

**A critical review of the formation of mono and dicarboxylated  
metabolic intermediates of alkylphenol polyethoxylates during  
wastewater treatment and their environmental significance**

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*Alkylphenoxyacetic acids, the metabolic biodegradation products of alkylphenol ethoxylates, are commonly found in wastewaters and sewage effluents. These persistent hydrophilic derivatives possess intrinsic estrogenic activity which can mimic natural hormones. Their concentrations increase through the sewage treatment works as a result of biodegradation and biotransformation and when discharged, can disrupt endocrine function in fish. These acidic metabolites represent the dominant alkylphenolic compounds found in wastewater effluent and their presence is cause for concern as, potentially through further biotransformation and biodegradation, they can act as sources of nonylphenol which is toxic and estrogenic. The review aims to assess the mechanisms of formation as well as elimination of alkylphenoxyacetic acids within conventional sewage treatment works with the emphasis on the activated sludge process. In addition, it evaluates the various factors influencing their degradation and formation*

*in both laboratory scale and full scale systems. The environmental implications of these compounds are considered as is the need for tertiary treatment processes for their removal.*

## **1. INTRODUCTION**

Alkylphenol ethoxylates (APEOs), introduced in the 1940s, are the second largest group of nonionic surfactants in commercial and industrial use. They are incorporated as additives in detergents, pesticide formulations, dispersing agents for wool scouring, hydrogen peroxide bleaching and dyeing processes. The worldwide production of APEOs was estimated at 500,000 tons in 1997 of which 80% were nonylphenol ethoxylates (NPEO) and 20% were octylphenol ethoxylates (OPEO) (Renner, 1997). Production of APEOs in Western Europe has declined between 2000 to 2002 from 116,000 to 83,000 tons respectively (Cefic, 2002). The usage of NPEO in the U.S. was approximately 130,600 tons in 2006 (ICIS Chemical Business Americas, 2007) whilst the consumption of these compounds in the growing Asian economies is expected to rise including an increased demand for nonylphenol (NP) in India. The Indian Union Commerce Ministry's statistics show that the importation of NP (and similar chemicals and their isomers) have grown from 818 tonnes in 2000, to 23,843 tonnes in 2006. Most, if not all, of NP would have been used in NPEO production to contribute to the estimated annual NPEOs consumption of between 40,000 and 44,000 tonnes in India (Dutta, 2008).

As a result of their use, these commercial APEOs, consisting of a complex mixture of ethoxy homologues and alkyl isomers (European Commission, 2002, 2003; Petrovic et

al., 2002a; Knepper et al., 2003) are discharged to sewage treatment works (STWs) or released directly into the aquatic environment (Gibson et al., 2005; Koh et al., 2005; Langford et al., 2004, 2005a). After secondary wastewater treatment usually more than 95 % of the complex mixtures are degraded to various stable and persistent metabolites such as short chain APEOs i.e. mono- and di-ethoxylates (AP<sub>1</sub>EO and AP<sub>2</sub>EO), alkylphenols (APs), alkylphenoxyacetic acids i.e. carboxyalkylphenol polyethoxycarboxylates (CAPECs) and alkylphenol ethoxycarboxylates (APECs) (Fig 1) which are frequently detected in various water bodies of Europe, North America, Japan and Asia (Table 1).

*Please insert Fig 1 here*

These metabolites are recognised as endocrine disruptors and unlike their parent compounds are also toxic to both marine and freshwater species (Comber et al., 1993; Jobling and Sumpter, 1993; McLeese et al., 1981; Purdom et al., 1994). These effects are dose dependent, as NP can cause intersexuality at “high” concentrations (concentrations exceeding 1 µg l<sup>-1</sup> in *in vivo* studies) (Gray and Metcalfe, 1997; Johnson and Sumpter, 2001), but not at low concentrations (Nimrod and Benson, 1998; Sumpter, 2002).

As a consequence of poor degradability and toxicity of these metabolites, APEOs have been replaced in household applications in most western countries with the exception of the U.S. and Japan, mainly by alcohol ethoxylates (Loos et al., 2007). Throughout northern Europe (Scandinavian countries, U.K. and Germany) a voluntary ban on APEO

use in household cleaning products began in 1995, and restrictions on industrial cleaning applications in 2000 (Renner et al., 1997). Furthermore, the European Union passed in 2003, an amendment to directive 76/769/EC; the marketing and use directive for NPEOs which was implemented into national laws of each EU member country by 2005. The directive states that NP and NPEO “may not be placed on the market or used as a substance or constituent of preparations in concentrations equal or higher than 0.1 % by mass”. This directive applies to most of the industrial applications including many of its uses in industrial cleaning, textile industries and in metal working. However, mainly because of lower production costs, APEOs are still being used in southern European countries in substantial amounts in institutional and industrial applications. There are no regulatory restrictions on the manufacture, processing or use of NPEOs in the U.S. (APEREC, 2004). Some efforts have been implemented by the U.S. Environment Protection Agency to encourage and recognise companies, facilities and others who voluntarily phase out or commit to phasing out the manufacture or use of NPEOs under the Safer Detergents Stewardship Initiative which is designed to protect aquatic life.

Despite the legal and voluntary ban on APEOs in the EU, these compounds are still found in some wastewater effluents, especially in industrialised regions. This is because the use of APEOs in some industrial applications is not restricted. In Catalonia, a heavily industrialized area in the northeast of Spain, industries (i.e. leather tanning, textile, pulp and paper industries) use these surfactants in substantial amounts and as a result they are discharged into the municipal sewer systems to be treated in municipal STW together with urban wastewaters (Petrovic et al., 2007). In these effluents, APECs were found to

be the dominant metabolites at  $250 \mu\text{g l}^{-1}$ . The dominance of these carboxylic metabolites including CAPECs is also observed in various aquatic environments i.e. in biologically treated wastewater, receiving rivers and the marine environment (Ahel et al., 1994b; Field and Reed, 1996; Marcomini et al., 1990; Ding and Chen, 1999; Ding and Tzing, 1998; Ding et al., 1999; Gross et al., 2004). As much as 66% and 63% of the total APEO metabolites detected in Italian sewage effluents were APECs and CAPECs (Di Corcia et al., 1998a, 2000). Table 1 summarises the concentrations of these metabolites found by some of the major worldwide investigations.

*Please insert Table 1 here.*

The high concentrations of APEC and CAPEC residues detected in the aqueous phase in environmental samples reveal that these metabolites are polar and recalcitrant to further biotransformation (Johnson and Sumpter, 2001; Hoai et al., 2004). These properties allow the metabolites, whose concentrations increase through STWs as a result of biodegradation and biotransformation to remain in the effluent of STWs and resist removal. It was observed that CAPEC residues persisted in an aliquot of effluent inoculated with microorganisms from an activated sludge STW for more than five months after their generation (DiCorcia et al., 1998a). Due to their recalcitrance and their hydrophilic character, they are also among the most frequently detected contaminants by the United States Geological Survey in surface waters (Kolpin et al., 2002).

Several workers have expressed concerns about these metabolites persisting in aquatic (Shang et al., 1999) and surficial marine sediments (Ferguson et al., 2000, 2001) where they may undergo remobilization and bioaccumulate into the food chain (Ferguson et al., 2000). A recent study showed that nonylphenol polyethoxy carboxylates (NPECs) contributed to 33.7 % of the levels of nonylphenolics found in the tissue of benthic invertebrates (Mayer et al., 2007). In Japan, it has been reported that the concentrations of NPECs in sewage effluents and rivers could be one or two orders of magnitude higher than those of NP (Isobe and Takada, 2004) suggesting that NPECs might be more important than NP in terms of endocrine disruption on the basis that the relative estrogenic potency of NPECs to NP is 0.63 (Servos et al., 2000).

Furthermore, through further biotransformation or biodegradation and during wastewater chlorination, carboxylated alkylphenoxy ethoxylates (CAPECs) can act potentially as sources of AP, short chain APEOs and halogenated APECs (such as Br-APECs, or Cl-APECs) which are estrogenic to fish and other aquatic organisms (Kinae et al., 1981; Reinhard et al., 1982; Ball et al., 1989; Ahel et al., 1994a, 1994b; Jobling et al., 1996). The formation of halogenated derivatives of the alkylphenols and acidic alkylphenols, mostly brominated compounds, were reported at  $\mu\text{g l}^{-1}$  levels in wastewater effluent and receiving river water after disinfection with chlorine in the presence of bromide ions in the wastewater treatment plant (Kinae et al., 1981; Reinhard et al., 1982; Ventura et al., 1988; Fujita et al., 2000). The toxicity and estrogenicity of halogenated APECs were found to retain a significant affinity for the estrogen receptors in *in vitro* tests and their

acute toxicity to *Daphnia magna* was higher than their nonbrominated precursors APEOs and APECs (Maki et al., 1998; Garcia-Reyero et al., 2004).

Detailed studies of these compounds are hindered by the lack of commercially available authentic standards (Montgomery-Brown and Reinhard, 2003) and the nature of both APECs and CAPECs. They partially coelute when using gas chromatography and their mass spectra share many features. This is a consequence of the complex branching pattern of the nonyl group in nonylphenol polyethoxylates which form numerous isomers that are difficult to separate and quantify. To date, CAPEC residues have been determined semi-quantitatively by comparing their summed selected ions with the base ion of a specific internal standard (Ding and Chen, 1999; Ding and Tzing, 1998; Ding et al., 1999, 1996; Montgomery-Brown et al., 2003), or through biodegradation test of APEOs (DiCorcia et al., 1998a, 2000) and NP<sub>2</sub>EC (nonylphenoethoxy carboxylates) (Jonkers et al., 2001). The identification and quantitation of CAPECs so far has been uncertain and subject to inaccuracies. Due to these difficulties, few reports have appeared concerning the abundance of both APECs and CAPECs and how APECs are transformed into CAPECs under environmental conditions.

The relatively high concentration, environmental persistence, hydrophilic nature and the potencies of these carboxylated APEOs suggest that APECs might be more important than APs in terms of endocrine disruption and hence have motivated on-going efforts to identify them. However, little is known about the occurrence and fate in the aquatic environment of APECs and CAPECs due to lack of commercially available authentic

standards. This review intends to assess and elucidate the mechanisms of formation as well as the elimination of the carboxylated alkylphenol ethoxylates within conventional STWs with emphasis on the activated sludge process. Concomitantly it aims to extend our knowledge by filling the void regarding their behaviour and fate within STWs, thus providing a significant tool to the wastewater industry treating and managing this particular class of endocrine disrupting chemicals (EDC) within STWs.



## 2. FACTORS INFLUENCING FORMATION AND DEGRADATION

### 2.1 *Small laboratory studies*

#### 2.1.1. “Abiotic” versus “Abiological”

Light dependence NPEC formation was reported by Wang et al (2006). Negligible concentrations of NPECs were found in the dark under particle free, sterile conditions whilst the production of NPECs under sterile conditions in the presence of light was  $24.7 \text{ nmol l}^{-1}$  and  $6.39 \times 10^2 \text{ nmol l}^{-1}$  at 12 h and 120 h respectively. The abiological production of NPECs was believed to arise from the photo-(catalytic) oxidation of NPEOs, as many compounds present in natural waters can act as photosensitizers like humic acids, Fe(III)-aquo complexes and nitrates (Ahel et al., 1994d). These compounds can participate in photocatalytic reactions and have a strong oxidation ability towards organic pollutants (Haag and Hoigne, 1985; Zepp et al., 1985; Mansour et al., 1997; Fukushima and Tatsumi., 2001). The half life of NP in lake water exposed to a mercury vapour lamp (intensity 10 times greater than natural sunlight) was approximately 45 minutes; in distilled water, its half life was approximately five times longer. Another factor to be taken into consideration in the photodegradation rate of APEOs is the light intensity. It was demonstrated that at depths of 20-25 cm, photodegradation rates were approximately 1.5 times slower than rates observed at the surface which was reported to be between 10 and 15 hours for NP (Ahel et al., 1994d).

In the presence of microorganisms, NPECs production increased after 12 h and 120 h to  $1.06 \times 10^2$  and  $8.81 \times 10^2 \text{ nmol l}^{-1}$  respectively (Wang et al., 2006). The formation rates increased by 4.3 and 1.3 times over the corresponding test periods. The increased NPECs

concentrations was attributed to the influence of the microorganism; as the production of NPECs under particle free, non-sterile and sterile conditions was much less compared to the amount of NPEOs degradation especially in non-sterile treatment. These authors suggest that either NPEC is further degraded relatively quickly, or that other degradation pathways exist. This is unsurprising as the NPEO degradation pathway does not necessarily produce NPECs as shown in Fig 2. The rates of NPEC formation inferred from the data from Wang et al (2006) where NPEC forms at a faster rate in the presence of microorganisms compared to the rates observed due to photolysis, then biodegradation as a breakdown mechanism is more significant than photolysis.

#### 2.1.2. Suspended Particle Matter

In a laboratory scale study to ascertain the influence of inorganic suspended particulate matter, Wang et al. (2006) observed decreased NPEC concentrations in the presence of these suspended inorganic particles. The presence of suspended particles is thought to encourage a possible shielding effect to light which limits the photo-oxidation and also allows some NPECs to associate with the solid phase (Fukushima and Tatsumi, 2001), although the partition coefficient values of NPECs are quite low. Additionally, they found that the adsorption of NPEOs to the inorganic particulates hindered its bioavailability to form NPECs. The impact of hindered bioavailability within the biological mixed liquors in the activated sludge process arising from inorganic particulate matter may not be significant since these particles are present in small amounts (typically less than 10 % of the biomass).

### 2.1.3. Effect of Organic Matter

Recent surveys conducted in Japan on aquatic environments demonstrated the occurrence of long-chain carboxylates in a relatively polluted river water, suggesting the involvement of biochemical oxygen demand (BOD) constituents (Ito et al., 2002). These studies indicated that BOD constituents may contribute to determining the degradation route of APEOs. As such, Hayashi et al. (2005) investigated the effect of organic matter such as yeast, glucose and methanol on the biodegradation of NP<sub>n</sub>EOs and their metabolites under a modified OECD 301E biodegradation test protocol. When organic matter was absent, small amounts of short chained NPECs were detected which arose from the oxidation of the short chained NPEOs. Biodegradation tests in the presence of organic matter as a carbon source using NP<sub>2</sub>EO, NP<sub>3</sub>EO, NP<sub>5</sub>EO and NP<sub>10</sub>EO as precursors resulted in the formation of the corresponding NPEOs-carboxylates i.e. NP<sub>2</sub>EC, NP<sub>3</sub>EC, NP<sub>5</sub>EC and NP<sub>10</sub>EC. This suggests that oxidation is independent of EO chain length. The NP<sub>10</sub>EC formed 85 % of the original concentration (molar based) on the 15th day. The biodegradation of NPEOs and its corresponding formation of NPECs in the presence of low growth bacterial population led Hayashi et al. (2005) to suggest that the NPECs may be generated by cometabolism of NPEOs when methanol is being utilised oxidatively as the sole carbon source. Several other workers have reported that increasing the amount of yeast extract enhanced the transformation of NP<sub>2</sub>EO to NP in cultured medium (Liu et al., 2006) and that of NP and NP<sub>1</sub>EO in river sediment (Fujii et al., 2000). Ushida et al. (2003) also described that a novel NP-degrading *Sphingobium amiense* sp. nov. could degrade NP in the presence of an organic nutrient such as yeast extract but could not use NP as a carbon source. An appropriate addition of

supplementary nutrients seems to be indispensable for promoting degradation; such supplementation corresponds to a biostimulation technique in the bioremediation of pollutants *in situ*.

## 2.2 Full scale studies

### 2.2.1. Unit treatment process type

The type of secondary treatment used at a STWs is important in determining the relative concentrations of the NPEO parent components and their metabolites present in effluent. Concentrations of  $NP_{1-3}EO > NPEC$  indicate that the predominant degradation route has been under anaerobic conditions, while if  $NP_{1-3}EO < NPEC$  then this would indicate that aerobic degradation is predominant (Tarrant et al., 2005, Barber et al., 2000; Petrovic and Barcelo, 2001).

Samples taken from several municipal sewage treatment works in Switzerland were analysed and various degrees of elimination for nonionic surfactants were observed (Giger et al., 1987). In general, nonylphenolic compounds were removed efficiently by the activated sludge treatment with increased carboxylated metabolite concentrations in all plants. The STW with the highest  $NP_{3-20}EO$ s,  $NP_{1-2}EO$  and NP removal i.e. 99 %, 78 % and 98 % respectively showed the lowest carboxylated metabolite formation (573 %). In contrast, the highest formation of NPECs of *ca.* 33200 % was accompanied by a net formation of  $NP_{1-2}EO$  (61%) and decreased elimination efficiencies for all nonylphenolic compounds.

In the same study Giger et al (1987) analysed the concentrations of metabolites in each step of the Uster STW. Primary treatment resulted in a reduction in the concentration of the lipophilic NP and NP<sub>1-2</sub>EO accompanied by a small increase in NPECs i.e. 3 %. A significant proportion of the NPECs, i.e. 58 %, was found in the secondary effluent and in the activated sludge. The activated sludge process reduced concentrations of NP, NP<sub>1-2</sub>EO and most significantly the NP<sub>3-20</sub>EO. Tertiary treatment had no effect on the more hydrophilic NP<sub>1-2</sub>EC. This supports the notion that NPECs are the biodegradation products of NPEOs. The same finding was also reported by Ahel et al (1994a) in effluent samples taken from various STWs in Switzerland.

A comprehensive study on the occurrence, transformation and elimination of NPEO compounds in eleven full-scale activated sludge sewage treatment works in the Glatt Valley, Switzerland was undertaken by Ahel and co-workers (1994a). The NP<sub>n</sub>EO oligomer distributions in the effluents preceding each treatment were significantly different. In primary effluents, the principal components were NP<sub>3-20</sub>EOs accounting for approximately 82 % of the total followed by NP<sub>1-2</sub>EOs (circa 12 %), 3 % NP and 3 % NP<sub>1-2</sub>ECs. However, in the secondary effluent, NP<sub>1-2</sub>ECs were found to be the most abundant (circa 46.1%) followed by NP<sub>3-20</sub>EOs which contributed to circa 28 % whereas NP<sub>1-2</sub>EOs accounted for circa 22 % and 4 % NP. This 14.9 fold concentration increase from primary to secondary effluents suggests that the biological activated sludge treatment favours the formation of NP<sub>1-2</sub>ECs.

Recently, Nakada et al. (2006) carried out a comprehensive study at a sewage treatment work in Japan. In their summer survey, NPEC removal efficiencies of 1, 33 and 31 % were found after the primary settling tank, aeration and final settling tank and disinfection tank respectively. This is in contrast to various workers who have reported a net increase in NPECs in the secondary effluents compared to influents.

Bennie (1999) summarized the levels of NPECs found in Canadian STW effluents and reported that the concentrations of NP<sub>1-2</sub>EC increased with increased degrees of treatment in contrast to the decline in NP<sub>1-2</sub>EO concentrations. They reiterated that the nature of the inputs and type and degree of treatment strongly influence the concentrations and relative proportions of NPEOs released in final effluents. This is in agreement with Barber et al. (2000). The median concentrations of NPECs in the effluents of various processes are as follows in descending order: STW only trickling filter > activated sludge STWs without trickling filter > activated sludge STWs with trickling filter > activated sludge STWs with granular activated carbon treatment (Barber et al., 2000). However, these data may be interpreted inaccurately since neither detailed mass balances nor removal efficiencies were reported.

This finding is consistent with earlier work (Ball et al., 1989) which observed in time cause experiments that in activated sludge OP<sub>2</sub>EC was a significant intermediate, whereas OP<sub>2</sub>EC accumulated only to a minor extent in the crude sewage. When comparing the two experiments, it appears that the dominant factors affecting formation and accumulation of OPECs in aerobic environments include composition and concentration

of microorganisms with the higher concentration of microorganisms in activated sludge possibly favouring the formation of OPECs.

From the various studies reported above, the removal efficiencies of the carboxylated compounds as well as other alkylphenolic metabolites are shown to vary with treatment process conditions. Other variables such as sludge age, temperature and hydraulic retention time are important parameters for organic degradation (Langford et al., 2005a, 2005b) and may also play an important role which is discussed below.

### *2.2.2. SRT and HRT*

The solid retention time or sludge age (SRT) is the mean residence time of microorganisms in the biological reactor and only organisms that can reproduce themselves during this time can be retained and enriched. High SRT will therefore allow the enrichment of slowly growing bacteria and consequently the establishment of a more diverse biocenosis with broader physiological capabilities (e.g. nitrification). It has been recognized that the complete biodegradation of surfactants requires a consortium of bacteria due to the limited metabolic capacities of individual species (van Ginkel., 1996; Langford et al., 2005b). Opportunities for commensalism and synergism to occur exist in a consortium. Such interactive effects lead to more effective biodegradation than is possible by any individual microorganism. This condition is often realised with a membrane bioreactor (MBR) which is a modification of the activated sludge process. In the few studies undertaken comparing MBRs to conventional activated sludge systems,

the higher removal efficiencies of alkylphenolic compounds were attributed to the extended length of SRT (Gonzalez et al., 2007; Terzic et al., 2005).

Overall NPEO eliminations of 74 % and 87 % accompanied by NPEC formations of 1471 % and 429 % were achieved in the conventional STW and MBR respectively (Terzic et al., 2005). The NPEC contributed to 67 % and 42 % of the total nonylphenolic composition within the secondary effluent and the MBR treated wastewater respectively. These differences have been attributed to the longer SRT in MBRs as compared to the conventional activated sludge STW which allowed the development of microbial consortia capable of biotransforming more persistent oligomers (Terzic et al., 2005). In another study, the conventional STW achieved overall elimination of nonylphenolic compounds of around 54 % with NP<sub>1,2</sub>EC accounting for up to 60 % of the total nonylphenolic compounds in the effluent. In the MBR system, the elimination efficiency of nonylphenolic compounds was 94 % and the percentage proportion of NP<sub>1,2</sub>ECs in MBR effluent was 35 %. Up to 73 % of NP<sub>1,2</sub>ECs were removed in the MBRs as compared to a net formation of more than 70 % occurring in the conventional activated sludge STW (Gonzalez et al., 2007). Additional tests were carried out to determine if the decreased concentrations arose from degradation or sorption onto the sludge. These workers confirmed that the better elimination in the MBR was a result of better degradation rather than from sorption. The better performance of the MBR was attributed to the higher sludge age and the lower sludge load, which gave more time for the degradation of the organic contaminants and/or to the better adaptation of the microorganisms. However, both studies did not provide the SRT for both processes



which leads us to question if SRT is really a significant factor as opposed to other operating parameters for the different removal performances observed in both processes. Furthermore, if NPEC is degraded dependency of SRT, the determination of a critical SRT would not be possible.

Clara et al. (2005) evaluated the impact of SRT on treatment removal efficiencies using conventional STWs and MBRs operated at different SRTs. The operation of STWs with SRTs suitable for nitrogen removal (SRT >10 days) exhibited increased removal potential for NPEOs and NPECs. Comparable results are reported by Ahel et al. (1994a) who observed the highest removal rates for NPEOs in low-loaded conventional municipal STWs operating at high SRTs. The NPEC productions of 1.4 % and 49 % corresponding to SRTs of 114 and 237 days respectively were observed in the conventional STW under other comparable operating conditions i.e. HRT, food to microorganism ratio and temperature. Similarly, increasing the operating SRT in the MBR from 10 days to 27 days resulted in increased NPECs production of 142 % to 604 % respectively. Negligible differences in the removal potential of the nonylphenolic compounds were observed between the conventional STW and MBRs which led these workers to conclude that the membrane did not allow further retention of nonylphenolic compounds and that there were no differences in treatment efficiencies between the two treatment techniques (Clara et al., 2005). This may not be the case for NPEC since observations were based on the sum of the short chained metabolites i.e. NP<sub>1-2</sub>EO, NP and NP<sub>1-2</sub>EC to which these authors reasoned that the interactions between the different fractions required an integrated evaluation. This is debatable since other major metabolites such as the

dicarboxylates are not even included. Moreover, the operating parameters for both processes were not similar to allow for a fair comparison.

From the limited literature, there is no doubt that SRT plays a role in the removal of NPECs. However, in the studies comparing conventional STW and MBRs, the improved removal of NPECs could not distinctly and solely be attributed to the SRT since other additional characteristics associated with MBRs have been expected to contribute to the enhanced removals. One of these is that MBRs have a low sludge load in terms of biochemical oxygen demand (BOD). In this situation, the bacteria are forced to mineralize poorly degradable organic compounds. The notion of loading has been demonstrated indirectly (Ahel et al., 1994a; Clara et al., 2005). The NP<sub>1-2</sub>EC formations of 240 % and 280 % were observed in low-loaded and high loaded conventional STWs respectively (Ahel et al., 1994a).

Another important process variable to consider is the hydraulic retention time (HRT). This is the amount of time the sewage spends in the biological reactor during activated sludge treatment and determines the exposure time a bacteria will have to degrade or adsorb a compound (Langford et al., 2005a). Since APECs are hydrophilic and are weakly sorptive, it could be predicted that longer HRT results in greater removal. The biological degradation of OP<sub>1</sub>EC was observed after 2 days by Fujita and Reinhard (1997) after exposure to groundwater microorganisms. OP<sub>1</sub>EC was utilized as a sole carbon source and after day 4, no OP<sub>1</sub>EC could be detected. In MBR studies, Gonzalez et al (2007) observed higher NPECs removal efficiencies than previous workers (Terzic et

al., 2005) and attributed this, amongst other possible factors, to the longer HRT used in their study, 10 hours compared to 5.7 hours (Terzic et al., 2005), which favoured a more complete degradation of NPEC. Although not explicitly noted by Clara et al. (2005), operating the MBR and conventional STW under similar conditions other than a change in HRT from 4 days to 13.6 days resulted in NP<sub>1-2</sub>EC formations of 126 % to 254 % respectively. This observation opposes the view made by Gonzalez et al (2007). The limited studies carried out on the effects of HRT on NPEC formations hinders our judgement to assess how significant the effect of HRT is.

### *2.2.3. Temperature*

Generally metabolic processes increase with temperature (Lester and Edge, 2001; Birkett and Lester, 2002). Greater removal of APEOs from effluent can be observed in summer however, this corresponds with an accumulation of their metabolites. To date, there is little information on APECs in these studies involving seasonal variations. If APECs are intermediate degradation metabolites of APEOs, then temperature should be of some relevance to the production of APECs.

Studies on the biodegradation of OPEO during the percolation of contaminated sewage through a trickling filter in Preston, U.K. have shown degradation from 20% up to 80% between the winter and summer seasons respectively (Mann and Reid, 1971). In the Glatt River study (Ahel et al., 1994a) NP and NP<sub>1-2</sub>EOs concentrations were measured over a year with lower concentrations being found during summer than in winter. In a study of the seasonal variation of concentrations of NP, NP<sub>1</sub>EO and NP<sub>2</sub>EO in the Glatt and Thur

Rivers, Switzerland significantly higher levels of all components were measured in samples taken in the winter months (Ahel et al., 2000). Similar findings were also observed more recently by Maruyama et al. (2000). Water samples were taken from three rivers in Tokyo, Japan. Concentrations of all nonylphenolic compounds were found to decrease with increasing water temperature. Maruyama et al. (2000) reported that the average chain length of NPEOs in winter was longer (5-8) than in summer (2-5) suggesting that higher water temperature and therefore greater bacterial activity may cause faster cleavage of ethoxylate chains.

Although collative conclusions from the previous studies (Mann and Reid, 1971; Stiff et al., 1973; Ahel et al., 1994a) indicate that temperature has a significant effect on APEOs degradation within full-scale and experimental wastewater treatment facilities, to date, only one study exists demonstrating clearly the effect of temperature in a wastewater treatment plant on the production of APECs. Nakada et al. (2006) studied the removal efficiency of an activated sludge STW in Kanagawa, Japan, in winter (January) and summer (July) of 2004 for NP, NPEOs and NPECs. They reported NPECs removal of approximately 50 % in summer and an overall formation of 10 % in winter.

### **3. METABOLIC PATHWAYS**

Despite the fact that the APEO degradation has been studied for 45 years, the mechanisms for certain APEO metabolites remain unknown. In fact one group of metabolites (CAPECs) was not even discovered until 1996 (Ding et al., 1996). The inclusion of terminal oxidative metabolic pathways as the dominant pathway in aerobic

APEO biodegradation was only realised in 2001 (Jonker et al, 2001; Sato et al., 2001). Furthermore, although there is no disagreement about the degradability of long chained APEOs, experimental evidence for the formation of their oxidative metabolites is inconsistent. The formation of mono and dicarboxylated metabolites within STWs during biological activated sludge treatment is a result of aerobic biodegradation of APEOs (Giger et al., 1981, 1984, 1987; Stephanou and Giger, 1982; DiCorcia et al., 1994). However, even under strict anaerobic conditions, some researchers have observed substantial oxidation of APEOs resulting also in the formation of APECs as intermediates (Field and Reed, 1999; Schroder, 2001; Ferguson and Brownawell, 2003). Field and Reed (1999) studied the occurrence of NPECs in anaerobically digested municipal and industrial sludges. NP<sub>1-4</sub>EC concentrations were in the range 27–113 mg kg<sup>-1</sup>, with NP<sub>2</sub>EC the most abundant oligomer, and with *ortho*-to-*para* isomer ratios  $\geq 1$ , which indicated the depletion of *para* NPEC isomers relative to *ortho* isomers during anaerobic sludge treatment. By contrast, sludge that had not undergone anaerobic treatment contained only *para* isomers. It is thus timely to review the proposed pathways and mechanisms reported in the literature.

### 3.1. NPECs

#### 3.1.1. Non oxidative model followed by subsequent oxidation

The biodegradation of the EO chain in the non-oxidative model proceeds through shift of hydroxyl group from the terminal to the penultimate carbon followed by dissociation of the resulting hemiacetal to form shorter EO chains with liberation of ethylene glycol (Fig 2 pathway 2). This hydroxyl shift model for anaerobic biodegradation of alcohol

polyethoxylates has been proposed by Wagener and Schink (1988). Furthermore, John and White (1998) reported that NPEO was biodegraded by *Pseudomonas putida* through the non-oxidative hydroxyl shift under both aerobic and anaerobic conditions. Since long chained APECs were not detected in these experiments, it was generally accepted that APEOs are biodegraded through this model to shorter ethoxy chain APEOs residues containing one or two ethoxylate units (Fig 2 pathway 2). This has been reported by several workers (Maki et al., 1994; Frassinetti et al., 1996; van Ginkel, 1996; John and White, 1998; Szymanski et al., 2001, 2003) who observed the biodegradation distribution pattern of NPEO with time. The results suggested that breakdown occurs through the successive exocission of the ethoxylate chain and not by direct scission between the second and third ethoxy groups.

Under aerobic conditions, the shortened APEOs i.e. AP<sub>1-2</sub>EOs undergoes  $\omega$ -carboxylation of the terminal alcoholic groups producing AP<sub>1-2</sub>ECs (Manzano et al., 1999; Ahel et al., 1994a, 1994b; Tanghe et al., 1998) (Fig 2 pathway 1). The degradation of NP<sub>15</sub>EO in river water resulted in the formation of NP<sub>2</sub>EO and ethylene glycol, followed by subsequent formation of NP<sub>2</sub>EC and NP<sub>1</sub>EC; NP<sub>1</sub>EO was not detected (Manzano et al., 1998, 1999).

### 3.1.2. Terminal oxidative model

The APECs formation pathway described above (Fig 2 pathway 2 followed by pathway 1) generally stood as long chained carboxylated ethoxylates were previously not detected. However, Jonkers et al (2001) observed long chain NPECs immediately upon beginning

their aerobic biodegradation studies in river water and concluded that the dominant pathway in aerobic NPEO biodegradation starts with the oxidation of the terminal alcohol on the ethoxyl chain (Fig 2 pathway 1) prior to shortening of the ethoxylate chain. No mechanism was given for the stepwise shortening of the long chain NPEC to short chain NPEC which is mainly NP<sub>2</sub>EC (Fig 2 pathway 4). Since small amounts of NP<sub>2</sub>EO were formed during the aerobic degradation, these workers postulated that NP<sub>2</sub>EO were produced in the presence of anaerobic microenvironments. These workers did not observe the formation of higher NPEOs to show conclusively that the primary degradation products were carboxylate derivatives as only 19% of the initial compound was recovered. Since mineralization of NPEO metabolites often occurs very slowly (if at all), it seems plausible that the 81% that was unrecovered was in the form of undetected metabolites. Recently, Zhang et al. (2007) studied the aerobic biodegradation of behaviours of two NPEC mixtures in two microcosms according to an OECD protocol and observed the shortening of long chained NPECs to NP<sub>2</sub>EC without NPEO and NP formation. These authors gave two possible reasons for the absence of NPEOs: 1. NPEO were not formed in the test; 2. NPEOs may be formed and removed (by sorption or degradation) at similar rates.

Another terminal oxidative biodegradation pathway proposed by Sato et al. (2001, 2003) proceeds through the oxidation of the terminal EO unit (Fig 2 pathway 1) followed by scission of the neighbouring ether bond to form the EO chains shortened by one EO unit with liberation of glyoxylic acid (Fig 2 pathway 3). These workers studied the biodegradation of OPEOs in a pure culture (*Pseudomonas putida* S-5) under aerobic

conditions and observed the formation of  $OP_nEO$  ( $n=2-8$ ) and their corresponding  $OP_nEC$ . This observation of the presence of intermediate carboxylates is also made by several authors (Maki et al., 1996; Maeda and Mikami, 1998; Hayashi et al., 2005).

Most of the studies where NPECs were not observed with the progressive shortening of the ethoxylated chain are presumably due to the lack of microbial consortia required for NPEC formation (Langford and Lester, 2003). Nguyen and Sigoillot (1997) found few Gram-negative bacteria that are able to degrade APEO with nine–ten ethoxy groups. Whilst some *Pseudomonas* strains degrade only down to four or five ethoxy groups, other species of bacteria which are unable to degrade the long chain APEO are able to degrade the APEO with four or five ethoxy groups down to the two ethoxy group compounds. Some NPEO degraders, such as *Pseudomonas* sp. Strain 14-1 and *Pseudomonas* sp. Strain TR01 degraded NPEOs to  $NP_2EO$  through oxidation of the EO chain to produce carboxylates as intermediate metabolites (Maeda and Mikami, 1998).

*Please insert Fig 2 here.*

### 3.2. CAPEs

Instead of undergoing  $\omega$ -carboxylation of the terminal alcoholic groups as described previously to produce  $AP_{1-2}ECs$ , the alkyl chain degradation can occur to the short chained APEOs. In one study of STW effluent, another class of intermediates with the alkyl chain carboxylated (CAPEs) was identified (DiCorcia et al., 1998a) (Fig 2 pathway 5). This class of metabolites was observed simultaneously with its dicarboxylated forms



i.e. CAPECs as described later. By comparing the CA<sub>6</sub>P<sub>2</sub>ECs spectrum with different spectra obtained from their test solution these authors attributed the formation of CAPEs to the oxidative attack of the alkyl side chain of the lower APEO oligmers with one and two ethoxyl units. The new chromatographically determined class had only the alkyl chain oxidised and they appeared with one and two ethoxy units (CAP<sub>1-2</sub>E) with CA<sub>6</sub>P<sub>2</sub>E being the most abundant identified isomer. The CAPEs were thought to arise from the biotransformed species of their isomeric class of NPEOs having the characteristic structure identified by the presence of a methyl group on the branched  $\alpha$ -carbon of the alkyl chain. Preferential oxidation of the alcoholic terminal of ethoxylate chain over the highly branched alkyl chain occurs due to steric hindrance. Hence CAPEs are rarely observed as compared to APECs and they are generated less extensively than the branched isomers. The detected CAPE did not persist as it disappeared in the biodegradation test solution after 170 days. However, a subsequent study carried out on “Cobis” STW located in Rome showed negligible amounts of CAPEs were present in the effluent. Instead CAPECs accounted for more than 63% of all NPEO breakdown products (DiCorcia et al., 2000).

### 3.3. CAPECs

The dicarboxylic metabolites of APEOs i.e. CAPECs were initially detected by Ding et al. in 1996. These metabolites form via  $\omega$ - oxidation of the alkyl side chain of the short chained APECs (Fig 3 pathway 6) where the terminal methyl group is converted into a carboxylic acid. Subsequently, shortening of the oxidised alkyl chain proceeds with  $\alpha$  or  $\beta$ - oxidation (Fig 3, pathway 7) and this leads to carboxylation on both side chains of

varying lengths (Fig 3). This is evident from the results of several workers (Ding et al., 1996; Ding and Tzing, 1998; DiCorcia et al., 1998b). Carboxylation of the alkyl side chain of NPECs observed from the product-ion mass spectra and the comparison of methylated and propylated CNPEC derivatives are suggestive of this. DiCorcia and co-authors (1998a) studied the biodegradation pathways of the branched alkyl chain of NPEOs under laboratory conditions by using the OECD screening test (OECD., 1980), using an aliquot of effluent inoculated with microorganisms from an activated sludge STW. They found a wide range of mono and di-carboxylated biotransformation products of NPEOs with various degrees of the alkyl chain length and ethoxylate branching. It was concluded that a group of COP<sub>2</sub>EC isomers were produced by the  $\omega$ -oxidation mechanism of the alkyl side chain of NP<sub>2</sub>ECs (Fig 3, pathway 6). By comparing intact APECs with their corresponding methylated forms they found additional evidence of the formation of other di-carboxylated degradation species i.e. CA<sub>6</sub>P<sub>2</sub>EC and CA<sub>5</sub>P<sub>2</sub>ECs. Thorough investigations allowed them to conclude that  $\omega/\beta$ -oxidation mechanisms, particularly of the alkyl side chains of NP<sub>2</sub>EC isomers, led to the formation of CA<sub>6</sub>P<sub>2</sub>ECs species (Fig 3, pathway 7).

*Please insert Fig 3 here.*

Amongst the dicarboxylic metabolites, carboxyalkylphenoxy monoethoxy carboxylates (CA<sub>x</sub>P<sub>1</sub>ECs, x = 6 and 8) and carboxyalkylphenoxy diethoxy carboxylates (CA<sub>y</sub>P<sub>2</sub>ECs, y = 6 and 8) are the two most common metabolites detected in various environmental samples (DiCorcia et al., 1998a; 2000). These dicarboxylic metabolites are postulated to

originate from NPEOs whilst the less abundant metabolites of C<sub>5</sub>APECs and C<sub>7</sub>APECs arise from OPEOs. Current evidence suggests that various CAPECs metabolites will only form after the ethoxylate chains are shortened independent of the initial breakdown pathway (DiCorcia et al., 1998a, 2000).

#### 3.4. Further transformation and ultimate degradation

It is generally thought that NP<sub>1</sub>ECs is transformed to NP only under anaerobic conditions (Ahel et al., 1994a; Minamiyama et al., 2006) and anoxic conditions (Ike et al., 2002). Recently Liu et al. (2006) reported the formation of NP from NP<sub>2</sub>EO with NP<sub>1</sub>EC and NP<sub>2</sub>EC as the intermediate metabolites which were further transformed to shorter NPEOs under aerobic conditions by *Ensifer* sp. strain AS08 and *Pseudomonas* sp. strain AS90. The pathway was through the removal of one EO unit to form shorter NPEO followed by further oxidation of the terminal alcohol group of the EO chain to carboxylic acid via aldehyde. This process is repeated until NP is formed. This is in agreement with a previous study which speculated that NP<sub>2</sub>EC could be further degraded and/or oxidised to NP<sub>1</sub>EO and/or NP<sub>1</sub>EC (Maki et al., 1996).

Under aerobic conditions, although NPEC are more resistant to biodegradation than the longer chain NPEO, they are ultimately biodegraded (Staples et al., 2001; Environment Canada and Health Canada, 2001). Staples and co-workers (1999) determined ultimate biodegradability, based on CO<sub>2</sub> formation using the OECD screening test (OECD., 1980), of NP<sub>1-2</sub>ECs and OP<sub>1-2</sub>ECs in a laboratory study by using bacterial seed inoculum from municipal and industrial activated sludge. Half-lives for NP<sub>1-2</sub>EC and OP<sub>1-2</sub>EC in

bacteria-seeded water were in the range of 18-22 and 12-18 days respectively. Using municipal settled sewage as an inocula for the OECD tests, OP<sub>1</sub>EC and NP<sub>1</sub>EC biodegradation reached 72 % and 59 % respectively. Mixed liquor collected from industrial STWs as an inocula exhibited similar levels of biodegradation for OP<sub>1</sub>EC and NP<sub>1</sub>EC of 65.3 % and 66 % respectively. Although all the compounds exceeded 60% of theoretical CO<sub>2</sub>, none of the tested compounds demonstrated 100% degradation over 28 days. These workers found greater biodegradation with OP<sub>2</sub>ECs and NP<sub>2</sub>ECs than their shorter chain counterparts i.e. OP<sub>1</sub>EC and NP<sub>1</sub>EC in municipal activated sludge. The mineralization is thought to involve the breakdown of the aromatic ring at the centre of the NPEO molecule prior to the loss of the final ethoxylate or carboxylate groups, since NP is not observed under aerobic conditions (Jonkers et al., 2001; Tanghe et al., 1999; Staples et al., 1999).

The CAPECs metabolites are recalcitrant to further biotransformation; they persisted in the test liquor more than 5 months after they were generated (DiCorcia et al., 1998a). These authors stated that the lack of further CAPECs biotransformation was not due to the lack of microorganisms since CAP<sub>2</sub>ECs to CAP<sub>1</sub>ECs conversion was observed. The persistence of CAPECs was also evident in studies investigating the amounts present in river waters and effluents from STWs. DiCorcia et al (2000) found that these dicarboxylated species were by far the most abundant metabolites found in the effluents of five STWs accounting for ca 66 %. In a recent study involving Taiwanese waters, CAPECs were found to be the dominant alkylphenolic compounds i.e. CAPECs (up to 94.6 µg l<sup>-1</sup>) followed by NPECs (up to 63.6 µg l<sup>-1</sup>) (Cheng et al., 2006b). A possible

explanation for the persistence of CAPECs is that these dicarboxylic acid molecules form strong intermolecular hydrogen bonds between their acidic ends resulting in greater stability. This phenomenon is analogous with the strong interactions between two acidic molecules forming a dimer which acts as one single molecule (Hill et al., 1993). The significance of this for CAPECs biodegradation is that it probably hinders or restricts further biodegradation of the aromatic ring. Additionally, these carboxylic acids possess the ability to resonate resulting in enhanced stabilizing effects on the  $\text{RCO}_2^-$  ends by the charge-delocalization species i.e. the negative charge is equally distributed between two oxygen atoms and this increased stability is comparable to that present in an aromatic system (Gutsche and Pasto, 1975).

## **4. DISCUSSION**

### *4.1. Environmental impact*

Both APECs and CAPECs are formed by degradation of longer-chain-length APEOs during wastewater treatment and they therefore, increase in concentration during the course of treatment and can reach levels considerably higher than those of AP or APEOs in final effluent. Typical concentrations of APECs and CAPECs found in the environment are up to  $931 \mu\text{g l}^{-1}$  and  $755 \mu\text{g l}^{-1}$  respectively (Table 1). The concentrations and occurrence of these APECs and CAPECs are important for three main concerns: 1. they may contribute to the estrogenicity of the discharges either individually or as a mixture effect; 2. they can be precursors of APs and short chained APEOs and 3.

formation of halogenated APECs which are more toxic during chlorination in direct water reuse purposes.

#### 4.1.1. Contribution to overall estrogenicity

Although APECs and CAPECs are less estrogenic than APs, there have been implications that they contribute to the estrogenicity of the waters either individually or as a mixture effect. In a risk assessment study, Bennie et al. (2001) estimated the total estrogenic potency of municipal effluents in Canada. When considered alone, the concentrations of NP would not exceed the threshold for estrogenic responses. If the potential estrogenic effects of the NP<sub>1-2</sub>EOs are added to the effect of NP then about 15 % of the sites were expected to exceed the threshold of 1 µg l<sup>-1</sup>. When NP<sub>1-2</sub>ECs were also added, almost 60 % of the municipal sites exceeded the threshold.

Elsewhere, it has been suggested that NPECs may contribute to the estrogenic activity measured at sites further downstream (5km away from the STW) (Sheahan et al., 2002). Because NPEO, NP and steroids are more susceptible to removal by sorption, these authors suspected that other hydrophilic chemicals may play a part. Since NP<sub>1-2</sub>ECs are estrogenic at least in trout (Jobling and Sumpter, 1993; White et al., 1994; Jobling et al., 1996), these authors suggest that the greater solubility of NPECs and its higher resistance to degradation under aerobic conditions would support the suggestion that it may be making a significant relative contribution to estrogenic activity downstream of the Keighley STW effluent discharge in the UK.

The relative estrogenic potencies for comparisons in both studies were based on the vitellogenin induction in trout hepatocytes data of Jobling and Sumpter (1993) in which the relative potency of NP<sub>1-2</sub>EC to estradiol vitellogenin induction in trout hepatocytes was reported as  $6.3 \times 10^{-6}$ . These authors also demonstrated that continuous exposure of three weeks to approximately 0.11  $\mu\text{M}$  of NP<sub>1</sub>EC ( $30 \mu\text{g l}^{-1}$ ) in sewage effluent caused an inhibition on the testicular growth of male rainbow trout (*O. mykiss*) (Jobling et al., 1996). Another study undertaken over for a period of 22 days found that concentrations of  $>10 \mu\text{g l}^{-1}$  of NP<sub>1</sub>EC produced statistically significant reduction in rainbow trout weight (Ashfield et al., 1998). Over a 35 day period, exposure to NP<sub>1</sub>EC at 1, 10 and 30  $\mu\text{g l}^{-1}$  resulted in significant increase in fish length and weight which indicated that NP<sub>1</sub>EC exhibit adverse effects to rainbow trout at these levels. Recent studies demonstrate the relative estrogenic potency of NP<sub>1</sub>EC is lower where NP<sub>1</sub>EC is reported to be 30 times less estrogenic than NP (Dussault et al. 2005) contrary to previous report by Jobling and Sumpter (1993). Therefore, if the lower potency is applied, fewer effluent discharges would exceed the threshold for estrogenic mediated responses arising from the presence of NPECs would be exceeded after 2005 (Table 1).

Given their high concentrations, CAPECs and NP<sub>2</sub>ECs could be significant if they have endocrine disrupting potential. Pure carboxylates and CAPECs of different isomeric structures would need to be tested for endocrine activity to produce the required data. It has been demonstrated that only the 4-tertiary isomers of nonyl and octylphenol and their metabolites have estrogenic potency (Routledge and Sumpter, 1997).

Therefore, if an assessment is made solely on the available potency data and current observed levels, NP<sub>1</sub>EC would be perceived as a very weak contributor to the endocrine problem. However, if their potential to form NP and other halogenated NPEC which are more toxic and estrogenic is taken into account, then the presence of these compounds could be significant. The concern about the ability of carboxylates to form NP is amplified by the recent findings of vitellogenin induction in juvenile rainbow trout following prolonged exposure to concentrations of NP as low as 1.05 µg l<sup>-1</sup> (Ackermann et al., 2002).

#### 4.1.2. Precursors of APs and short chained APEOs

Recently, Loos et al (2007) analysed the levels of NP and NPECs in discharges, effluent and river receiving waters from the textile industry and their corresponding STWs in Belgium and Italy. These workers report higher concentrations of NP in receiving rivers compared to the effluents and attributed this to NP being the final degradation product of NPEO surfactants. This suggests that the carboxylated metabolites could potentially contribute to NP levels. High levels of NP in river sediments, even at distances of up to 10 km from the point of STW effluent discharges, were reported by Isobe et al. (2001) who concluded that the water of the Sumidagawa River could be potentially hazardous to fish. They suggested that the ubiquitously high concentrations of APs in the riverine sediments could be attributed to the large proportion of wastewater effluent in the river water. It was also postulated that increased NP concentrations may arise from NP<sub>1</sub>EO which is anaerobically degraded even though these hydrophobic metabolites were released in



small amounts. The study failed to identify the quantities of NPECs which can also anaerobically degrade to form NP (Minamiyama et al., 2006). The notion of NPECs contributing to the NP levels detected in the environment has been suggested by Conn et al. (2006). These workers attributed the apparent production of NP in wetland systems, a predominantly mixed redox environment, to the presence of NPECs in the effluent which was released into the system.

#### 4.1.3. Halogenated APECs

It has been suggested that APECs should be regarded as potentially very important groundwater contaminants at river water infiltration sites. Coupled with the predominance of these carboxylic compounds in river water samples, the higher solubility of APECs and their resistance to biodegradation in aerobic conditions (Ahel et al., 1987) are possible reasons for their higher mobility in aquifers compared to APEOs. However, it has been concluded that additional investigations are necessary to draw firm conclusions about the behaviour of APECs during infiltration (Ahel et al., 1994c).

Another consequence of the persistence of APECs and CAPECs is that these compounds will find their way into drinking water treatment plants. In some cases mono and dicarboxylated metabolites have been found in groundwater and drinking water samples (Ventura et al., 1988, 1992; Petrovic et al., 2001, 2002b). Pre-chlorination was found to reduce the concentration of short-ethoxy chain NPECs and NPEOs by 25–35% and this arises partly from their transformation to halogenated derivatives (Petrovic et al., 2002b). After pre-chlorination, halogenated nonylphenolic compounds represented approximately

13% of the total metabolite pool, of which 97% were in the form of brominated acidic metabolites. Little is known about the environmental significance and toxicology of brominated and chlorinated alkylphenolic compounds. Reinhard and co-workers (1982) suspected that the occurrence of mutagenicity in wastewater is correlated with the formation of brominated alkylphenolic byproducts; however, their preliminary experiments conducted with BrAPECs failed to confirm this hypothesis. Maki et al. (1998) determined that both BrNPEOs and BrNPECs show higher acute toxicity to *Daphnia magna* than do their nonbrominated precursors NPEOs and NPECs. The 48 h 50 % lethal concentrations (LC50s) of NP<sub>2</sub>EO, BrNP<sub>2</sub>EO, NP<sub>2</sub>EC and BrNP<sub>2</sub>EC were reported as 0.148, 0.067, 0.990 and 0.141 mg l<sup>-1</sup> respectively. A recent study, employing recombinant yeast assay (RYA) and enzyme linked receptor assay (ELRA) for the determination of estrogenic and anti-estrogenic activity, showed that halogenated compounds acted as weak estrogens when compared to NP but retained a significant affinity for the estrogen receptors. This suggests that they may be still able to disturb the hormone imbalance of exposed organisms as the halogenated NPECs behaved as estrogenic antagonists in the RYA (Garcia-Reyero et al., 2004). In addition, an increased cytotoxicity for the carboxylated derivatives in both yeast and mammalian cells was detected. Although derivatization mask the apparent estrogenicity of nonylphenol, the resulting compounds still represent a potential hazard since they are still able to bind the estrogen receptor and to influence the physiological response to estrogens.

The reported levels of these halogenated compounds in the aqueous environment are scarcely available to accurately carry out any risk assessments. However if the levels are

similar to those found in drinking waters, ranging from  $<2-105 \text{ ng l}^{-1}$  in Spain (Petrovic et al., 2001), then these compounds do not pose any harm. This is not surprising given the strict legislations to ensure high requirements on the quality of drinking water. In circumstances where there are no legal requirements, these levels might be of significant concern to aquatic lives in rivers, near the coastal areas or salt mines, containing higher levels of bromine and chlorine ions since bromination of APEO biodegradation products occur relatively readily in water originating from such sources (Maki et al., 1998).

#### *4.2. The need for tertiary treatment?*

It seems from the literature gathered from both small scale and field studies that microbial actions on NPEC are vital to their formation and removal. Factors that affect microbial well being (temperature, starvation- organic content), the diversity (SRT), the exposure time (HRT) for these hydrophilic compounds play a more significant role than physical conditions.

Several studies have been carried out to investigate the use of tertiary treatments to reduce carboxylated metabolites either chemically or physically. Recently, using a laboratory-scale reactor operating on ultrapure water, Ike et al. (2002) determined the effectiveness of ozone treatment for the degradation of NPEO metabolites, this followed the order:  $\text{NP}_1\text{EC} \gg \text{NP} > \text{NP}_1\text{EO}$ . Acidic metabolites were completely degraded within 4 to 6 minutes (initial concentration,  $0.4-1.0 \text{ mg l}^{-1}$ ), the NP concentrations were reduced by 75–80% in 6 minutes, while only 25–50 % of  $\text{NP}_1\text{EO}$  was eliminated in the same time. Studies on the decomposition of NPEOs, NPECs and NP by electron beam irradiation,

ozone and combined ozone/ electron beam irradiation treatment in laboratory batch conditions using spiked tap water were carried out by Petrovic et al. (2004). The results indicated that single electron beam irradiation is the most efficient process leading to a rapid breakdown of all nonylphenolic compounds, including NPEOs recalcitrant metabolites, such as NP and NP<sub>1</sub>EC. The higher OH radical concentration generated by the combined ozone/ electron beam irradiation process did not result in improved decomposition of the compounds studied, while ozone alone achieved the lowest OH radical production and least efficient decomposition of alkylphenolic compounds. However, using advanced oxidation processes (AOP) to treat the carboxylic metabolites would seem economically undesirable as the degradation efficiency of an AOP is often limited by the radical scavenging capacity of the matrix of the treated water. In a subsequent study carried out by Petrovic et al. (2007) using electron beam irradiation to treat sewage effluent, NP elimination was less efficient and they attributed this to the simultaneous elimination of NP (present in STW effluent) and its formation as intermediate products of NPEC degradation. The complex wastewater matrix, as indicated by the high COD and BOD values, slowed down electron beam irradiation induced decomposition of alkylphenolic compounds and required higher doses. For comparison, radiolytic decomposition of 500 µg l<sup>-1</sup> of long ethoxy chain NPEOs in spiked tap water required a 1 kGy dose (Petrovic et al., 2004); for naphthalene disulfonic acids, a 96% degradation yield at 10 µg l<sup>-1</sup> in a tap water was observed at 2 kGy (Gehring et al., 2005). For complex industrial effluents, the necessary dose to remove 90% of the most organic compounds and more than 70% of their toxicity (determined by *Vibrio fischeri* Microtox test and Microcrustacean *Daphnia similis* test) was found to be

20 kGy (Duarte et al., 2002; Moraes et al., 2004). This means that the concentration of dissolved organic carbon will play an important role in the sufficient degradation of the NPECs from wastewater and that economic considerations have to underpin the feasibility of the process for wastewater treatment. The other drawbacks of using AOP to treat alkyphenolic surfactants and their metabolites in water and wastewater have been reviewed by Ikehata and El-Din (2004).

A further method of removing carboxylic metabolites from aqueous streams involves the use of physical separation such as membranes. Nanofiltration/reverse osmosis (NF/RO) membrane filtration processes have all been recognized as important technologies which can be used to remove a wide range of contaminants including endocrine disruptors. The adsorption and release process of several endocrine-disrupting chemicals (EDCs) during NF/RO filtration processes was examined by Nghiem and Schäfer (2006). Results indicated that the membrane can serve as a large reservoir for EDCs. Their release may be possible during membrane cleaning or as a result of erratic pH variation during operation. Treatment of membrane cleaning solution should be considered carefully when EDCs are amongst the target contaminants in NF/RO membrane filtration. Other workers demonstrated that the physicochemical properties of the membranes and the solutions play vital roles in their rejection efficiencies (Chiu et al., 2006). Lower rejection efficiencies were found in experiments that employed low concentrations of compounds (Kimura et al., 2003). This is applicable also to membranes within membrane bioreactor systems (MBRs). Other considerations to take into account are the regeneration cost of

membranes, as they are prone to fouling, and their downtime. Additionally, as NF/RO serves to improve the effluent quality, these compounds would concentrate within the reject stream and thus careful consideration and management of the reject stream are essential.

Considering the drawbacks of using physical and chemical methods as possible tertiary treatment, it seems that more efforts should be placed on optimising operating parameters of biological treatment plants. The use of enhanced biological treatment is an option worth considering. Enhanced biological treatment such as biological nutrient removal (BNR) systems possess some of the conditions required for the removal of these carboxylated metabolites such as the increased SRT or HRT as described previously. Under aerobic and anaerobic conditions, the breakdown of alkyphenolic carboxylates will be encouraged. However concern for the presence of the breakdown end-product NP arises in BNR systems. Anaerobic degradation of carboxylic metabolites leads to the formation of NP which is more toxic. Studies on the anaerobic biodegradation of NP indicate that sulfate-reducing bacteria constitute a major microbial component in the anaerobic degradation of NP, but that the methanogen and eubacteria microbial populations are also involved. This result was similar to that reported by Chang et al. (2004), who studied the anaerobic degradation of NP in river sediment, and supports the idea of using anaerobic microbes to remove NP from sludge. However the degradation rate declined when the concentration of NP increased. This has been attributed to the increased levels of toxicity at higher NP concentrations.

The ideal way to remove carboxylates from wastewater would be to oxidise them completely. Two ways may be feasible. Firstly, to increase the aerobic section of the wastewater treatment plant to increase the exposure time of these metabolites to aerobic conditions. Secondly, to use strong oxidation conditions as used in industrial wastewater treatment processes using tertiary non-biological processes. Ultimately, the question arises at what environmental costs, in terms of energy consumption and CO<sub>2</sub> release, will this be accomplished at and will the solution cause greater detrimental effects to the environment than APEOs themselves.

#### *4.3. Environmental and economic analysis*

The Environment Agency of England and Wales has instigated recently a “Demonstration Programme” which includes evaluating tertiary treatment methods for the removal of endocrine disrupting chemicals including APEOs. The economical and environmental costs of proposed tertiary treatments have been evaluated recently (Jones et al., 2007). Table 2 shows the ranges of the equipment operational costs for each of the process options which were designated by the Environment Agency of England and Wales.

*Please insert Table 2 here.*

Table 2 includes the cost of utilizing drinking water technologies to treat urban wastewater and demonstrates that it is likely to be costly since the additional capital expense required is almost as much as the total capital cost of a standard plant. The capital cost of the sand filter and membranes exceeded the cost of the basic activated

sludge (AS) plant by £1.5 million. The potential operating costs of the extra treatment processes are also significantly higher than standard treatment, with treatment via MF and RO being more expensive than GAC (granular activated carbon) and O<sub>3</sub> (ozone) (Table 2). There is however, an economy of scale in the cost per m<sup>3</sup> of sewage treated via the advanced treatments, as cost was found to decrease as the size of the plant increased.

An important factor to consider when utilizing advanced treatment technologies is the inevitable environmentally undesirable increase in energy consumption. At present this demand would be met mainly from non-renewable sources. An energy intensive tertiary process plant would, contribute therefore, a large amount of CO<sub>2</sub> to the atmosphere, with associated ramifications for global warming and climate change. In addition, there would be increased sludge production associated with advanced treatment which would have to be safely disposed of, ideally in an environmentally sustainable manner. At present it is not clear how this could be achieved. Taken together these issues can increase significantly the economic (and environmental) cost of this type of treatment (Jones et al., 2007).

Studies attempting to calculate the environmental benefits of removing endocrine disrupting compounds from the wastewater stream have been commissioned (AEA Technology Environment, 2004). The report was based on introducing various technologies to reduce the levels of these compounds in 48 of Yorkshire Water's wastewater treatment works in the Yorkshire Water region, England with an estimated population equivalent of 4 million. To comply with the Water Framework Directive



2000/60/EC (European Commission, 2000), it is reported that additional electrical energy of 35 GWh would be required per annum for pumping and producing ozone and UV light. The electricity use alone would lead to an annual release of around 175 and 1 kilotonnes of CO<sub>2</sub> and SO<sub>2</sub> (combustion of fossil fuel) respectively.

A paradigm of wastewater treatment is that increasing effluent quality can only be environmentally beneficial; this should be carefully considered as the benefits of improved effluent quality are often outweighed by the negative effects on the wider environment when process construction, operation and the increased sludge production from the advanced treatment technologies are taken into account. Using advanced treatment methods to treat sewage is likely to reduce pollution, but will also incur large financial and environmental costs (Jones et al., 2007).

Before upgrading existing sewage treatment works with advanced treatment technologies, potential alternative options need to be considered, including preventive and remediation measures. Known dangerous chemicals should be regulated and their production minimised, avoiding contamination of municipal sewage and aquatic environment. New chemicals should be tested rigorously with standard methods for reliable and comparable results. Looking at the cost of removing such compounds, it seems a better option to eliminate the problem at the source i.e. limiting APEO production or to use substitutes rather than to subsequently treat them.

## **5. CONCLUSIONS**

- The current NPEO production and consumption in EU has declined, however, in other parts of the world notably in Asia and the U.S. substantial levels of NPEOs are still in use.
- High levels of persistent alkylphenolic carboxylated derivatives are detected in sewage effluents.
- The hydrophilic nature of these compounds means they are highly mobile and can enter the aquatic environment in significant concentrations.
- Although weakly estrogenic, the presence of these carboxylates can contribute significantly to the overall estrogenicity found in the environment as ad mixture effect.
- Under appropriate conditions, these hydrophilic compounds can further transform to form NP which is toxic and estrogenic.
- Tertiary treatment options seem able to remove these compounds but at undesirable economic and environmental costs.
- Improving biological treatment processes and optimising their operating conditions (i.e. SRT, HRT) may hold the key to increase the removal of these metabolites.

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List of Tables

Table 1. Occurrence of alkylphenolic carboxylated metabolites in municipal treatment plants and rivers.

Sampling location	Surfactant	Medium		References	
		Influent concentration ( $\mu\text{g l}^{-1}$ )	Effluent concentration ( $\mu\text{g l}^{-1}$ )		
U.S.	OP <sub>1</sub> EC	-	4.9-11	Ball and Reinhard, 1985	
	OP <sub>2</sub> EC	-	24-84		
Switzerland	NP <sub>1</sub> EC	ND	ND-224	Ahel et al., 1987	
	NP <sub>2</sub> EC	ND-14	71-233		
	NP <sub>1-2</sub> EC	80-270	290-930		
Italy	NP <sub>1</sub> EC	ND	1.5-3.9	DiCorcia et al., 1994	
	NP <sub>2</sub> EC	ND	5.1-9.4		
U.S.	NP <sub>1</sub> EC	-	7.6-29.4 ND-140 <sup>†</sup>	Field and Reed, 1996	
	NP <sub>2</sub> EC	-	64.1-144 ND-931 <sup>†</sup>		
	NP <sub>3</sub> EC	-	24.8-105 ND-172 <sup>†</sup>		
	NP <sub>4</sub> EC	-	9.7-29.2 ND-26.7 <sup>†</sup>		
	CAPEC	-	58		
Canada	NP <sub>1-2</sub> EC	2-17	9-44	Lee and Peart, 1998	
	OP <sub>1-2</sub> EC	0-8	1-29		
	NP <sub>1</sub> EC	0.9-8.3	3.2-703	Lee et al., 1998	
	NP <sub>2</sub> EC	1.7-20.1	11.1-565		
	OP <sub>1</sub> EC	0.7-1.14	0.29-4.61		
	OP <sub>2</sub> EC	0.14-1.62	0.54-7.74		
	NP <sub>1</sub> EC	2.0-15	1.9-22		WTI, 1998
	NP <sub>2</sub> EC	1.9-35	11-32		
	OP <sub>1</sub> EC	0.56-1.33	0.05-7.37		

Taiwan	OP <sub>2</sub> EC	0.24-2.3	0.12-4.0	
	NP <sub>1-3</sub> EC	-	19.2-99.2	Ding and Tzing, 1998
	CNP <sub>1-2</sub> EC	-	14.7-67.2	
Italy	NP <sub>3-20</sub> EC	-	1-15	DiCorcia et al., 2000
	CA <sub>3-8</sub> PEC	-	1-24	
U.S.	NP <sub>1-4</sub> EC	-	0.1-140*	Barber et al., 2000
			0.1-120**	
Spain	Tot NPEC	0-80	ND-270	Sole et al., 2000
Japan	NPEC	3	6/34	Fujita et al., 2000
Germany	NP <sub>1</sub> EC	-	0.17-5.8	Spengler et al., 2001
Spain	NP <sub>1</sub> EC	13	58	Petrovic et al., 2001
	NP <sub>2</sub> EC	6.5	22	
	OP <sub>1</sub> EC	2.0	25	
	OP <sub>2</sub> EC	8.5	19	
Spain	NP <sub>1</sub> EC	1-65	4-105	Petrovic et al., 2002a
	OP <sub>1</sub> EC	<0.05	<0.05-3	
Japan	NP <sub>1-2</sub> EC	0.1-0.2	2-2.9	Isobe and Takada, 2004
Austrian	NP <sub>1-2</sub> EC	0.36-2.3	0.19-8.5	Clara et al., 2005
			0.58-4.9 <sup>a</sup>	
Japan	CA <sub>5-8</sub> P <sub>1</sub> EC	-	0.14-1.82	Hoai et al., 2006
	NP <sub>1-10</sub> EC	0.07-4.7	ND-23 <sup>#</sup>	Komori et al., 2006
		0.05-3.9	ND-10 <sup>##</sup>	
Spain	NP <sub>1</sub> EC	<5-14	7-21	Gonzalez et al., 2007
			4 <sup>a</sup>	
	NP <sub>2</sub> EC	30-95	44-179	
			20 <sup>a</sup>	
Belgium	NP <sub>1</sub> EC	-	0.01-2.4	Loos et al., 2007
	NP <sub>2</sub> EC	-	0.001-0.77	
	NP <sub>3</sub> EC	-	ND-1.0	
	OP <sub>1</sub> EC	-	0.001	
	OP <sub>2</sub> EC	-	ND-0.18	

Italy	NP <sub>1</sub> EC	-	2.8-4.5	Loos et al., 2007
	NP <sub>2</sub> EC	-	0.36-0.91	
	NP <sub>3</sub> EC	-	1.4-2.6	
	OP <sub>1</sub> EC	-	0.001-0.002	
	OP <sub>2</sub> EC	-	0.043-0.13	
U.S.	NP <sub>1-2</sub> EC	-	133 <sup>#</sup>	Barber et al., 2007
Spain	NP <sub>1</sub> EC	-	10.7	Petrovic et al., 2007
	NP <sub>2</sub> EC	-	115	
	OP <sub>1</sub> EC	-	11.6	
	OP <sub>2</sub> EC	-	11.3	

### Rivers

		Aqueous concentration ( $\mu\text{g l}^{-1}$ )		
Switzerland	NP <sub>1</sub> EC		<1-45	Ahel et al., 1994b
	NP <sub>2</sub> EC		2-71	
	NP <sub>1</sub> EC		8.4-20	
U.S.	NP <sub>2</sub> EC		20.6-28.7	Field and Reed, 1996
	NP <sub>1</sub> EC		ND-2.0	
	NP <sub>2</sub> EC		ND-11.8	
Taiwan	NP <sub>1-3</sub> EC		17.6-105	Ding and Tzing, 1998
	CNP <sub>1-2</sub> EC		66.5-138	
	Tot NPEC		16.4-292	
	Tot CNPEC		19.2-755	
Japan	NP <sub>1-2</sub> EC		0.11-2.8	Isobe and Takada, 2004
Taiwan	NP <sub>1-3</sub> EC		ND-63.6	Cheng et al., 2006a
	CAP <sub>1-2</sub> EC		0.7-94.6	
Japan	CA <sub>5-8</sub> P <sub>1</sub> EC		0.12-2.69	Hoai et al., 2006
Belgium	NP <sub>1</sub> EC		0.41-1.9	Loos et al., 2007
	NP <sub>2</sub> EC		0.14-0.62	
	NP <sub>3</sub> EC		0.06-0.79	



Italy	OP <sub>1</sub> EC	0.002	Loos et al., 2007
	OP <sub>2</sub> EC	0.012-0.14	
	NP <sub>1</sub> EC	0.28-2.2	
	NP <sub>2</sub> EC	0.03-1.3	
	NP <sub>3</sub> EC	0.02-1.0	
	OP <sub>1</sub> EC	0.001-0.008	
	OP <sub>2</sub> EC	0.005-0.42	

†: Paper mill effluent; \*: Samples collected 1997; \*\*: Samples collected in 1998; #: Samples collected in 2002; ##: Samples collected in 2003; <sup>a</sup>: membrane bioreactor; ND: Not detected.

Table 2. Total Costs of the Three Sizes of Works Calculated by Application of the TR61 Procedure (Adapted from Jones et al., 2007)

Treatments Used	Works size (PE)	Capital cost for standard STP (£ million)	Capital cost of advanced STP treatment steps (£ million)	Capital cost of digestion & dewatering (£ million)	Total capital cost (£ million)	Operating cost per year for standard STP & sludge treatment (£ million)	Operating cost per year of advanced treatment (£ million)	Total operating cost per year (£ million)	Total cost per m <sup>-3</sup> wastewater Treated (£)
<b>Option 1</b> (AS + sand filter, GAC & ozone)	5,000	2.03	0.70	0 (remote treatment)	2.73	0.19	0.02	0.21	3.17
	50,000	6.94	2.70	4	13.64	0.14	0.14	0.28	1.55
	200,000	20.70	8.00	12	40.70	0.62	0.54	1.16	1.17
<b>Option 2</b> , (AS + sand filter, MF & RO membranes)	5,000	2.03	1.30	0 (remote treatment)	3.33	0.19	0.12	0.31	3.89
	50,000	6.94	9.57	4	20.51	0.14	0.94	1.08	2.41
	200,000	20.70	22.20	12	54.9	0.62	3.61	4.23	1.65

List of Figures

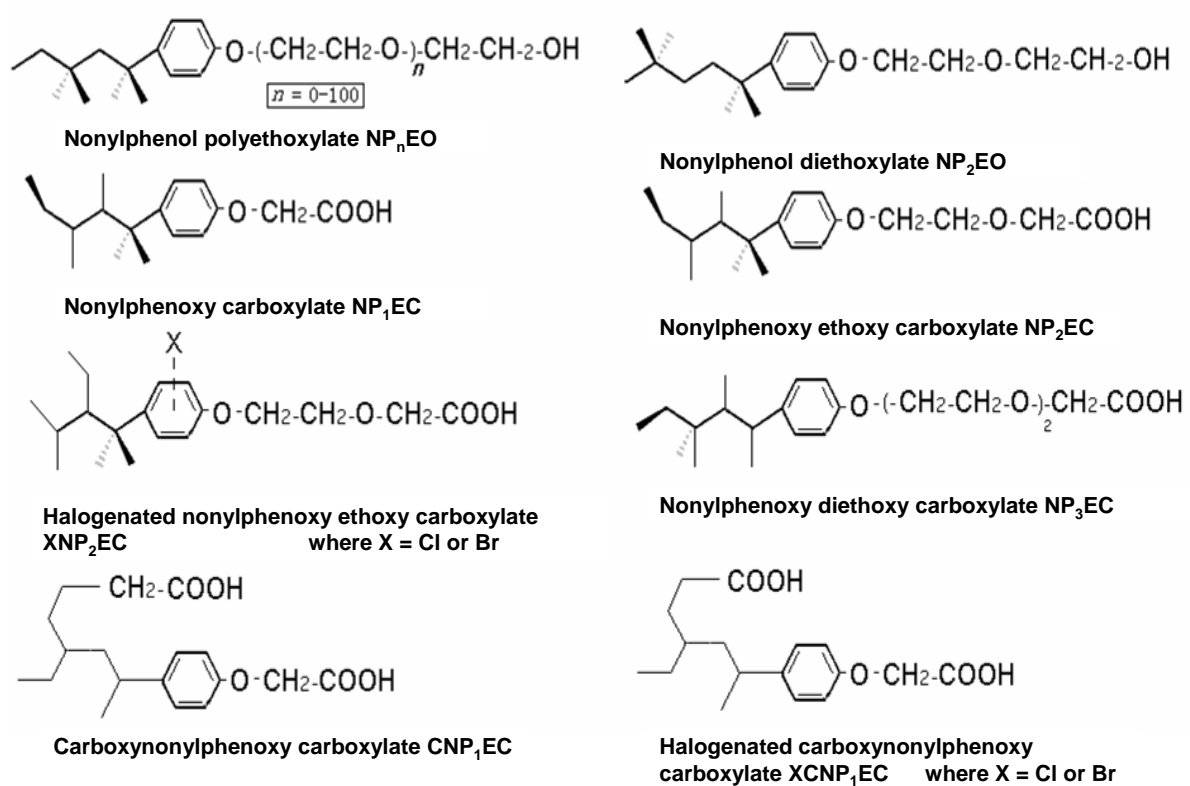


Fig. 1. Structures of some of the common metabolites of APEOs found in STWs influent.

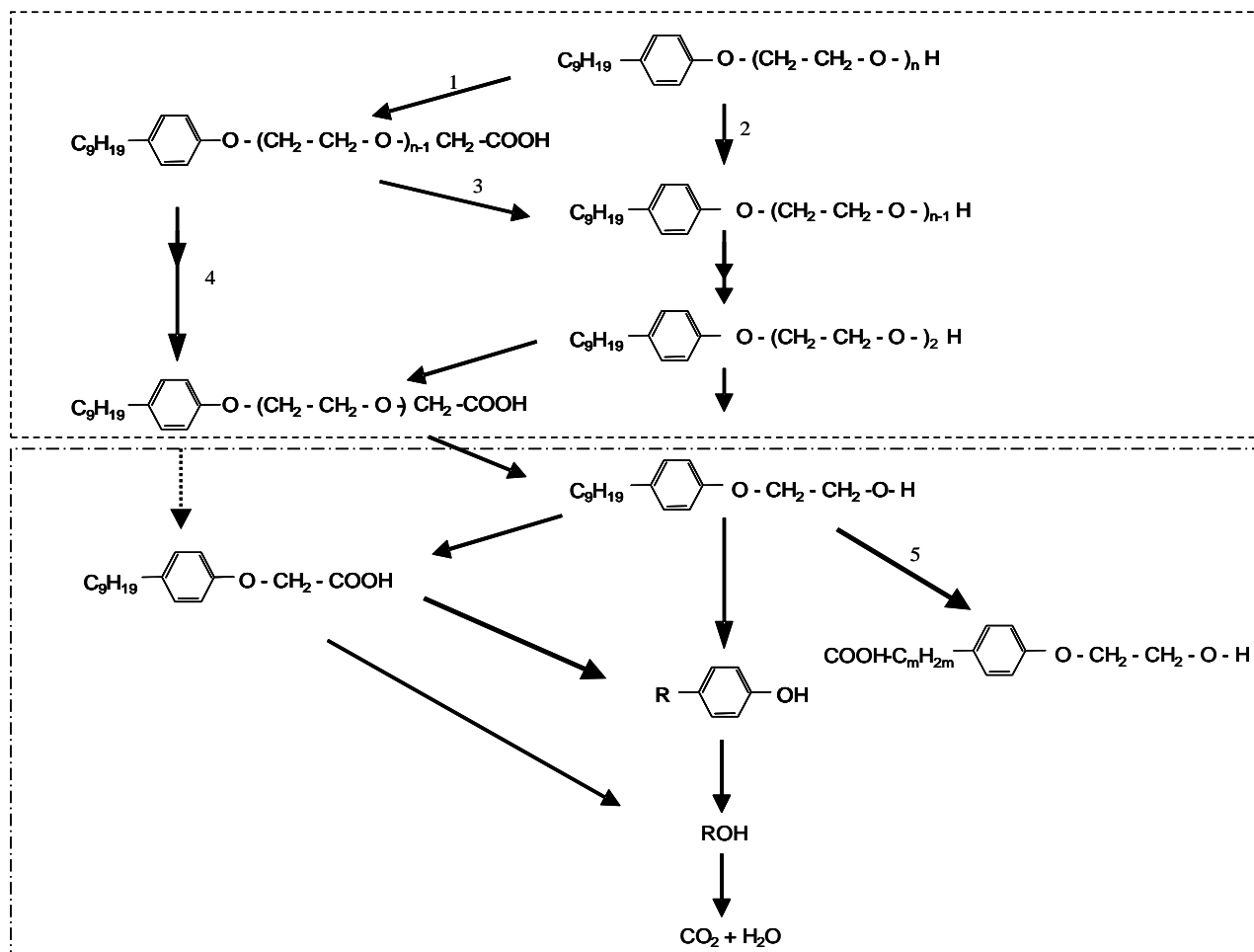


Fig. 2. Proposed pathways for the formation and destruction of mono carboxylated species ( $n = 2-20$ ).

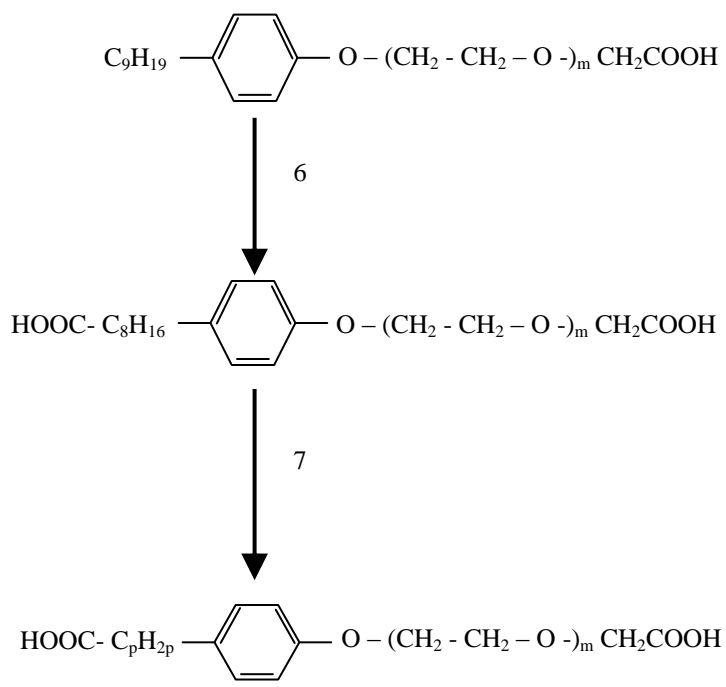


Fig. 3. Formation of dicarboxylated species ( $m = 0-2$ ,  $p = 0-7$ ) (Adapted from Montgomery-Brown and Reinhard, 2003).