ENVIRONMENTAL ENGINEERING SCIENCE
Volume 20, Number 5, 2003


# Occurrence and Behavior of Alkylphenol Polyethoxylates in the Environment

**John Montgomery-Brown and Martin Reinhard***

*Department of Civil and Environmental Engineering*
*Stanford University*
*Stanford, CA 94305-4020*

## ABSTRACT

Alkylphenol polyethoxylates (APEOs) are nonionic surfactants whose degradation metabolites have become ubiquitous in the aquatic environment. The environmental significance of APEO metabolites and the threat they pose to wildlife is still a matter of debate. Even though advanced analytical procedures are available, researchers have been unable to obtain a complete mass balance during biodegradation studies. The ultimate fate of APEOs and their metabolites is not adequately understood. Biodegradation is believed to be the dominant attenuation process, but photodegradation may also play an important part. The synthesis of single isomer standards for APEOs and their metabolites is necessary for accurate quantification, estrogenicity experiments, and biological and chemical fate studies.

**Key words:** alkylphenol polyethoxylates; environment

## INTRODUCTION

ALKYLPHENOL POLYETHOXYLATES (APEOs) are nonionic surfactants that have been used for more than 40 years in household and industrial detergents, petroleum refining, pulp and paper production, crop protection chemicals, and plastics and textiles manufacturing (Talmage, 1994). The worldwide demand for APEOs is greater than 500 million kg/year (Naylor, 1995); of this total, approximately 80% are nonylphenol polyethoxylates (NPEOs) and 15–20% are octylphenol polyethoxylates (OPEOs) (Staples *et al*, 1999).

APEOs are composed of a polyethoxy chain and an alkyl chain connected to a benzene ring (Fig. 1). The alkylphenol (AP) is formed through the acid-catalyzed Friedel-Crafts alkylation of phenol with an alkene. The phenol is preferentially alkylated at the *para* position; *ortho* isomers make up less than 10% of a technical APEO mixture (Talmage, 1994). Octyl and nonyl groups are made from diisobutylene and tripropylene, respectively; octyl groups are predominantly 1,1,3,3-tetramethylbutyl, while nonyl groups are mixtures of branched isomers. The AP is then reacted with ethylene oxide; the resulting product is a mixture of APEOs having various polyoxyethylene chain lengths (Talmage, 1994). In this article, APnEO will be used to represent an alkylphenol

*Corresponding author: Civil & Environmental Engineering, Stanford University, Stanford, CA 94305-4020; *Phone*: 650-723-0308; *Fax*: 650-723-7058; *E-mail*: reinhard@ce.stanford.edu

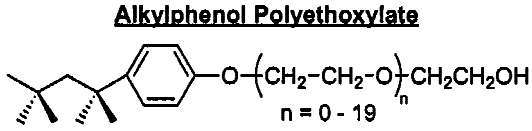


**Figure 1.** Structure of an alkylphenol polyethoxylate.

polyethoxylate with *n* ethoxy units; most commonly, APEOs have between 1 and 20 ethoxy (EO) units. The analytical detection, identification, and quantification of APEOs and their metabolites has been hindered by a lack of standards; even the available standards are mixtures of various structural isomers. For example, using high-resolution gas chromatography-mass spectrometry (GC-MS), Wheeler *et al.* (1997) determined that there were 22 distinct *para*-nonylphenol (NP) isomers in a technical NP mixture.

During biological treatment, APEO metabolites like short-chained APnEOs ($n$ = 1–3), alkylphenol polyethoxycarboxylates (APECs), carboxyalkylphenol polyethoxycarboxylates (CAPECs), and APs are produced. Halogenated APEOs and APEO metabolites can also be formed during wastewater chlorination (Reinhard *et al.*, 1982; Ball, 1985; Ding *et al.*, 1996). Structures for several of the aforementioned metabolites are shown in Fig. 2. APEO metabolites were among the most commonly detected wastewater-derived contaminants detected by the United States Geological Survey (Kolpin *et al.*, 2002) during a national survey of lakes, rivers, and streams in the United States. Because these metabolites are relatively recalcitrant in the environment (Fujita *et al.*, 1996; DiCorcia *et al.*, 1998; Shang *et al.*, 1999), can bind to and accumulate in soils and sediments (Khim *et al.*, 1999; Ferguson *et al.*, 2001), can bioaccumulate in plants and animals (Ahel *et al.*, 1993; Staples *et al.*, 1998), and can be estrogenic to wildlife at low concentrations (Jobling *et al.*, 1996; Routledge *et al.*, 1998), their ubiquitous presence in the environment may be cause for concern.

In 1999, the Water Quality Research Journal of Canada featured several comprehensive reviews on the occurrence, persistence, and aquatic toxicity of APEOs and their metabolites in the environment (Bennie, 1999; Maguire, 1999; Servos, 1999); the analytical techniques used in the detection of these compounds were also extensively reviewed (Lee, 1999). A more recent review covered the APEO removal in activated sludges (Johnson and Sumpter, 2001). Because recent research has focused on (1) determining the estrogenic potency of APEOs *in vivo*, (2) developing analytical techniques capable of detecting both neutral and acidic APEO metabolites, and (3) obtaining a better understanding of degradation pathways in environmental matrices, this review will focus on the advances and current trends in these areas.

## ENVIRONMENTAL SIGNIFICANCE

Indigenous fish exhibiting symptoms of endocrine disruption can be found in a number of aquatic environments (Jobling *et al.*, 1998). For example, a report by Harries *et al.* (1997) found that fish caught at a wastewater treatment plant (WWTP) outfall had abnormal ratios of sex steroid hormones and exhibited histological changes in their reproductive organs. Another study was able to attribute the feminization of male reproductive organs in fish to WWTP effluent exposure (Rodgers-Gray *et al.*, 2001). Natural estrogens play important roles in the development of male and female reproductive organs and in metabolic regulation, even at very low concentrations (Servos, 1999). Therefore, they, and their synthetic analogues, might be responsible for the observed endocrine disruption in fish. Another possibility may be estrogen mimics (i.e., compounds able to elicit estrogenic responses in test organisms). In 1991, while culturing hu-

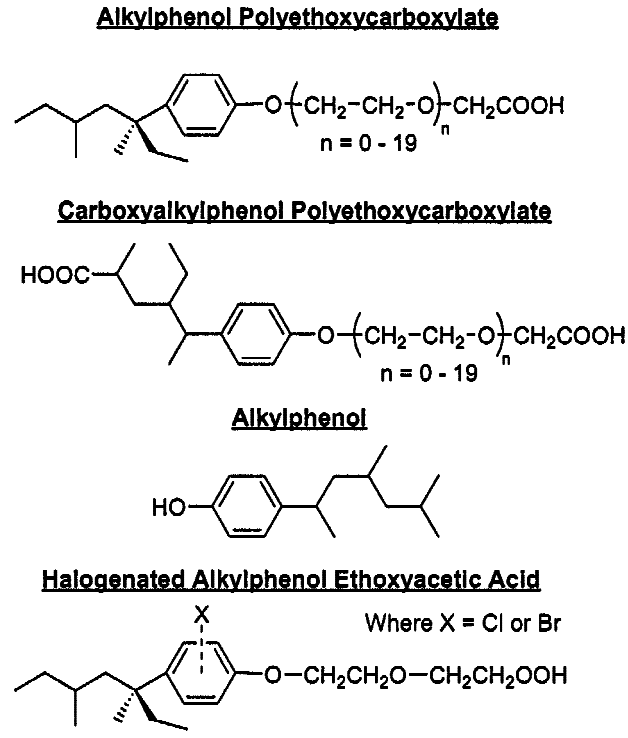


**Figure 2.** Potential structures of several APEO metabolites (NPEC, CA8PEC, NP, and a halogenated NP2EC, respectively).

man breast cancer cells, Soto *et al.* (1991) discovered that *para*-nonylphenol was a weak estrogen. Because of the structural similarities between some nonylphenol isomers and natural and synthetic estrogens (Fig. 3), it is likely that nonylphenol can compete with 17$\beta$-estradiol for the estrogen binding receptor in vertebrates. Thus, it seems reasonable that APEOs and their metabolites could also be weak estrogens. Furthermore, the high concentrations of APEOs and their metabolites ($\mu$g/L) in WWTP effluents relative to other estrogens and estrogen mimics (ng/L), suggests that these compounds could play an important role in the observed endocrine disruption in fish.

Through various *in vitro* and *in vivo* tests, it was shown that APEO metabolites like APs and short-chained APnEOs ($n$ = 1–3) are active environmental estrogens at low microgram per liter concentrations (White *et al.*, 1994; Jobling *et al.*, 1996). To compound the problem, the estrogenic responses to these xenoestrogens may be additive (Sumpter and Jobling, 1995; Routledge *et al*, 1998). Estrogenicity tests are commonly used to determine the overall estrogenic potency of water samples (Khim *et al.*, 2001b; Korner *et al.*, 2001). Of all APEO metabolites, octylphenol (OP) is the most potent xenoestrogen, and can induce significant effects in fish at concentrations of 3 $\mu$g/L (Jobling *et al.*, 1996); technical-grade nonylphenol (NP) is active at concentrations of 8.3 $\mu$g/L (Harris *et al.*, 2001). At concentrations below 100 $\mu$g/L, APECs and APnEOs ($n > 2$) do not appear to elicit estrogenic responses in fish (Metcalfe *et al.*, 2001; Nichols *et al.*, 2001); estrogenic studies on CAPECs have yet to be performed. The lack of standards for APEO metabolites (especially individual isomers) has prevented researchers from accurately assessing structure–activity relations and the impact of these chemicals in the environment.

Because all vertebrates produce 17$\beta$-estradiol, and it is very potent at low concentrations (ng/L), this hormone was selected as the basis for determining the estrogenicity of xeno- and synthetic estrogens. *In vitro* tests indicate that, compared to 17$\beta$-estradiol, APEOs and their metabolites are weak xenoestrogens; for example, Jobling *et al.* (1996) found that the potencies of APEO metabolites were 1,000 to 10,000 times less than that of 17$\beta$-estradiol. However, *in vivo* experiments have found that OP is only 100 to 1,000 less effective than 17$\beta$-estradiol; the increased potency of OP was postulated to result from bioaccumulation (Routledge *et al.*, 1998; Thorpe *et al.*, 2000). Conclusions based on *in vivo* experiments may be more relevant than those from *in vitro* experiments because living organisms may be better able to inactive or excrete natural plant and animal estrogens than xenoestrogens like APEO metabolites (Johnson and Sumpter, 2001). However, *in vivo* estrogenic responses are affected by the organisms being used, their sex (i.e., males are more sensitive than females), and their developmental stage (i.e., developing organisms are more sensitive than adults) (Jobling *et al*, 1996; Routledge *et al.*, 1998).

In general, the APEO metabolite concentrations observed in the environment (Table 1) are below the levels known to cause estrogenic effects in fish and other wildlife. However, OP and NP formation from the degradation of other APEO metabolites (e.g., APECs) could result in OP and NP concentrations capable of inducing estrogenic effects. Furthermore, because several APEO metabolites can accumulate and persist in the environment, and bioaccumulate in wildlife, it is difficult to assess what concentrations pose a significant risk for endocrine disruption.

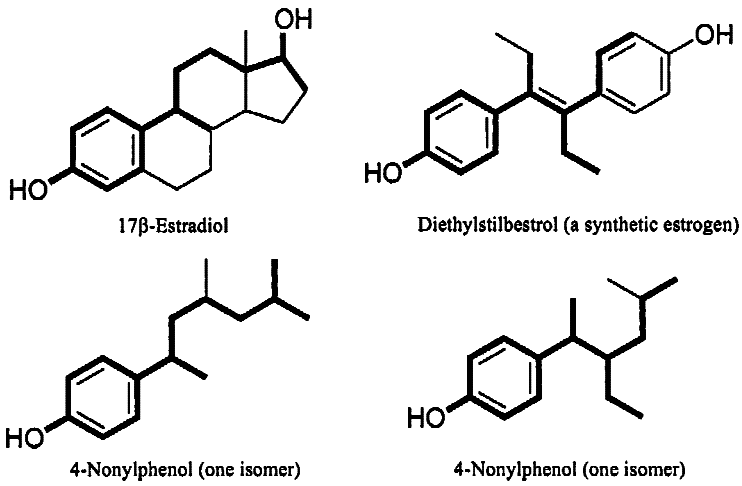


**Figure 3.** Structural similarities between two NPEO metabolites and 17-$\beta$-Estradiol (a human estrogen) and Diethylstilbestrol.

**Table 1.** Occurence of APEOs and APEO metabolites in the environment.

| *Location* | *Compound* | *Concentration* | *Reference* |
|---|---|---|---|
| 1° WWTP effluent[a] | NP(1–18)EO | 96–430 μg/L | Ahel *et al.* (2000) |
| 2° WWTP effluent[a] | NP(1–18)EO | 2–8 μg/L | |
| WWTP effluent[b] | NP(1–2)EO | 0.4–8.9 μg/L | Rodgers-Gray *et al.* (2001) |
| | NP | 1.2–2.7 μg/L | |
| | OP | 30–190 ng/L | |
| WWTP effluent[b] | NP1EO | 45 ± 16 μg/L | Lye *et al.* (1999) |
| | NP | 3.00 ± 0.85 μg/L | |
| WWTP effluent[c] | CAPEC | 58 μg/L | DiCorcia *et al.* (1998) |
| WWTP effluent[c] | NP(1-2)EO | 1.7–6.6 μg/L | DiCorcia *et al.* (2000) |
| | NPEC | 0.6–15 μg/L | |
| | CAPEC | 6.31–40.56 μg/L | |
| WWTP effluent[d] | NP2EO | bd–5.5 μg/L | Spengler *et al.* (2001) |
| | NP1EC | 0.17–5.8 μg/L | |
| | NP | 0.33–2.3 μg/L | |
| WWTP effluent[d] | NP | 111 ng/L | Kuch and Ballschmiter (2001) |
| | OP | 14 ng/L | |
| WWTP effluent[e] | NP | < 1.0–33 μg/L | Hale *et al.* (2000) |
| WWTP Sediments[e] | NP | < 5–12,400 μg/L dw | |
| Septage[e] | NP | > 1,000 μg/L | Rudel *et al.* (1998) |
| Land-applied sewage sludge[e] | NP(1–2)EO | < 1.5–408 mg/kg dw | La Guardia *et al.* (2001) |
| | NP | 5.4–887 mg/kg dw | |
| | OP | < 0.5–12.6 mg/kg dw | |
| Sewage sludge[e] | NP(1–4)EC | 27–113 μg/g dw | Field and Reed (1999) |
| Rivers, streams, and lakes[e] | NP(1–2)EO | < 1–20 μg/L | Kolpin *et al.* (2002) |
| | NP | < 0.5–40 μg/L | |
| | OP(1–2)EO | < 0.2–2 μg/L | |
| River water[f] | NP(2–8)EO | 2–35 nM | Maruyama *et al.* (2000) |
| River water[d] | NP | 6–135 ng/L | Kuch and Ballschmiter (2001) |
| River sediments[b] | NP1EO | 160–3970 ng/g dw | Lye *et al.* (1999) |
| | NP | 30–9050 ng/g dw | |
| | OP | 2–340 ng/g dw | |
| Estuary water[e] | NP(1–3)EO | 697 ng/L | Ferguson *et al.* (2000) |
| | NP | 201 ng/L | |
| | OP (1–3)EO | 31.9 ng/L | |
| | OP | 3.27 ng/L | |
| Estuary water[e] | NP(1–3)EO | 0.22–1.05 μg/L | Ferguson *et al.* (2001) |
| | OP(1–3)EO | 7–40 ng/L | |
| Estuary sediments[e] | NP(1–3)EO | 3.15 μg/L | Ferguson *et al.* (2000) |
| | NP | 846 ng/g dw | |
| | Cl- and Br-NP | 13.8 ng/g dw | |
| | OP(1–3)EO | 79.7 ng/L | |
| | OP | 8.11 ng/L | |
| Estuary sediments[e] | NP(1–3)EO | 0.05–30 μg/g dw | Ferguson *et al.* (2001) |
| | Cl- and Br-NP | nd–45.6 ng/g dw | |
| | OP(1–3)EO | 5–90 ng/g dw | |
| Estuary sediments[g] | NP | 113–3890 ng/g dw | Khim *et al.* (1999) |
| | OP | 4–179 ng/g dw | |
| Estuary sediments[g] | NP | < 1–19.9 ng/g dw | Khim *et al.* (2001a) |
| | OP | < 1–2.91 ng/g dw | |
| Marine sediments[h] | NP(1–19)EO | 1.5 μg/g dw | Shang *et al.* (1999) |

**Table 1.** (Cont'd)

| *Location* | *Compound* | *Concentration* | *Reference* |
|---|---|---|---|
| Edible mollusks[i] | NP | 67–696 ng/g fw | Ferrara *et al.* (2001) |
| | OPEO | nd–0.43 ng/g fw | |
| | OP | 2.7–18.6 ng/g fw | |
| Fish tissue[b]; | NP1EO | 190–940 ng/g fw | Lye *et al.* (1999) |
| Mature *Platichthys flesus* | NP | 5–55 ng/g fw | |
| Drinking water[d] | NP | 2–15 ng/L | Kuch and Ballschmiter (2001) |
| | OP | 0.15–5 ng/L | |
| Food[d] | NP | 0.1–19.4 $\mu$g/kg fw | Guenther *et al.* (2002) |

dw, dry weight; fw, fresh weight; bd, below detection; nd, not detected.
[a]Switzerland; [b]United Kingdom; [c]Italy; [d]Germany; [e]United States; [f]Japan; [g]Korea; [h]Canada; [i]Adriatic Sea.

## ANALYTICAL METHODS

### *Sample preparation*

*Water.* For water-based samples, the concentration technique most often used is reverse-phase solid-phase extraction (SPE) (Ding and Tzing, 1998; DiCorcia *et al.*, 2000; Ferguson *et al.*, 2001). For reverse-phase SPE, columns are most often filled with C18-coated silica particles (Jonkers *et al.*, 2001; Schroder, 2001; Spengler *et al.*, 2001), graphitized carbon black (Ahel *et al*, 2000; DiCorcia *et al.*, 2000), or hydrophobic polymers (e.g., XAD-16) (Maruyama *et al.*, 2000). Differential elution from the SPE cartridge is often used for the separation and enrichment of acidic and neutral compounds (DiCorcia *et al.*, 2000; Castillo *et al*, 2001). Acidic metabolites can be separated from the reverse-phase SPE eluate by silica gel chromatography (Spengler *et al.*, 2001; Korner *et al.,* 2001). Typical SPE recoveries range from 78 to 95%. Other techniques used to concentrate APEOs and their metabolites from water are evaporation (Ding *et al.*, 1996) and liquid–liquid extraction (Potter *et al.*, 1999; Ahel *et al.*, 2000).

*Soils and sediments.* Extraction of APEOs and their metabolites from soils, sediments, and sludges can be accomplished using Soxlet extraction (Khim *et al.*, 1999), enhanced solvent extraction (Field and Reed, 1999; Shang *et al.*, 1999; Ferguson *et al.*, 2000), and steam distillation (Tanghe *et al.*, 1999). Recoveries range from 60 to >95%. Aminopropyl and cyanopropyl normal phase SPE columns have been used to clean up sediment extracts (Shang *et al.*, 1999; Ferguson *et al.*, 2000).

### *Analytical separation and detection*

*Liquid chromatography.* Several liquid chromatography (LC) techniques have been developed for the separation of APEOs and their metabolites. The advantages of liquid chromatography include its versatility and its ability to analyze nonvolatile, high molecular weight samples without derivatization; its main drawback is its low resolution. For example, normal phase (silica and aminopropyl-coated silica solid phases) LC techniques can only separate APEOs based on the length of their ethoxylate chain. Reverse phase (C8- and C18-coated silica solid phases) can only separate APEOs based on the length and structure of their alkyl chain. Therefore, normal-phase LC cannot distinguish between OP1EO and NP1EO, and reverse-phase LC cannot distinguish between NP1EO and NP10EO. Recently, however, Gunderson (2001) developed a reverse-phase technique, using graphitized carbon black, which could resolve the 22 isomers found in technical grade NP into 12 groups. This method could also differentiate between the different isomers found in NPEO mixtures, but more work is necessary to achieve better separation of different ethoxylate chain lengths.

Fluorescence detectors are increasingly being used for the detection of APEOs and their metabolites because of their higher selectivity and sensitivity than UV diode array detectors. However, as with UV adsorption, the relative response factors for fluorescence change considerably with molecular weight; therefore, it is necessary to know the oligomeric composition of the mixture when determining total NPEO concentrations using fluorescence spectroscopy (Ahel *et al.*, 2000). The excitation and emission wavelengths most commonly used are ~225 and ~305 nm, respectively (Tanghe *et al.*, 1999; Ahel *et al.*, 2000; Gundersen, 2001). The UV absorption wavelengths most commonly used are ~230 and ~277 nm (Brand *et al.*, 1998; Potter *et al.*, 1999).

For LC mass spectrometry (MS), reverse-phase C8 and C18 analytical columns are most frequently used (Ferguson *et al.*, 2000; Jonkers *et al.*, 2001; Schroder, 2001).

The two most common ionization techniques used for APEOs and their metabolites are atmospheric pressure chemical ionization (APCI) (Castillo *et al.*, 2001; Schroder, 2001) and electrospray ionization (ESI) (DiCorcia *et al.*, 2000; Ferguson *et al.*, 2001; Jonkers *et al.*, 2001). ESI is more popular because of its wide applicability to large and small, polar and nonpolar compounds, as well as singly and multiply charged species. For ESI LC-MS, parent APEOs are generally detected by monitoring the positive ions produced during ionization while APEO metabolites are detected by monitoring the negative ions produced during ionization (DiCorcia *et al.*, 2000; Ferguson *et al.*, 2000; Jonkers *et al.*, 2001).

*Gas chromatography.* Capillary column GC has been used for the analysis of underivatized and derivatized APEOs and their metabolites (Ding *et al.*, 1996; Wheeler *et al.*, 1997; Spengler *et al.*, 2001). Despite its higher resolution, and its ability to distinguish between alkyl chain isomers and ethoxylate chain length, GC is becoming less common as it is limited to short-chained ($n$ = 1–4) APEO metabolites because of their low volatility. Derivatization is frequently used to increase the volatility of APECs and CAPECs; it can also reduce matrix effects, enhance chromatographic separation, and facilitate mass spectral identification. APECs and CAPECs are most commonly derivatized to their methyl (Ball *et al.*, 1989; Field and Reed, 1999), propyl (Ding and Tzing, 1998; Wild and Reinhard, 1999), and pentafluorobenzoyl (Kuch and Ballschmiter, 2001) esters.

GCs may be used in conjunction with flame ionization detectors (Wheeler *et al.*, 1997; Guenther *et al.*, 2002), electron capture detectors (ECD) (Kuch and Ballschmiter, 2001), and mass spectrometers (Ding and Tzing, 1998; Ejlertsson *et al.*, 1999; Field and Reed, 1999). For GC-MS, both electron ionization (EI) (Ding and Tzing, 1998; Spengler *et al.*, 2001) and chemical ionization (CI) (Field and Reed, 1999; Kuch and Ballschmiter, 2001) are used. Because APEO molecules and their metabolites fragment readily, it is often difficult to identify the molecular ion using EI. CI results in less fragmentation and greater molecular ion abundances, and is hence, very useful when used in conjunction with EI for the identification of APEOs and their metabolites. CI techniques most commonly use methane as a reagent gas; however, ammonia is very useful for APEOs and their metabolites because it gives very intense [$M^+$-$NH_4^+$] peaks (Field and Reed, 1999). Positive ions are most commonly detected in both EI and CI GC-MS (Ding and Tzing, 1998; Field and Reed, 1999). In CI GC-MS, negative ion detection is occasionally used to obtain very low detection limits (Ejlertsson *et al.*, 1999; Kuch and Ballschmiter, 2001).

*Mass spectrometry.* Triple-stage quadrupole (DiCorcia *et al.*, 2000; Jonkers *et al.*, 2001) and ion-trap (Ding and Tzing, 1998; Tanghe *et al.*, 1999) mass spectrometers are becoming more common because they provide more detailed information than single-stage quadrupole mass spectrometers. APEO metabolites are identified using full-scan spectra and quantified using single or multiple ion monitoring. Quantification of APEOs and their metabolites by MS is difficult because of the lack of standards. Even when standards are available (as for NP), quantification is still difficult because each NP isomer may have a different response factor. Because the response factors for AP1EC and AP2EC are similar, most researchers assume that the response factors for other metabolites (e.g., CAPECs) will not differ significantly (DiCorcia *et al.*, 2000; Schroder, 2001).

## SOURCES OF APEOS AND APEO METABOLITES

Approximately 55% of the annual APEO production is used in industry (e.g., plastics, textiles, and paper manufacturing), 30% for industrial cleaning products, and the remaining 15% for consumer products (Talmage, 1994). Thus, approximately 200 million kilograms of APEOs enter WWTPs per year. APEO removal in WWTPs varies widely (60 to >98%; Thiele *et al.*, 1997) because of the different source waters, operating conditions, and treatment technologies used. In a study using Swiss WWTPs, Ahel *et al.* (1994a) determined that approximately 40% of the incoming APEO mass is mineralized; the remainder was biotransformed to a mixture of metabolites (despite the recent discovery of CAPECs, these percentages are likely still reasonable because any CAPECs present in the effluent would have been quantified along with the APEC metabolites).

These hydrophobic and hydrophilic metabolites are discharged into the environment via sewage sludge and wastewater effluents (Ahel *et al.*, 1994a). Although municipal and industrial WWTP effluents have traditionally been considered the main sources of APEO metabolites in the environment, recent research suggests that other sources may also be important. For example, Hale *et al.* (2000), found high concentrations (>100 $\mu$g/kg) of sediment-bound APEO metabolites near storm water discharges, civilian and military shipyards, and other government and industrial facilities. Septic systems may also be significant sources of APEO metabolites; all septage sampled by Rudel *et al.* (1998) had NP concentrations greater than 1,000 $\mu$g/L.

## OCCURRENCE DATA

Table 1 summarizes the observed concentrations of APEOs and their metabolites in the environment since 1999; for a summary of occurrence data prior to 1999, see Bennie (1999).

### *Water*

APEO metabolites have become almost ubiquitous in the aquatic environment due to their extensive industrial and domestic use. For example, during a national recognizance of streams, lakes and rivers in the United States, the United States Geological Survey found that APEO metabolites (NP, NP1EO, NP2EO, OP1EO, and OP2EO) were detected approximately 70% of the time and accounted for approximately 36% of the total concentration of 95 different antibiotics, pharmaceuticals, nonprescription drugs, steroids and hormones, and wastewater-related compounds (Kolpin *et al.*, 2002); if acidic APEO metabolites were included, the frequency of detection and total concentration would likely have been significantly higher. APEOs and their metabolites have also been detected in estuaries (Ferguson *et al.*, 2001), groundwater (Ding *et al.*, 1996), and even drinking water (Kuch and Ballschmiter, 2001).

APECs and CAPECs are frequently the most abundant APEO metabolites present in WWTP effluents. For example, DiCorcia *et al.* (2000) determined that CAPECs accounted for approximately 65% of the APEO metabolites in Italian WWTP effluents; the concentrations of these metabolites ranged between 6.31 and 40.56 $\mu$g/L. Few researchers have examined the occurrence and distribution of halogenated APEO metabolites (Ding *et al.*, 1996; Ferguson *et al.*, 2001; Petrovic *et al.*, 2001).

### *Sewage sludges and sediments*

In WWTPs, hydrophobic APEO metabolites tend to sorb to sewage sludges. Tanghe *et al.* (1998) found that between 60 and 90% of spiked NP, a hydrophobic (log $K_{OW} \sim 4.2$) APEO metabolite, sorbs to 3–4 g/L of sewage sludge. Even relatively hydrophilic metabolites like APECs have been found in sewage sludges (Ahel *et al.*, 1994a; Field and Reed, 1999). Interestingly, approximately 45% of the NPECs detected by Field and Reed (1999) were *ortho* isomers. This may indicate that *ortho* isomers are more recalcitrant than *para* isomers.

Sorbed APEO metabolites have also been found in estuarine, lacustrine, and riverbed sediments (Table 1). In anoxic marine sediments from the Strait of Georgia, Canada, Shang *et al.* (1999) found very little biodegradation of NPEOs; half-lives were estimated to be greater than 60 years. Therefore, estuarine, lacustrine, marine, and riverbed sediments may have accumulated large amounts of APEO metabolites, and could serve as reservoirs for these compounds.

In sediments and sludges, it is important to analyze for long-chained APnEOs ($n > 3$) in addition to short-chained APnEOs ($n = 1$–3). For example, more than half of the sediments analyzed by Shang *et al.* (1999) had NPEOs with more than two ethoxy units; this lead these researchers to conclude that analyses, which only focus on short ethoxy chain oligomers in sediments or sludges, may underreport total NPEO concentrations by a factor of 2.

## DEGRADATION IN THE ENVIRONMENT

Research on the physiochemical properties of APEOs and their metabolites and the impact these properties have on the environmental fate of these compounds is limited. Due to the lack of standards, even less is known about APECs and CAPECs. Table 2 summarizes the observed and estimated physical properties for several APEO metabolites. Based on these properties, volatilization (Dachs *et al.*, 1999) and sorption to suspended solids and sediments may be important physicochemical removal methods for some metabolites (APs and short-chained APnEOs). Other removal mechanisms include chemical and biological processes; chemical removal is dominated by photochemical degradation and biological removal is accomplished by APEO uptake and transformation by micro-organisms (Maguire, 1999).

### *Biodegradation*

APEO biodegradation is believed to be the dominant removal process for these compounds in the environment. Despite the fact that the degradation of these compounds has been studied for 40 years, the degradation mechanisms for certain APEO metabolites remain unknown. In fact, one group of metabolites (CAPECs) was not even discovered until 1996 (Ding *et al.*, 1996). Furthermore, although there is no disagreement about the degradability of long-chained APEOs, experimental evidence for the degradation of their metabolites is inconsistent—some experiments indicate that APEO metabolites are readily biodegradable (Staples *et al.*, 1999; Montgomery-Brown *et al.*, 2002), others indicate that they are very recalcitrant (DiCorcia *et al.*, 1998; Shang *et al.*, 1999).

It is generally accepted that APEO biodegradation begins by the rapid stepwise shortening of the ethoxy chain (Fig. 4). Degradation of the branched alkyl chain may proceed as outlined in Fig. 5. Little is known about the

**Table 2.** Observed and estimated physical properties for several APEO metabolites.

| | *NPnEO* (n = *1–4*)[a] | *NP*[b] | | *NP1EO*[b] | *NP2EO*[b] | *NP1EC*[b] | *CAP1EC*[b] |
|---|---|---|---|---|---|---|---|
| Solubility (mg/L) | 3–9 | 1.7 ± 0.56 | | 2.0 ± 0.63 | 1.2 ± 0.38 | 1.8 ± 0.59 | 30 ± 9.6 |
| Log $K_{ow}$ | ~4.2 | 4.8–5.3 | | 4.5–5.4 | 4.5–5.4 | 4.3–5.3 | 3.0–3.9 |
| Henry's Law constant at 25°C ($m^3$ atm/mol) | | $8.6 \times 10^{-6} \pm 1.1 \times 10^{-6}$ | $3–4 \times 10^{-5}$[c] | $1.6 \times 10^{-6} \pm 1.9 \times 10^{-7}$ | $4.9 \times 10^{-9} \pm 6.1 \times 10^{-10}$ | $1.5 \times 10^{-7} \pm 1.9 \times 10^{-8}$ | $2.2 \times 10^{-13} \pm 2.8 \times 10^{-14}$ |

[a]Maguire (1999); [b]ranges, averages, and standard deviations determined using four isomers determined using Reinhard and Drefahl (1999); [c]Dachs *et al*. (1999).

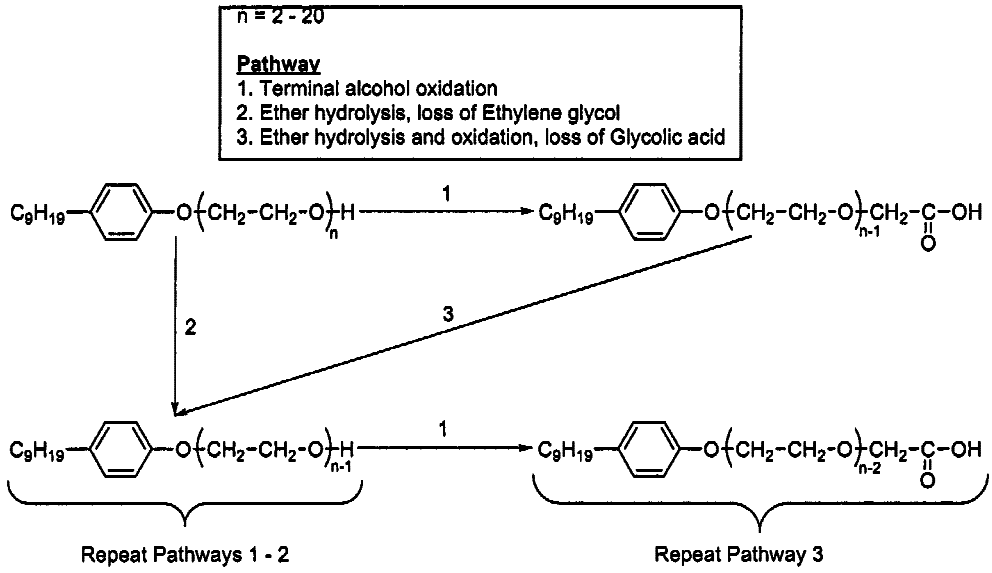


**Figure 4.** Ethoxy side-chain transformation. The exact structure of the alkyl chain is not specified.

biodegradation pathways that lead to the mineralization of APs and carboyxlated APEO metabolites (Fig. 6).

*Ethoxylate chain degradation.* Degradation of the ethoxylate chain appears to be the first APEO biotransformation process. As seen in Fig. 4, transformation of the parent APEO can occur by two pathways: terminal alcohol oxidation, or ether hydrolysis (Fig. 4, pathways 1 and 2, respectively). Because long-chained carboxylated ethoxylates had not been detected, the primary ethoxylate chain shortening pathway for under both aerobic and anaerobic conditions, was believed to be stepwise ether hydrolysis accompanied by the loss of ethylene glycol (Fig. 4, pathway 2). This process proceeds rapidly until short-chained APnEOs ($n$ = 1–3) are formed. As the ethoxy side chain is shortened, APEO metabolite solubility decreases and their persistence increases. Under anaerobic conditions short-chained APnEOs ($n$ = 1–3) are converted to APs (Fig. 6, pathway 8); under aerobic conditions, they are oxidized (Fig. 4, pathway 1) (Thiele *et al.*, 1997; Maguire, 1999). Carboxylation increases both the solubility and persistence of these metabolites (Ahel *et al.*, 1994b).

As with most biological processes, biodegradation

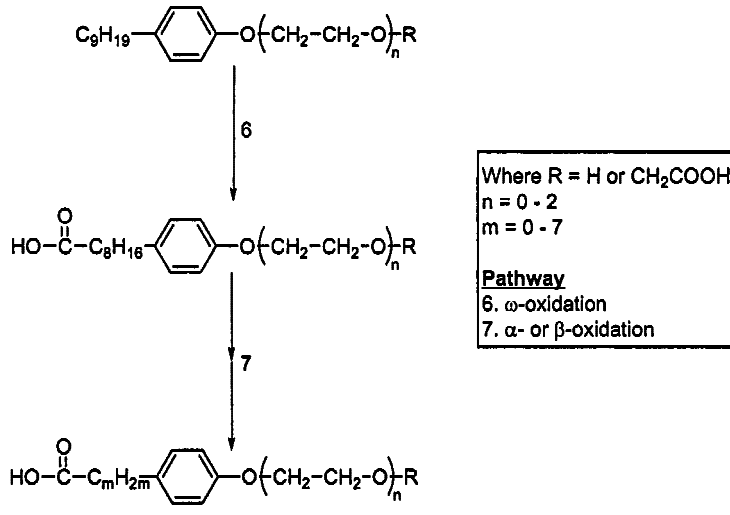


**Figure 5.** Alkyl side-chain transformation. The exact structure of the alkyl chain is not specified.

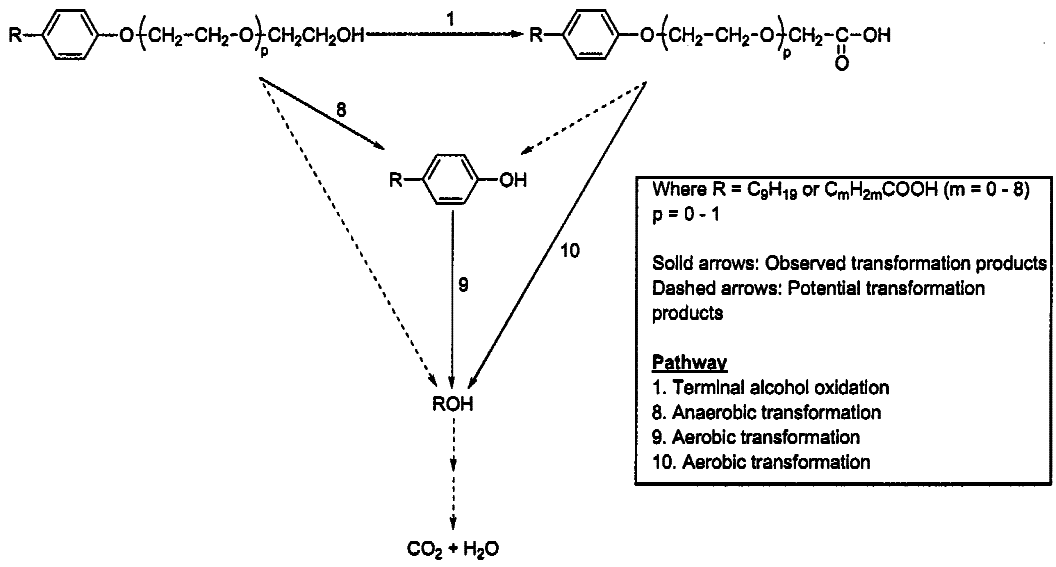


**Figure 6.** Mineralization. The exact structure of the alkyl chain is not specified.

rates are significantly affected by temperature. Generally, higher rates and lower concentrations are observed in the summer, while lower rates and higher concentrations are observed in the winter (Ahel *et al.*, 1996; Maruyama *et al.*, 2000). Recent research indicates that the length of the ethoxy chain also depends on temperature; in rivers around Tokyo, Japan, APEO metabolites generally had between two and five ethoxy units in the summer and between five and eight units in the winter (Maruyama *et al.*, 2000).

Recently, Jonkers *et al.* (2001) concluded that the dominant pathway in aerobic APEO biodegradation is the oxidation of the terminal alcohol on the ethoxy side chain (Fig. 4, pathway 1), not, ether hydrolysis. This conclusion was made because long-chained (up to $n = 15$) APnECs were observed almost immediately upon beginning their experiment. Stepwise shortening of the carboxylated ethoxy chain might proceed by terminal ether hydrolysis (Fig. 4, pathway 3). Because only small amounts of AP2EO were detected, it is plausible that the APEOs observed in the environment are produced in anaerobic microenvironments. The researchers were only able to account for 19% of the initial APEO—this suggests either complete APEO mineralization or the formation of undetected metabolites.

Under anaerobic conditions, the dominant transformation process appears to be stepwise ether cleavage to short-chained ($n = 1$–3) APEOs and APs (Fig. 4, pathway 2). Interestingly, even under strict anaerobic conditions, some researchers have observed substantial oxidation of the ethoxylate chain (Fig. 4, pathway 1) (Ball *et al.*, 1989; Schroder, 2001). Because short-chained APEOs and APs appear to be the ultimate products under anaerobic conditions, it is plausible that ethoxycarboxylates formed, or deposited, under anaerobic conditions, are later transformed by hydrolysis of the terminal ether bond (Fig. 4, pathway 3).

*Alkyl chain degradation.* Although alkyl chain degradation (Fig. 5) can occur concurrently with ethoxy chain shortening, the highly branched nature of this chain often limits the rate and extent of its biodegradation. The initial step in alkyl chain degradation is $\omega$-oxidation (Fig. 5, pathway 6), a process by which a terminal methyl group is converted into a carboxylic acid. After oxidation, the alkyl chain can be shortened by $\alpha$- or $\beta$-oxidation. $\beta$-Oxidation occurs unless there is a methyl group on the $\beta$-carbon (i.e., a tertiary carbon); in these cases, $\alpha$-oxidation takes over (Swisher, 1987). Conceivably, this process could continue until the entire alkyl chain has been degraded. Alkyl chains with quaternary carbon atoms (e.g., OP) are generally very difficult to degrade, as only a few pathways have been documented for the biotransformation of quaternary carbon atoms (Swisher, 1987).

Using a GC/TSQ-MS with CI and EI capabilities, Ding *et al.* (1996) detected 10 dicarboxylated APEO metabolites (i.e., CAPECs) in a WWTP effluent. Another study in Italy found that the dominant APEO metabolites in WWTP effluents are CAPECs (DiCorcia *et al.*, 2000). In these effluents, CA6PECs and CA8PECs (i.e., those having a total of seven and nine carbon atoms in the alkyl chain) accounted for approximately 87% of the CAPEC class. The researchers postulated that these metabolites

were formed by $\omega/\beta$-oxidation and $\omega$-oxidation, respectively, of the alkyl side chain. CA5PECs and CA7PECs were postulated to result from the $\omega/\beta$- and $\omega$-oxidation, respectively, of OPEOs. CA5PECs may also result from the $\omega/\beta$-oxidation of certain NPECs (Fig. 7). CAP2ECs were approximately twice as abundant as CAP1ECs; interestingly, Ding *et al.* (1996) observed that CAP1ECs were more abundant.

Similar results were found in batch aerobic biodegradation studies (DiCorcia *et al.*, 1998). Interestingly, although there was no significant accumulation of NP1EC, a substantial amount of CAP1ECs were formed or during the experiment. Over time, there was a slow disappearance of CAP2ECs and a concurrent increase in CAP1ECs. However, no decreases were noted in total CAPEC concentrations in more than 5 months; this suggests that CAPECs may be very recalcitrant in the environment. However, results from a Soil Aquifer Treatment site in Mesa, AZ, indicate that CAPECs are rapidly transformed during infiltration into the subsurface (Montgomery-Brown *et al.*, 2003). As DiCorcia *et al.* (1998) were unable to account for all the NPEO they added, they postulated the presence of undetected metabolites.

Based on recent results, and due to the lack of selectivity of the early GC and HPLC techniques used to identify APEO metabolites, it is likely that many of the APECs previously identified were, in reality, CAPECs.

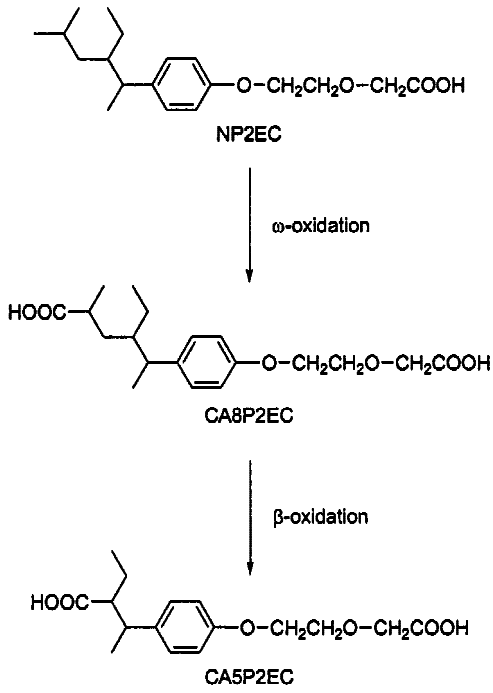


**Figure 7.** One possible $\omega/\beta$-oxidation route for a NP2EC isomer.

*Mineralization.* Evidence for aromatic ring degradation is mounting. For example, during the aerobic degradation of NPEO, Schroder (2001) observed the disappearance of NPEC without the formation of NP or carboxylated alkyl chain metabolites (CAPECs). Potter *et al.* (1999) studied the aerobic static-die away of NPEOs and their metabolites in estuarine water samples. NP2EC was the dominant degradation product and was relatively persistent, but it was eventually transformed; the mechanism whereby the NP2EC was transformed is unknown, but no NP was detected. Other experiments monitored the amount of carbon dioxide produced during the aerobic biodegradation of OP1EC, OP2EC, NP1EC, and NP2EC and determined that only 15 to 35% of the initial carbon was not mineralized (Staples *et al.*, 1999, 2001); these results suggest some biodegradation of the aromatic moiety.

Despite these findings, little effort has been made to detect transformation metabolites or determine the biodegradation pathways. Because of this, our understanding of APEO mineralization in the environment is very limited (Fig. 6). Under aerobic conditions, biodegradation of the aromatic moiety is likely the dominant APEO biotransformation mechanism. For example, Fujita and Reinhard (1997) determined that OP1EC was rapidly transformed in stoichiometric quantities, through ring opening, to 2,4,4-trimethyl-2-pentanol. This alcohol was resistant to further degradation. An aerobic column study by Wild and Reinhard (1999) also observed very rapid OP1EC transformation; OP1EC concentrations decreased by more than 90% in less than 30 min. However, residual OP1EC concentrations (0.3–3 $\mu$g/L) persisted for more than 2 months; the authors suggest that perhaps OP1EC concentrations were too low to support microbial growth. Data indicated that approximately 8.3 mol of dissolved oxygen were required per mol of OP1EC. Because the complete mineralization of 1 mol of OP1EC would require 16.5 mol of dissolved oxygen and no intermediates were detected, it was proposed that the remaining material was incorporated into bacterial biomass (Wild and Reinhard, 1999).

Further evidence for ring-opening reactions came from branched NP degradation studies using a liquid culture of *Sphingomonas* sp. Tanghe *et al.* (1999) found that one isolate began degrading NP by phenyl ring scission and preferentially metabolized *para*-NP over *ortho*-NP isomers. This preferential degradation might explain the prevalence of *ortho*- vs. *para*-isomers observed in sewage sludges by Field and Reed (1999). Between 6.2 and 7.4 mol of oxygen were required per mol of NP oxidized;

these values are similar to those obtained for OP1EC (Fujita and Reinhard, 1997; Wild and Reinhard, 1999). There was no distinct accumulation of products, but the researchers detected various branched 5 to 10 carbon alcohols, ethers, and esters; other than NP, no aromatic compounds were detected.

In contrast, while studying OP biodegradation using inoculums from both rural and urban locations along a U.K. river, Johnson *et al.* (2000) concluded that the alkyl side chain was degraded prior to phenyl ring scission because the rate of OP transformation was greater than the rate of phenyl ring mineralization. In fact, these researchers only observed ring opening once during their experiments. The half-life for OP in aerobic microcosms was between 7 and 50 days; lower degradation rates were observed when using inoculums from rural areas. Their data indicated that OP is not more persistent at low concentrations (~0.5 $\mu$g/L) than at higher concentrations. No degradation was observed in anaerobic bed sediments.

Under anaerobic conditions, many researchers have observed the formation of APs from short-chained APnEOs ($n$ = 1–3) (Fig. 6, pathway 8); it is plausible that short-chained APnECs or CAPnECs ($n$ = 1–3) could also be transformed into APs, but thus far, this has not been observed. Because neither the breakdown of the aromatic moiety nor transformation of the alkyl chain have been observed under anaerobic conditions (Ejlertsson *et al.*, 1999; Johnson *et al.*, 2000; Schroder, 2001), it is believed that APs are the ultimate products anaerobic APEO biodegradation.

### *Photodegradation*

Very little research has focused on the importance of photodegradation in the fate of APEOs, and their metabolites, in the environment. The main factors that influence APEO photodegradation rates are light intensity and the presence of photosensitizers. Early research by (Ahel *et al.*, 1994c) determined that APEO degradation rates decrease substantially with depth. At the surface of the lake, the half-life for NP was between 10 and 15 h; at depths of 20–25 cm [dissolved organic carbon (DOC) ~4 mg-C/L], photodegradation rates were approximately 1.5 times slower. Ahel *et al.* (1994c) also determined that naturally occurring photosensitizers like nitrate, Fe(III)-aquo complexes and humic acids play a major role in the photodegradation of APEOs and their metabolites. For example, the half-life for NP in lake water (DOC ~4 mg-C/L) exposed to a mercury vapor lamp ($\lambda > 280$ nm; intensity ~10× greater than natural sunlight) was approximately 45 min; in distilled water, its half-life was approximately five times longer.

*Photodegradation pathways.* Relatively little is known about the photochemical pathways whereby APEOs, and their metabolites, are degraded in the environment. These pathways are dependent on the photosensitizer and whether the resulting solution is heterogeneous or homogeneous. Early research focused on using titanium dioxide particles to enhance APEO photodegradation rates (Hidaka *et al.*, 1988, 1990; Pelizzetti *et al.*, 1989). The first step in the photodegradation of APEOs appeared to be sorption of the aromatic ring or the ethoxylate chain onto the surface of the titanium oxide. In this system, the surface of the titanium dioxide both facilitated the photoformation of hydroxyl radicals and concentrated these radicals relative to the bulk solution (Pelizzetti *et al.*, 1989). The researchers determined that the next step involved the competitive attack of a hydroxyl radical on the aromatic ring (OH· addition to the ring) and the ethoxylate chain (H abstraction) (Hidaka *et al.*, 1988; Pelizzetti *et al.*, 1989). Several OH· attacks on the aromatic ring were required to effect ring cleavage; ring opening may also occur through the addition of molecular oxygen to a hydroxy cyclohexadienyl radical (Pelizzetti *et al.*, 1989). Complete APEO mineralization was observed (Pelizzetti *et al.*, 1899; Hidaka *et al.*, 1990) without the formation of APs (Pelizzetti *et al.*, 1989).

More recently, researchers have focused on the homogeneous photodegradation of APEOs using trivalent iron (Brand *et al*, 1998; Castillo *et al*, 2001). Trivalent iron was chosen as a photosensitizer because Fe(III)–aquo complexes are very efficient at forming hydroxyl radicals through the following reaction:

$$Fe(OH)^{2+}_{(aq)} + h\,\nu \rightarrow Fe^{2+}_{(aq)} + OH\cdot_{(aq)}$$

The hydroxyl radical produced in this reaction can react with the alkyl chain, the ethoxy chain, or the aromatic ring of APEOs or their metabolites. In contrast with earlier results using titanium dioxide, neither research group observed OH· addition to the aromatic ring. Rather, they observed that the hydroxyl radical preferentially attacked the ethoxy chain, and resulted in shorter chained APEOs. Other intermediates and metabolites observed were: APECs, alkylphenol formate and aldehyde ethoxylates, alkyl chain carboxylated APEOs (CAPEOs), and CAPECs (Brand *et al.*, 1998; Castillo *et al*., 2001). Alkyl chain degradation and ring opening were only observed in the presence of Fe(III) (Castillo *et al.*, 2001); no mechanisms for these transformations were proposed. During prolonged irradiation (~24 h) more than 90% of the initial NPEO was transformed; no degradation products were detected (Brand *et al.*, 1998; Castillo *et al.*, 2001). In the absence of Fe(III), degradation rates were much slower (Castillo *et al.*, 2001).

Because both of the photodegradation mechanisms described above rely on hydroxyl radicals, it should be possible to estimate the importance of these radicals on the

degradation of APEOs and their metabolites in surface waters. However, estimating the half-lives of APEOs and their metabolites using steady-state hydroxyl radical concentrations in surface waters does not support experimental results. This suggests that other factors and processes likely play an important role in the photo-oxidation of APEOs and their metabolites.

## FUTURE RESEARCH NEEDS

Over the past couple of years, our analytical detection capabilities have increased dramatically, allowing the detection and identification of APEO metabolites previously missed. The detection of these metabolites has permitted a greater understanding of APEO degradation in the environment. Future research should concentrate on:

1. Biodegradation of APEOs and their metabolites. Current research is still divided about the ultimate fate of APEOs metabolites in the environment. A better understanding of the biodegradation pathways and the enzymatic regulation of these pathways is necessary. For example, how common are ring-opening pathways? Why are NP and other APEO metabolites persistent under anaerobic conditions when other phenolic compounds are degradable? Can CAPECs be further degraded? Can a single bacterium mineralize APEOs or is a consortium of bacteria required? Why do low concentrations of APEO metabolites persist in the environment?
2. Photodegradation of APEOs and their metabolites. Further research is necessary to determine whether photodegradation plays an important role in the attenuation of APEOs and their metabolites in the environment, and if so, to determine the pathways.
3. Analytical detection capabilities. The lack of mass balances in several experiments and the detection of CAPECs only 6 years ago, suggests that there are perhaps other metabolites that have yet to be detected. Further improvements to analytical detection techniques would lower detection levels, improving accuracy, and perhaps permit researchers to determine whether APEOs and their metabolites are ubiquitous in the environment.
4. Attempts should be made to synthesize isomeric standards for APEOs and their metabolites. These standards could then be used to determine the physical properties of each metabolite, in the analytical detection and positive identification of APEO metabolites, and in structure–activity estrogenic studies.
5. Estrogenicity of APEOs and their metabolites. There is still debate about the estrogenicity of these compounds and whether they are responsible for the symptoms of endocrine disruption observed in wild fish.
6. Environmental occurrence of APEOs and their metabolites. Only a few studies have looked for halogenated APEO metabolites. Because these metabolites can be formed during the chlorination of WWTP effluents, their environmental occurrence could be high.

## ACKNOWLEDGMENTS

Funding was provided by the Environmental Protection Agency (EPA) and American Water Works Association Research Foundation (AWWARF) through a grant to the National Center for Sustainable Water Supply at Arizona State University in Tempe, AZ.

## REFERENCES

AHEL, M., GIGER, W., and KOCH, M. (1994a). Behavior of alkylphenol polyethoxylate surfactants in the aquatic environment. 1. Occurrence and transformation in sewage-treatment. *Water Res.* **28**(5), 1131–1142.

AHEL, M., GIGER, W., MOLNAR, E., and IBRIC, S. (2000). Determination of nonylphenol polyethoxylates and their lipophilic metabolites in sewage effluents by normal-phase high-performance liquid chromatography and fluorescence detection. *Croatica Chem. Acta* **73**(1), 209–227.

AHEL, M., HRSAK, D., and GIGER, W. (1994b). Aerobic transformation of short-chain alkylphenol polyethoxylates by mixed bacterial cultures. *Arch. Environ. Contaminat. Toxicol.* **26**(4), 540–548.

AHEL, M., McEVOY, J., and GIGER, W. (1993). Bioaccumulation of the lipophilic metabolites of nonionic surfactants in fresh-water organisms. *Environ. Pollut.* **79**(3), 243–248.

AHEL, M., SCHAFFNER, C., and GIGER, W. (1996). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment. 3. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. *Water Res.* **30**(1), 37–46.

AHEL, M., SCULLY, F.E., HOIGNE, J., and GIGER, W. (1994c). Photochemical degradation of nonylphenol and nonylphenol polyethoxylates in natural-waters. *Chemosphere* **28**(7), 1361–1368.

BALL, H.A., REINHARD, M., and MCCARTY, P.L. (1989). Biotransformation of halogenated and nonhalogenated octylphenol polyethoxylate residues under aerobic and anaerobic conditions. *Environ. Sci. Technol.* **23**(8), 951–961.

BALL, H.A. and REINHARD, M., Ed. (1985). *Water Chlorination: Chemistry, Environmental Impact, and Health Effects.* Chelsea, MI: Lewis Publishers.

BENNIE, D.T. (1999). Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates. *Water Qual. Res. J. Can.* **34**(1), 79–122.

BRAND, N., MAILHOT, G., and BOLTE, M. (1998). Degradation photoinduced by Fe(III): Method of alkylphenol ethoxylates removal in water. *Environ. Sci. Technol.* **32**(18), 2715–2720.

CASTILLO, M., PENUELA, G., and BARCELO, D. (2001). Identification of photocatalytic degradation products of nonionic polyethoxylated surfactants in wastewaters by solid-phase extraction followed by liquid chromatography-mass spectrometric detection. *Fresenius J. Anal. Chem.* **369**(7–8), 620–628.

DACHS, J., VANRY, D.A., AND EISENREICH, S.J. (1999). Occurrence of estrogenic nonylphenols in the urban and coastal atmosphere of the lower Hudson River estuary. *Environ. Sci. Technol.* **33**(15), 2676–2679.

DICORCIA, A., CAVALLO, R., CRESCENZI, C., and NAZZARI, M. (2000). Occurrence and abundance of dicarboxylated metabolites of nonylphenol polyethoxylate surfactants in treated sewages. *Environ. Sci. Technol.* **34**(18), 3914–3919.

DICORCIA, A., COSTANTINO, A., CRESCENZI, C., MARINONI, E., and SAMPERI, R. (1998). Characterization of recalcitrant intermediates from biotransformation of the branched alkyl side chain of nonylphenol ethoxylate surfactants. *Environ. Sci. Technol.* **32**(16), 2401–2409.

DING, W.H., FUJITA, Y., AESCHIMANN, R., and REINHARD, M. (1996). Identification of organic residues in tertiary effluents by GC/EI-MS; GC/CI-MS and GC/TSQ-MS. *Fresenius J. Anal. Chem.* **354**(1), 48–55.

DING, W.H., and TZING, S.H. (1998). Analysis of nonylphenol polyethoxylates and their degradation products in river water and sewage effluent by gas chromatography ion trap (tandem) mass spectrometry with electron impact and chemical ionization. *J. Chromatogr. A* **824**(1), 79–90.

EJLERTSSON, J., NILSSON, M.L., KYLIN, H., BERGMAN, A., KARLSON, L., et al. (1999). Anaerobic degradation of nonylphenol mono- and diethoxylates in digestor sludge, landfilled municipal solid waste, and landfilled sludge. *Environ. Sci. Technol.* **33**(2), 301–306.

FERGUSON, P.L., IDEN, C.R., and BROWNAWELL, B.J. (2000). Analysis of alkylphenol ethoxylate metabolites in the aquatic environment using liquid chromatography-electrospray mass spectrometry. *Anal. Chem.* **72**(18), 4322–4330.

FERGUSON, P.L., IDEN, C.R., and BROWNAWELL, B.J. (2001). Distribution and fate of neutral alkylphenol ethoxylate metabolites in a sewage-impacted urban estuary. *Environ. Sci. Technol.* **35**(12), 2428–2435.

FERRARA, F., FABIETTI, F., DELISE, M., BOCCA, A.P., and FUNARI, E. (2001). Alkylphenolic compounds in edible molluscs of the Adriatic Sea (Italy). *Environ. Sci. Technol.* **35**(15), 3109–3112.

FIELD, J.A., and REED, R.L. (1999). Subcritical (hot) water/ethanol extraction of nonylphenol polyethoxy carboxylates from industrial and municipal sludges. *Environ. Sci. Technol.* **33**(16), 2782–2787.

FUJITA, Y., and REINHARD, M. (1997). Identification of metabolites from the biological transformation of the nonionic surfactant residue octylphenoxyacetic acid and its brominated analog. *Environ. Sci. Technol.* **31**(5), 1518–1524.

FUJITA, Y., DING, W.H., and REINHARD, M. (1996). Identification of wastewater dissolved organic carbon characteristics in reclaimed wastewater and recharged groundwater. *Water Environ. Res.* **68**(5), 867–876.

GUENTHER, K., HEINKE, V., THIELE, B., KLEIST, E., PRAST, H., *et al.* (2002). Endocrine disrupting nonylphenols are ubiquitous in food. *Environ. Sci. Technol.* **36**(8), 1676–1680.

GUNDERSEN, J.L. (2001). Separation of isomers of nonylphenol and select nonylphenol polyethoxylates by high-performance liquid chromatography on a graphite carbon column. *J. Chromatogr. A* **914**(1–2), 161–166.

HALE, R.C., SMITH, C.L., DEFUR, P.O., HARVEY, E., and BUSH, E.O. (2000). Nonylphenols in sediments and effluents associated with diverse wastewater outfalls. *Environ. Toxicol. Chem.* **19**(4/pt. 1), 946–952.

HARRIES, J.E., SHEAHAN, D.A., JOBLING, S., MATTHIESSEN, P., NEALL, M., *et al.* (1997). Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environ. Toxicol. Chem.* **16**(3), 534–542.

HARRIS, C.A., SANTOS, E.M., JANBAKHSH, A., POTTINGER, T.G., TYLER, C.R., *et al.* (2001). Nonylphenol affects gonadotropin levels in the pituitary gland and plasma of female rainbow trout. *Environ. Sci. Technol.* **35**(14), 2909–2916.

HIDAKA, H., IHARA, K., FUJITA, Y., YAMADA, S., PELIZZETTI, E., *et al.* (1988). Photodegradation of surfactants. 4. Photodegradation of non-ionic surfactants in aqueous titanium-dioxide suspensions. *J. Photochem. Photobiol. A-Chem* **42**(2–3), 375–381.

HIDAKA, H., YAMADA, S., SUENAGA, S., ZHAO, J., SERPONE, N., *et al.* (1990). Photodegradation of surfactants. 6. Complete photocatalytic degradation of anionic; cationic and nonionic surfactants in aqueous semiconductor dispersions. *J. Mol. Catal.* **59**(3), 279–290.

JOBLING, S., NOLAN, M., TYLER, C.R., BRIGHTY, G., and SUMPTER, J.P. (1998). Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* **32**(17), 2498–2506.

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. *Environ. Toxicol. Chem.* **15**(2), 194–202.

JOHNSON, A.C., and SUMPTER, J.P. (2001). Removal of endocrine-disrupting chemicals in activated sludge treatment works. *Environ. Sci. Technol.* **35**(24), 4697–4703.

JOHNSON, A.C., WHITE, C., BHARDWAJ, L., and JURGENS, M.D. (2000). Potential for octylphenol to biodegrade in some English rivers. *Environ. Toxicol. Chem.* **19**(10), 2486–2492.

JONKERS, N., KNEPPER, T.P., and DE VOGT, P. (2001). Aerobic biodegradation studies of nonylphenol ethoxylates in river water using liquid chromatography-electrospray tandem mass spectrometry. *Environ. Sci. Technol.* **35**(2), 335–340.

KHIM, J.S., KANNAN, K., VILLENEUVE, D.L., KOH, C.H., and GIESY, J.P. (1999). Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. Instrumental analysis. *Environ. Sci. Technol.* **33**(23), 4199–4205.

KHIM, J.S., LEE, K.T., KANNAN, K., VILLENEUVE, D.L., GIESY, J.P., *et al.* (2001a). Trace organic contaminants in sediment and water from Ulsan Bay and its vicinity, Korea. *Arch. Environ. Contamin. Toxicol.* **40**(2), 141–150.

KHIM, J.S., LEE, K.T., VILLENEUVE, D.L., KANNAN, K., GIESY, J.P., *et al.* (2001b). In vitro bioassay determination of dioxin-like and estrogenic activity in sediment and water from Ulsan Bay and its vicinity, Korea. *Arch. Environ. Contamin. Toxicol.* **40**(2), 151–160.

KOLPIN, D.W., FURLONG, E.T., MEYER, M.T., THURMAN, E.M., ZAUGG, S.D., *et al.* (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: A national reconnaissance. *Environ. Sci. Technol.* **36**(6), 1202–1211.

KORNER, W., SPENGLER, P., BOLZ, U., SCHULLER, W., HANF, V., *et al.* (2001). Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 2. Biological analysis. *Environ. Toxicol. Chem.* **20**(10), 2142–2151.

KUCH, H.M., and BALLSCHMITER, K. (2001). Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. *Environ. Sci. Technol.* **35**(15), 3201–3206.

LA GUARDIA, M.J., HALE, R.C., HARVEY, E., and MAINOR, T.M. (2001). Alkylphenol ethoxylate degradation products in land-applied sewage sludge (biosolids). *Environ. Sci. Technol.* **35**(24), 4798–4804.

LEE, H.B. (1999). Review of analytical methods for the determination of nonylphenol and related compounds in environmental samples. *Water Qual. Res. J. Can.* **34**(1), 3–35.

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. *Environ. Sci. Technol.* **33**(7), 1090–1014.

MAGUIRE, R.J. (1999). Review of the persistence of nonylphenol and nonylphenol ethoxylates in aquatic environments. *Water Qual. Res. J. Can.* **34**(1), 37–78.

MARUYAMA, K., YUAN, M., and OTSUKI, A. (2000). Seasonal changes in ethylene oxide chain length of poly(oxyethylene)alkylphenyl ether nonionic surfactants in three main rivers in Tokyo. *Environ. Sci. Technol.* **34**(2), 343–348.

METCALFE, C.D., METCALFE, T.L., KIPARISSIS, Y., KOENIG, B.G., KHAN, C., *et al.* (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). *Environ. Toxicol. Chem.* **20**(2), 297–308.

MONTGOMERY-BROWN, J., DREWES, J., FOX, P., and REINHARD, M. (2003). Behavior of alkylphenol polyethoxylate metabolites during soil aquifer treatment. Wat. Res. **37,** 3672–3681.

NAYLOR, C.G. (1995). Environmental fate and safety of nonylphenol ethoxylates. *Textile Chemist Colorist* **27**(4), 29–33.

NICHOLS, K.M., SNYDER, E.M., SNYDER, S.A., PIERENS, S.L., MILES-RICHARDSON, S.R., *et al.* (2001). Effects of nonylphenol ethoxylate exposure on reproductive output and bioindicators of environmental estrogen exposure in fathead minnows, *Pimephales promelas. Environ. Toxicol. Chem.* **20**(3), 510–522.

PELIZZETTI, E., MINERO, C., MAURINO, V., SCLAFANI, A., HIDAKA, H., *et al.* (1989). Photocatalytic degradation of nonylphenol ethoxylated surfactants. *Environ. Sci. Technol.* **23**(11), 1380–1385.

PETROVIC, M., DIAZ, A., VENTURA, F., and BARCELO, D. (2001). Simultaneous determination of halogenated derivatives of alkylphenol ethoxylates and their metabolites in sludges, river sediments, and surface, drinking, and wastewaters by liquid chromatography-mass spectrometry. *Anal. Chem.* **73**(24), 5886–5895.

POTTER, T.L., SIMMONS, K., WU, J.N., SANCHEZOLVERA, M., KOSTECKI, P., *et al.* (1999). "Static die-away of a nonylphenol ethoxylate surfactant in estuarine water samples." *Environ. Sci. Technol.* **33**(1), 113–118.

REINHARD, M., and DREFAHL, A. (1999). Toolkit for estimating physiochemical properties of organic compounds. New York: John Wiley and Sons, Inc.

REINHARD, M., GOODMAN, N., and MORTELMANS, K.E. (1982). Occurrence of brominated alkylphenol polyethoxy carboxylates in mutagenic wastewater concentrates. *Environ. Sci. Technol.* **16**(6), 351–362.

RODGERS-GRAY, T.P., JOBLING, S., KELLY, C., MORRIS, S., BRIGHTY, G., *et al.* (2001). Exposure of juvenile roach (*Rutilus rutilus*) to treated sewage effluent induces dose-dependent and persistent disruption in gonadal duct development. *Environ. Sci. Technol.* **35**(3), 462–470.

ROUTLEDGE, E.J., SHEAHAN, D., DESBROW, C., BRIGHTY,

G.C., WALDOCK, M., *et al.* (1998). Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. *Environ. Sci. Technol.* **32**(11), 1559–1565.

RUDEL, R.A., MELLY, S.J., GENO, P.W., SUN, G., and BRODY, J.G. (1998). Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage, and groundwater on Cape Cod, Massachusetts. *Environ. Sci. Technol.* **32**(7), 861–869.

SCHRODER, H.F. (2001). Tracing of surfactants in the biological wastewater treatment process and the identification of their metabolites by flow injection-mass spectrometry and liquid chromatography-mass spectrometry and -tandem mass spectrometry. *J. Chromatogr. A* **926**(1), 127–150.

SERVOS, M.R. (1999). Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. *Water Qual. Res. J. Can.* **34**(1), 123–177.

SHANG, D.Y., MACDONALD, R.W., and IKONOMOU, M.G. (1999). Persistence of nonylphenol ethoxylate surfactants and their primary degradation products in sediments from near a municipal outfall in the strait of Georgia; British Columbia; Canada. *Environ. Sci. Technol.* **33**(9), 1366–1372.

SOTO, A.M., JUSTICIA, H., WRAY, J.W., and SONNENSCHEIN, C. (1991). *p*-Nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. *Environ. Health Perspect.* **92,** 167–173.

SPENGLER, P., KORNER, W., and METZGER, J.W. (2001). Substances with estrogenic activity in effluents of sewage treatment plants in southwestern Germany. 1. Chemical analysis. *Environm. Toxicol. Chem.* **20**(10), 2133–2141.

STAPLES, C.A., NAYLOR, C.G., WILLIAMS, J.B., and GLEDHHILL, W.E. (2001). Ultimate biodegradation of alkylphenol ethoxylate surfactants and their biodegradation intermediates. *Environ. Toxicol. Chem.* **20**(11), 2450–2455.

STAPLES, C.A., WEEKS, J., HALL, J.F., and NAYLOR, C.G. (1998). Evaluation of aquatic toxicity and bioaccumulation of C8- and C9-alkylphenol ethoxylates. *Environ. Toxicol. Chem.* **17**(12), 2470–2480.

STAPLES, C.A., WILLIAMS, J.B., BLESSING, R.L., and VARINEAU, P.T. (1999). Measuring the biodegradability of nonylphenol ether carboxylates, octylphenol ether carboxylates, and nonylphenol. *Chemosphere* **38**(9), 2029–2039.

SUMPTER, J.P., and JOBLING, S. (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ. Health Perspect.* **103**(S7), 173–178.

SWISHER, R.D. (1987). *Surfactant Biodegradation,* 2nd ed. New York: M. Dekker.

TALMAGE, S.S. (1994). *Environmental and Human Safety of Major Surfactants: Alcohol Ethoxylates and Alkylphenol Ethoxylates.* Boca Raton, FL: Lewis Publishers.

TANGHE, T., DHOOGE, V., and VERSTRAETE, W. (1999). Isolation of a bacterial strain able to degrade branched nonylphenol. *Appl. Environ. Microbiol.* **65**(2), 746–751.

THIELE, B., GUNTHER, K., and SCHWUGER, M.J. (1997). Alkylphenol ethoxylates: Trace analysis and environmental behavior. *Chem. Rev.* **97**(8), 3247–3272.

THORPE, K.L., HUTCHINSON, T.H., HETHERIDGE, M.J., SUMPTER, J.P., and TYLER, C.R. (2000). Development of an *in vivo* screening assay for estrogenic chemicals using juvenile rainbow trout. (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **19**(11), 2812–2820.

WHEELER, T.F., HEIM, J.R., LATORRE, M.R., and JANES, A.B. (1997). Mass spectral characterization of p-nonylphenol isomes using high-resolution capillary GC-MS. *J. Chromatogr. Sci.* **35**(1), 19–30.

WHITE, R., JOBLING, S., HOARE, S.A., SUMPTER, J.P., and PARKER, M.G. (1994). Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* **135**(1), 175–182.

WILD, D., and REINHARD, M. (1999). Biodegradation residual of 4-octylphenoxyacetic acid in laboratory columns under groundwater recharge conditions. *Environ. Sci. Technol.* **33**(24), 4422–4426.