 70% ; muscle, 80%; algae, 80%.

Table 8: A comparison of oestrogenic effect levels and predicted levels of NP in biota, using bioconcentration factors from Tables 3 and 4

tested for their oestrogenic effects, but more than 2 ethoxy groups seemed to prevent oestrogenic activity.

Further evidence of the hormonal mode of action of alkylphenolic toxicity comes from research done by Hewstone (1994), who applied solutions of calcium alkylphenates (used as lubricating oil additives) to the skin of male New Zealand White rabbits. The application of a 25% solution of calcium alkylphenate to 10 rabbits at the rate of 2 ml kg⁻¹ day⁻¹, 5 days a week for 4 weeks led to a 70% drop in testicular weight and all 10 rabbits stopped producing sperm. Even after a 30 day recovery period two out of five rabbits were still not producing sperm (Hewstone, 1994).

6.1 Implications of oestrogenic effects on the determination of safe environmental levels of alkylphenol ethoxylates

It is clear that 4-NP is oestrogenic *in vitro* at concentrations of around 1 μM (220 $\mu\text{g l}^{-1}$), and 4-OP is oestrogenic at concentrations of around 0.1 μM . Unfortunately, few attempts have been made to measure the levels of alkylphenolic compounds (APCs) in organisms (Table 3). Most of these results indicate possible NP concentrations of 1 - 2 μM in the organs of organisms tested, similar to the oestrogenic effect levels, though most of these organisms

were taken from a river with a low level of NP contamination, only 3.9 $\mu\text{g l}^{-1}$ (Ahel *et al.*, 1993). Contamination measured in samples of river water has varied between 0 - 1000 $\mu\text{g l}^{-1}$, though there is insufficient published UK data available to evaluate a likely level for a river in the UK.

If it is assumed that the level of NP in a river is 10 $\mu\text{g l}^{-1}$, which seems to be a sensible guess for an industrialised area, the expected concentration of NP in the biota can be estimated using the known bioconcentration factors (Table 8). This table shows that at a typical environmental concentration of NP, it is possible most of the organisms are accumulating NP levels high enough to have an oestrogenic effect, if an organ concentration of 1 μM is taken as the threshold of effects.

Table 8 also estimates what environmental concentration of NP would be necessary to ensure that the concentration in the organs of exposed organisms was only 1 μM . The results, around 0.1-4. $\mu\text{g l}^{-1}$, are at the low end of measured NP concentrations currently in rivers. If a safety factor of 10 is used, i.e. that expected organ concentrations should be less than 0.1 μM , then environmental concentrations of NP must be kept below 0.01 - 0.4 $\mu\text{g l}^{-1}$. Oestrogenic effects are also likely to be cumulative, so exposure to NP, OP, OPnEO, OPnEC, NPnEO, NPnEC, should be summed in setting any safety standard, taking into consideration the higher oestrogenic effects of OP. The evidence of studies on sewage works suggest that it would be very difficult to treat effluent containing APCs effectively enough to ensure that the sum of APCs in the effluent was low enough to pass any standard calculated on this basis. It is therefore unlikely that this safety limit could be achieved without the phasing out of all APC use. The uncertainty about the extent of oestrogenic effects in whole organisms, particularly in developing embryos, is arguably a reason for setting even lower safety levels.

There is already evidence that sewage effluent in the UK is oestrogenic, as male rainbow trout placed under sewage outfalls have been found to produce the egg-yolk protein vitellogenin (Purdom *et al.*, 1994), an *in vivo* version of the test used by Jobling and Sumpter (1993). It is considered that this effect may be partly due to the synthetic oestrogens used in the contraceptive pill, though these are probably not present in sufficient quantities, so it is likely that alkylphenol ethoxylates also have a role (MAFF, 1994). The oestrogenic effect persists in rivers receiving sewage effluent, gradually reducing as the effluent is diluted. The River Lea in north-west London, which is used as a source of drinking water, has been particularly closely studied, though 'many other rivers receive equivalent rates of sewage effluent inputs and would therefore be expected to show similar results to the Lea study' (MAFF, 1994). Male trout placed in some parts of this river produce 570,000 times the quantity of vitellogenin produced by control fish, a level above that found in female trout just prior to ovulation.

Another feature of the River Lea is a very high level of tumours and other gross morphological disorders in Bar-

bels, a bottom dwelling fish (Tyler and Everett, 1993). A quarter of fish examined were found to have these disorders, compared to 0% and 2% in the rivers Teme and Kennet respectively, both of which receive far less sewage input. The cause of these tumours is not known, though does not seem to be because of increased age, as the ages of fish were checked during the survey. The river in the area where the fish were caught can be up to 80% sewage effluent in a dry summer. The Thames NRA region is currently studying the levels of alkylphenolic compounds in the River Lea, but has not yet released any information (Thames NRA, personal communication).

6.2 Implications of oestrogenic effects on human health

The oestrogenic effects of alkylphenol ethoxylates have also got implications for human health. In the UK, 30 % of drinking water is abstracted from lowland rivers, many of which will have received sewage treatment effluent (Sumpter and Jobling, 1993). Levels of nonylphenolic compounds in drinking water in the US have already been found to reach almost $1 \mu\text{g l}^{-1}$, but no research has been done on the quantity absorbed and accumulated by those drinking this water.

The insecticide chlordecone (Kepone) shows similar behaviour to alkylphenols, accumulating in liver and adipose tissue, and eliciting oestrogenic activity (Soto *et al.*, 1991). Workers exposed to this insecticide can suffer reproductive effects such as low sperm counts and sterility. In addition, the oestrogenic effects of chlordecone on MCF₇ cells occur at similar concentrations to those of alkylphenols, suggesting that alkylphenols will be a similar health hazard if target cells are exposed to μM levels of these compounds (Soto *et al.*, 1991).

Concern about the possible effects of oestrogens on humans has been increasing recently, as a large number of synthetic chemicals and chemicals from plants have been found to be oestrogenic (Stone, 1994). There appears to have been a drop in the human sperm count in industrialised countries over the last 50 years, though this data is subject to many uncertainties. There has also been a two to four-fold increase in testicular cancer in industrialised countries in the last 50 years. As this cancer affects mainly young men, this increase is not due to ageing populations. One piece of evidence connecting this increase in testicular cancer with oestrogens is the discovery that the male offspring of pregnant mice exposed to high levels of oestradiol have an increased rate of testicular cancer (Stone, 1994). It has been suggested that there may be some connection between sperm abnormalities in London and the water supply (Ginsburg *et al.*, 1994).

7. Conclusions

Alkylphenol ethoxylate detergents were introduced into the UK in 1944; 50 years later their persistence, bioaccumulation and oestrogenic effects are clear. The data presented in this assessment indicates that these chemicals should be phased out as fast as possible. They do not degrade adequately in sewage treatment, they persist in

the environment, they breakdown to form toxic intermediates and they may have oestrogenic effects at current environmental concentrations.

It is surprising that none of the UK Government-commissioned reports in the last few years (Clarke *et al.*, 1992; DoE, 1992; CES, 1993) have incorporated any measurements of environmental levels of APCs. The 1993 report for the Department of the Environment, 'Uses, Fate and Entry to the Environment of Nonylphenol Ethoxylates' (CES, 1993), is supposed to be 'a twelve week study into the uses, fate and entry to the environment of nonylphenol ethoxylates.', in order to 'collate information on NPEs in order to assist in the development of UK policy.' It is hard to see how UK policy can be developed without any information on environmental levels of APCs in the UK.

These reports were produced just before the oestrogenic effects of APCs became a public issue, though after the work on breast cancer cells by Soto *et al.* (1991). They therefore compare known environmental levels of APCs (not in the UK) with those levels known to be toxic. This leads to conclusions such as:

'The committee concluded that, against a background of declining use and with reported environmental levels significantly less than the measured NOEC ...for Daphnia....one of the two most sensitive species reported in the literature, there is no reason for concern regarding the environmental impact of NPE in UK waters. In the wider context, the proposal that NPE should be included in the OECD Risk Reduction Programme would appear to be inappropriate. So would the proposals being discussed in the Paris Commission that the use of these substances be phased out' (DoE, 1992)

The Paris Commission, the administrative body of the Paris Convention, which deals with the pollution of the North East Atlantic by industrial wastes, took a fundamentally different approach:

'[The Paris Commission] is concerned about NPEs because of their wide use and relatively stable degradation metabolites which may have potential toxicity to organisms and ability to bioaccumulate. In addition, the Paris Commission considers that despite the fact that NPEs have been in use for over 40 years there is little known about the consequences of their continued use' (CES, 1993).

As a result of this, the contracting parties to the convention, including the UK, have agreed to phase out the use of NPEs as industrial cleaning agents by the year 2000, and to reduce all discharges to the environment (PARCOM Recommendation 92/8, CES, 1993). No commitments have been made with regard to octylphenol ethoxylates, uses other than cleaning or other alkylphenolic compounds.

Once oestrogenic effects are incorporated into an environmental assessment, there is no longer a safety margin of approximately 100 fold between environmental levels and those known to be toxic, as could be claimed in the past. The assessment above strongly suggests that there may be *no* safety margin between current environmental

levels and the threshold of oestrogenic effects.

7.1 Replacement of alkylphenol ethoxylates

In most applications, APEOs can be replaced by linear alcohol ethoxylate surfactants, which are readily biodegradable (CES, 1993). However, direct replacement can be difficult in some situations.

Switzerland has already banned the use of alkylphenol ethoxylate surfactants (Maki *et al.*, 1994). In the UK it is predicted that some industries including textiles, cleaning, metal cleaning, paints and agrochemicals may have replaced APEOs by the year 2000, whereas there is no timetable for their replacement in emulsion polymers and phosphate esters (CES, 1993). Even within the textile industry, it is apparent that NPEOs may continue to be used in wool scouring until they are banned, due to problems finding adequate alternatives (CES, 1993).

7.2 Future research needs

In spite of the slow moves towards phasing out of APEOs, it is important for research to continue into their fate and effects, since their recalcitrance means that they will remain in the environment for some time after they are no longer in use. Some possible areas for future research are given below:

1) More information is needed on levels of alkylphenol ethoxylates and their metabolites in rivers and estuaries, drinking water and in organisms, including humans. Standardised analytical procedures are needed for the detection of APs, APnEOs and APnECs.

2) Research is needed into the *in vivo* oestrogenic effects of nonylphenol, including whether existing environmental levels are high enough to show an effect, and what metabolism of APCs occurs within organisms. This research should be used to rapidly establish safety levels for APCs in drinking water, rivers and estuaries.

3) The full pathway for the breakdown of alkylphenols should be established, to ensure that no other intermediates are accumulating in the environment.

4) The oestrogenic components of sewage effluent need to be characterised - this research is currently being undertaken by the National Rivers Authority (NRA, Personal Communication).

5) Research on alternatives to alkylphenols and alkylphenol ethoxylates should be pursued in order to enable a rapid phasing-out of the use of these chemicals from all applications.

7.3 Some comments

The gradual accumulation of environmental information on APEOs during the past 50 years provides a good example of the changes in attitudes to the environment during this period, and points out many deficiencies in past and current approaches:

1) It is wrong to use chemicals if their environmental fate and toxicity are not known. A chemical should not be

used for 50 years whilst knowledge of its environmental fate is minimal.

2) It was established in the early 1960's that branched-chain surfactants were not well degraded in sewage works, so branched-chain alkylbenzene sulphonates were phased out. Why are we still using branched-chain APEO surfactants?

3) Primary biodegradation is an archaic and misleading concept. Its only role in surfactant assessment is as a predictor of foaming. It gives no indication of the accumulation of intermediates, which may be more toxic than the original. It is essential that breakdown pathways are established, and intermediates tested for toxicity and bioaccumulation. This approach has been used by Neilson *et al.* (1994) to evaluate the hazard to the aquatic environment of paper bleaching effluents. In my view, the term 'Primary Biodegradation' should not be used, as it only serves to mislead those who do not know its real meaning.

4) It is not sensible to produce a report on the effects of a chemical on the UK environment without measuring levels of this chemical in the UK environment.

5) When chemicals are being assessed, it is important to keep an open mind on possible mechanisms of toxicity, and the literature should be searched for possible biochemical effects of the compound under assessment, its metabolites and similar compounds. The oestrogenic effects of *p*-alkylphenols were established in 1938 - 6 years before the introduction of NPEOs in the UK. These effects were not connected with NPEOs until 1991 - 53 years later.

6) Existing toxicity assessment procedures do not adequately account for chronic exposure, or for chemicals with subtle biochemical effects. Hormonal effects are probably best evaluated using cell culture, while more research is needed into other potential toxic mechanisms, such as those involving the immune or the nervous system.

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