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THE ROLE OF BIODEGRADATION AND SORPTION IN DETERMINING THE FATE OF NONIONIC SURFACTANTS IN THE ENVIRONMENT

By

Kathleen A. Wight

A THESIS

Submitted to
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in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Environmental Engineering

1997

ABSTRACT

THE ROLE OF BIODEGRADATION AND SORPTION IN DETERMINING THE FATE OF NONIONIC SURFACTANTS IN THE ENVIRONMENT

By

Kathleen A. Wight

Research studies related to nonionic surfactants -- alcohol ethoxylates and alkylphenol polyethoxylates -- are reviewed. Surfactants are defined; unique structural characteristics are identified. The importance of molecular structure, biodegradation kinetics, physical-chemical properties, and environmental conditions in determining behavior and fate are discussed. Possible biodegradation pathways are presented. Data from the most recent environmental studies are highlighted. Environmental levels of surfactants and measurements from toxicity studies are compared. Results indicate that alcohol ethoxylates biodegrade readily to products that are environmentally safe. However, metabolites of the alkylphenol polyethoxylates -- alkylphenols, alkylphenol mono- and diethoxlyates, and carboxylated alkylphenol mono- and diethoxylates, sometimes halogenated -- accumulate and are an environmental concern in some areas. The paucity of knowledge about the interrelationship between biodegradation and sorption, and the environmental impact of these derivatives are addressed.

To all who have given me wings to soar with the eagles, especially to Dad who taught me about dreaming and to Mom who taught me realism; their undying support provides more strength than they will ever know.

ACKNOWLEDGMENTS

The writing of this paper would not have been possible without the technical assistance and support of the many people named below. I include this section at the risk of inadvertently forgetting to name individuals who have been instrumental in assisting me with any of this paper's many phases. In this event, I apologize to any of you whom I may forget. My omission makes your contribution no less important.

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LIST OF ABBREVIATIONS

1°	primary
2°	secondary
AE_p	alcohol ethoxylate (p = no. of ethoxy units)
AES	alcohol ethoxy sulfate
AP	alkylphenol
APc	alkylphenolic compounds
APc	alkylphenolic compounds
APE_p	alkylphenol ethoxylate (p = no. of ethoxy units)
APE_pC	carboxylated alkylphenol ethoxylate (p = no. of ethoxy units)
AS	alkyl sulfate
ASL	activated sludge
BCF	bioconcentration factor
BHL	biodegradation half-life
BOD	biochemical oxygen demand
br-C_mE_p	designation for branched alcohol ethoxylate with 'm' carbon groups and 'p' ethoxy units
C_mE_p	designation for alcohol ethoxylate with 'm' carbon groups and 'p' ethoxy units
DCPIP	2,6-dichlorophenolindophenol
DDPE	dodecylphenol ethoxylate
DEEDMAC	diethanolester dimethylammonium chloride (cationic surfactant)
DEG	diethylene glycol
DNPE	dinonylphenol ethoxylate
DOC	dissolved organic carbon
DPE	decylphenol ethoxylate
EC_{50}	effect concentration at which a 50% reduction in growth rate is observed
EG	ethylene glycol
EO	ethoxy group (-CH_2-CH_2-O-)

F
G
G
H
H
H
I &
I R
LA
LC
LC
LC
LO
MA
MC
ME
MI
ML
NA
NA
NM
NO
NP
NP
NP
NP
NP
OB
OP
OP
PA
PB-L
PE
PP
PP
PP

FAB/MS	fast atom bombardment with mass spectrometry
GA	glucose amide (nonionic surfactant)
GC/MSD	gas chromatography with mass selection detection
HC	hydrocarbon
HPLC	high pressure liquid chromatography
HRT	hydraulic retention time
I & I	institutional and industrial
IR	infrared
LAS	linear alkylbenzene sulfonate
LC	liquid chromatography
LC/MS	liquid chromatography with mass spectrometry
LC_{50}	lethal concentration at which > 50% of tested organisms die
LOEC	lowest observed effect concentration
MATC	maximum accepted toxicant concentration
MCRT	mean cell-residence time
MEG	monoethylene glycol
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
NAD	nicotinamide adenine dinucleotide (oxidized cofactor)
NADH	reduced nicotinamide adenine dinucleotide (reduced cofactor)
NMR	nuclear magnetic resonance
NOEC	no observed effect concentration
NP	nonylphenol
NP-HPLC	normal-phased high pressure liquid chromatography
NPc	nonylphenolic compounds ($NPE_{1\text{-}20}$, $NPE_{1\&2}C$, NP)
NPE_p	nonylphenol ethoxylate (p = no. of ethoxy units)
NPE_pC	carboxylated nonylphenol ethoxylate (p = no. of ethoxy units)
OES	optical emission spectroscopy
OPE_p	octylphenol ethoxylate (p = no. of ethoxy units)
OPE_pC	carboxylated octylphenol ethoxylate (p = no. of ethoxy units)
PAE	primary alcohol ethoxylate
PB-LC/MS	particle beam-liquid chromatography with mass spectrometry
PEG	polyethylene glycol
ppb	parts per billion
ppm	parts per million
ppq	parts per quadrillion

ppt	parts per trillion
QSAR	quantitative structure-activity relationship
RP-HPLC	reversed-phase high pressure liquid chromatography
RSM	resuspended solid materials
RT	residence time
SAE	secondary alcohol ethoxylate
sec-C_mE_p	secondary alcohol ethoxylate with 'm' carbon groups and 'p' ethoxy units
SIM-GC/MS	selected ion monitoring gas chromatography with mass spectrometry
STP	sewage treatment plant
TBS	tetrapropylenebenzene sulfonate
TF	trickling filter
TLC	thin layer chromatography
TLC/RAD	thin layer chromatography with radio scanner
UV	ultraviolet
WWTP	wastewater treatment plant

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Introduction

For close to half a century synthetic detergents have been the subject of biodegradation and environmental fate research studies. Not long after the initiation of their mass production in late 1946, the effect of synthetic detergents in the aeration tanks of activated sludge sewage treatment plants was obvious, as layers of sticky, bulky, smelly suds up to eight feet thick developed in some cases (*1*). Plant operators measured less dissolved oxygen in the wastewater (*2*), thus more organics in the plant effluent, and observed less settling and filtration of solid sewage material (*1*). The general public was alerted to this situation when foam appeared and remained in receiving natural waters downstream from outlets of wastewater treatment plants (*1-3*). Concern about the long term effects of the new synthetic detergents in our sewage treatment plants, surface waters, and ultimately, our drinking water increased as their use increased. Soon studies identified surfactants, specifically the highly branched, biorefractory tetrapropylenebenzene sulfonates (TBSs), as the detergent components that were causing these adverse conditions. Pressured by consumers and water treatment personnel, surfactant producers replaced TBS with linear alkylbenzene sulfonate (LAS), a more biodegradable surfactant, by mid-1965 (*1*). Since then, other surfactants -- nonionic and cationic -- have come increasingly into use.

Coincident with efforts to find readily biodegradable components, the development of methods to test biodegradability and to determine the environmental fate and safety of detergent components, have continued to be the focus of research studies in the detergent industry. Surfactants, comprising approximately 25% by weight of detergent formulations (*4*), have received most of the attention. Surfactants are noted for their success in a wide range of products which have diverse uses in household, personal care,

institutional, and industrial markets (*5-7*). Total worldwide surfactant consumption during 1995 was approximately 8.4 million metric tons (Table 1). The North American share of this total was about 30%, 2.5 million metric tons. More than half of the 1995 world total, approximately 4.9 million metric tons, was used in consumer products in the form of detergents, dishwashing and cleaning agents, cosmetics and toiletries (*8*). Most of the remainder was used in a variety of industrial applications, such as lubricants, pastes, waxes, plastics, cements, plaster, and hydraulic fluids (*5, 8*). Surfactant consumption continues to grow, particularly in Asia and the third world countries (Table 1).

Table 1. Worldwide Consumption of Surfactants[1]

A. By Region	1995 (10^6 metric tons)	2005 (10^6 metric tons)	Change (%)
North America	2.5	2.8	12
W. Europe	1.8	2.2	22
Asia	2.6	4.0	54
Other Regions	1.5	2.3	53
Total	8.4	11.3	35
B. By End Use			
Household	4.3	5.8	35
Personal Care	0.6	0.9	50
I & I/Industrial	3.5	4.6	31
Total	8.4	11.3	35

[1] Ref. (*8*); soap not included.

The sense of urgency in understanding the fate of surfactants is attributed to:

1) their widespread use and release by households, industries and institutions, in large quantities (U.S. consumption of household and personal care products was over 6.8 million metric tons in 1993 (*8*));
2) knowledge gained through biodegradation and environmental fate studies that the metabolites of some surfactants, particularly the alkylphenol polyethoxylates (APEs), appear to persist and

accumulate in the environment (*9-17*), sometimes as halogenated derivatives (*18-23*);

3) increased awareness that some of the persistent APE metabolites are more toxic or more likely to bioaccumulate than their parent compounds (*24-27*); and

4) concern that some persistent APE metabolites, acting as estrogen mimics, may cause irreversible ecotoxicological damage in as yet unknown ways (*28-34*).

Predicting the environmental fate of any chemical relies upon gaining knowledge about the biological, chemical, and physical processes that determine its transformation (change in chemical structure) and disposition (no structural change) in the environment. A myriad of occurrences are possible -- deposition, volatilization, precipitation vs. dissolution, vertical and horizontal mixing, diffusion, sorption, sedimentation, bioturbation, resuspension, and photochemical, chemical, and biological reactions (*35*). Designed to be applied in water, surfactants are highly soluble and negligibly volatile. Thus biological reactions and sorption followed by sedimentation play major roles in determining fate in the environmental compartments in which detergent components are found (*6, 15, 36, 37*). Bioturbation, resuspension, volatilization, and photochemical reactions may be minor processes in the aquatic environment.

This paper reviews the results of recent research related to nonionic surfactants. Surfactants are described, with emphasis on the major ones found in synthetic detergents. Reasons for the evolving significance of nonionic surfactants are enumerated. The environmental compartments that are affected by the use of detergent surfactants are identified. The importance of biodegradability tests and biodegradation kinetics in determining

environmental acceptability is discussed, as is the importance of sorptive processes. Possible biodegradation pathways are presented. The most recent findings from environmental studies are highlighted, primarily those not discussed in previous comprehensive reviews (*6, 27*). A summary of data measured in environmental analyses is provided. Emphasis is placed on the role of microbial degradation, while also considering the effects of sorption. A discussion about the results of studies that have detected halogenated derivatives of alkylphenol ethoxylates in treated wastewater, surface waters, and drinking water is included. Integral to this review is a discussion of the manner in which molecular structure, physical-chemical properties, and environmental conditions determine surfactant fate. Concerns about the persistence, accumulation, and toxicity of surfactants and their metabolites are addressed.

Surfactants

Definition/Major Categories

A surfactant, short for "surface active agent", functions as a coupling agent between two surfaces, bringing them closer together because of the way the molecules orient themselves at the interface of the two surfaces (*1*). Surfactant molecules tend to concentrate at air-water, solid-water, or non-aqueous liquid-water interfaces (*6*). This tendency exists because structurally, surfactants are amphiphiles, with polar (hydrophilic) and non-polar (hydrophobic) moieties. The molecules orient themselves so that the hydrophilic (water-attracting) ends are in the water and the hydrophobic (water-avoiding) ends are positioned as far away as possible in the opposite direction (*1, 6*). Differences in the ionic properties (electrical charge) of the hydrophilic groups in water are the basis for classifying surfactants into one of four large groups -- nonionic, anionic, cationic, or amphoteric (zwitterionic). Within these groups surfactants can be further classified based on the means of linkage between the hydrophilic and hydrophobic moieties. The hydrophobic component may take one of several forms, but does not affect the classification of the surfactant into the four large groups named above (*6, 7*).

Structure

Hydrophobic groups are derived from fatty acids, olefins, alcohols, alkylbenzenes, alkylphenols, paraffins, or polyoxypropylenes. A hydrocarbon of between eight and twenty carbon groups is usually seen in the hydrophobic moiety of surfactants. The derivatization process may produce linear or branched alkyl groups. The benzene or phenol ring can be linked anywhere along the alkyl chain; ring substituents may be present in the *para-*, *ortho-*, or *meta*-position. A large number of various structures is

possible from the many available combinations. Additionally, the character of some hydrophobes (e.g. alcohols and alkylphenols) may be modified by adding propylene oxide. The longer the polyoxypropylene polymer, the more hydrophobic the compound. All hydrophiles possess polar groups that make them soluble in water. Hydrophilic groups may or may not ionize in aqueous solution. The many uses of surfactants derive naturally from the permutations and combinations that are possible with the large selection of hydrophobic and hydrophilic groups, and the ways of linking them together (*6*). Figure 1 provides examples of configurations for the three surfactant categories that are most often used in detergents.

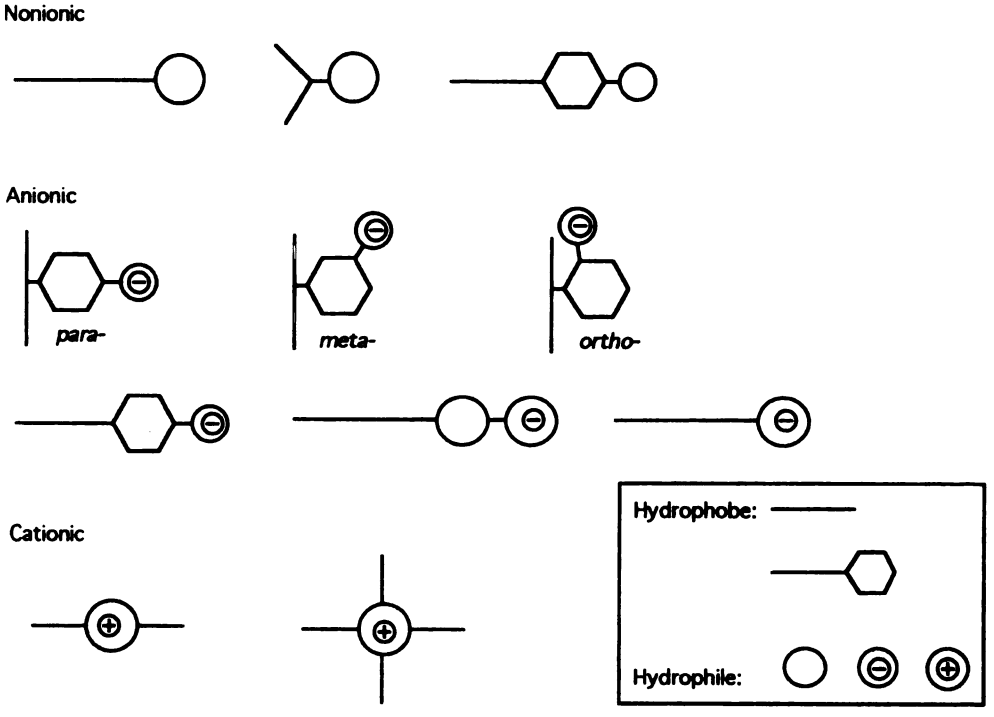
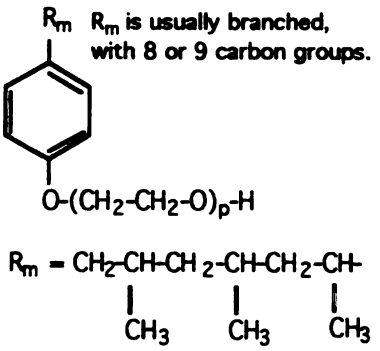


Figure 1. Examples of hydrophobe/hydrophile arrangements in detergent surfactants. Adapted from (*5*).

Nonionic surfactants do not dissociate into ions in aqueous solution. Anionic and cationic surfactants dissociate into negative and positive ionic fractions, respectively. The negative ion carries the surface active properties of the anionic surfactant and the positive ion carries the surface active properties of the cationic surfactant. A nonionic group commonly seen is polyoxyethylene ($(-O-CH_2-CH_2)_n-OH$). Anionic groups are sulfonate ($-SO_3^-$), sulfate ($-OSO_3^-$), and carboxylate ($-CO_2^-$). Quaternary ammonium ($-R_4N^+$) is an example of a cationic group. Amphoteric surfactants are molecules that carry both a positive and a negative charge in aqueous solution. Whether they have anionic or cationic properties is dependent on medium pH and composition (*5, 6*). Amphoteric surfactants are not generally used in detergents (*4*). Example molecular structures of the five surfactants (2 nonionic, 3 anionic) used most worldwide are shown in Table 2.

In this paper, C_mE_p denotes a primary linear AE molecule that consists of an average of m alkyl carbons and p EO units. Sec-C_mE_p or br-C_mE_p is used if the alkyl moiety is secondary or highly branched, respectively. APE_p (p=1-20) denotes an alkylphenol polyethoxylate that has p ethoxy groups. Oxidation of the terminal ethoxy unit during biodegradation may result in a carboxylated derivative. APE_pC denotes a carboxylated alkylphenol polyethoxylate that has p-1 ethoxy groups terminated with an acetic acid group. AP refers to an alkylphenol (no substituents). The prefixes OP-, NP-, DP-, and DDP- are used in place of AP- to define octyl-, nonyl-, decyl-, and dodecylphenol, respectively.

Table 2. Major Synthetic Detergent Surfactants

Common name	Acronym	Chemical structure	m,n = No. of carbon gps in hydrophobe; p = No. of ethoxylate gps. in hydrophile;
Nonionic surfactants			
Alcohol ethoxylates -primary -secondary	AE PAE SAE	R_m-O-(CH_2-CH_2-O)$_p$-H C(Rm)(Rn)-O-(CH_2-CH_2-O)$_p$-H	Primary: m = 8 - 18;[1] Secondary: 8 ≤ m+1+n ≤ 18;[1] All AE: p = 1 - 25 [2] p_{avg} = 6 - 11 [2]
Alkylphenol polyethoxylates	APE		m = 8 - 12;[1] p = 1 - 20 [3] p_{avg} = 9 - 10 [1]
Anionic surfactants			
Linear alkylbenzene sulfonates	LAS		10 ≤ m+1+n ≤ 15 [4,5]
Alcohol ethoxy sulfates	AES	R_m-O-(CH_2-CH_2-O)$_p$-SO_3Na	m = 12-15 [6] p = 3-7 [4] p_{avg} = 2-4 [6]
Alkyl sulfates	AS	R_m-O-SO_3Na	m = 8-18 [7]

Adapted from (*5*). Other references: [1] (*27*); [2] (*38*); [3] (*39-41*); [4] (*6*); [5] (*42*); [6] (*43*); [7] (*44*).

Detergency

Surfactants function as cleaners because as they concentrate at interfaces, they reduce the surface tension of water. At the air-water interface foam is produced, as seen in treatment plants and natural waters subsequent to their introduction (*1, 6*). Surface active agents may possess other properties -- wetting, dispersing, and emulsifying -- to various degrees. In a cleaning agent, or detergent, all three properties exist to approximately equal degrees (*1*). The wetting property lowers the solid-water interfacial tension, thus displacing air from solid surfaces and allowing penetration of a water solution into fabric where it can reach soiled material. At the solid-water and non-aqueous liquid-water interfaces, the dispersing property separates agglomerated particles of oil and dirt; the emulsifying property links water molecules with these particles, thus holding them in suspension (Figure 2). Soil removal occurs because the hydrophobic ends of the surfactant molecule surround soil particles as they are attracted to them. Simultaneously, the hydrophilic ends are attracted back into the wash water, lifting the soil particles away from the fabric. This action, together with the mechanical agitation of a washing machine, provides effective soil removal and suspension while preventing redepositon (*1, 5, 6*).

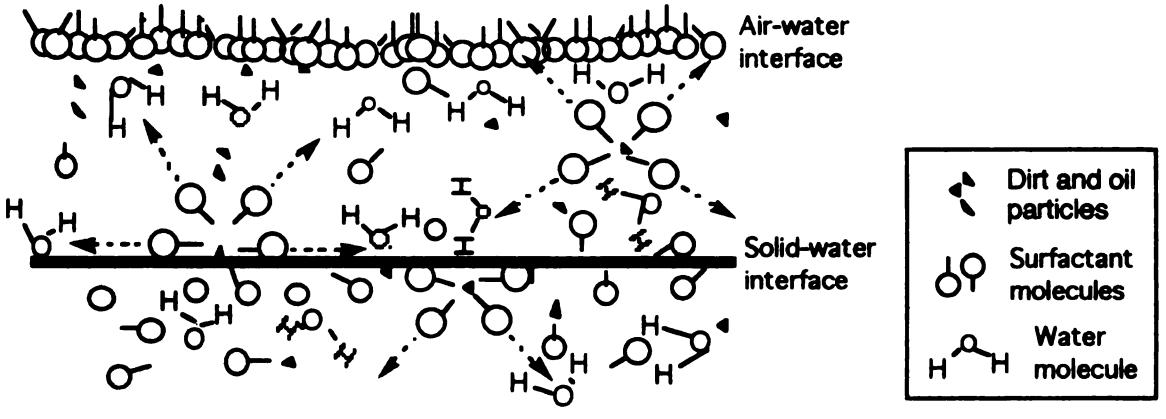


Figure 2. Surfactants clean by wetting, dispersing and emulsifying.

Nonionic Surfactants

Worldwide consumption of the most used nonionic surfactants -- alcohol ethoxylates (AEs) and alkylphenol polyethoxylates (APEs) -- was close to 1.4 million metric tons during 1995. The U. S. share of this was 227 thousand metric tons, over 16%. Though anionics enjoyed about 75% of market share in 1995, several developments in the synthetic detergent market, some interrelated, are eroding the anionic surfactant market share in favor of nonionic surfactants:

1) nonionics are superior to anionics in cleaning synthetic fibers (*3*);
2) nonionics clean better in cold water, which is preferred over hot water both for cleaning man-made fibers (*3*) and for conserving energy (*45*);
3) the nonionics, especially the linear primary alcohol ethoxylates (PAEs), continue to gain favor over the linear alkylbenzene sulfonates (LASs) because of their rapid biodegradability (*45*);
4) the gradual shift from dry powder to liquid detergents (*45, 46*) favors increased usage of nonionic surfactants (*45, 47*);
5) pushed by speculation that an upcoming U. S. Department of Energy directive will require more energy efficient, horizontal axis washing machines that will use 75% less water (*8, 48*), detergent manufacturers look to nonionics to satisfy requirements for a surfactant that will perform well in cold water with minimal foaming and superb rinsing capabilities (*6, 45*); and
6) detergent formulations, driven by cost and therefore likely to change according to fluctuating prices of raw materials, periodically favor the alcohol moiety used in alcohol ethoxylates over the alkylbenzene moiety used in LAS (*3, 48*).

During 1995 worldwide consumption of AEs and APEs was 726 and 635 thousand metric tons, respectively (*8*). The largest market for the AEs, mainly linear PAEs, is household laundry detergents and cleaning products (*27, 49*). Additionally, a large share of linear PAEs in the United States provides the alcohol ethoxy moiety of alcohol ethoxy sulfates (*27, 50*). Secondary and branched AEs, as well as the linear PAEs, are used in other specialized household cleaners, personal care products, and institutional and industrial (I & I) cleaners (*27, 49*). The APE's primary market in the U. S. is the industrial processing sector (55%). The remaining 45% is split between institutional cleaners (30%) and household and personal care products (15%) (*27*). Applications for both AEs and APEs exist in agriculture (*5, 18*), pulp and paper (*10*), textile and tanning (*20*), and other process industries (*27*). The AEs are gaining a stronger position in the nonionic market because concern about the persistence, accumulation, and toxicity of APE metabolites is forcing replacement of APEs with AEs in the I & I, household, and personal care market sectors (*45*).

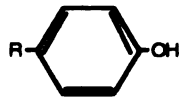
Alcohol ethoxylates -- manufacture

The manufacture of alcohol ethoxylates requires the reaction of alcohols with ethylene oxide. The alcohols, usually comprised of 8-18 carbon groups, are most often linear primary, but may also be linear secondary or branched (*27*). Linear alcohols that originate from vegetable or animal sources (oleochemicals) have an even number of 12 - 18 carbon groups (*38*). The number of carbon atoms (usually between 11 and 15) in those that are derived from crude oil (primarily ethylene) or natural gas (petrochemicals) can be even or odd (*27, 38, 51*). The number of ethoxy units found in the AEs typically used in detergents varies from one to between 15 and 25, with an average of 6 to 11 units (*38*) (Table 2).

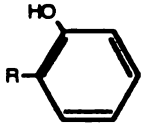
Biodegradability is not an issue in choosing between raw materials (*51*). Results of life cycle inventories indicate that both sources -- oleochemicals and petrochemicals -- have environmental trade-offs (*52*). Therefore, cost of production and availability, not biodegradability or other environmental considerations (e.g.: air emissions, waste byproducts, consumed energy), are determining factors (*45, 53*).

Alkylphenol polyethoxylates -- manufacture

The manufacture of alkylphenol polyethoxylates requires a base-catalyzed reaction of alkylphenols with ethylene oxide. The alkylphenol is produced by reacting branched olefins with phenol (acid-catalyzed). The alkyl group may exist in a variety of isomeric configurations. More than 90% of the alkylphenol products exist in the *para*-isomer form with less than 10% as *ortho*-isomers:



para-alkylphenol



ortho-alkylphenol .

Joined to the alkylphenol via an ether linkage is a hydrophilic chain with 1 to 100 ethoxy (EO) units (*27*). In spite of concerns about their environmental persistence, accumulation, and toxicity, use of the APEs continues because they are excellent performers with relatively low production costs (*5*). Octyl- , nonyl- , and dodecylphenols are found most frequently in commercially available APEs (*3, 5*). Use of 4-nonylphenol polyethoxylates is predominant in detergents (*5, 27*). Propylene trimer is the alkene used in the manufacture of the nonylphenol polyethoxylates (*5, 27, 54*); the hydrophile commonly consists of between 1 and 20 EO units (39, 40, 41), averaging 9 to 10 units per molecule (*27, 40*) (Table 2). Trace amounts of 2-nonyl-, 4-octyl-, and 4-decylphenol may be present as impurities in commercial 4-nonylphenol (*13, 55*) .

Environmental Compartments of Concern

Detergent surfactants, used and disposed of in wash water, or their metabolic products are found in sewage distribution systems, sewage treatment plants (STPs), surface waters, sediments, and groundwater. Figure 3 provides a pictorial diagram of the environmental compartments affected by the release of surfactants. Each of the major compartments is discussed briefly.

Sewer Distribution Systems

The safety assessment of surfactants begins with analysis of behavior in sewage distribution systems. Sanitary sewers carry domestic and industrial wastewater to treatment plants for removal of solids and organics prior to release to surface waters. Flows from residences, commercial buildings, institutions, recreational facilities, and industries (*56*) are channeled to interceptor and/or main sewers by way of collecting sewers before reaching the wastewater treatment plant. Sewer systems are usually designed to carry flows at velocities between 0.6 and 3 meters per second. Depending on the size of the area being served and distance from point of entry to treatment plant, minutes to hours may pass during a waste material's transit through a sewer distribution system (*57*). The extent to which sewers contribute to removal from the water phase is dependent on percent solids (affects sorption) and residence time (affects biodegradation).

Sewage Treatment Plants

Most sewage treatment facilities are designed to provide secondary as well as primary treatment. Some also offer tertiary, or advanced, treatment. *Primary treatment* removes settleable or floating solids physically. *Secondary treatment* removes dissolved organics biologically or chemically; activated sludge is the biological system used most worldwide. *Advanced treatment* combines operations and processes to remove other constituents, such as

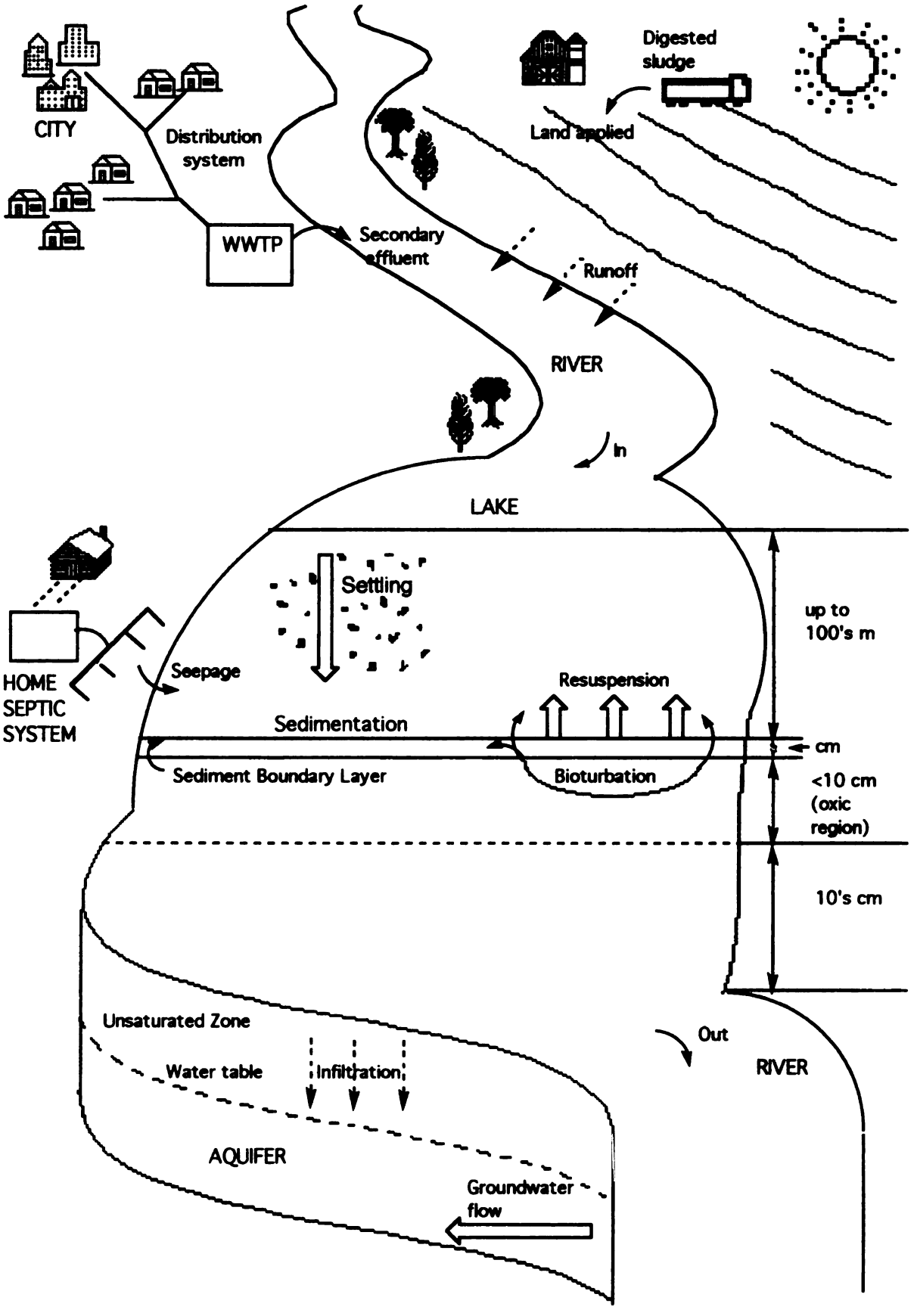


Figure 3. Environmental compartments affected by detergent surfactants. Adapted from Ref. (*58, 59*).

nutrients or priority pollutants, that are not removed during secondary treatment. Figure 4 is a generalized flow chart of the activated sludge sewage treatment plant seen commonly in the United States (*56*). Biofilters would replace the aeration tank in a flow chart for a trickling filter plant. An important aspect of the sewage treatment process is *sludge digestion and disposal.* Stabilization of sludge occurs during sludge digestion, which further decomposes organic and inorganic matter. The process is commonly designed to occur anaerobically, although aerobic digestion is also used, usually for smaller wastewater treatment facilities. Ultimately secondary effluents are disposed of in surface waters. Though there are many ways to dispose of digested sludge (*56*), the effects of land application only will be discussed in this paper. Appendix A provides a more detailed description of the major processes in sewage treatment facilities. Approximate times for the various waste processing segments are provided in Table 3. Times will vary under different load, temperature, and other environmental conditions (*56*). Typical ranges of suspended solids concentrations, found in a variety of environmental matrices, are provided in Table 4.

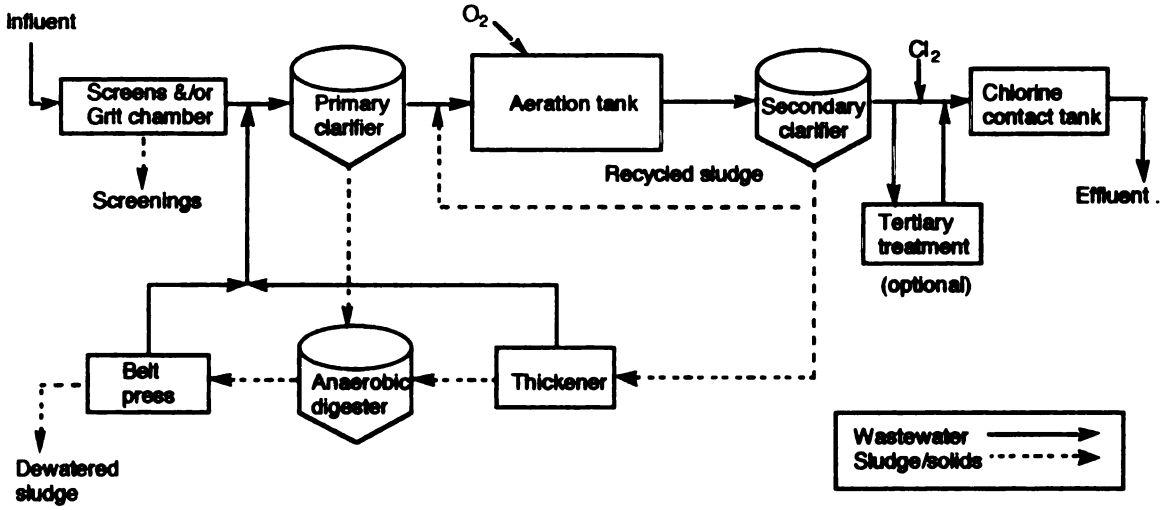


Figure 4. Generalized flow diagram of an activated sludge treatment plant. Adapted from Ref. (*56*).

Table 3. Activated Sludge Treatment Plant Retention Times

Process	Time Range
Primary	
-- Primary settling tank	1.5-2.5 hrs
Secondary	
-- Aeration tank (hydraulic)	3-8 hrs
-- Aeration tank (cell)	5-15 days
-- Clarifier	3-4 days
Contact Tank	15-45 mins
Anaerobic Digester	
-- Standard	30-90 days
-- High rate	10-20 days

Ref. (*56*).

Table 4. Concentrations of Suspended Solids in Selected Media

Medium	Concentration Range (mg/L)
Untreated domestic wastewater	100 - 350[1,2]
Aeration tank (MLVSS)	1,200 - 6,400[1,3]
Primary and waste activated sludge	30,000 - 80,000[1,4]
Secondary sludge (w/primary settling)	5,000 - 15,000[1,4]
Septage (cess pools and septic tanks)	2,000 - 100,000[1,5]
Surface water	0.5 - 600[6]

[1] Ref. (*56*). [2] Table 3-16, p. 109. [3] 0.80 * MLSS (*56, p. 390 and Table 10-5, p. 550*). [4] Table 12-8, p. 774. [5] Table 14-22, p. 1092. [6] Ref. (*60*).

Surface Waters and Sediments

Surface waters of interest in this paper include rivers, tributaries, lakes, and estuaries. Secondary effluents, usually discharged into a river or its tributaries, are the predominant source of surfactants in surface water. Behavior and fate studies focus on surfactant concentration levels (in effluent, surface water and sediment samples), stream flow, dilution factors, colloidal material, suspended solids concentrations, bio-transformation/ sorption rates, sedimentation rates, bioconcentration factors, and toxicity measurements.

The upper limit of 600 mg/L given for the concentration of suspended solids in surface waters (Table 4) may be low in some instances. According to (*61*), solids concentrations in natural water systems range between 10 and 10,000 mg/L, where 10 mg/L represents the lower limit of suspended solids concentrations in rivers and estuaries, and 10,000 mg/L represents the lower limit of solids concentrations found in sediment beds. Therefore it is conceivable that turbulence caused by high winds or heavy flow in a natural water system could increase suspended solids to levels above 600 mg/L.

Groundwater

Although surface waters, as the sink for secondary effluents discharged from STPs, have received most of the attention in environmental fate studies, they account for less than 5% of the earth's total freshwater supply. Approximately 95% of the freshwater used for domestic, commercial and industrial purposes is obtained from aquifers. Due to this distribution of freshwater supplies, groundwater is ever more important as a resource to meet increasing demands worldwide (*59*). This explains the concern about groundwater contamination, and recent investigations that have examined the behavior and fate of pollutants and microorganisms in groundwater (*62-73*).

Groundwater regions are subject to long-term contamination because their characteristics -- lesser amounts of mixing and dilution, low temperatures, and reduced microbial diversity and population sizes -- are not favorable to high rates of biodegradation (*64*). Behavior and fate predictions about movement (advection and dispersion), sorption (retardation), biotransformation, and resulting concentration levels through subsurface regions are complicated by the heterogeneous nature of aquifer material. The complex relationship between hydro-geological processes and contaminant behavior and fate dictates an interdisciplinary approach to groundwater research. Sampling must be much more extensive than in surface waters to ensure that contaminant plumes are properly identified. Subsurface residence times can vary from a relatively short period of several hours (*62*) to a very prolonged period of up to thousands of years (*59*). Studies begin by making simplifying assumptions that address only the major mechanisms at work in the groundwater environment. Given appropriate residence times and environmental conditions favorable to microbes, sorption and biological transformation are again the major removal mechanisms of surfactants in these regions.

The Role of Biodegradation in Assessing Environmental Safety

Environmental safety is assessed by evaluating data that measures ecological hazard and exposure (RISK = HAZARD * EXPOSURE). Results from toxicology studies, discussed in a later section, provide information for the HAZARD part of the risk assessment equation. Results from biodegradation and environmental fate studies provide information about the EXPOSURE part of the equation. Acceptance of the risk assessment is more probable as certainty increases that study results reflect what happens in the environment. A variety of approaches, test methods, and analysis techniques for determining biodegradation pathways, and extent and rate of biodegradation have evolved from concern about surfactant safety. Determination of biodegradation pathways is very complex and must be carried out in the laboratory under closely controlled conditions (*2*). Determination of the extent (biodegradability) and rate (kinetics) of biodegradation is conducted under both laboratory and field conditions (*74-78*). Environmental fate studies consider other factors, such as sorption, that may affect a compound's ultimate disposition. A host of terms related to chemical structure, biodegradability, water treatment, and affected environmental compartments has evolved from biodegradation, environmental fate, and toxicology studies. A glossary of these terms is provided in Appendix B.

To the extent that biodegradation is a major removal mechanism in the environment, thus reducing exposure, the assessment of biodegradability through standardized tests continues to be of primary importance when considering the introduction of all new component molecules to detergent formulations (*79-84*). Guidelines established by the Organization for Economic Cooperation and Development (OECD) suggest a tiered approach (three test levels), that acknowledges the inadequacy of laboratory testing to evaluate

ecological impact. The OECD guidelines are used widely by the detergent industry. The first level test is stringently designed and least resembles environmental conditions. Though the test is carried out under aerobic conditions, the test substrate is offered as the only source of carbon; the test substrate concentrations are higher and the active biomass concentrations are lower than found in the environment. Compounds that achieve ultimate biodegradation to acceptable levels within a specified time period during the first level screening test are classified as readily biodegradable. Conditions for the next two test levels are adjusted so that they are progressively more environmentally relevant. Compounds that meet the second level test requirements are classified as inherently biodegradable. Conditions in the third and final test in the series simulate the real world as closely as possible. This test assesses the rate as well as the extent of biodegradation (*82, 83*).

Degradation Kinetics

Microorganisms fulfill their requirements for energy and growth by catabolizing inorganic and organic compounds. Transformations occur in the presence of enzymes that organisms produce to fulfill their own metabolic needs (*85*). The rate of transformations are dependent on the level of requisite enzymatic activity, which is a result of microbial growth (*86*). Mathematical expressions describe the rate at which microorganisms grow, the rate at which breakdown occurs (substrate utilization), and the relationship between the two (*85*).

- Growth rate of microorganisms:

  $\mu = \mu_m S/(S+K_s) - b$ [1] $\mu_m = k_m Y_m$ [2]

- Substrate utilization:

  $dS/dt = - k_m X S/(S+K_s)$ [3]

- Relationship between growth rate and substrate utilization:

$$\mu = Y_m ((-dS/dt)/X) - b \quad [4]$$

where:

b = decay coefficient, day $^{-1}$

k_m = maximum specific rate of substrate utilization, day $^{-1}$

K_s = half-velocity coefficient, mg/L

μ = specific growth rate, day $^{-1}$

μ_m = maximum specific growth rate, day $^{-1}$

S = rate-limiting substrate concentration, mg/L

t = time, days

X = concentration of active microorganisms, mg/L

Y_m = maximum organism yield, mg organisms / mg substrate.

The minimum substrate concentration is defined to be the point at which growth equals decay:

$$S_{min} = K_s (b/(\mu_m - b)) = K_s (b/(Y_m k_m - b)) \quad [5]$$

Equations [1 -5] apply when an individual species is utilizing a single substrate for energy and growth. All other requirements are in ample supply and the substrate is rate-limiting. If the substrate concentration is above S_{min}, then net growth will occur. The substrate is a primary substrate (*85*). This set of circumstances can be set up easily in the laboratory when requirements for an individual species are known, but it rarely occurs in nature.

Environmentally, a variety of organisms are offered a mixture of organic and inorganic compounds, at concentrations well below S_{min}. The extent to which a population of organisms is established (X), the amount of substrate present (S) and the reaction velocity (K_s) determine the process by which the transformation occurs. In addition to the case where a single primary substrate is present, any one or a combination of several scenarios is

possible, and are determined in part by the interrelationships between X, S, and K_s. Possible transformation processes are summarized below (*85*).

1) $S >> S_{min}$ – S is the primary substrate, and net growth occurs;
2) $S << S_{min}$ – S is a secondary substrate;
   a) the substrate concentration is too low to support growth, but in the presence of a primary substrate that supports growth, the organism may still be capable of consuming (transforming) the secondary substrate;
   b) there may be several secondary substrates – S_1, S_2, S_3, ..., S_n – all at very low concentrations; versatile organisms might utilize many of the low concentration substrates simultaneously, such that they generate enough energy to satisfy both maintenance and net growth requirements;
3) co-metabolism occurs when an organism does not obtain energy from a transformation that is catalyzed by enzymes the organism produces; a molecule is transformed fortuitously.

Further examination of the substrate utilization equation [3] yields two different relationships when the substrate concentration S is extremely large or small relative to the half-velocity coefficient K_s.

$$dS/dt = - k_m X \text{ when } S >> K_s \quad [6]$$

$$dS/dt = - (k_m/K_s) X S \text{ when } S << K_s \quad [7] \qquad k' = k_m/K_s \quad [8]$$

Note that substrate utilization is first order (k_m is the rate constant) with respect to the concentration of microorganisms when the substrate concentration is large. Substrate utilization is second order (k' is the rate constant, equation [8]) with respect to substrate and organism concentrations when the substrate concentration is small (*85, 87*). The measurements of surfactant concentrations that are recorded in Tables 10, 22 - 24, 26, and 27

provide evidence that nonionic surfactant concentrations in surface waters, sediments, and groundwaters are usually well below 1 ppm. Therefore, the processes described above for secondary substrate utilization and co-metabolism generally apply for the transformation of surfactants.

Many factors, not yet understood, affect the rates of biodegradation in primary and secondary substrate utilization, and co-metabolism. Enzymes, produced by microorganisms while meeting their metabolic needs, catalyze reactions, but a very complex set of rules has evolved to govern enzyme induction, repression, inhibition, and activation (*85*). Together with varying environmental conditions such as substrate mix, microbial population, organic content, nutrient availability, suspended solids, pH, temperature, acidity, and salinity, there are too many factors that can cause wide variations in biodegradation rates from one location and time to another to allow reliable prediction.

In spite of the above limitations, some researchers have attempted to determine biodegradation rates using a more direct and simplified approach (*75*). The many complexities of biodegradation processes are ignored. The test compound is the sole source of carbon and energy (*75, 88*) and may be radiolabelled (^{14}C) (*75, 78, 86, 89-92*). Compounds are tested in various environmental matrices. Over a range of concentration levels spanning up to several orders of magnitude, measurements of CO_2 or $^{14}CO_2$ evolution and C or ^{14}C substrate removal have yielded curves that follow zero (*78, 86, 90*), first (*75, 78, 86, 88-90, 92*), or mixed-order (*75, 86, 90*) kinetics. At lower concentrations that are representative of environmental levels, CO_2 evolution and substrate removal are typically best described using first order kinetics, as depicted in Equations [9 -10] below. The rate constant is determined with a non-linear regression model. CO_2 or $^{14}CO_2$ evolution data and substrate (C or

^{14}C) removal data is analyzed using a basic exponential product formation equation and an exponential decay equation, respectively (*89-91*).

- Formation:

$$P = 0 \quad for\ t \leq c$$
$$P = P_0(1 - e^{-k_1(t-c)}) \quad for\ t > c \qquad [9]$$

where:

P = CO_2 produced

P_0 = maximum amount of CO_2 produced (theoretical)

t = time (days)

c = lag time (days)

k_1 = production rate constant (day^{-1}).

- Decay:

$$C = C_0 \quad for\ t \leq c$$
$$C = C_0(e^{-k_2(t-c)}) \quad for\ t > c \qquad [10]$$

where

t and c are as defined above

C = amount of substrate remaining in solution

C_0 = initial substrate concentration

k_2 = decay rate constant (day^{-1})

First order equations similar to equation [9] have been found to describe the ultimate biodegradation of compounds in both soil and aquatic test systems (*75*). Equation [9] can describe ultimate biodegradation when environmental concentrations are low because net growth (production of biomass) occurs only at higher concentrations, after energy needs for maintenance have been fulfilled. In keeping with the previous discussion following equation [8] about the interrelationship of substrate utilization, substrate and organism concentrations, and the rate of biodegradation, the two rate constants, k_1 and

k_2, are really pseudo first order rate constants, with $k_1 = k_1' X_1$, and $k_2 = k_2' X_2$. That is, k_1' and k_2' are second order rate constants with respect to substrate and organism concentrations (*75, 91*).

When pseudo first order kinetics applies, another approach in safety assessment evaluations may consider the ratio of a compound's biodegradation half-life (BHL) and its residence time (RT) in a particular environmental compartment. The biodegradation half-life is the time it takes for half the substrate of interest to biodegrade. The residence time is the length of time the compound is in the particular environmental compartment of concern. The ratio -- BHL/RT -- provides an estimate of the amount of biodegradation that occurs. If the ratio is equal to one, about half the material will biodegrade. As the ratio becomes very much smaller or greater than 1, then greater or lesser amounts of removal via biodegradation, respectively, will occur (*76, 81, 93-95*).

Once the biodegradation rate constant has been estimated, the BHL can be obtained by solving equation [9] for t such that:

$$t_{1/2} = 0.693/k_1 \qquad [11]$$

Though calculation of the BHL/RT ratio may provide a relative sense of the effect of removal by way of biodegradation, it cannot be the only criteria by which surfactant safety is evaluated. The value calculated for the biodegradation half life is only a first approximation, just as the rate constant calculated by fitting data to equations [9 and 10] is a first approximation (*75*), The residence time in a given aquatic or soil system can be estimated, but it can vary widely with the passage of time, from one location to another, based on flow velocity, settling velocity, suspended solids, and clay/organic content.

Other Factors Affecting Biodegradation

Many other aspects must be considered in biodegradation studies. A compound's biodegradability or, conversely, its recalcitrance is a function of both the conditions of its environment and its molecular structure. Table 5 lists factors that affect the extent and rate of biodegradation of organic compounds in the laboratory and in nature. The absence of any one of these may prevent a compound from degrading.

Table 5. Factors Affecting Extent and Rate of Biodegradation of Organic Compounds [1]

1. Accessibility of substrate to biodegrading organisms
2. Presence of requisite enzymes
   - -- Enzyme must exist (assumes prior exposure to similar molecular structure)
   - -- Enzyme must be available to act on substrate
     - - induced if it is not constitutive
     - - not inactivated by adsorption or inhibition
   - -- Cell walls must be permeable to compound if enzyme acts intracellularly
3. Availability of requisite substances for energy and growth (water, organics, nutrients, minerals, electron acceptors)
4. Presence of favorable environmental conditions (pH, salinity, alkalinity, temperature, osmotic pressure, toxicity)
5. Presence of molecular structure(s) that enzyme(s) can act on

[1] Ref. (*96-99*)

Physical-chemical properties also influence all reactions and processes that occur. Applied early in a new chemical's life, physical-chemical property constants provide a first determination of a chemical's behavior and fate by defining characteristics such as solubility, partitioning, and sorption (*76*). Constants that are derived in the laboratory, under highly structured and well controlled conditions, may not represent what occurs environmentally. Varying test conditions can yield widely different results. Constants for surfactant metabolites that have been determined in various studies are provided in Table 6.

Table 6. Nonionic Surfactant Physical-Chemical Properties

Constant Alkylphenol ethoxylates	NP	NPE_1	NPE_2	NPE_3	$NPE_{1-3}C$	OP	Ref.
Distribution coefficient [3] (L/kg)	10,500	1,800	900	--[1]	500	--[1]	(*29*)
Sorption constants [4] (L/kg)	10,000 - 26,000	3,900 - 11,000	1,300 - 6,900	--[1]	--[1]	--[1]	(*15*)
log K_{ow}[2]	4.2	--[1]	--[1]	--[1]	--[1]	3.7	(*24*)
log K_{ow} (@ 20.5° C)[2]	4.48 ±0.12	4.17 ±0.15	4.21 ±0.18	4.20 ±0.11	4.07[5]	4.12 ±0.10	(*100*)
log K_{hw} (@ 20.5° C)[2]	3.7 ±0.04	3.4 (n=1)	3.3 (n=1)	--[1]	--[1]	3.0 ±0.04	(*100*)
Aqueous solubility (mg/L @ 20.5°C)	5.43 ±0.17	3.02 ±0.07	3.38[8] ±0.12	5.88 ±0.13	--[1]	--[1]	(*101*)
Aqueous solubility (μmol/L @ 20.5°C)	24.7 ±0.77	11.4 ±0.27	11.0[8] ±0.39	16.7 ±0.34	--[1]	--[1]	(*101*)

	OP	OPE_1	OPE_2	OPE_3			
Aqueous solubility (mg/L @ 20.5° C)	12.6 ±0.50	8.0 ±0.18	13.2 ±0.21	18.4 ±0.55			(*101*)
Aqueous solubility (μmol/L @ 20.5°C)	61.2 ±2.43	32.0 ±0.72	44.9 ±0.71	54.4 ±1.63			(*101*)

Alcohol ethoxylates	AE	$C_{10}E_p$ p=3,5,8	$C_{12}E_p$ p=3,5,8	$C_{14}E_p$ p=3,5,8	$C_{16}E_p$ p=5,8		
Sorption constants (K_{sw})[2] (L/kg)		40-130	250-1230	2950-3550	4750-6200		(*102*)
log K_{sw} (@ 28° C)[2]		1.61-2.10	2.41-3.09	3.47-3.55	3.68-3.79		(*102*)
Sorption constants (K_{sw})[2] (L/kg)	$C_{15}E_9$ 350-2100[6]						(*103*)
log K_{ow}[2]	2.47[7]						(*76*)

[1] Not available. [2] K_{ow} = octanol-water partition coef.; K_{hw} = hexane-water partition coef.; K_{sw} = sediment-water partition coef. [3] Ratio between concentration in activated sludge and 2° effluent. [4] As related to activated sludge samples. [5] Estimated; calculations provided in Appendix C; method in Ref. (*35*). [6] Range measured in 4 sediments. [7] Structure not identified; temperature not provided. [8] Ahel and Giger feel this result is low (experimental error); if adjusted to fit linear regression model the values would be 4.30 mg/L and 14.0 μmol/L.

Experimentally obtained K_{ow} values could not be found for the carboxylated alkylphenol ethoxylates. The K_{ow} for NPE_1-3C that is provided in Table 6 was estimated by adjusting the K_{ow} for NPE_1-3, 4.20, (*100*), based on a fragment constant that has been determined for a carboxylic acid group (*35*). More details about the calculation are provided in Appendix C.

Values for the aqueous solubility constants in Table 6 depict an anomaly that deserves comment. AP is considered to be the most lipophilic of the APE derivatives. This is corroborated by larger distribution coefficient and octanol-water partition constant (log K_{ow}) values for NP than for the lower oligomers. However, the usual inversely proportional relationship between increasing distribution coefficient and log K_{ow} and decreasing solubility does not apply. Instead, the water solubility values are considerably higher for NP and OP than for any of the lower oligomers. Ahel and Giger attribute this to the dominating role that the slightly acidic phenol group plays vs. the alcohol group in the ethoxylate chain (*101*).

Another approach to solubility estimates considers the effects of pure rather than mixed chemicals on solubility. The solubility values in Table 6 were derived in pure water with pure chemicals. In order to more closely simulate environmental conditions, Ahel and Giger determine solubilities experimentally for a mixture of nonylphenolic compounds as found in two commercial surfactants, and arrive at very different results, Table 7. Their experimentally obtained solubility results compare closely with solubilities calculated using an expression presented by Stumm and Morgan (*104, p. 734*). Stumm and Morgan's expression calculates solubility for a mixture of chemicals, assuming that each individual component behaves ideally, according to:

$$\text{Solubility of mixture} = X_1S_1 + X_2S_2 + \ldots + X_pS_p = \Sigma X_iS_i,\ i = 1\text{-}p, \quad [12]$$

where X_i is the mole fraction of component i, and S_i is the pure water solubility of the pure component i. NP is not found in the commercial mixtures, but a look at the results for NPE_{1-4} demonstrates that an individual component's contribution to solubility is largely dependent on its mole fraction -- the smaller or larger the mole fraction, the smaller or larger its respective contribution to solubility.

Table 7. Comparison of Pure and Mixed Aqueous Solubilities of NPE_{1-4} [1]

	Concentration (μmol/L)				
	Pure[2]	Marlophen 83[3]		Imbetin N/7A[4]	
Compound		experimental[5]	calculated	experimental[5]	calculated
NPE_1	11.4	2.1	2.0	8.0	8.6
NPE_2	14	5.0	5.6	2.4	2.8
NPE_3	16.7	4.7	4.7	0.5	0.8
NPE_4	19.3	2.1	2.7	nd[6]	nd[6]
ΣX_pNPE_p Sol	--	13.9	15.0	10.9	12.2

[1] Adapted from Ref. (*101*). [2] Values from Table 6. [3] Original mix mole fractions for NPE_1, NPE_2, NPE_3, and NPE_4 = 0.174, 0.401, 0.281, and 0.142, respectively. [4] Original mix mole fractions for NPE_1, NPE_2, and NPE_3 = 0.75, 0.20, and 0.05, respectively. [5] Determined using generator column method. [6] Not detected.

Sorption has a profound effect on the extent and rate of surfactant biodegradation. While biodegradation of a compound may ultimately lead to complete mineralization (conversion to CO_2 and water), sorption decreases bioavailability as a surfactant is transferred from the aqueous phase to a solid phase. Through sorption a compound is removed from the water column as it adsorbs to particulate material, then settles to become associated with sludge or sediment. When sorption is significant, its effects must be factored into safety assessments. A variety of approaches are found in the literature. Eadsforth and Moser, 1983, (*105*) compared chromatographic methods for determining partition coefficients for a wide variety of chlorinated and non-chlorinated

organic compounds. Ahel and Giger (*100*) used HPLC to determine coefficients for low oligomer APEs (Table 6), and proposed a method for estimating coefficients for high oligomer APEs. Kiewiet *et al.* (*102*) used sorption data to write a quantitative structure-activity relationship (QSAR) equation that estimates a sediment-water partition coefficient based on alkyl and ethoxylate chain lengths. Cano and Dorn (*103*) compared sorption of an AE ($C_{15}E_9$) for various sediments. Brownawell *et al.* (*106*) characterized the mechanisms involved in surfactant adsorption according to molecular structure, solution composition, and sorbent properties. They determined adsorption isotherm constants for nonionic, anionic, and cationic surfactants on five sediment and soil types obtained from the EPA. Scow and Hutson (*107*) examined the relationship of diffusion and sorption to biodegradation kinetics while still others have considered the interactions between adsorption and desorption on partitioning in effecting removal from the water column (*61, 108*),

Ideally, environmental studies should be executed in the field in the appropriate environmental compartments at realistic concentrations. This approach is impractical for satisfying all research needs related to surfactants because of the complexities of the interrelationships and the variability of conditions that are possible environmentally. However, recent improvements in analysis techniques provide more reliable results from environmental samples and have eliminated some of the uncertainty in the exposure part of the environmental risk assessment equation. After presenting proposed biodegradation pathways and a brief summary of conclusions drawn from pathway and kinetic research, findings from analyses of environmental samples with respect to surfactant behavior in appropriate environmental compartments will be reviewed.

Biodegradation Pathways

The literature reveals a variety of possible biodegradation pathways for AEs and APEs. This paper briefly discusses the pathways seen most commonly (Figures 5 - 9). Evidence of the mineralization of AEs or APEs by a single organism could not be found in the literature. It is more probable that the requisite enzymes for mineralizing nonionic surfactants are produced by a mixed consortium of microorganisms. Aerobic and anaerobic organisms that are capable of growing on nonionic surfactants or polyethylene glycols (PEGs) are listed in Table 8. If available, information about experimental conditions, original source of organisms, points of attack, and byproducts is provided. *Candida maltosa* is the only yeast in the list; it utilizes linear 4-1-nonylphenol as sole carbon and energy source by degrading the alkyl chain. Detailed coverage of reaction mechanisms and enzymology is beyond the scope of this paper. Reviews that offer additional perspectives can be found in Cox, (*109*), Cain, (*2*), and Swisher, (*6*).

Evidence of nonionic surfactant biodegradation is available for a diverse set of compartments and conditions. Most surfactant biodegradation research has been conducted with mixed cultures enriched from activated or digested sludge (aerobic or anaerobic). Cultures from soils, sediments, and surface waters have been used to a lesser extent. Environmental compartments that have been simulated in the laboratory include: semi-continuous activated sludge (SCAS) system and simulated surface water from the system (*88*); continuous flow activated sludge (CAS) system (*17, 74*); sludge digester (*110, 111*); creek sludge (*111*); lake water (*74*); river water (*75, 89, 90*); estuary water (*86, 90*); marine water (*112*); sediment (*106*); and soil (*92, 106*).

Table 8. Organisms Capable of Growing on Nonionic Surfactants or PEG

Organism	Compound	Remarks: Culture (Pure or Mixed)/ Conditions/ Source/ Point of attack/ Byproduct	Ref.
Pseudomonas P400	$C_{12}E_{23}$ (Brij 35)	P; aerobic; soil;	(*113*)
Pseudomonas sp. strain 14-1	AE (C_mE_p), NPE_p	P; aerobic; dyeing wastewater;	(*114*)
Pseudomonas sp.	NPE_p	P; aerobic; EO chain degraded; to 90% TOC -- authors speculate that NPE_2 accum'd, but provide no evidence;	(*115*)
Pseudomonas sp. strain TR01	C_5E_3, $C_{10}E_8$	P; activ'd sludge; EO and alkyl chains degraded; byproducts: short chain fatty acids;	(*116*)
	$C_{16}E_{20}$	P; activ'd sludge; partial EO chain degraded;	
	$OPE_{9.5}$, $NPE_{9.5}$	P; activ'd sludge; EO chain degraded via oxidative p'way (glycolate and glyoxalate assimilated); byproducts: NPE_2 and NPE_2C;	
Nocardia	$R\phi E_6$ (APE_6) R = H; CH_3; R = *n*-C_4 (lin); R = *sec*-C_4 (br);	P; growing cells: aerobic; resting cells: aerobic, anaerobic; enrichment culture; EO chain only degraded; growth on lin C_4 very slow; no growth on br C_4; when R = H, $H\phi E_1$ accumulates;	(*9*)
Cylindrocarpon	$H\phi E_1$	P; aerobic; enrichment culture;	
Pelobacter venetianus	$C_{16}E_{20}$ (Brij 58),	P; anaerobic; marine, Venice River sediment; partial EO chain degraded; fate of alkyl chain not mentioned;	(*117*)
KoB35 (~*Pelobacter propionicus*); KoB58 (~*Acetobacterium* sp.); ~*Methanospirillum hungatei*; ~*Methanothrix soehngenii*	$C_{10\text{-}12}E_{7.5}$, $C_{12}E_{23}$ (Brij 35), APE ($C_{10\text{-}12}\phi E_9$); studied in (*111*)	M; anaerobic - fixed bed rctr; polluted creek sediment or anoxic digested sludge; KoB35 and KoB58 grow on EO chain, *M. hungatei* & *M. soehngenii* on fatty acids; AE byproducts: CH_4, CO_2, traces of acetate and propionate; APE byproducts: CH_4, acetate, alkylphenol residues	(*111*, *118*)
KoB35, KoB58 (see above entry)	$C_{12}E_{23}$ (Brij 35), $C_{16}E_{20}$ (Brij 58), PEGs	P; anoxic digested sludge; EO chain only degraded; isolates did not reduce nitrate, sulfate, sulfur, thiosulfate, or sulfite; byproducts: acetate, propionate (KoB35 only), long chain fatty acids (from AE's);	(*118*)
Candida maltosa	4-1-NP (lin)	P; aerobic sludge from trtmt of textile plant wastewaters; alkyl chain degraded; byproduct: 4-acetylphenol	(*119*)

Table 8. (cont'd)

Methanobacterium, Desulfovibrio *Methanosarcina* sp.	EG, DEG, 9EG PEGs: 22EG, 450EG	M; anaerobic digester sludge; byproducts: acetate and methane;	(*120*)
Desulfovibrio desulfuricans, *Bacteroides* sp.	EG, DEG, 3EG, 4EG PEGs: DEG to 450 EG; $C_{12}E_{23}$ (Brij 35)	P; anaerobic digester sludge; ether bonds cleaved by extracellular hydrolysis or hydrogenation; byproducts: acetate, ethanol, hydrogen;	(*121*)

Field studies have been conducted in STPs (*11, 13-16, 18, 23, 29, 39, 40, 49, 54, 55, 122-131*), fresh surface waters (*12, 14, 19-22, 40, 49, 54, 131-136*), groundwater (*62, 68*), marine water (*137, 138*), drinking water (*19-22, 40, 62, 132*), and sludges, soils, and sediments (*54, 62, 130, 133*).

The impact of environmental conditions on extent and rate of biodegradation has been studied by modifying the temperature (*10, 17, 36, 41, 75, 89*), substrate concentration (*17*), test inoculum (*75, 88, 111*), and dissolved O_2 levels (*75, 110, 111, 118*). The latter three references provide results for studies completed under anaerobic conditions. Key points of these and other earlier works are presented in more detail in Swisher (*6*) and Talmage (*27*).

Alcohol Ethoxylates

Figure 5 depicts a general scheme for the biodegradation of alcohol ethoxylates. AEs can be attacked initially in one of three places (*2, 6*) by way of:

I) ω-oxidation on the terminal methyl group of the hydrophobe end (*77, 110, 139, 140*);

II) hydrolysis, directly or oxidatively, at the central ether bond joining the hydrophobic and hydrophilic moieties (*74, 77, 86, 110, 139-141*);

III) step-by-step elimination of the ethoxy (EO) units of the hydrophilic end (*6, 113, 117, 124, 140*).

Further discussion about early pathway research is provided in Appendix D.

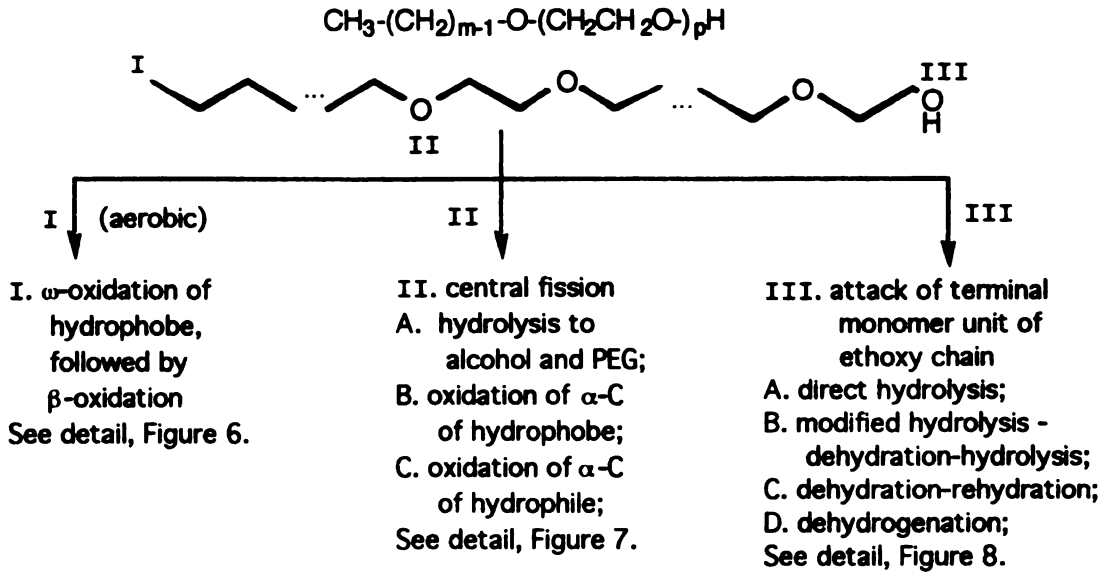


Figure 5. Major proposed biodegradation pathways for AEs.

Molecular structure and environmental conditions, including the presence of oxygen and the nature of the organisms or enzymes present, determine whether attack is most likely to occur at point I, II or III (*142*) (Figure 5). It is generally agreed that the hydrophobic alkyl chain of AE degrades more quickly than the hydrophilic ethoxy chain, and that the rate of primary biodegradation is fastest when the hydrophobic moiety is primary and linear, which is usually the case for AEs used in detergents. Given an AE with a linear primary hydrophobe, attack is more likely to occur at points I or II than III; yet no evidence could be found in the literature whether hydrolysis (I) or central fission (II) occurs more readily in the environment. Delayed degradation and much slower rates of degradation that occur with a secondary or branched hydrophobe (*141*) (deficiency of requisite enzymes) increase the likelihood that the ethoxy chain (III) will be attacked first.

Omega-oxidation of the hydrophobe

The reactions in Figure 6 are similar to those seen frequently in the literature for straight chain alkanes (*143-149*). The first oxidation reaction activates the carbon of the terminal methyl group to allow insertion of an oxygen atom. Elemental oxygen, a hydroxylating NADH-dependent mixed function oxidase (monooxygenase), and a reduced cosubstrate (rubredoxin or cytochrome P450) must be present to initiate the first step of ω-oxidation, which converts the terminal methyl group to an alcohol group. The next two oxidation steps are dehydrogenation reactions which convert the alcohol to an aldehyde, then to a carboxylic acid. The final step preparing the chain for β-oxidation is esterification, which converts the carboxyl group to a thioester (-S-CoA) group. The alkyl chain is now prepared for β-oxidation, a sequence of four reactions that removes two carbon groups, the terminal S-CoA group and its α-carbon, from the side chain. B-oxidation repeats until there are one or two carbons remaining in the chain. In this example, the final intermediate shown is similar to monocarboxylated polyethylene glycol (PEG).

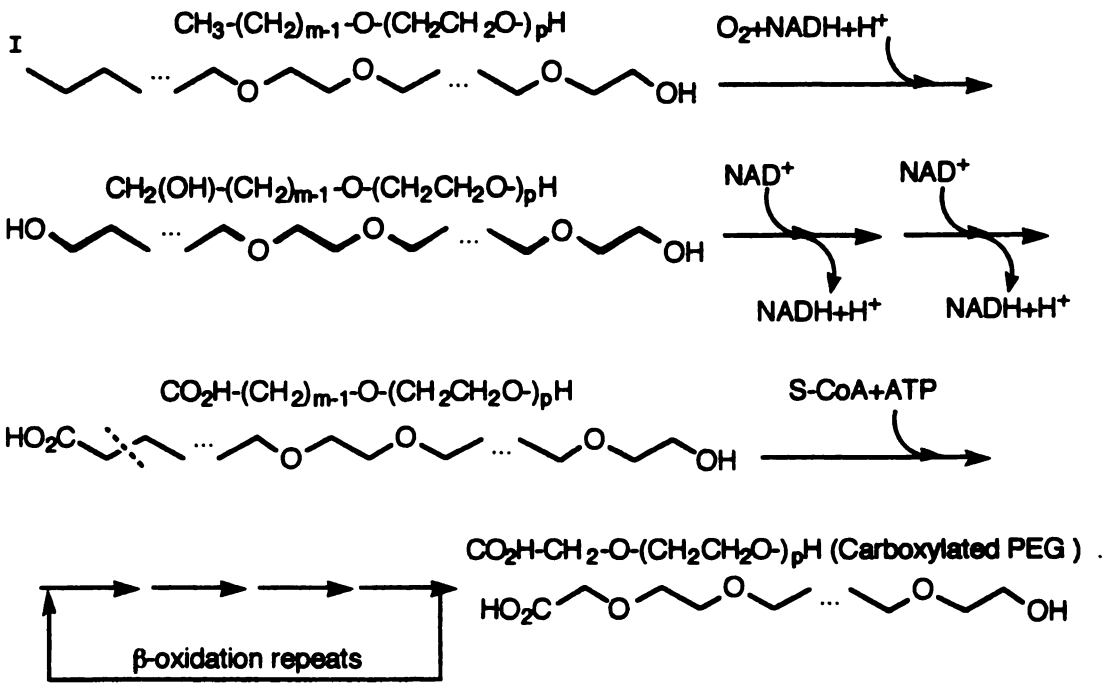


Figure 6. Proposed biodegradative ω-oxidation pathway for AEs.

Central fission

Figure 7 depicts three major alternative hydrolytic central fission pathways. The most direct pathway, designated A, is a simple hydrolytic reaction, which produces an alcohol and PEG. Hydrolysis also occurs in the remaining two pathways, but a hydroxyl group is added to the α-carbon of the alkyl or ethoxy end (designated B and C, respectively) before cleavage occurs. As shown, each step of the pathway leading to hydrolysis offers two alternative reactions. The hydroxyl group is added to the hydrophobic or hydrophilic moiety either by activation of the α-C and oxygen insertion similar to the first ω-oxidation step described above for the alkyl chain, or dehydrogenation at the α-β bond is followed by insertion of a water molecule. The hydroxylated intermediate is a hemiacetal which is unstable and may be easily hydrolyzed, forming an aldehyde and an alcohol. Alternatively, dehydrogenation may occur, converting the hydroxyl group to a carbonyl

group, thus forming an ester (*10*) which may be hydrolyzed. In this case the products are an acid and an alcohol. The specific products formed are dependent on which α-C is hydroxylated (alkyl or ethoxy chain) and whether hydrolysis of the hemiacetal or esterification followed by hydrolysis occurs. More information can be found in Swisher, (*6*). The products of two possible reaction sequences are shown at the bottom of Figure 7.

Elimination of the ethoxy chain

The final major pathway is depicted in Figure 8. It has been generalized to show that biodegradation of the ethoxy chain is thought to be similar for AEs, APEs, and PEGs. All pathways depicted in Figure 8 propose that the ethoxy units are cleaved by hydrolysis. As in Figure 7, the pathway designated A depicts direct hydrolysis. Non-oxidative or oxidative modification of the ethoxy unit precedes hydrolysis in the remaining pathways. Pathways designated B and C are non-oxidative; the pathway designated D is oxidative.

Direct hydrolysis

Haines and Alexander (*150*) and Schink and Stieb (*117*) suggest that a direct hydrolytic cleavage reaction occurs in the biodegradation of PEG in both aerobic (*150*) and anaerobic (*117*) systems, though neither group explains the cleavage mechanism.

Haines and Alexander experimented with a strain of *Pseudomonas aeruginosa* isolated from soil. By producing an enzyme that catalyzes depolymerization extracellularly, their organism can degrade mono-, di-, tri-tetra, and polyethylene glycols with up to 450 units (average molecular weight 20,000). They identified diethylene glycol as a product of hydrolysis, and concluded that monoethylene glycol or possibly oligomers with 2 to 8 units of ethylene glycol may also be products. If hydrolysis is indeed the mechanism,

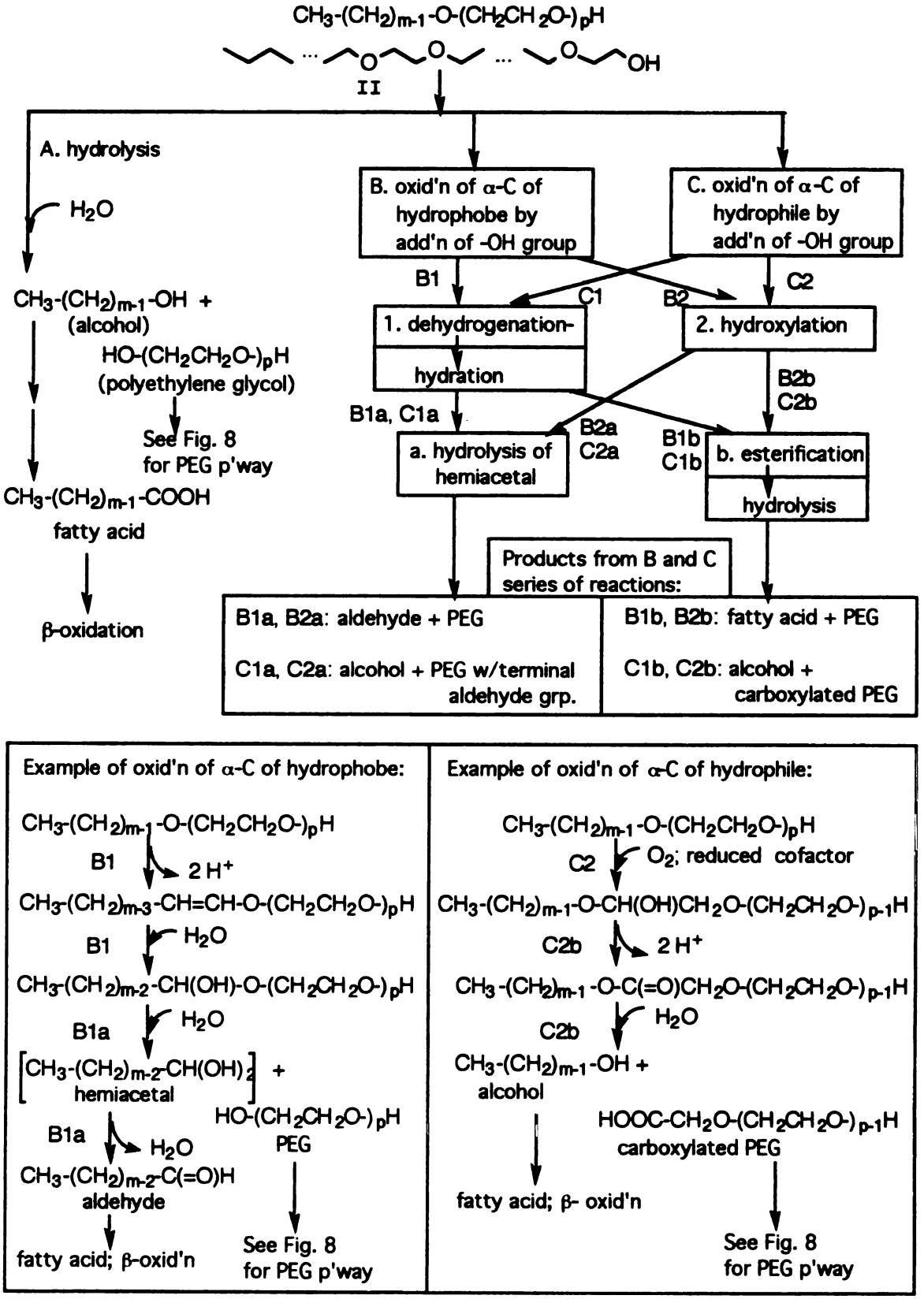


Figure 7. Proposed central fission pathways for AEs.

the hydrolase has never been characterized,. The organism was lost (*117*), and further work supporting their conclusions about hydrolytic cleavage by an extracellular enzyme could not be found.

Schink and Stieb hypothesized two pathways, both intracellular, involving direct or indirect hydrolysis and ending in the production of equimolar amounts of ethanol and acetate from acetaldehyde via a disproportionation reaction. The direct hydrolysis pathway is shown in the anaerobic portion of Figure 8, pathway A. The strictly anaerobic organisms (Gra PEG 1, Gra PEG 2, and Ko Peg 2) used by Schink and Stieb consume dimers, oligomers (up to and including 8 units), and polymers with as many as 450 units of ethylene glycol. Schink and Stieb could not explain how the 20,000 molecular weight PEGs could permeate the cytoplasmic membrane. Monoethylene glycol (MEG) appeared to block the uptake mechanism and inhibited further degradation when any of the three strains were used alone. However MEG was consumed by a fourth anaerobic strain, Gra EG 12, when grown in coculture with Gra PEG 1, Gra PEG 2, or Ko Peg 2. The strains were not able to reduce sulfate, sulfur, thiosulfate, or nitrate. The indirect hydrolysis pathway proposed by Schink and Stieb suggests that hydrolysis follows modification of the terminal ethoxy unit by a dehydration-rehydration reaction, discussed below and shown in Figure 8, pathway C.

Ethylene glycol biodegradation

A variety of possible pathways have been presented in the literature for the further transformation of ethylene glycol (EG). Anaerobically, or when the environment is stressed by minimal oxygen levels, EG will undergo a rearrangement to 1,1-ethanediol (*6, p. 678, Eq. 7.40 & 7.41, 118*), Figure 8, pathway A. Schink and Stieb (*117*) present degradation steps for ethylene glycol under anaerobic conditions that include dehydration, enol-keto

tautomerization, and disproportionation of acetaldehyde to equimolar amounts of ethanol and acetic acid, shown in Figure 8 (following the rearrangement step in pathway A). Aerobically Wiegant and de Bont's, (*151*) pathway is similar, except that it does not include disproportionation. All of the acetaldehyde would be oxidized to acetic acid, then to acetyl-S-CoA, before entering the tricarboxylic acid cycle and the glyoxylate bypass. Steber and Wierich's pathway, not shown, proposes a series of four oxidation reactions, all dehydrogenations producing oxalate, which is further degraded to CO_2 and formate by a decarboxylation reaction (*140*). Steber and Wierich also consider the possibility of a pathway of a more circuitous nature, not shown, that leads to entry into one or a combination of other common metabolic pathways -- the dicarboxylic acid cycle and glycerate pathway (*140, 152-155*), or Embden-Meyerhof-Parnas pathway (*144*).

Modified hydrolysis

Pearce and Heydeman, (*156*), isolated a strain of *Acinetobacter*, S8, that could effect cleavage, in the presence or absence of oxygen, in oligomers having between two and four units. Using diethylene glycol (DEG) as their starting compound, dehydration precedes hydrolytic cleavage (pathway B, Figure 8), producing ethylene glycol (EG) and acetaldehyde. Disproportionation as presented in the discussion above did not appear to occur. Not shown -- ethylene glycol accumulates; acetaldehyde is quickly converted to relatively large quantities of ethanol by an unknown reduction mechanism (aerobically and anaerobically) and smaller amounts of acetic acid (aerobically only). C_2 units are further utilized in a variety of common metabolic pathways, as discussed above.

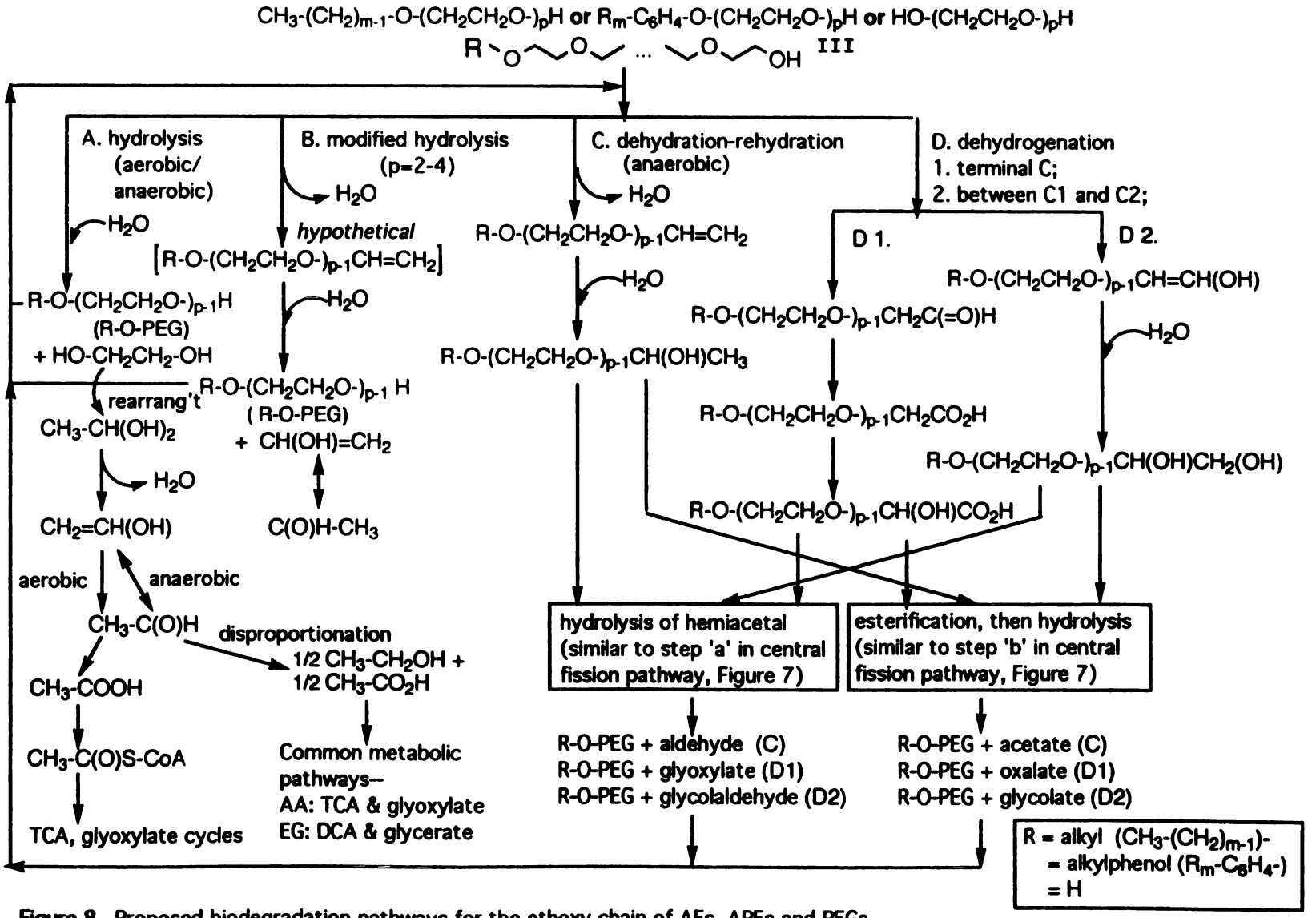


Figure 8. Proposed biodegradation pathways for the ethoxy chain of AEs, APEs and PEGs.

Dehydration-rehydration

Another anaerobic scheme, hypothesized for linear alcohol ethoxylates (*118*) and analagous to a PEG biodegradation pathway proposed earlier (*117*), involves dehydration and rehydration of the terminal ethoxy unit, forming a hemiacetal (pathway C, Figure 8). As with the central fission pathways, the hemiacetal could undergo hydrolysis or esterification before hydrolysis. Under this scheme, an alkyl-PEG or PEG, shortened by one ethoxy unit, and aldehyde or acetate is produced.

Oxidative hydrolysis

Oxidative hydrolysis begins with dehydrogenation that attacks either the terminal carbon (*157, 158*) or the 1-2 carbon bond (*113*) of the terminal ethoxy unit (pathway D, Figure 8). Payne and Todd, (*157*), were the first to identify a flavoprotein dehydrogenase that is active with a secondary alcohol ethoxylate possessing nine ethylene glycol units (secondary $C_{11\text{-}15}E_9$). Ferricyanide serves as electron acceptor. Payne and Todd were unable to identify products, and therefore made no conclusions about the reaction mechanism effecting cleavage. Nor did they suggest whether cleavage or dehydrogenation occurs first. However, they did determine that ethylene glycol is not metabolized by the dehydrogenase in extracts. Kawai *et al.*, 1978, found a dehydrogenase linked with 2,6-dichloro-phenolindophenol (DCPIP) that oxidizes ethylene glycols with from two to 450 units. Attack of the aldehyde that is formed leads to a second dehydrogenation reaction that produces a carboxyl group on the terminal ethoxy unit. To lend additional support to this pathway, Watson and Jones, (*159*), and Steber and Wierich, (*140*) identified carboxylated ethoxy intermediates in their research. Watson and Jones were successful in degrading PEGs with up to 34 units (average molecular weight 1500); Steber worked with linear $C_{18}E_7$ and PEG 400 (average

molecular weight 400, nine units). Thélu *et al.*, (*113*) identified a dehydrogenase that degrades PEGs up to PEG 400. Their dehydrogenase is DCPIP-linked, but initiates removal of one hydrogen from each of the 1- and 2-carbons, thus creating a double bond between the carbon groups in the terminal ethoxy unit. The addition of a water molecule across the bond introduces a hydroxyl group on the 2-carbon, thus forming a hemiacetal, which is either hydrolyzed directly or esterified prior to hydrolysis.

Additional studies

Research examining ether cleavage reactions has also been conducted for alkoxyalkanes (*160*), chlorophenoxy-acetates (*161-166*), chloromethyl-phenoxyacetates (*163, 167*), methoxybenzoates (*168-170*), and methoxyphenyl and methylene-phenyl compounds (*171, 172*).

Alkylphenol Polyethoxylates

The major pathway in the biodegradation of the alkylphenol poly-ethoxylates, APE_p, inferred from both lab (*15, 23, 139, 173, 174*) and field (*15*) experiments, is step by step elimination of the most terminal monomer unit of the hydrophilic ethoxy chain, Figure 9. As already discussed, the alkyl structure of the alkylphenol polyethoxylates is usually highly branched, with an average of eight (octylphenol polyethoxylate - OPE) or nine (nonylphenol polyethoxylate - NPE) carbon groups. Dimethyl branches are not unusual, resulting in quaternary carbons that are not likely to be attacked readily by the same enzymes that effect conversion of an alkyl chain to a fatty acid (via ω-oxidation) in preparation for β-oxidation (*2*). Unlike the alcohol ethoxylates, central fission does not occur, probably because such a reaction is blocked by the presence of the highly branched alkyl chain together with the aromatic ring (*139*). Swisher argues that steric hindrance alone (from the branched alkyl group) explains the resistance to central fission (*6*).

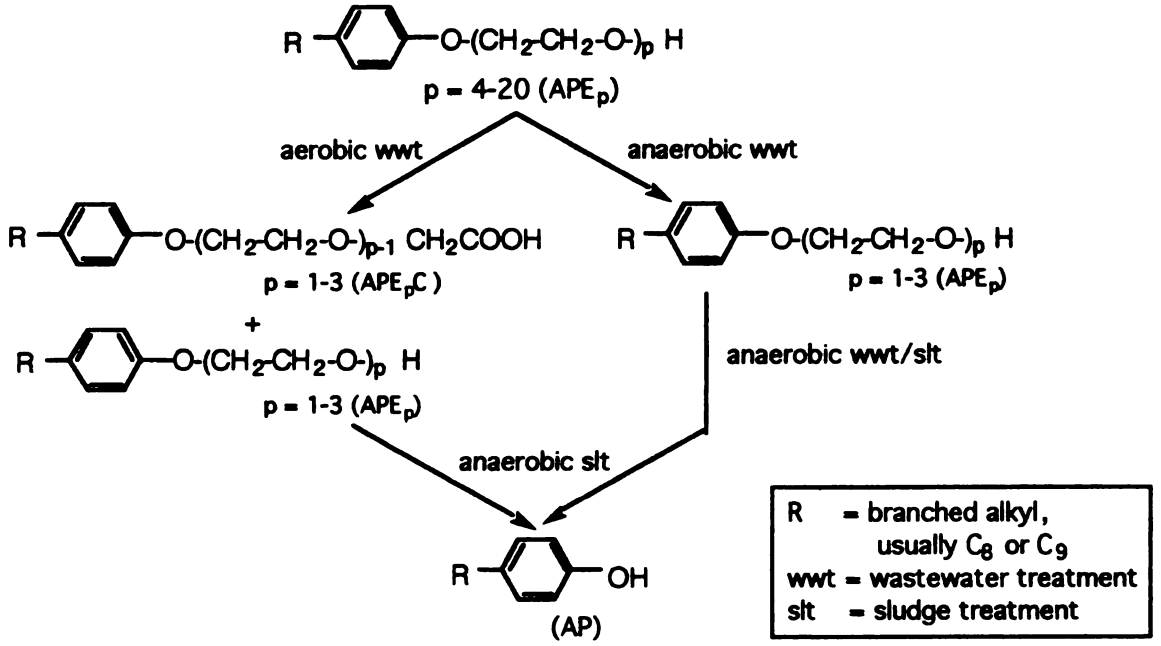


Figure 9. Proposed biodegradation pathways for APEs. Adapted from (*15*).

Mineralization of the APE structure, i.e. biotransformation to CO_2 and water, will occur only if enzymes are present to effect shortening of the ethoxy chain, elimination of the shortened ethoxy chain, ring cleavage, and finally oxidation of the alkyl branch. Evidence of the isolation of a single organism that produces enzymes capable of catalyzing all of these activities could not be found in the literature. Organisms that attack the ethoxy chain have been isolated, as depicted in Table 8. The mechanism for terminal ethoxy monomer elimination has never been definitely determined, but it is thought to be a hydrolytic (*117, 140, 150*) or oxidative-hydrolytic (*156*) reaction, similar to that already described for polyethylene glycols. Again, the reaction can occur aerobically (*116, 140, 150, 156*) or anaerobically (*6, 117*). Reported results vary, but generally removal of the ethoxy monomers continues down to one, two, or three ethoxy units (*15, 23, 174*). Under aerobic conditions carboxylation may occur either on a terminal carbon group on the alkyl end (provided that the hydrophobe is not heavily branched), (*139, 174*), or on the

terminal ethoxy group (*15, 23, 116, 126, 127, 175*). APECs with over 3 EO units have been detected in environmental samples, raising speculation about when carboxylation occurs (*18, 20, 23, 132, 176*). The lower ethoxamers, whether carboxylated or not, tend to accumulate prior to being biotransformed during the next stage of biodegradation, conversion to an AP or carboxylated AP.

The alkylphenol, if formed, is also resistant to biodegradation. Giger *et al.* found that NP accumulation is greatest in anaerobically treated sludge (*13*). Research results providing evidence for a mechanism that transforms branched alkylphenols could not be found. Steric hindrance most likely prevents attack of the alkyl moiety. If biodegradation proceeds further, it is probably by way of oxidation of the aromatic structure, which prepares the ring for cleavage by transforming the AP to an alkylcatechol (*144*). The branched substituent may be degraded by a combination of steps involving disruption of the quaternary structure (*177, 178*) and β- and α-oxidation steps (*147*).

Recently Corti *et al.*, 1995, (*119*), isolated a strain of the yeast *Candida maltosa*, LMAR 1, from aerobic sludge samples, that transforms a linear alkylphenol, 4-(1-nonyl)phenol (Table 8). The predominant metabolite is 4-acetylphenol. Corti *et al.* conclude that a combination of β-oxidation and decarboxylation reactions occur to reduce the length of the alkyl chain. At some point, further degradation must proceed via aromatic cleavage. Though other metabolites are present, but not identified, it is unclear whether or at what stage during biodegradation the aromatic ring is cleaved.

Pathways, Extent and Kinetics of Biodegradation -- Generalizations

The material covered thus far is summarized below. Figure 10 is a diagram of the relative ease (rate and extent) of biodegrading several AE and APE surfactants. It is a pictorial representation of the narrative that immediately follows. The comparisons are relative, not absolute. Note that the surfactants are grouped into three clusters:

1) linear AEs and AEs with a single methyl branch;
2) linear secondary AEs, very high oligomer linear PAEs, linear APEs, and branched PAEs;
3) quaternary branched AEs and APEs; branched APEs.

The position of the branched PAEs in the second cluster is based on the assumption that they are highly branched. A minor amount of branching could easily place them at the beginning of the second cluster, before the linear secondary AEs.

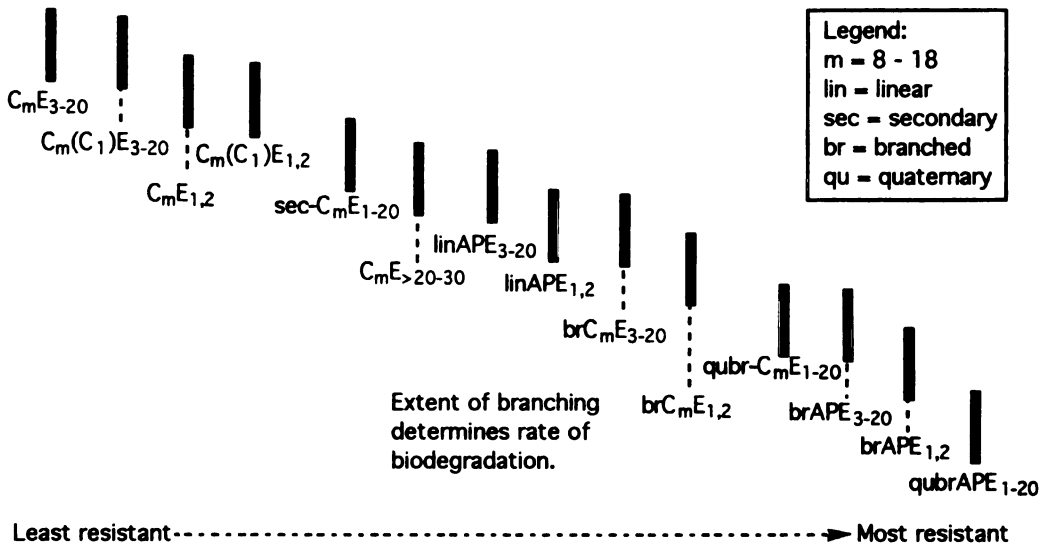


Figure 10. Relative ease of biodegrading AEs and APEs. Adapted from Ref. (*46, 179*).

The following conclusions can be drawn:

1) The structure of the hydrophobe (linear, branched, phenolic) determines the rate and extent of biodegradation (*10, 17, 46, 141, 173, 180*).
   a) linear and singularly branched hydrophobes:
      i) linear AE surfactant molecules undergo rapid and extensive primary biodegradation with removal of the alkyl chain (*10, 17, 74, 140, 141*); the length of the alkyl chain generally has little effect on the rate and extent of biodegradation of linear primary AE (*10, 89, 90, 141, 179*);
      ii) a single methyl branch on an alkyl chain does not significantly affect the rate or extent of biodegradation (*10, 17, 179*);
      iii) a primary linear AE will biodegrade more rapidly and to a greater extent than a linear secondary AE (*10, 139, 141*);
   b) the presence of a phenyl group in the hydrophobe slows the rate and decreases the extent of biodegradation significantly (*139, 141, 173, 179*); a hydrophobic structure with branching and a phenyl group degrades very slowly and incompletely (*10, 139, 173*);
   c) as hydrophobic branching increases, the rate and extent of biodegradation continues to decrease (*17, 141, 174, 179*); a hydrophobe with a phenyl group and quaternary branching is most resistant (*141, 174*).
2) Degradation of hydrophilic moieties of ethoxylate (EO) surfactants is slow to begin and proceeds slowly once begun (*10, 74, 140*).
   a) EO chains with < 20 units tend to biodegrade more rapidly and extensively; biodegradation is slower and less extensive when the

chains are more than 20 or 30 EO units long (*10, 179*); however Larson did find comparable biodegradation rates for long (22 EO units) and short (3 and 9 EO units) AE homologs (*89*);

b) the EO chain associated with a linear hydrophobe will biodegrade significantly more rapidly and extensively than the EO chain associated with a branched, phenolic hydrophobe(10); APEs with very short EO chains (< 3 EO groups) are most hydrophobic, most likely to partition to organic matter, and therefore least likely to biodegrade.

Environmental Analysis and Safety Assessment

The development of specific and sensitive analysis techniques, for the detection and quantitation of nonionic surfactants in environmental samples at very low concentrations, has been very difficult (*181*). Analysis of the polyethoxylate nonionic surfactants is more complex than analysis of anionic and cationic surfactants because:

1) the number of possible molecular structures is greater;
2) analytical response of intermediates cannot be easily differentiated from the parent surfactant; and
3) interference from other substances is frequently a problem. (*6, p. 140*)

Within the last several years, techniques have been developed that can be used to determine the compositions of both the alkyl and ethoxylate moieties of alcohol ethoxylates at very low concentrations in environmental samples (*41, 181, 182*). Recent reviews summarize the extraction/enrichment, clean-up, separation (chromatographic), and detection methods that are currently available to complete specific and sensitive analyses of:

1) AEs in aqueous and solid environmental matrices (*38*); and
2) AE and APE homolog, isomer, and oligomer distributions in environmental aqueous samples (*182*).

The reader is referred to these articles if more information is desired regarding current nonionic surfactant chromatographic analysis techniques.

Concerns about environmental persistence and accumulation of APE metabolites to toxic levels, particularly alkylphenols and alkylphenol mono- and diethoxylates, compelled much earlier development of analysis techniques for detecting APE and its metabolites in environmental samples. Nonylphenol polyethoxylates have received the greatest amount of attention because as the

most widely used of the APEs they are most likely to enter the environment as a result of their use in detergent and industrial products (*27, 39*) Walter Giger, working with other researchers, has been studying the behavior and environmental fate of alkylphenol ethoxylates since the early 1980's (*11-13, 55, 126, 127, 129, 130, 183*). They observe that the APEs and their intermediates can be categorized into four major groups according to their behavior -- the parent alkylphenol polyethoxylates (APE_{3-20}), alkylphenol mono- and diethoxylates ($APE_{1\&2}$), alkylphenols (AP), and alkylphenoxy carboxylic acids ($APE_{1\&2}C$) (*15*). Hydrophobicity, sorption and toxicity increase as the ethoxy units are eliminated from the hydrophilic end, down to two or fewer ethoxy units. Sorption can become more important than biodegradation in determining the fate of APE metabolites, particularly if the concentration of suspended solids is high.

Aided by the development of more sensitive analysis techniques, which allow for the detection and quantitation of organic compounds in a variety of environmental matrices at ever lower concentrations (ppb, ppt, ppq) (*40, 49, 131, 176, 181, 184*), more attention is being devoted now than ever before to the removal processes that determine environmental behavior and fate. Results of field studies support the findings of laboratory studies related to surfactant pathways, biodegradability, and kinetics (*18, 40, 75, 77, 78, 185*). Findings from field studies are reviewed next, with respect to the appropriate environmental compartments of concern.

Alcohol Ethoxylates

Alcohol ethoxylates easily pass stringent laboratory screening tests for ready biodegradability, and therefore are considered to be environmentally safe. Due to their reputation and the relatively recent development of analysis techniques that can be used to correctly identify a variety of AE homologs and oligomers at the very low concentrations present in environmental samples, AE environmental studies have been less extensive than APE studies. AEs are increasingly used to replace alkylphenol polyethoxylates in domestic, institutional, and industrial products as concern about the environmental effects of APEs becomes greater. As the use of AEs increases, questions are being raised about their environmental safety if used in much larger quantities on a widespread basis. Highlights from the available AE environmental studies follow.

Sewer distribution systems

Matthijs *et al.* (*78*) used results from lab and field studies to demonstrate the importance of the sewer in removing a representative sampling of surfactants. They completed sewage die-away studies in the laboratory with AEs, alcohol ethoxy sulfates (AESs), and diethanolester dimethyl ammonium chloride (DEEDMAC) - a cationic surfactant. Half-lives and rate constants were estimated by nonlinear regression analysis assuming first order kinetics (Table 9A). Supporting evidence for results from the laboratory studies is provided by comparing actual measured environmental concentrations of AES, glucose amide (GA - a nonionic surfactant), and boron to predicted concentrations based on consumption, water usage, and population data for the Netherlands. Measured concentrations for AES and GA are each 47% lower than the predicted concentrations (Table 9B). Confirmation of this procedure lies in the fact that the concentration measured for boron, as a conservative

tracer, is very close to the predicted level (within 1%). AEs would be eliminated in the sewer at least to the same extent as AESs, given their lower estimated half-life and the fact that lab studies tend to be a conservative estimate of what is likely to happen environmentally (*185-187*). The half-life for complete biodegradation of AES in activated sludge plants is estimated to be less than one hour (*78*).

Table 9. Environmental Analyses in Sewer Distribution Systems[1]

Component A) Lab	Observed Kinetics	Estimated 1/2-life (hr)[2]	Lab Test	Analysis
AE ($C_{14}E_3$)	zero order	~ 3	sewage die-away	TLC/RAD[4]
AES ($C_{14}E_3SO_3$)	first order	~ 4	sewage die-away	TLC/RAD[4]
DEEDMAC	mixed	6-10	sewage die-away	TLC/RAD[4]

B) Field	Predicted Concentration (µg/L) [3]	Measured Concentration (µg/L)	Removal (%)	Analysis
AES	3400	1800	47	LC/MS[5]
GA	426	227	47	FAB/MS[6]
Boron	893	900	0	OES[7]

[1] Ref. (*78*), Netherlands; statistics not provided. [2] First order kinetics assumed. [3] Based on consumption data, population count, and water usage. [4] Thin layer chromatography with radioscanner. [5] Liquid chromatography with ion spray mass spectrometry. [6] Flow injection fast atom bombardment with mass spectrometry. [7] Inductively coupled plasma optical emission spectroscopy.

The above approach does not consider removal that occurs in the sewer lines due to sorption. A more valid approach to ascertain that biodegradation occurs in sewer distribution systems would be to determine the change in total concentration by measuring concentration levels of dissolved and particulate fractions for the organic compounds of interest at both input and output points in the sewer distribution system.

Sewage treatment plants

The following generalizations are highlights from three research studies that were conducted recently in four, three, and seven municipal, mechanical-biological sewage treatment plants in the United States (*49*), Italy (*40*), and the Netherlands (*125*), respectively. The U. S. study (*49*) investigates removals in two activated sludge (A/S) and two trickling filter (TF) plants. The biological means of removal is not identified for the study performed in Italy (*40*). All seven plants included in the study for the Netherlands are activated sludge plants (*125*). The results for one of the TF plants are excluded from the general summary statements in this paper because a major rain event that occurred before and during the sampling period affected measurements adversely. The data is, however, included in the tables and figures below. Environmental levels cannot be directly compared because test conditions, sampling methodology, analyte measurements and analysis techniques are different (*27*).

Primary treatment

Alcohol ethoxylate concentrations entering municipal sewage treatment plants vary from less than one to less than 7 mg/L (Table 10, Figure 11) (*40, 49, 125*). Average removal during primary treatment can vary from 6 (*49*) to 28% (*49, 125*). The 6% reading was obtained for the Oskaloosa, Iowa TF plant, the U. S. plant that did not experience the rain event mentioned above. Average removal during primary treatment is highest in the seven Dutch plants, averaging about 28% of the total influent load (*125*). AE concentrations were measured in the primary effluents for only five of the seven plants. Removals for the five plants range between 10 and 41%. Extent of removal is dependent largely on the settleable solids in the primary clarifier (*49, 125*), although with a two hour average detention time (Table 3) biodegradation also

contributes to removal. During primary treatment, the AE concentrations are reduced to between less than 1 and about 4 mg/L (Table 10, Figure 11) (*49, 125*). Removal efficiencies during primary treatment are not provided by Crescenzi *et al.*, perhaps because primary treatment is not provided in the Italian plants.

Table 10. Environmental Analyses in Sewage Treatment Plants and Receiving Waters (μg/L)

Comp	Raw infl low/high (avg±sd)	1° effl low/high (avg±sd)	2° effl low/high (avg±sd)	Recv'g water low/high (avg±sd)	Tap water	Analysis	Ref.
USA[1]							
$C_{12\text{-}15}E_p$ (A/S)	3210/ 3670 (3440± 325)	2670/ 2820 (2745± 106)	11/71 (41±42)	18/37 (28±13)	--	GC/MSD w/HBr deriv'n[8]	(*49*)
$C_{12\text{-}15}E_p$ (TF)	680[4]/ 2670 (1675± 1407)	950[4]/ 2500 (1725± 1096)	50[4]/114 (82±45)	30/34 (32±3)	--	GC/MSD w/HBr deriv'n[8]	(*49*)
Italy[2]							
$C_{12\text{-}18}E_p$	217.8/ 1151[5] (508.7 ±312)	--	0.03/ 12.86[5] (4.0±4.9)	2.52[6]	0.024[6]	LC-ES-MS[9]	(*40*)
Netherlands[3]							
$C_{12\text{-}15}E_p$	1190/ 5500 (2978 ±1236)	1020/ 3440[7] (1949 ±646)	<3.0/ 11.5[5] (<4.5 ±2.0)	--	--	RP-HPLC w/pic deriv'n; UV det'n[10]	(*125*)
$C_{12\text{-}18}E_p$	1480/ 6350 (3415 ±1365)	1240/ 3930[7] (2290 ±743)	<4.1/ 21.7[5] (<7.5 ±4.0)	--	--	RP-HPLC w/pic deriv'n; UV det'n[10]	(*125*)

[1] Infl & effl: 24 hr, 3 day flow-weighted composite samples from 2 activated sludge (A/S) and 2 trickling filter (TF) plants (late summer, early fall); rec'g waters: grab samples. [2] Infl & effl: 24 hr composite samples, 2 or 3 days, from 3 mech'l-biol'l plants; rec'g waters: one grab sample. [3] 24 hr composite samples, 3 consecutive days, from 7 A/S plants; measured aqueous & solid fractions. [4] Low TF readings occurred during rain event. [5] Different dates and plants for high & low readings. [6] One sample. [7] Five plants sampled. [8] Gas chromatography with mass selection detection; HBr derivatization. [9] Liquid chromatography coupled to mass spectrometry via an electrospray interface. [10] Reversed-phase high pressure liquid chromatography; phenyl isocyanate derivatization; UV detection.

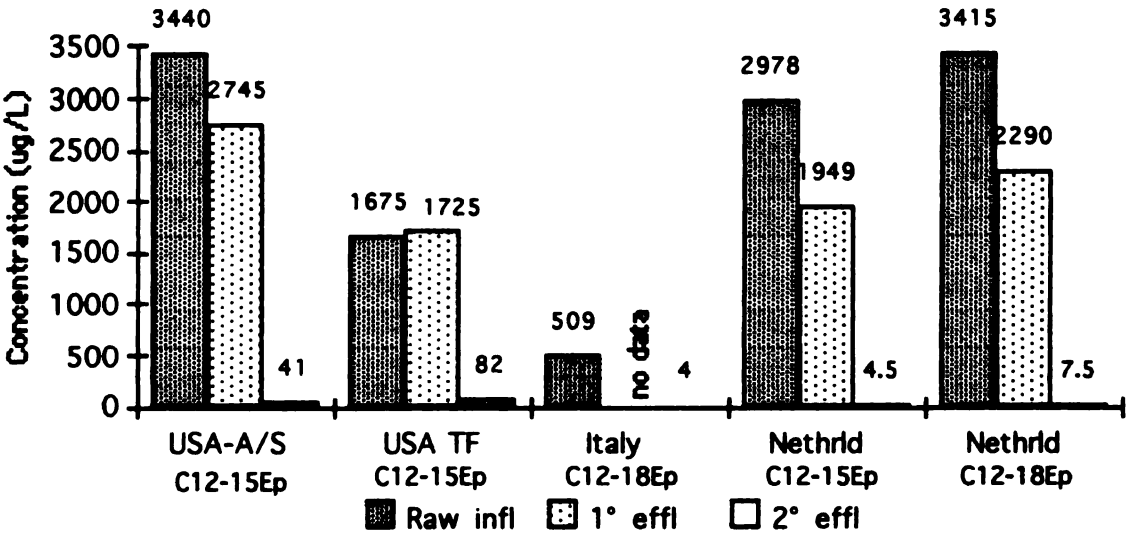


Figure 11. Concentrations of AEs in raw influents, and primary and secondary effluents. Values from Table 10.

Sorption is a large factor in removal from the water phase, as more than 50% of AEs can become associated with the solid phase (Table 11, Figure 12) (*125*). Recent investigations of AEs attempt to determine whether the length of the alkyl or ethoxy chain affects sorption. Fendinger *et al.* (*49*) conclude that an AE with a long alkyl chain is no more likely to be removed by sorption than an AE with a short alkyl chain. However their research is limited to AE molecules having between 12 and 15 carbon groups. Crescenzi *et al.* and Kiewiet *et al.* address behavior of AE molecules with up to 18 carbon groups. They observe that the longer alkyl chains, C_{16} and C_{18}, are more likely to be associated with the solid phase and thus exist in the aqueous phases at lower concentrations (*40, 125*). Table 11 summarizes data (*125*) that demonstrates the importance of sorption as a removal mechanism during primary treatment. A greater fraction of the AEs is found in the aqueous phase of the primary effluent after solid settling.

Table 11. Liquid/Solid Phase Fractions of Raw and Settled Influent [1]

Component	Raw influent Range: (Avg±sd: liq/sol [2] liq/sol) [3]	Settled influent Range: (Avg±sd: liq/sol [2] liq/sol) [4]
C_{12}-C_{15}	0.28-0.37/0.72-0.63 (0.33±0.03/0.67±0.03)	0.44-0.61/0.56-0.39 (0.52±0.07/0.48±0.07)
C_{12}-C_{18}	0.26-0.35/0.74-0.65 (0.30±0.03/0.70±0.03)	0.38-0.57/0.62-0.43 (0.47±0.08/0.53±0.08)

[1] Ref (*125*). [2] Different plants for settled vs. raw influent. [3] n=6. [4] n=4.

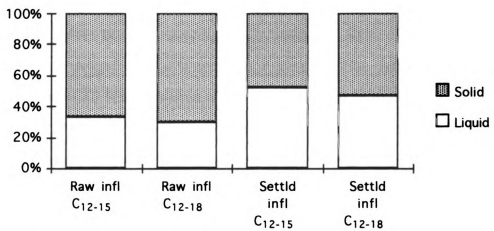


Figure 12. A greater proportion of AEs is in the aqueous phase following 1° treatment. Values from Table 11.

Secondary treatment

Secondary treatment reduces AE concentrations to between less than 1 and 114 μg/L (Table 10) (*40, 49, 125*). Biodegradation is the major means of removal during secondary treatment. Average removal efficiencies during secondary treatment range from 95 (*49*) to over 99% (*40, 125*). Chromatograms and related mass spectra of the oligomeric distribution of AEs in raw and untreated water suggest to Crescenzi *et al.* that central fission and oxidation of the alkyl chain, not ethoxy unit elimination, are the most likely biodegradation pathways. Radiolabeled compounds were not used in any of the

three studies to determine CO_2 production or biodegradation pathways. Fendinger *et al.* used ^{14}C labeled compounds, $C_{13}E_3$ and $C_{13}E_9$, to evaluate the efficiency of the extraction and extract cleanup steps in their study (*49*).

The removal efficiency of linear primary alcohol ethoxylates is not affected by lower temperatures in laboratory (*17, 36*) and field (*188*) studies. Stiff reports on the results of a study using the porous pot technique with $C_{12-15}E_9$ and $C_{9-11}E_8$ (*36*). The AEs are removed efficiently under both winter and summer conditions, which were simulated by running tests at three temperatures -- 8, 12, and 15°C. Kravetz *et al.* decreased temperatures from 25 to 15 to 8°C while testing linear $C_{12-15}E_7$, branched $C_{13}E_7$ and NPE_9 in a continuous activated sludge flow-through system (*17*). Effluent CTAS values are constant at all temperatures when $C_{12-15}E_7$ (lin) is biodegraded. On the other hand, CTAS values increase significantly for $C_{13}E_7$ (br) or NPE_9 (br) when the temperature is dropped to 8°C. Turner *et al.* report on results of a field trial in biological filters (*188*). Influent and effluent temperatures were below 12.2°C throughout their study. Within the filters, temperatures between 5 and 10°C were measured. Yet the removal efficiency is between 96 and 98% for $C_{14-15}E_7$ and $C_{14-15}E_{11}$.

Surface waters

Thus far AE levels in natural waters have been well below toxic thresholds (*27, 142, 189*), and relatively little effort has been devoted to their study. AE concentrations measured in environmental samples of surface waters are provided in Table 10 (*40, 49*). Not surprising, lower concentrations are observed in receiving waters that have higher stream flows (higher dilution factors) (*49*). Environmental levels cannot be directly compared because test conditions, sampling methodology, analyte measurements and analysis techniques vary (*27*). Other researchers have also detected alcohol

ethoxylates in river water and drinking water (*19, 20, 22*), although quantitative information is not available.

To determine whether or not alcohol ethoxylates biodegrade at trace concentrations after release to the environment, Larson and Games (*90*) and Vashon and Schwab (*86*) conducted laboratory tests with environmental levels of AEs in fresh water and estuarine water, respectively. Larson and Games (*90*) observed the biodegradation of ^{14}C-labeled compounds in Ohio River water. The compounds, $C_{12}E_9$ and $C_{16}E_3$, were each radiolabeled either at the α-carbon of the alkyl chain or uniformly labeled in the EO chain, resulting in four separate groups. From measurements of the rate of $^{14}CO_2$ production, they determined biodegradation rates for each of the four materials separately. From ^{14}C activity converted to $^{14}CO_2$, remaining in solution, and incorporated into biomass, they concluded that biodegradation of all four AE groups follows first order kinetics, occurs rapidly, and is essentially complete. The rate and extent of biodegradation of the alkyl and EO chains for $C_{16}E_3$ are about equal. The alkyl and EO chains of $C_{12}E_9$ biodegrade to the same extent, but the rate of $^{14}CO_2$ production with the $C_{12}E_9$ EO chain labeled is half the rate of $^{14}CO_2$ production with the $C_{12}E_9$ alkyl chain labeled. The biodegradation half-life based on the mean value of the biodegradation rate constant of all four groups is approximately 35 hours.

Vashon and Schwab (*86*) conducted a similar experiment with ^{14}C-labeled AE compounds at trace concentrations in Escambia Bay water. They used $C_{12}E_9$ labeled uniformly in the EO chain and $C_{16}E_3$ labeled at the α-carbon of the alkyl chain. Again biodegradation is rapid and extensive; first order kinetics apply at environmentally relevant concentrations. Central fission is indicated as the primary pathway. Biodegradation rates are lower in salt water

than in fresh water. Calculated half-lives equal 2.3 and 5.8 days for the respective $C_{16}E_3$ alkyl carbon and $C_{12}E_9$ ethoxylate carbon compounds.

Sorption also plays a role in the fate of AEs in surface waters. Kiewiet *et al.* (*102*) determined sediment-water partition coefficients for a variety of AEs ($C_{10\text{-}16}E_{3\text{-}8}$) and found that sorption increases with increasing alkyl and ethoxylate chain length (Table 6). However the increase is greater with addition of carbon groups to the alkyl chain than with the addition of ethoxy units. Though Cano and Dorn (*103*) agree with Kiewiet *et al.* that sorption is a hydrophobic mechanism, they found that sorption decreases with increasing ethoxylate chain length. This is reasonable since more oligomers would increase the polarity, thus increasing the solubility. They examined the sorption behavior of $C_{15}E_9$ to four different sediment samples (Table 6). They did not compare AEs that vary in size hydrophobically. More studies are required to further evaluate effect of molecular structure and environmental conditions on biodegradation and sorption.

Groundwater

Evidence of a study conducted to determine the behavior and fate of alcohol ethoxylates in groundwater could not be found, no doubt due to the reputation that AEs enjoy as readily biodegradable compounds in STPs and surface waters. Research conducted by Knaebel *et al.* in 1990 (*92*) provides evidence that AEs biodegrade readily in soils that have not been previously exposed to anthropogenic surfactants, as well as in sludge amended soils. Knaebel *et al.* applied low concentrations of a 70%-30% mixture of $C_{12}E_9$ and $C_{12}E_8$ AE surfactants to 11 different soil types gathered from various U. S. locations. They used surfactants that were uniformly labeled in the ethoxylate chain to track $^{14}CO_2$ production during mineralization. Measured CO_2 production was between 30 and 69%. The application of four wetting/drying

cycles increased the rate of mineralization. Similar behavior can be assumed in the event that AEs are introduced to groundwater.

Summary

Removal of alcohol ethoxylates begins in sewer distribution systems. AEs are removed efficiently during primary and secondary treatment in sewage treatment plants. Average total removals (primary and secondary treatment) are high, ranging from 96% (TF plant in the U. S. (*49*)) to almost 100% (activated sludge plants in the Netherlands (*125*)). Measured removals confirm quantitatively (*40, 49*) the high removals predicted in laboratory biodegradation testing and bench scale activated sludge system tests (*17, 74*). Central fission and ω-oxidation of the hydrophobe are more likely to occur than step-by-step elimination of the ethoxy units (*40*). Lower operating temperatures do not decrease the efficiency of removal.

Alcohol ethoxylate concentrations in surface waters in the U. S. and Italy are less than 40 μg/L. Laboratory studies indicate that alcohol ethoxylates biodegrade rapidly and extensively in both fresh and estuarine waters at these concentrations.

The presence of alcohol ethoxylates in groundwater is not a concern environmentally.

More studies are required to further evaluate the effect of AE molecular structure and environmental conditions on the interrelationships between biodegradation, sorption, and AE removal in STPs and surface waters.

Alkylphenol Polyethoxylates

Worldwide agreement does not exist regarding the extent to which APE metabolites accumulate (*41, 54, 116, 124, 127*), thereby effecting ecotoxicological damage (*29, 54, 62, 131, 133*). Giger and co-workers have concluded that the accumulation of NPE metabolites, particularly NP, creates an environmental risk. Kubeck (*131*) and Naylor (*54*) have concluded that NPE metabolites do not accumulate and are not an environmental risk. Conclusions and an analysis of results of studies from the two viewpoints are below. Highlights of other investigations by other researchers, in other geographical areas, are also provided.

In terms of the four major categories identified previously, researchers generally agree that the parent surfactant (APE_{3-20}) is removed, while the metabolites ($APE_{1\&2}$, AP, $APE_{1\&2}C$) persist to varying degrees during treatment. The investigations of Ahel, Giger, and co-workers into the behavior and fate of these four groups allowed them to determine:

1) varying behaviors dependent on wastewater treatment conditions (*15, 16*);

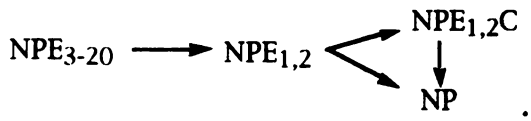
2) mass and molar fluxes (*29, 39, 128*);

3) solubility constants (*101*) and partitioning coefficients (*100*).

Most recently Ahel, Giger, and co-workers published three articles which are comprehensive summaries of their accumulated data and their conclusions about the behavior and fate of APEs and refractory metabolic products (*29, 62, 133*). The three phases of their study examine fate and behavior of nonylphenol polyethoxylates in sewage distribution systems and treatment plants (*29*), river water (*133*), and groundwater (*62*). Most of the generalizations that follow are taken from their three part comprehensive study.

Sewer distribution systems

Ahel *et al.* demonstrated that biodegradation of NPEs begins in the sewer with data collected from the Uster sewage treatment plant (STP) (*29, Figure 3*). They compared distribution curves for raw influent, primary and secondary effluent, and a commercial NPE_9 mixture (Marlophen 810) used most commonly in laundry detergents (Figure 13). They measured molar concentrations for each oligomeric configuration, NPE_{0-18}. The distribution of ethoxy (EO) groups per molecule of nonylphenol for Marlophen 810 resembles a normal probability distribution, with a peak at 9 EO groups. Less than 1% by weight of the Marlophen 810 molecules carry one or two ethoxy groups (*127*). NP and NPEC are not present in the commercial mix (*29*). The raw influent, however, is distributed bimodally such that two peaks are obvious, at NPE_1 (highest peak) and NPE_8. NP, NPE_1, and NPE_2 are considerably higher in the raw influent than in the original surfactant mix: 260 vs. 0 $\mu mol/m^3$, 530 vs. 20 $\mu mol/m^3$, and 180 vs. 20 $\mu mol/m^3$ for NP, NPE_1 and NPE_2, respectively. NPE_3 is slightly higher at 55 vs. 40 $\mu mol/m^3$. The distribution of the NPE_{4-20} oligomers is similar to that for the commercial mix, but is shifted down and to the left. While providing evidence that biodegradation does begin in the sewer prior to entry into the water treatment plant, this also corroborates the proposed biodegradation pathway for the APEs, that is, step-by-step elimination of the ethoxy units according to:



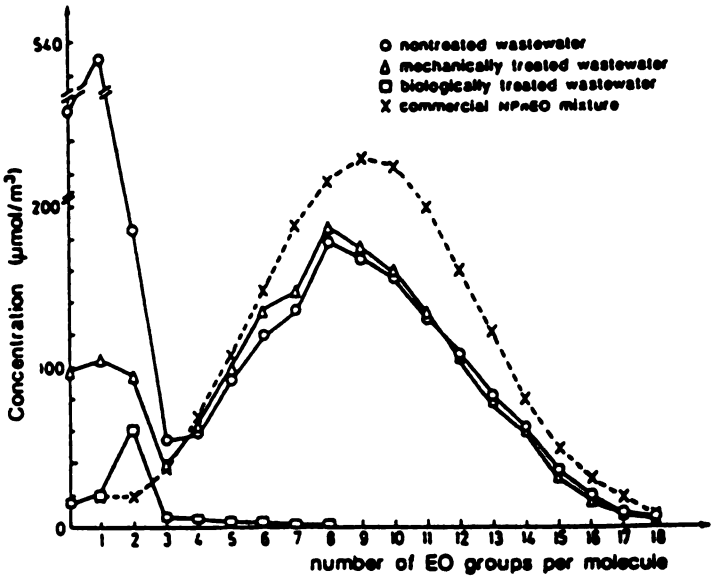


Figure 13. Distribution of individual NPE_n oligomers in raw sewage, primary effluent and secondary effluent of the sewage treatment plant Uster and a commercial mixture Marlophen 810. Reprinted from (*29, Figure 3*), with permission from M. Ahel.

A more accurate means to determine whether or not biodegradation occurs in the sewer is to compare measurements of the concentrations of nonylphenolic compounds in raw sewage samples at sewer input ports with concentrations at sewer outlets. Measurements of this type could not be found in the literature.

Sewage treatment plants

The bulk of data analyzed in the first phase of Ahel's 1994 report was collected during the 1980's from 11 plants located along a 35 km stretch of the Glatt River in Switzerland. At times Ahel and co-workers chose data from an individual plant or from two plants so as to make comparisons. Two-hour composite, 24-hour composite, and grab samples were measured. Previously published data is included in the tables and figures below for illustrative purposes; the nature and origin of the data is provided as appropriate.

The values provided in Tables 12 and 13 are rough estimates that have been derived based on information taken from several sources. Table 12 provides molar flows (mol/day) for the Zürich-Glatt STP. The values for the primary and secondary effluents are based on measurements from 24-hour composite samples (*29, Table 4*). The total raw influent value is estimated based on Ahel's assumption (*29, Figure 8A*), that 20% of total influent nonylphenolic compounds (NPc) settles during primary treatment and is routed to the anaerobic digester in the primary sludge. The raw influent values for the four major categories are estimated based on data provided and statements made in the text of articles (*15, text, 29, text and Figure 3*). The values for digested sludge are estimated based on grab sample measurements of total nonylphenolic derived compounds and nonylphenols in Zürich-Glatt and Uster (*29, Table 8*). Figure 14 is a bar chart of Table 12 values.

Table 12. NPE Molar Flows in Zürich-Glatt STP (mol/day (% of Total))

Category	Raw infl [1] mol/day(%)	1° effl [2] mol/day(%)	2° effl [2] mol/day(%)	Dig'd sldg [3] mol/day(%)
NPE_{3-20}	83. (63.)	76.1 (55.9)	6.3 (4.6)[5]	tr[6]
$NPE_{1,2}$	40. (28.)	20.2 (14.9)	4.0 (2.9)	2.0 (1.5)
NP	10 (7.)	6.3 (4.6)	0.9 (0.7)	29.0 (21.5)
$NPE_{1,2}C$	3. (2.)[4]	6.3 (4.6)[4]	19.8 (14.8)	tr[6]
Tot'l NPc	136. (100.)	108.9 (80.0)	31.0 (23.0)[7]	31.0 (23.0)[8]

[1] Ref. (*29, Figure 3*): Values estimated. [2] Ref. (*29, Table 4*): Table 4 value * 45,000 m^3/day. [3] Ref. (*29, Table 8 and Figure 8*): Values estimated. [4] Ref. (*15, Figure 12*); slightly higher levels of $NPE_{1,2}C$ measured in Uster 1° effluent than raw influent. [5] NPE_{9-20} not present; NPE_{3-8} present in trace amounts. [6] Only trace levels present. [7] Discharged to surface waters. [8] Usually land applied, or land filled.

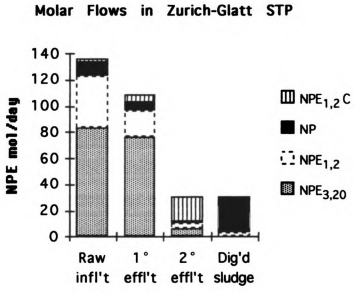


Figure 14. NPE molar flows in the Zürich-Glatt STP. Values from Table 12.

The reader is cautioned that measurements from composite and grab samples do not correlate. For example, composite sample measurements indicate that the overall elimination of NPc during secondary treatment in the Zürich-Glatt STP is 71% (*29, Table 4*); grab sample measurements indicate that the overall elimination of NPc during both primary and secondary treatment is only 58% (*29, Table 8*). The estimates of distribution ranges provided in Table 13 are an attempt to account for this. Also, Ahel *et al.* (*29*) estimate that the environment ultimately receives 60-65% or more of all NPc (molar-based) that are introduced to sewage treatment plants. Between 30 and 40% of this (20-25% of total NPc) is released in the form of digested sludge; the remainder (35-45% of total NPc) is discharged in secondary effluents to surface waters. Table 13 values predict a less severe environmental impact than those estimated by Ahel *et al.* The values in columns 2-4 of Table 13 consider the elimination data of 24-hour composite samples of 11 Glatt River treatment

plants (*29, Table 4*); the digested sludge values in column 5 consider results from grab samples (*29, Table 8*). Ahel *et al.* found that the overall NPc elimination efficiencies during secondary treatment in the 11 plants ranged between 26 and 79% (molar-based) (*29, Tables 2 and 4*); the values in Table 13 of this paper indicate that between 30 and 75% elimination of total NPc during secondary treatment is achieved, or between 15 and 50% is discharged to the environment in secondary effluents. Differences in the relative importance of biodegradaton and sorption at each stage of sewage treatment for the four major nonylphenolic groups are depicted in Figure 15.

Table 13. % Distribution of Nonylphenolic Compounds (Molar-Based)[1]

Category	Raw infl	1° effl	2° effl	Dig'd sldg[2]
NPE_{3-20}	57-63	55-62	3-10[4]	tr[5]
$NPE_{1,2}$	28-30	15-18	2-12	1-2
NP	3-10	5-6	1-5	15-24
$NPE_{1,2}C$	<1-4[3]	1-5[3]	8-25	tr[5]
Tot'l NPc	≤100	80-90	15-50[6]	15-25[7]

[1] Unless stated otherwise, values estimated from 11 plant study; Ref. (*29*): text, Figs. 3 & 8, Tables 2, 4 & 8. [2] Ref. (*29, Table 8 and Figure 8*): Values estimated. [3] Ref. (*15, Figure 12*); slightly higher levels of $NPE_{1,2}C$ measured in Uster 1° effluent than raw influent. [4] NPE_{9-20} not present; NPE_{3-8} present in trace amounts. [5] Only trace levels present. [6] Discharged to surface waters. [7] Usually land applied, or land filled.

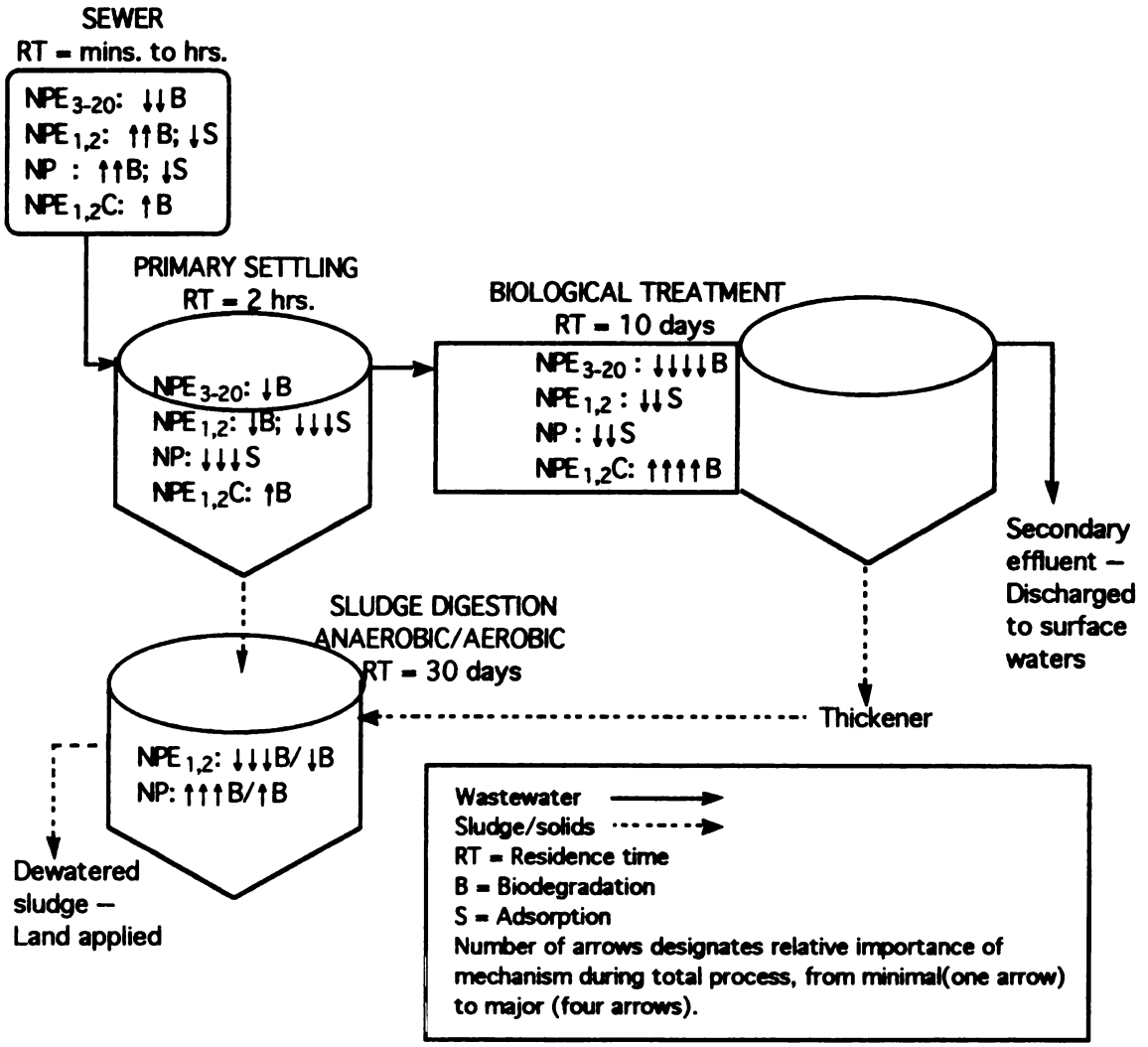


Figure 15. Relative importance of biodegradation and sorption as removal (↓) and accumulation (↑) mechanisms during water treatment – by major category.

Primary treatment

With a 2 hour retention time (Table 3) and high percent suspended solids in the primary tank, sorption is the major removal process, as depicted by the distribution curves for the Uster plant (*29*), Figure 13. The lowest and most lipophilic oligomers ($NPE_{1\&2}$) and NP, are considerably lower in the primary effluent than in the raw influent, down to approximately 100 μmol/m^3 from 180 - 530 μmol/m^3. NPE_3 is slightly lower, at 40 μmol/m^3. The NPE_{4-18} oligomers change very little, demonstrating that biodegradation is not

a major removal mechanism in the primary treatment stage. This is illustrated in Figures 13 and 15.

Secondary treatment

The hydraulic retention time in the Zürich-Glatt STP is about 6 hours (*133*); the mean cell residence time is not provided for any of the 11 plants studied, but the times provided in Table 3 are probably a close approximation (10 days). This allows ample time for biodegradation, the major removal process, to occur. The major findings are summarized below.

- Nonylphenolic compounds are not the most biodegradable part of DOC; they are eliminated to a lesser degree than is overall BOD (70 ± 15% vs. 86 ± 9% (weight-based)). Overall elimination efficiency of the NPEs and metabolic products during secondary treatment in the 11 plants studied by Ahel *et al.* varied from 43 to 87% (weight-based), and from 26 to 79% (molar-based) (*29, Table 2*).
- NPE_{3-20} is reduced substantially in the aeration tank, leaving only trace amounts of the NPE_{3-8} oligomers in the secondary effluent (Tables 12 and 13, Figure 14).
- $NPE_{1\&2}C$ is the predominant product in secondary effluents (*29, 116, 190*). Formation of $NPE_{1\&2}C$ during aerobic biological treatment occurs in all plants so that levels are 2.1 - 7.6 times higher in secondary than primary effluent (Tables 12 and 13, Figure 14). The apparent need for anaerobic conditions for transformation of $NPE_{1,2}C$ (*13, 15, 29, 55*) and a relatively low distribution coefficient (500 L/kg for $NPE_{1,2}C$ vs. 10,500 L/kg for NP (Table 6)) contribute to its persistence and resulting high levels in secondary effluents.

- Persistence of NPE metabolites increases with increasing lipophilicity, or shortening of the EO chain. The fate of the lipophilic products -- NP, NPE_1 and NPE_2 -- is influenced by solid/aqueous phase partitioning. The effect of formation of NP and $NPE_{1\&2}$ in the aqueous phase of secondary treatment is diminished by sorption due to much higher partitioning coefficients -- 10,500, 1800, and 900 L/kg measured in the Uster plant for NP, NPE_1 and NPE_2, respectively. Maintenance of a high suspended solids concentration (mixed liquor volatile suspended solids), typical in the activated sludge process (Table 4), facilitates the sorption of these compounds, thus removing them from the aqueous phase (Tables 12 and 13, Figures 14 and 15). Sorbed to sludge, they are further biodegraded during sludge digestion. If the sludge is later applied to agricultural land or placed in a landfill, any metabolites remaining may leach into groundwater, wash into surface waters as runoff, or be mobilized with colloidal material, perhaps increasing risk in additional environmental compartments.
- Related to the above, the order of resistance to biodegradation is NP, NPE_1, NPE_2 (most to least) (*12, 126*). Sorption, toxicity, and bioconcentration increase (NPE_2 < NPE_1 < NP) as rates of biodegradation decrease (*24-27, 54, 142*).

- The elimination rates of products of NPE_{3-20} vary widely (Table 14). The highest elimination rates are seen in low-load plants. $NPE_{1,2}$ is formed more quickly than eliminated (-19%) in one high load plant. $NPE_{1,2}C$ is formed more quickly than eliminated (-660 to -110%) in all treatment plants. Elimination of NP varies from 9 to 94%.

Table 14. Range of Product Elimination Efficiencies (Aqueous Phase) -- Secondary Treatment[1]

Product	% Elimination - Range (Lowest to Highest)
$NPE_{1,2}$	-19 to 80
$NPE_{1,2}C$	-660 to -110
NP	9 to 94

1 Ref. (*29*); from study of 11 plants.

- Lower temperatures result in lower overall elimination rates for NPEs in both lab and field experiments (Table 15). Studies that determine the effect of changing temperatures on biodegradability have been conducted in bench-scale systems simulating activated sludge treatment (*17, 41*), in a porous pot apparatus (*36*), and in the field in both biological filtration (*36*) and trickling filter systems (*124*). Naylor found only a 2-5% reduction in removal efficiencies due to seasonal effects, but specific temperatures are not provided (*54*).

Biodegradability in natural environments cannot be predicted based on fluctuations in temperature alone. It is possible that organisms more tolerant of lower temperatures exist in some environmental compartments into which surfactants are discharged. In the event that acclimation has occurred, an increased population count or increased enzymatic activity of the degrading organism may result, and the biodegradation rate may actually increase when lower temperatures are introduced in a compartment (*75*).

Table 15. Temperature Effects on the Extent of APE Removal

Compound/ Influent Conc.	System/ Test Method	Temperature (° C) or period	Extent of Removal	Ref.
NPE_9/ 10 mg/L	Bench-scale activated sludge/CTAS[1]	15-25	≥ 92	(*17*)
NPE_9/ 10 mg/L	Bench-scale activated sludge/CTAS[1]	8	28 - 83	
NPE_9/ 50 mg/L	Bench-scale activated sludge/ HPLC-FD[2]	25	> 93	(*41*)
NPE_9/ 10 mg/L	Bench-scale activated sludge/HPLC-FD[2]	8	80	
OPE_{8-9}/ 7 mg/L	Pilot-scale activated sludge/Wickbold	10.5-14.0	79-88	(*36*)
OPE_{8-9}/ 20 mg/L	Porous pot activated sludge/TLC[3]	15	95	(*36*)
OPE_{8-9}/ 20 mg/L	Porous pot activated sludge/TLC[3]	11	67-70	
OPE_{8-9}/ 20 mg/L	Porous pot activated sludge/TLC[3]	8	48-53	
OPE_{8-9}/ 10 mg/L	Biological filtration/ Preston/TLC[3]	March-May	20	(*36*)
OPE_{8-9}/ 10 mg/L	Biological filtration/ Preston/TLC[3]	May-September	up to 80	
OPE_{8-9}/ 10 mg/L	Biological filtration/ Preston/TLC[3]	September-December	down to 20	
OPE_{8-9}/ 10 mg/L	Biological filtration/ Langley/TLC[3]	Nov '73 - Mar '74; 7.6-10.0	20-40	(*36*)
OPE_{8-9}/ 10 mg/L	Biological filtration/ Langley/TLC[3]	Summer '74	80-90	
OPE_{8-9}/ 10 mg/L	Biological filtration/ Langley/TLC[3]	Nov '73 - Mar '74; 9.2-11.2	~ 80-90	
$APE_{7.3}$ 0.77±0.06 mg/L	Trickling filter/ HPLC-UV or FD[2]	March '85	70 ± 3	(*124*)
$APE_{7.6}$ 0.73±0.06 mg/L	Trickling filter/ HPLC-UV or FD[2]	Sept. '85	75 ± 3	

[1] CTAS - Cobalt thiocyanate active substances. [2] HPLC-UV or FD - High pressure liquid chromatography-ultraviolet or fluorescence detection. [3] TLC - Thin layer chromatography.

Different conclusions are presented by Kubeck and Naylor (*54, 131*), who insist that:

1) the rate of biodegradation is not affected by the length of the ethoxylate chain (*131*);
2) all NPE_{1-18} oligomers are removed satisfactorily during sewage treatment (92.5 - 99.8%), even under high sludge loading conditions (*54*);
3) NP is not sorbed preferentially to sludge during treatment (*54, 131*).

Research results that support the conclusions of Kubeck and Naylor for environmental samples could not be found. However, researchers working in laboratories around the world have published results and conclusions similar to those of Ahel, Giger, and co-workers:

1) Lower oligomers (especially NPE_2 and NPE_2C) predominate after treatment of APEs and carboxylated APEs (*9, 18, 21, 23, 41, 116*); additionally, Swisher (*6, pp. 721-724*) cites the work of several researchers, in addition to Giger, who identified NPE_2 as an intermediate that accumulates when the higher oligomer NPEs biodegrade (*192-198*);
2) NP and the lower oligomers are preferentially sorbed during primary and biological treatment (*39, 128, 142, 194, 199*).

Closer examination of the results and conclusions published out of both laboratories follows.

- In the published results of their 1990 study, Kubeck and Naylor conclude that NP and $NPE_{1\&2}$ do not accumulate during sewage treatment. They determine that improper work-up procedures in the research completed by W. Giger and co-workers cause the distribution of NPE to be skewed toward the lower oligomers. They identify the use of polyethylene storage

bottles, nitrogen or solvents contaminated with oxygen, interfering substances in the extract, or insufficient nitrogen streaming during blow-down as possible causes. They insist that NP and the lower oligomers ($NPE_{1\&2}$) are eliminated as efficiently as the higher oligomers. The data of overall elimination efficiencies, collected in May, 1988, from two plants (East and West) in High Point, North Carolina, and reported in their 1990 article, appear to support their conclusions (Table 16). However, their use of weight-based rather than molar-based measurements distorts their comparisons between higher and lower oligomers in terms of their reactivity (biological and chemical) (*29*). That is, distribution of the lower oligomers will appear to be less, relative to the higher oligomers, on a weight basis than on a molar basis. In spite of this, NPE oligomer concentrations in the West High Point plant depict a distribution that is skewed toward the lower oligomers -- NPE_1, NPE_2, and NPE_3 (*131, Figure 9*).

Table 16. Removal of NPE_{1-18} in Two North Carolina STPs[1] (µg/L)

Category	Date (mo/yr)	Raw infl low/high	2° effl low/high	%elimination
NPE_{1-18}	5/88	1600[2]/2520[3]	51[3]/104[2]	93[2]/98[3]
NPE_1	5/88	36[3]/69[2]	2.6[3]/3.4[2]	93[3]/95[2]
NP	5/88	nm[4]	0.8[2]/2.5[2]	--

[1] Ref. (*131*); statistics not provided. [2] Eastside plant -- industrial: textile processing and furniture manufacturing. [3] Westside plant -- domestic. [4] Not measured.

- Concentrations of individual oligomers measured at and near the Govalle, Texas plant, and published in the same article, also do not support their conclusion that the NPE oligomer distribution is not skewed to the lower oligomers. On the contrary, graphs of effluent and river water data all depict elevated quantities of NPE_1, NPE_2, and NPE_3 relative to the higher oligomers. The authors attribute the skewness to "the possibility of interferences", but no further explanation is provided.

- Additional data, collected as part of a later study from High Point and six other plants, depict similarly high removals overall (*54*). Nine of the 14 results published by Naylor are displayed in Table 17 for illustrative purposes. Two mid-range results from each of the East and West High Point, NC plants, and an earlier sampling (8/90) from the Small City, Midwest USA plant are excluded. Naylor provides graphs of NPE oligomer distributions for three of the fourteen treatment plant results, designated with '*' in Table 17. All three distributions reflect generally higher concentrations for NP and the lower oligomers -- NPE_1, NPE_2, and NPE_3 -- to varying degrees (*54, Figures 5-7*).

Table 17. Removal of NPE_{1-18} in Seven U. S. STPs[1] (µg/L)

Plant	Date (mo/yr)	NP + NPE infl	NP + NPE effl	% elim'n	% in dewat'd sludge
Burlington, NC (East)	5/92	384 + 903	14.4 + 54	94.6	
Burlington, NC (South) *	5/92	359 + 2619	15.3 + 39	98.2	
High Point, NC (East-high)	5/88	-- + 1780	1.3 + 79	95.5	
High Point, NC (East-low)	4/93	14 + 274	<0.2 + <5	98.3	
High Point, NC (West-high)	7/93	978 + 3140	0.2 + <5	99.8	
High Point, NC (West-low)	1/93	124 + 487	1 + <5	99.0	
Small City, Midwest USA *	3/91	28 + 1110	4.9 + 80	92.5	0.1
Wood Pulp Mill, USA	6/90	24 + 8450	3.3 + 201	97.6	
Paper Mill, USA *	4/93	516 + 33700	8.4 + 261	99.2	1.7

[1] Ref. (*54*); avg of 4 assays; statistics not provided.

- Only NPE_{1-18} are measured consistently in the Kubeck and Naylor studies. In the 1990 study, NP is not measured in influents; nor is NP measured in all effluents and river water samples. Carboxylated NPEs and total nonylphenolic compounds are not measured in either study. According to the distribution of nonylphenolic compounds in the four major categories obtained by Ahel *et al.*, (Tables 12 and 13), Kubeck and Naylor miss a significant portion of the NPc in the secondary effluent by excluding measurements of the carboxylated NPEs from their studies.

- High remaining levels of lower oligomers in secondary effluent and river water are not explained adequately, given the authors' conclusions that NP and the lower oligomers do not accumulate during biological treatment. Though not consistent, high levels of varying combinations of NP, NPE_1, NPE_2, and NPE_3 do occur in influent (*54*) and effluent samples (*54, 131*), as well as in downstream samples in river water (*131*). The cause is attributed to anaerobic degradation in sewer lines (*54*) for influents, or matrix effects (*54, 131*) for effluents and river water.
- Kubeck and Naylor conclude that NP and the lower oligomers ($NPE_{1\&2}$) do not accumulate in secondary effluents. Note that NP does not accumulate in the secondary effluent according to the results of any study. Research results for plants along the Glatt River indicate that $NPE_{1\&2}$ accumulate in only one Swiss plant that is operating under high load conditions, to 19% (Table 14) (*29*).
- It is unknown whether or not a portion of the APE metabolites was inadvertently missed during the detection phase of analysis because of the difference in disinfection systems between the U. S. and Swiss plants. Chlorination is commonly used in the United States as a means for disinfecting treated wastewaters (*56*). On the other hand, Switzerland does not use chlorination (*200*). The samples in the U. S. studies were taken from the final effluent, after the chlorination step (*201*), and may have carried halogenated derivatives. Other researchers studying halogenated APE derivatives have used GC/MS (*18, 20-22, 190*) or FAB, perhaps supplemented with FAB/MS or FAB-CID/MIKE (*19-22*), in the detection phase of analysis. Giger, Kubeck, and Naylor used UV detection. Though UV detection may be an effective means of identifying and quantitating halogenated APE derivatives, characteristic wavelengths for absorbance

would most likely be different than those for the nonhalogenated APE derivatives. Neglecting to observe results for all of the appropriate wavelengths would distort APE removal efficiencies on the high side. It is recommended that future studies consider this difference. Studies related to halogenated APE derivatives will be discussed in more detail in a later secton.

As a final observation about the conclusions of Ahel and Giger, there is no basis for their correlation between efficient elimination of total NPc and low loading rates/favorable nitrifying conditions (*15, 16, 29*). Elimination efficiencies are affected by hydraulic retention times, mean cell residence times, and the concentration of suspended solids, as well as by loading rates. However, both research groups (Ahel-Giger and Kubeck-Naylor) failed to recognize the importance of the mean cell residence time as a design parameter that can determine the NPE elimination rate and extent of nitrification during activated sludge treatment. Treatment plant operating parameters are not provided in any of the studies.

In Table 18, a plant with a low sludge loading rate and favorable nitrifying conditions (longer mean cell residence time, θ_c) is compared to a high load/non-nitrifying treatment plant (shorter mean cell residence time, θ_c) (*29*). In the low load plant, the higher oligomers (NPE_{8-17}) are eliminated almost completely. Rate of elimination gradually decreases for the lower oligomers, ranging between 31 and 98%. A 77% elimination rate is attained for NP. This is because nitrification, while not directly related to NPE_D removal, is favored at longer mean cell residence times, which also favor NPE_D degraders. Elimination efficiencies are lower in the high load treatment plant. Rates of elimination are 79-90%, 21-84%, and 37% for NPE_{8-17}, NPE_{3-7}, and NP, respectively; formation of $NPE_{1\&2}$ occurs more quickly than its removal.

Table 18. Variations in Oligomer Behavior During Secondary Treatment[1]

Low load/nitrifying ($\theta_c \geq 8$ days)	High load/non-nitrifying ($\theta_c < 8$ days)
• NPE_{8-17}: ≥ 98% elimination.	• NPE_{8-17}: 79-90% elimination.
• NPE_{1-7}: 31-98% elimination.	• NPE_{3-7}: 21-84% elimination.
• NP: 77% elimination.	• $NPE_{1,2}$: -3, -5% (form'n > elim'n).
	• NP: 37% elimination.

[1] Ref. (*29*); comparison of two plants.

Sludge digestion

As discussed earlier, measurements of samples from digested sludge do not correlate with measurements of samples drawn from primary and secondary effluents. Measurements of NPE metabolite concentrations in digested sludge are not synchronized with the incoming load of nonylphenolic compounds. Average residence times can vary from two hours to ten days to 30 days for primary treatment, secondary treatment, and sludge digestion, respectively. Additionally, grab samples rather than composite samples are collected for analysis of digested sludge. Under these conditions, it is not possible to synchronize the withdrawal of samples from the various treatment stages. Though an attempt has been made in Table 13 to estimate the distribution of NPE metabolites in digested sludge relative to the primary and secondary treatment stages, it is important to keep this disparity in mind while studying the fate of NPE metabolites.

According to the results of research completed by Giger, Ahel and co-workers, NP occurs in high concentrations in aerobically and anaerobically digested sludge of STPs that treat wastewater carrying nonylphenolic compounds. Its accumulation in digested sludge is a result of:

1) adsorption of NP and $NPE_{1\&2}$ to solids during primary and secondary treatment;
2) transformation of $NPE_{1\&2}$ to NP during sludge stabilization;
3) persistence of NP under anaerobic conditons.

Highlights of NPE research related to sludge digestion follow.

- Partitioning of NP is controlled by the concentration of suspended solids -- the greater the concentration of suspended solids, the greater the partitioning (Table 19) (*39, 128*). The effect of the concentration of suspended solids in primary treatment is illustrated by the average percent removal range for total NPc that is estimated in Table 13 (10 - 20%). Higher removal, closer to 20%, will be realized when the suspended solids concentration during primary treatment is correspondingly high. Once the lipophilic metabolites -- NP, NPE_1 and NPE_2 -- are sorbed and settle during primary treatment, biodegradation ceases to be the major mechanism for their removal. They persist because with relatively high sorption coefficients, especially for NP, solid partitioning in the sludge will be favored over biodegradation. They may undergo further biodegradation during sludge digestion.

Table 19. Relationship of Concentration of Suspended Solids to Fraction of NP Partitioning to Solids

Medium	Concentration of suspended solids [1] (mg/L)	Fraction of NP partitioning to solids [1,2] (%)
Digested sludge	50,000	~ 100
Raw sludge	14,000	~ 100
Activated sludge	1700	> 98
Raw sewage	87	~ 10
Primary effluent	48	~ 10
Secondary effluent	8	bd [3]

[1] Ref. (*128*). [2] Ref. (*39*). [3] Below detection.

- Transformation of $NPE_{1,2}$ to NP is favored under anaerobic conditions (*13*). Brunner *et al.* confirm that more NP is present in sludges treated anaerobically than aerobically by measuring and comparing NP, NPE_1 and NPE_2 concentrations in sludge samples from 29 STPs (*128*). Using 1986 data, the mean concentrations for NP were 1.27 and 0.30 g/kg dry matter in anaerobic (24 plants) and aerobic (5 plants) sludge samples, respectively. Furthermore, levels of NPE_1 and NPE_2 were lower in plants that treat sludge anaerobically. NPE_1 and NPE_2 mean concentrations in anaerobic samples were 0.19 ± 0.12 and 0.06 ± 0.04 g/kg dry matter, respectively, vs. 0.36 ± 0.28 and 0.13 ± 0.11 g/kg dry matter, respectively, in aerobic samples.
- Once formed, NP persists, remaining sorbed unless exposed again to aerobic conditions. Data developed by Ahel *et al.* (*29*) show that more than 90% of the NP that is discharged to the environment is associated with digested sludge. From another perspective, the extrapolation of studies in the Uster and Zürich-Glatt plants indicates that a relatively large fraction (15 - 25%) of the total nonylphenolic compounds that enter an STP leave the plant in the digested sludge, a large part of which is NP (Table 13).
- Reintroduction of sludge to an aerobic environment may occur following stabilization, with sludge disposal. If the sludge is land applied or landfilled, NP may biodegrade if conditions are favorable. If NP is desorbed, it may infiltrate or leach into groundwater or enter surface waters as run-off (Figure 3). Alternatively, NP may remain sorbed to solids (colloids), and in this way become mobilized. Research on the effects of colloids is in its infancy and the body of evidence is relatively small, emphasizing the need for more studies that will ascertain the environmental fate and behavior of NP as a result of sludge disposal.

Contrary to the conclusions of Ahel *et al.*, Naylor (*54*) concludes that metabolites of NPE do not accumulate in sludge and are not an environmental risk. Comments about Naylor's research, as related to the sorption of NPE metabolites in sludge, follow:

- In his 1995 study, Naylor provides the percent of NPE metabolites sorbed to sludge for only two plants (*54*). Results are reported in Table 17. More measurements are required to validate these readings.
- Measurement of NPE metabolites in dewatered sludge yields very low values, 0.1% and 1.7% (predominantly NPE_2) of total influent load, compared to 20 - 25% as determined in research published by Ahel *et al.* (*29, Table 8*). This implies much lower partitioning coefficients for NP and the lower oligomers than indicated in other research (*39, 128, 142*). A possible explanation for the low percent sorption values is experimental error. If NPE metabolites are not desorbed from sludge or glassware during extraction, then the amount of NPE partitioning to sludge will be underestimated.

World data - STPs

Table 20 provides results of several research studies that have been completed worldwide to determine levels of NPE and metabolites in raw, mechanically treated, and biologically treated effluents. Figure 16 is a bar chart of Table 20 values. Measurements from the Ahel *et al.* (*29*) study are included, with weight-based rather than molar-based concentration values. The data provides a comparison of measurements from varying parts of the world. Environmental levels cannot be directly compared because test conditions, sampling methodology, analyte measurements and analysis techniques are different (*27*).

Table 20. Concentration of NPE and Its Metabolites in STPs (μg/L)

Location	NPE_{3-20} (±std dev)	$NPE_{1\&2}$ (±std dev)	NP (±std dev)	$NPE_{1\&2}C$ (±std dev)	Analysis/ Ref.
Switzerland (NE)					HPLC[4]/(*29*)
11 plant avg., Glatt R.					
1° effluent	1181	173	43	43	
2° effluent	112	88	16	184	
% elimination	90.5	49.1	62.8	-327.9	
Switzerland (W)					SIM-GC/MS[5] /(*14*)
Lk Geneva					
Vidy STP 2° effluent	--	387.8	35.8	--	
Nyon STP 2° effluent	--	61.5	44.6	--	
Prangins STP 2° effluent	--	106.1	69.3	--	
Italy					LC[6]/(*176*)
Ostia					
Raw influent		213.3[1] (±10.8)	18.6 (±11.3)	--	
2° effluent		10.3[1] (±7.5)	1.8 (±1.0)	39.6[2] (±35.2)	
% elimination		95.2	90.3	bp[3]	
USA					PB-LC/MS[7]/ (*184*)
New Jersey					
Plant A - 2° effluent	40[8]	--	--	--	
Plant B - 2° effluent	13[9]	--	--	--	
Palo Alto STP		$OPE_{1,2}$	$OPEC_{3-4}$	$OPEC_{1,2}$	GC/MS[10]/ (*18*)
5 day average		4.5[11] (±2.0)	8.0[11] (±2.8)	56.1 (±25.7)	

[1] Includes NPE_{3-20} [2] $NPE_{1\&2\&3}C$; $NPE_{1\&2}C$= 22 (± 10) μg/L (quantitative); $NPE_{>3}C$ = 18 (± 28) μg/L (semi-quantitative). [3] Biodegradation product: formation > elimination. [4]High pressure liquid chromatography; 11 plant average of 24-hour composite samples (statistics not provided). [5] Selected ion monitoring gas chromatography/ mass spectrometry; mean values of two determinations (statistics not provided). [6] Liquid chromatography; annual average of 12 monthly 24-hour composite samples. [7] Particle beam-liquid chromatography/mass spectrometry (statistics not provided). [8] Semi-quantitative; NPE_4. [9] Semi-quantitative; NPE_4 = 1 μg/L; NPE_5 = 12 μg/L. [10] Gas chromatography/ mass spectrometry; 5 day average of flow-weighted composites from three grab samples per day, @ 9am, 2 and 7 pm. [11] Value inflated because 0.5 μg/L used for three readings that were below detection level of 0.5 μg/L.

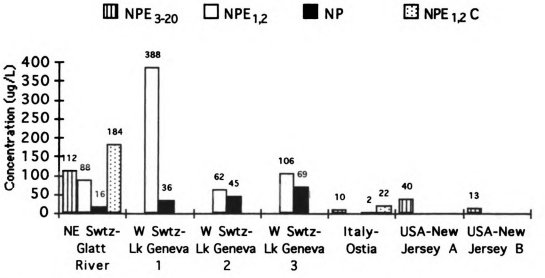


Figure 16. Concentrations of NPE and metabolites in secondary effluents. Values from Table 20.

Surface waters

As is the case with sewage treatment plants, research studies address the behavior and fate of the APE surfactants in terms of the four categories defined previously -- APE_{3-20}, $APE_{1\&2}$, AP, $APE_{1\&2}C$. Again, biological degradation and solid/aqueous phase partitioning are the major processes that determine the behavior and fate of nonylphenolic compounds in river systems (water column and sediment). Hydrodynamic conditions (longitudinal and transverse dispersion) also must be considered (*133*). Volatilization (*199, 202*) and photochemical reactions (*133, 203*) may play lesser roles in reducing environmental levels of surfactant metabolites. A review of the major findings about NPE behavior and fate in surface waters, with emphasis on the Ahel *et al.* (*133*) river study, is provided below.

For the second part of their study, Ahel *et al.* drew secondary effluent samples and river water samples (one day, 12 successive 2-hr. composite samples) at several stations along the Glatt River during February (winter) and August (summer), 1984. Sampling times were set so as to obtain the same water package; STP and river sampling were synchronized. During other seasons grab samples of river water and sediment were taken from different locations along the river (*133*).

Water column

The elimination process that begins in the STP continues in the river. Less than 5% of total NPc enters the river at its inlet, where Lake Greifensee flows into the Glatt River. The remaining load is introduced in the secondary effluent that is discharged to the river from STPs. Almost 88% of total NPc load is released to the Glatt River within the first 15 km of its 35 km length, and most of this is released between 12 and 14 km from the inlet (*101*). Measurements of NPE and metabolites in river waters are provided in Tables 21

(mol/day) and 22 (μg/L). The average hydraulic retention time of the majority (75%) of total NPc input to the Glatt River is estimated to be between 10 and 15 hours, long enough to effect biological transformation (affected by light intensity and duration, amount of rainfall, and temperature) and physical-chemical processes. An estimated 15 - 20% of the water at the outlet of the river is treated wastewater (*133*).

- Ahel *et al.* found that 24% overall elimination (molar flow) of NPE and metabolites occurs in the river along the 35 km stretch studied (Table 21). This falls below the range of elimination efficiencies that were measured in the Glatt River STPs (26 - 79%).
- Similarly to secondary effluents, the presence of $NPE_{3\text{-}20}$ oligomers is minimal. Biodegradation is the major process by which the parent surfactant is removed from the water column. Bio-transformation of the parent surfactant, $NPE_{3\text{-}20}$, and its products, $NPE_{1\&2}$, to the carboxylated form occurs rapidly in the aerobic environment of river water during transport, according to the pathway:

  $$NPE_{3\text{-}20} \rightarrow NPE_{1,2} \rightarrow NPE_{1,2}C.$$

  The distribution of total NPc introduced in the secondary effluent changes in favor of the carboxylated products -- $NPE_{1,2}C$ -- up from 51 to 86%, while the parent compound, $NPE_{3\text{-}20}$, and the noncarboxylated metabolites, $NPE_{1\&2}$ and NP, decrease to 3, 9, and 2%, respectively (Table 21, Figure 17). $NPE_{1\&2}C$ persist.

Table 21. Inflow and Outflow of NPEs in the Glatt River

Component	Inflow (2° effl) (mol/d)	Outflow (mol/d)	Elimination (%)	Inflow Distribution (%)	Outflow Distribution (%)
NPE_{3-20}	23.4	2.8	88	22	3
$NPE_{1,2}$	24.3	7.2	70	22	9
NP	5.3	2.0	62	5	2
$NPE_{1,2}C$	55.2	70.1	-27	51	86
NPc	108.2	82	24	100	100

Ref. (*133*).

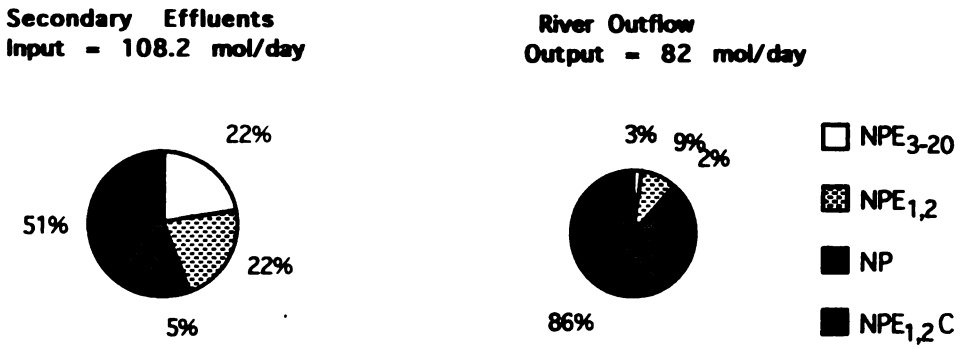


Figure 17. Distribution of NPE compounds in secondary effluents and Glatt River water. Adapted from (*133*).

- Large fluctuations in concentrations of NPE and metabolites occur along the river (Table 22), varying with location, season, and time of day. The high measurements for $NPE_{1\&2}$ (69 μg/L) and NP (45 μg/L) are outliers; of the samples measured, 219 and 109 measurements fall in the range 0 - 30 μg/L and 0 - 8 μg/L, respectively.

Table 22. Environmental Analyses of NPEs in River Water and Sediment -- by Major Category [1]

Component	Number of samples	River water low/high (μg/L - ppb)	River sediment low/high (μg/g - ppm) [2]
NPE_{3-20}	--[3]	<1./7.1	--[4]
$NPE_{1,2}$	220	<0.3/69[5]	0.29/11.25
NP	110	<0.3/45[6]	0.51/13.1
$NPE_{1,2}C$	96	<1/71	--[4]
NPc		NA	1.27/24.35

[1] Ref. (*133*). [2] Based on dry weight of sample. [3] Not provided. [4] Not measured. [5] One NPE_1 sample was 69 μg/L; 219 samples were < 30 μg/L. [6] One NP sample was 45 μg/L; 109 samples were ≤ 8μg/L.

- Longitudinal profiles based on product concentrations vs. mass flows (both winter and summer data) depict the role that dilution plays, together with biodegradation and physical-chemical processes, in decreasing aquatic concentration levels.
  - -- Concentrations of all NPE products increase with the input of secondary effluents up to the test station located 15 km downstream. Between the test stations that are 15 and 35 km downstream, the concentrations of all products decrease, Figure 18.
  - -- Mass flows also increase up to the test station that is 15 km downstream, but they are more likely to remain relatively flat or increase between the 15 and 35 km test stations, Figure 18.
- The effect of improved efficiencies of elimination that occur in the STPs during warmer weather are reflected in the seasonal longitudinal profiles

measured from the river water, Figure 18. River water temperatures during winter and summer samplings are 4.8 - 5.6 and 18 - 20°C, respectively. Summer readings of the NPE product concentration levels and mass flows are generally lower at all downstream test stations. Improved elimination efficiencies in the river water due to warmer temperatures are also evident. Dilution attributed to seasonal variations in river flow affects concentration levels minimally, if at all.

- -- NP: Data measurements are slightly higher during the cold season, Figure 18. NP remains relatively flat during both seasons, probably because sorption, a process that is much less sensitive to temperature, is the mechanism for its removal rather than biodegradation.
- -- $NPE_{1,2}$: Winter measurements are 2 - 7 times higher than summer measurements. The winter mass flow increases about 15% in the last 20 km stretch of the river, while the summer mass flow decreases 30%, Figure 18.
- -- $NPE_{1,2}C$: A longitudinal profile for $NPE_{1,2}C$ is not provided for the winter months. Summer longitudinal profiles portray a 15% increase in mass flows beyond the 15 km point, probably due to higher biodegradation rates associated with warmer temperatures, Figure 18.

- Nonylphenol ethoxylates and their derivatives are the predominant organic compounds in the Glatt River system, exceeding other hydrocarbons (HCs), chlorinated HCs and chlorinated phenols (*12*). Concentrations of the individual oligomers of nonylphenolic compounds are one order of magnitude higher than other compounds, such as tetra-chloroethylene, 1,4-dichloro-benzene, 1,3-dimethylbenzene and pentachlorophenol, measured in another study (*65*).

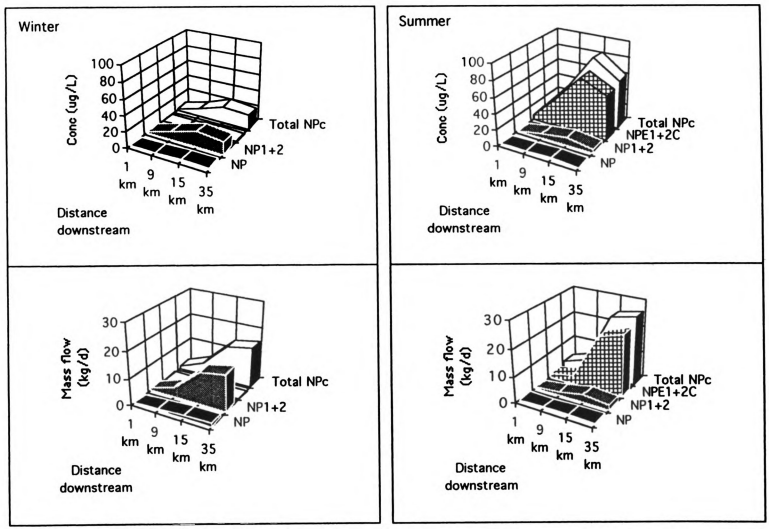


Figure 18. Longitudinal profiiles (concentration and mass flows) of measured NPE derivatives and total measured NPc. Adapted from Ref. (*133*).

Water column and sediment

Partitioning to organic material, suspended solids, or colloids, and subsequent settling remove NP and $NPE_{1,2}$ from the water column. Photochemical degradation (*203*) and volatilization (*199, 202*) may be removal mechanisms in surface waters that have very little organic material, or very low concentrations of suspended solids or colloidal material. However, the high concentration of colloids, suspended solids, and organic material, typical of many surfactant relevant environmental compartments (Table 4) favors sorption over photolysis and volatilization. Although the fraction of organic material, and concentrations of colloidal material and suspended solids along the Glatt River are not reported, Ahel *et al.* report the following:

- Higher concentrations of NP, NPE_1, and NPE_2 are measured in mud than in sand collected at the same location (*133*). Partitioning is greater in the mud because of its higher organic content. The concentration levels in Table 22 provide evidence that the concentration of NP and the lower oligomers ($NPE_{1,2}$) is about three orders of magnitude greater in Glatt River sediment than in the river water.
- Algae growth is so thick in parts of the Glatt River that it is removed mechanically at the end of every summer (*26*). Ahel *et al.* have measured concentrations of NP, NPE_1 and NPE_2 in algae as high as 25, 80, and 29 ppm (mg/kg), respectively.
- Not only does the algae offer an environment onto which NP and the lower NPE oligomers can sorb, but its accumulation leads further to a disposal problem when it is removed annually from the river (*26*).

- Longitudinal profiles of NP, NPE_1 and NPE_2 in river water and sediment are similar (Figures 18 and 19). The sediment concentration levels of all three metabolites decrease between the 15 and the 35 km sediment test stations. $NPE_{1,2}C$ values are not provided for sediment.
- NP persists under anaerobic conditions prevalent in sediments. It remains sorbed to organic material in the sediment unless turbulence (fast water flow or bioturbation) effects resuspension or aerobic conditions, in which case it may biodegrade. The extent to which NP accumulates needs to be determined. Ahel *et al.* measured the ratio of NP in sediment to NP in water to be between 364 and 5100 (*133*).

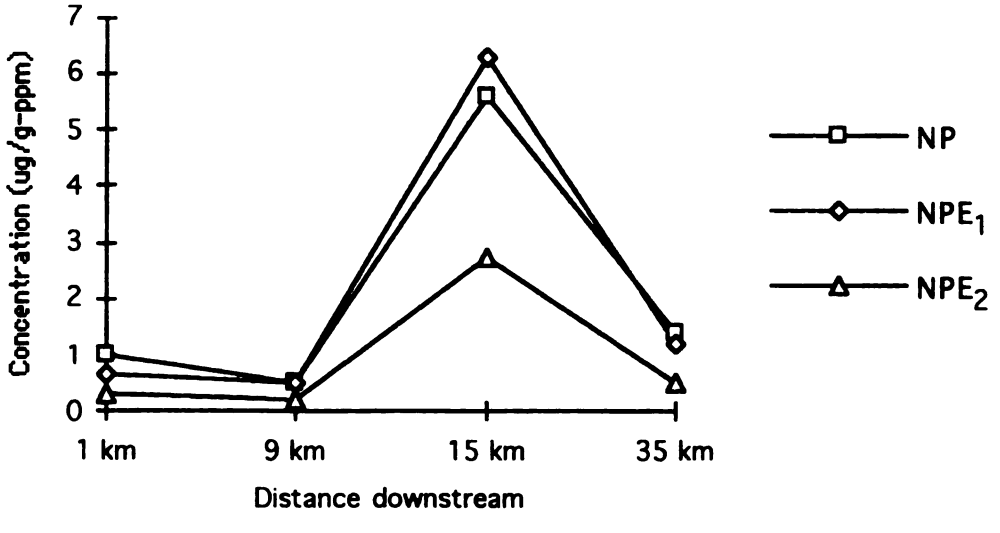


Figure 19. Longitudinal concentration profiles of NP, NPE_1, and NPE_2 in sediment. Adapted from Ref. (*133*).

World data - Surface waters

Tables 23 and 24 and Figures 20 and 21 provide information about NPc concentrations that have been measured for surface water samples collected worldwide. The data cannot be directly compared because test conditions, sampling methodology, analyte measurements and analysis techniques are different (*27*).

The studies reported in Table 23 were completed in Switzerland and the United States, in fresh water environments. A wide range of aqueous concentrations is evident for NP (Table 23), from as high as 3000 ppb in a small creek that receives the overflow from an anaerobic treatment pond of a carpet yarn mill (*136*) to as low as below detection levels (0.11 ppb) (*54*). In the former study, NP concentrations in samples drawn from several downstream locations decrease rapidly as distance from the treatment pond increases, so that only 2 ppb NP is measured in a Savannah River sample taken 9.6 km from point of discharge. As discussed above, this is due primarily to hydrodynamic conditions, dilution, and sorption. Concentrations in sediment samples also vary (Tables 22 and 23), ranging from 13,100 ppb (*133*) to below detection levels (2.99 ppb) (*54*) in samples measured in Switzerland and the United States, respectively.

Table 23. Environmental Analyses of NPcs in Fresh Water and Sediment [1,2]

Location		NPE_{3-20}	$NPE_{1\&2}$	NP	$NPE_{1\&2}C$	Analysis/ Ref.
Switzerland						
Glatt River						HPLC/(*133*)
Low/high		<1. /7.1	<0.3/69	<0.3/45	<1./71	
Switzerland	Distance/					
Lk Geneva	depth					SIM-GC/
Average (n = 2)	(m)					MS/(*14*)
@ Vidy	200/ 6	--[3]	9.7	2.9	--[3]	
@ Nyon	5-10/4-5	--[3]	2.4	1.2	--[3]	
@ Prangins	5-10/4-5	--[3]	8.3	3.4	--[3]	
USA						GC/MS/FID/
Georgia [4]	Distance [5] (km)					(*136*)[4]
Discharge [6]		--[3]	--[3]	4000	--[3]	
Small creek	1.6	--[3]	--[3]	3000	--[3]	
Small river	2.4	--[3]	--[3]	200	--[3]	
Small river	7.2	--[3]	--[3]	30	--[3]	
Savannah River	9.6	--[3]	--[3]	2	--[3]	
USA						
Thirty rivers						
study-water						HPLC/(*54*)
Highest		14.9	1.8	0.64	--[3]	
Average (n = 100)		2.0	0.2	0.12 [7]	--[3]	
Thirty rivers						
study-sediment			(µg/kg)	(µg/kg)		HPLC/(*54*)
Highest		--[3]	175[8]	2960	--[3]	
Average (n = 80)		--[3]	18[8]	162[9]	--[3]	

[1] Data reported in µg/L unless designated otherwise. [2] Statistics not provided. [3] Not measured. [4] NP concentrations in textile waste and receiving waters. [5] Downstream from textile mill's anaerobic treatment pond discharge. [6] From textile mill's anaerobic treatment pond. [7] NP was below detection (0.11 ppb) in more than 70% of samples. [8] NPE_1 only measured. [9] NP was below detection (2.99 ppb) in 28% of samples.

Table 24 provides data from research that examines NPc behavior and fate in marine environments. The data is collected from two locations that border the Adriatic Sea, in Croatia (*137*) and Italy (*138*). Again the range of concentrations that are measured in the two studies span up to three orders of magnitude.

The Venice lagoon receives treated wastewaters from domestic and industrial sources. This is evident in the lower concentrations for NPE_{1-13} and

its derivatives -- NP, $NPE_{1\&2}$. Concentrations in water, associated with RSM (resuspended solid material), and in sediment cores are reported in Table 24.

Croatia discharges untreated municipal wastewater into Sibenik Harbor, as reflected by the very high average concentrations of NPE_p (p=0-20) released from four sewer line outlets, Table 24. Analysis of wastewater samples yields NPE oligomeric distributions that are characterisic of NPE found in heavy duty detergents; the maximum of the oligomeric distribution is at 10 EO units/NPE molecule. Partitioning followed by sedimentation and dilution are the major processes by which NPEs are removed from the water column closest to the sewer outlets. The effect of partitioning is examined by measuring the NPE concentrations of dissolved vs. particulate fractions in the sewer lines and at the sewer outlets. A significant amount, up to 60% in some samples, is removed from the water column when it rapidly sorbs to particulate matter in the sewer line. Figure 20a is a pictorial representation of the change in total concentration and the dissolved and particulate fractions as the wastewater moves through the sewer line to its point of discharge. The sewer line and outlet concentrations of NPE associated with suspended solids based on the dry weight of suspended solids are also provided in Table 24, and are illustrated in Figure 20b. The sewer line and outlet concentrations of NPE based on the dry weight of suspended solids are very similar, indicating that desorption is either not occurring or is negligible. Additionally, oligomeric distributions of the dissolved and particulate fractions demonstrate the importance of lipophilic partitioning in determining behavior and fate of NPEs in wastewaters. The oligomeric distribution of the dissolved fraction is similar to the total sample, but the central and maximum point is shifted to the higher oligomers; oligomers with between six and eight EO units/NPE molecule are predominant in the particulate fraction.

The decrease observed in total concentrations at the sewer outlet is the result of dilution upon mixing with marine water in the harbor. The effects of further dilution are evident in measurements of estuarial NPE concentrations, which decrease exponentially as distance from the outlet increases from 1 to 200 m, Table 24. Concentrations of suspended solids drop from 122 - 884 mg/L in the wastewater to between 1 and 5 mg/L in the estuary, ruling out sorption as a major process in removing additional NPE from the estuarial water column.

Table 24. Analyses of NPcs in Marine Environments -- Aqueous/Solid Partitioning

	Dissolved fraction (μg/L±sd) low/high	Particulate fraction (μg/L±sd) low/high	Total conc'n (μg/L±sd) low/high	Dry weight conc'n (mg/kg) low/high	Distance /depth (m/m)
Croatia[1]					
Sibenik Harbor, Krka R estuary					
7 municipal sewage lines[2]					
NPE_p	371±231 127/783	209±115 50/406	580±290 177/1189	871±468 300/1500	
4 outlets[2]					
NPE_p	252±146 124/408	50±31 16/83	302±177 140/491	850±574 500/1700	
In estuary					
NPE_p[3]	--[10]	--[10]	126±92 43/257	--[10]	1/0.5
NPE_p[4]	--[10]	--[10]	0.4±0.2 <0.1/0.7	--[10]	25-200/ 0.5
NPE_p[4]	--[10]	--[10]	0.2±0.1 <0.1/0.4	--[10]	25-200/ 6.0
	Conc'n in water-ppb (μg/L±sd) low/high	**Conc'n on RSM[6]-ppm (μg/g±sd) low/high**		**Conc'n in sediment-ppb (ng/g±sd) low/high**	
Italy [5]					
Venice lagoon					
NPE_{1-13}[7]	1.8±1 0.5/4.5	--[10]	--[10]	--[10]	
$NPE_{1\&2}$	--[10]	1.4±2[8] 0.2/8.1	--[10]	35±24[9] 13/91	
NP	--[10]	0.89±1[8] 0.1/5.6	--[10]	14±9[9] 5/42	

[1] Ref. (*137*); analysis - HPLC (normal- and reversed-phase). [2] March 1991 samples; untreated water. [3] July and August 1990 samples; measurements from two outlets. [4] July and August 1990 samples. [5] Ref. (*138*); analysis - HPLC; data collected during February, April, July, and October 1987. [6] RSM: resuspended solid material. [7] 5 stations measured during 3 sampling sessions; April, July, October 1987. [8] Based on dry weight of solid material; 5 stations measured during 8 sampling sessions; February, April, July, October 1987, each at low and high shear stress. [9] Based on dry weight of solid material; 5 stations measured during 4 sampling sessions; sediment core samples = 5 cm. [10] Not measured.

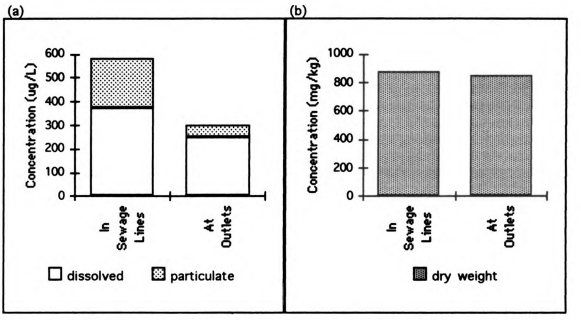


Figure 20. NPE_{1-20} marine environment concentrations, from untreated sewer waters discharged to Sibenik Harbor, Croatia: (a) in dissolved and particulate fractions based on water sample volume, and (b) based on dry weight of suspended solids. Adapted from (*137*).

Figure 21 depicts the effect that stratification in the water column has on NPE_p concentrations in Sibenik Harbor, at some distance 25m or more from the sewer outlets. The highest concentrations are observed at the air-water surface microlayer and the brackish water-sea water interface. The interface between the brackish and saline layers serves as a barrier to vertical transport; higher NPE_p concentrations within its boundaries are due partly to its high organic content. Wind currents explain the range of depths possible for the interface, and the overlap in the depth variations of the top and bottom saline layers. The top layer (0 - 6 m) is brackish, and has higher NPE_p concentration values (1.1 - 6 μg/L). The NPE_p concentration in the bottom, saline layer (0.2 - 40 m) is much lower (<0.1 - 0.7 μg/L). Two determinations provide evidence that biodegradation occurs in the estuary waters, although at lower rates than in fresh water. Warmer temperatures favor biodegradation. Winter and summer samplings indicate that the most abundant oligomers in

estuary water are NPE_{10-11} and NPE_{7-9}, respectively. Additionally, the proportion of $NPE_{1\&2}$ relative to total NPE_p concentration is higher in estuary water than in wastewater samples. The authors conclude that biodegradation, rather than sorption, is the more likely removal process in the estuary because at less than 5 mg/L the concentration of particles does not favor partitioning. Volatilization and photolysis may be minor removal processes that the authors did not address.

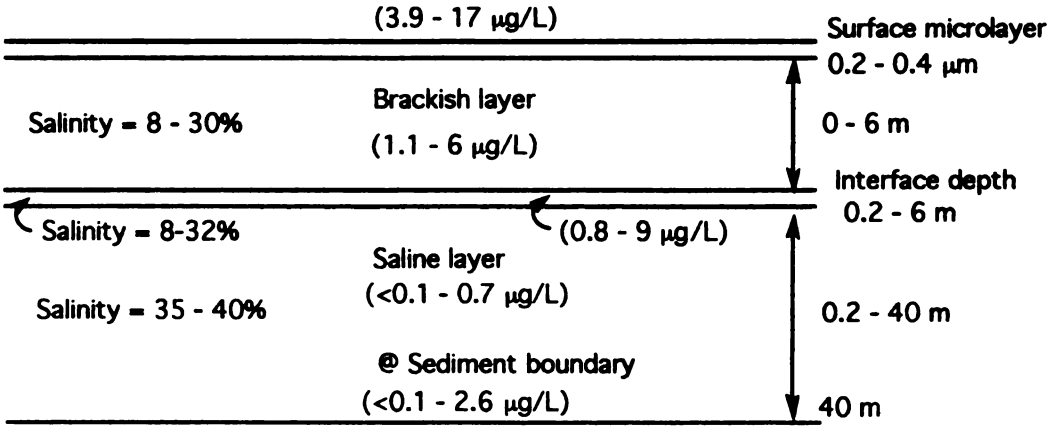


Figure 21. Concentrations of NPE_p in the vertical profile of Sibenik Harbor, Croatia.

Groundwater

Ahel *et al.* chose two sites for the third phase of their study, involving the Glatt and Sitter Rivers in northeastern Switzerland. Their study addresses the fate and behavior of NPE metabolites in aquifers with relatively short residence periods (*62*). The aquifers receive water that infiltrates through a river bed. Relatively high velocities -- Glatt: 0.1 - 0.3 m/hr (*65*); Sitter: 4 m/hr during pumping (*62*) -- account for the short residence times. The NP and $NPE_{1\&2}$ data were collected over a period of 14 months (July, 1984 to August, 1985) at approximately monthly intervals; up to seventeen samples were collected. Only two samples of $NPE_{1\&2}C$ were collected, on May 14 and 20, 1986. Samples of river water and groundwater were drawn from observation wells placed at 2.5, 5, 7, 13, and 130 m intervals along the groundwater flow line (*65*). The plunger pump used to draw the samples was placed approximately 0.5 m below the water table to ensure that infiltrated Glatt River water was analyzed (*65*). Figure 22 provides a 0 - 13 m longitudinal concentration profile of average concentrations of the samples gathered from the Glatt River site for each of the five metabolites. Table 25 is a tabular version of the data plotted in Figure 22. The values in Table 25 are in terms of percent elimination; approximate residence times that correspond to the first four observation wells are also provided for minimum and maximum velocities measured in the aquifer.

Samples were drawn from the Sitter River site to determine whether variations observed in pollutant concentrations are similar in river water and groundwater. Grab samples were collected in September, 1985, at two hour intervals for 24 hours. $NPE_{1\&2}C$ are not measured. Analysis of sample measurements from the Sitter River site confirm the findings from average

measurements in the Glatt River study. The findings are summarized as follows:

- NP, NPE_1 and NPE_2 are eliminated efficiently from the aqueous phase, on the average. Most of the elimination occurs within the first 2.5 m of the river bed (Figure 22 and Table 25). High organic matter and probably a higher population of microorganisms both contribute to the removal efficiency.
- NPE_1C and NPE_2C are eliminated more slowly and to a lesser extent -- both within the first 2.5 m from the river bed and over the total distance (Figure 22). NPE_1C and NPE_2C continue to be the predominant metabolites of NPE in groundwater. They appear to be more mobile than the other NPE products.
  - -- NPE_1C and NPE_2C are more soluble yet more resistant to biodegradation than the noncarboxylated oligomers.
  - -- The difference is even more remarkable if one considers that the averages for NPE_1C and NPE_2C are based on two samples collected during May, 1986, a warmer season. If cold weather results had been included in the calculations the inputs (measurements in river water at 0 m) would have been higher. More studies are necessary to determine how colder temperatures affect the rate and extent of elimination efficiencies, though it is likely that slower rates and lower elimination efficiencies would prevail.
- Dilution also plays a role in eliminating NPE metabolites from this aquifer, owing to its relatively high velocities.
- Order of elimination efficiency from highest to lowest is:

  $NPE_2 \geq NPE_1 > NP > NPE_2C > NPE_1C$

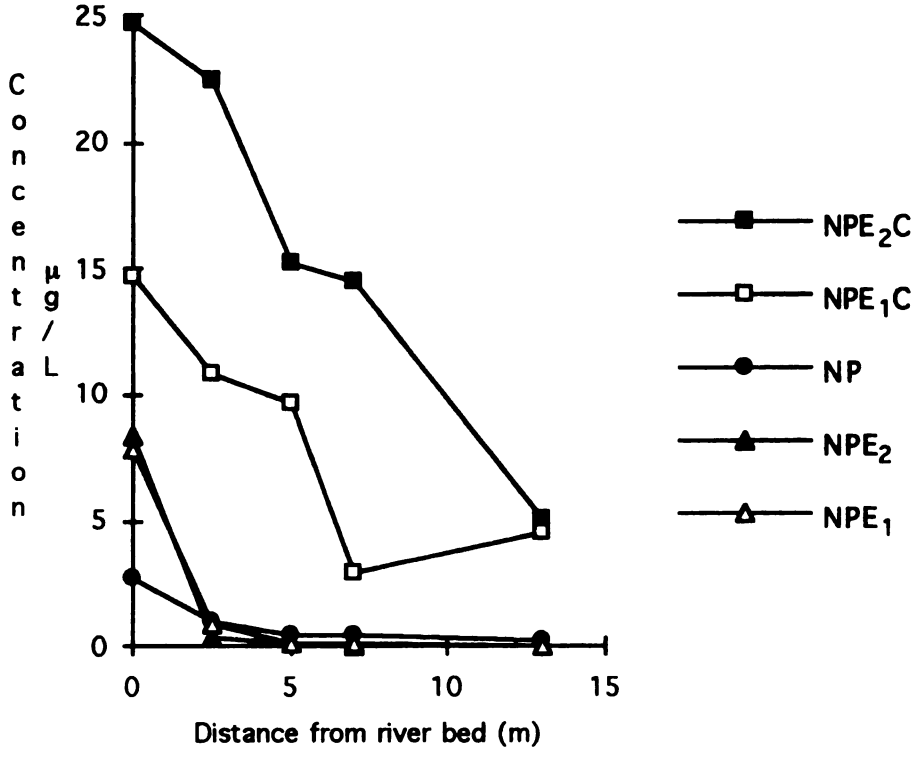


Figure 22. Longitudinal profile of average concentrations measured in Glatt River and underlying aquifer, at 0.5 m below the water table. Adapted from (*62*)

Table 25. NPc Elimination in Groundwater Underlying the Glatt River

Distance from river bed (m)	2.5	5	7	13
Component		% Elimination		
NPE_2C [1]	9.3	38.1	41.3	79.4
NPE_1C [1]	25.9	34.0	80.3	69.4
NP [2]	64.4	85.2	83.7	92.6
NPE_2 [2]	96.1	98.1	99.3	>99.9
NPE_1 [2]	88.3	97.7	98.8	99.5
	Approximate residence time at above distance (days)			
vel = 2.4m/day	> 1	> 2	< 3	5-6
vel = 7.2m/day	> 0.3	< 0.7	< 1	< 2

[1] Based on 2 samples. [2] Based on 16 samples.

- Concentration measurements of NP, NPE_1 and NPE_2 in winter samples of river water are 2 - 4 times higher than those taken during warmer seasons. Figure 23 provides NP, NPE_1 and NPE_2 concentration levels that were determined in river and observation well samples during October, 1984, and January and June, 1985. Throughout the winter season, elimination efficiencies of NP and NPE_1 are lower at the 2.5 m observation well. According to the January, 1985 data, the elimination efficiency of NPE_2 does not appear to be affected in winter at the 2.5 m observation point. Seasonal variations are mixed for the metabolites at the 5 m well. In general, the metabolites are eliminated more efficiently during the warmer months. This illustrates the importance of biotransformation as an elimination mechanism, and the role that temperature plays in affecting biodegradation rates. A range in temperatures (4 - 20°C) was only detected within the first seven meters of the aquifer. Seasonal variations in temperature did not affect elimination efficiencies for any of the metabolites at the 7, 13 and 130 m wells, where the temperature is fairly constant at 10 - 12°C.

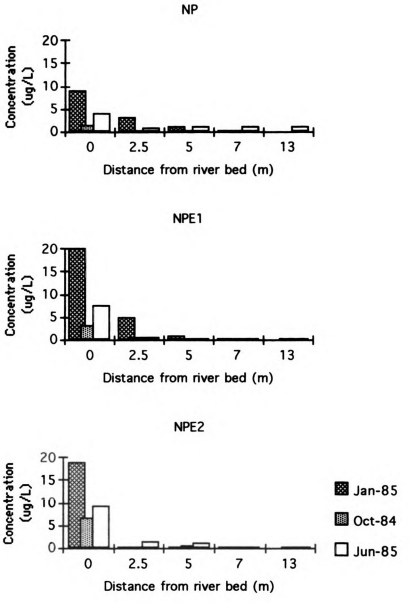


Figure 23. Seasonal variations in NP, NPE_1, and NPE_2 concentrations, measured in river water and groundwater, 0.5m below water table.

- Wide fluctuations in concentration levels of NP and NPE_2 occur during the winter months. The data is provided in Table 26 and portrayed in Figure 24. February, 1985 measurements of NP are high -- 29 and 33 μg/L at the 2.5 and 13 m wells, respectively. A high NPE_2 measurement of 23 μg/L, from December, 1984, is depicted for the well that is 5 m downstream. More knowledge about biological transformations and transport in aquifers is necessary to ascertain the reasons for the wide variations. The authors provide two possible explanations:
  - -- A highly polluted "package" of water might move into the river bed such that the concentration of pollutant exceeds the ability of the surrounding aquifer to adsorb it and/or biotransform it. Breakthrough of the pollutant would cause a huge increase in concentration.
  - -- The likelihood that anaerobic conditions might establish within the first few meters of the aquifer could cause rapid biotransformation of $NPE_{1\&2}$ to NP.

Table 26. Fluctuations in NPE Product Concentrations -- High Readings from the Glatt River and Underlying Aquifer (μg/L)

Product	0 m	2.5 m	5 m	7 m	13 m	130 m
NP	26	29 [1]	4.4	3.4	33 [1]	7.2
NPE_1	20.0	4.8	4.9	4.4	1.7	3.4
NPE_2	21	1.6	23 [1]	0.7	0.1	1.7

[1] Extraordinarily high readings.

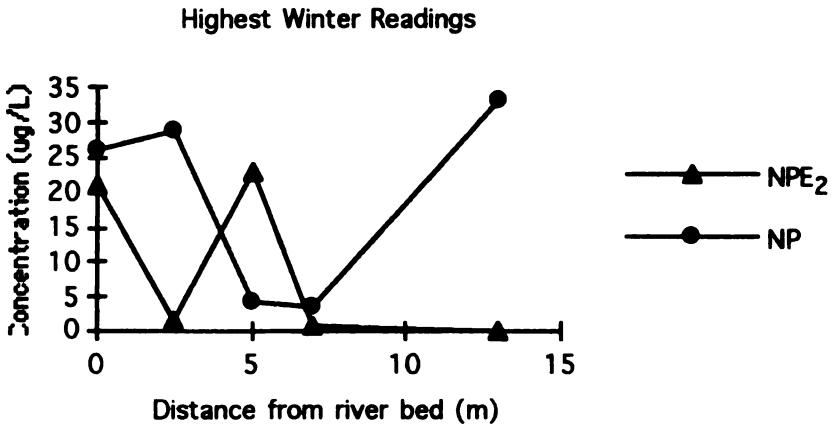


Figure 24. NP and NPE_2 concentrations fluctuate in aquifer during winter months.

- The extent to which sorption affects transport and ultimately elimination in groundwater can be estimated by considering the retardation factor:

$$R_f = 1 + 3.2*f*f_{OC}\ (K_{OW})^{0.72}\ \rho(1-\varepsilon)/\varepsilon$$

It is calculated as a function of organic matter and the octanol/water partition coefficient (*62, 65, 100*). To obtain low and high end retardation values within 5 m of the river bed, Ahel *et al.* assumed that the fraction of organic carbon in the aquifer (f_{OC}) is 0.001 - 0.01, and that the fraction of grain size < 125 mm (f) is 0.2 - 0.4. Density of the aquifer material, ρ, is 2.5 g/cm^3 and total porosity, ε, is 0.2. Using log K_{OW} values equal to 4.48 and 4.12 for NP and $NPE_{1\&2}$, respectively, Ahel *et al.* obtained retardation factors ranging from 11.7 to 216 (NP) and 6.9 to 119 ($NPE_{1\&2}$). If log K_{OW} is equal to 4.2 (the approximate value in Table 6 for $NPE_{1\&2}$) (*100*), the estimated retardation factor range is between 7.8 and 136. This is still at least 63% below the range calculated for NP. Ahel *et al.* (*62*) suggest that biotransformation of $NPE_{1\&2}$ to NP explains the apparent greater transport capabilities of NP over $NPE_{1\&2}$ in spite of a retardation factor for NP that is higher by a factor of 1.5 to 1.8 (*62*). Transport of colloidal material associated with NP may also explain its apparent mobility.

- High retardation factors extend the length of time that NP and $NPE_{1\&2}$ remain in the portion of the aquifer closest to the river bed. This enhances opportunities for biotransformation, while also keeping the pollutants close to the surface of the river bed. Whether biotransformed or not, they may reenter the river water by resuspension or desorb and be transported with groundwater flow (Figure 3).

Concerns that nonylphenolic compounds may adversely affect groundwater quality are justified in light of data collected at pumping stations located long distances from NPc sources. Water samples from a pumping station located 130 m from the Glatt River provide evidence that NP and $NPE_{1\&2}$ have not been completely removed (Table 26) (*62*). $NPE_{1\&2}C$ was not measured. A study, conducted at three field sites of an aquifer that receives infiltrated water from the Sava River near Zagreb, Croatia, also yields detectable quantities of NPE derivatives -- NP, $NPE_{1\&2}$, and NPEC (Table 27) (*68*). The reader will note that the values are much smaller than those recorded in Table 26. They are included for the sake of comparison, and to emphasize that the carboxylated NPE derivatives, according to the data collected from Sites 1 and 2, persist, and therefore demand further study. Likewise, $NPE_{1\&2}C$ was detected (not quantified) during analysis of sewage contaminated groundwater from a site on Cape Cod, Massachusetts (*63*).

Table 27. NPc Concentrations in Groundwater in Zagreb, Croatia (μg/L)[1]

	Site 1		Site 2		Site 3	
Product	Sava River	120 m	Landfill	30 m	Waste-water	15 m
NP	0.70	<0.10	<0.10	--[2]	10.3	0.28
NPE_1	0.40	<0.10	<0.10	--[2]	34.0	0.03
NPE_2	0.20	<0.10	<0.10	--[2]	1.90	0.01
NPEC	5.00	0.20	0.05	0.05	0.29	0.004

[1] Taken from (*68, Table 4*). [2] Not determined.

Summary

Primary biodegradation of alkylphenol polyethoxylates (APE_{3-20}) generally proceeds rapidly in sewage treatment plants. Lower temperatures reduce elimination efficiencies during the winter months. Biodegradation proceeds by step-by-step elimination of the hydrophilic ethoxy units, down to two or fewer units (AP and $APE_{1\&2}$). Under aerobic conditions, common in activated sludge processes, carboxylation occurs on the α-carbon of the terminal ethoxy unit such that $APE_{1\&2}C$ are produced and accumulate. High quantities of carboxylated alkylphenol ethoxylates (8 - 25% of total introduced APE_p), and lesser quantities of APE_{3-8} and $APE_{1,2}$ (up to approximately 10 and 12% each, respectively, of total introduced APE_p) are released to surface waters in secondary effluents. AP and $APE_{1\&2}$ that are not carboxylated or released in secondary effluents sorb to suspended solids and settle with the sludge in the primary or secondary clarifier (Figure 4). $APE_{1\&2}$ are biodegraded to AP during sludge digestion; biotransformation of $APE_{1\&2}$ to AP is favored under anaerobic conditions. Between 15 and 25% of alkylphenolic compounds introduced to an STP are released to the environment in digested sludge. Alkylphenolic compounds remaining in the digested sludge are AP (approximately 95% or 15 - 24% of the total APE introduced) and $APE_{1\&2}$ (5% or 1 - 2% of the total APE introduced). Land application of the digested sludge may initiate further breakdown of AP under aerobic conditons, or lead to mobilization of AP that is associated with colloidal material.

The elimination of APE and its metabolites from surface waters must be studied in terms of their behavior in association with particles and sediment, as well as in the water column. Biotransformation, sorption and dilution are the primary processes at work in the water column. Volatilization and photolysis are less important. Higher biodegradation rates that occur with

higher temperatures result in lower concentrations overall during summer months; lower concentrations are introduced to surface waters and observed in surface waters downstream of discharge points due to greater elimination efficiencies. The biotransformation of APE_{3-20} to $APE_{1\&2}$ to $APE_{1\&2}C$ occurs under aerobic conditions in the water column. Elimination efficiencies are lower in surface waters than in STPs owing to shorter residence times, dilute concentrations, and smaller microbial populations. The rate of biodegradation in marine water appears to be lower than in fresh water. $APE_{1\&2}Cs$ persist and accumulate. Most fresh and marine water NPE metabolite concentration values are between 0 and 10 μg/L. The highest fresh water value reported is for NP at 4000 μg/L (effluent from an anaerobic treatment pond). The NP concentration quickly decreases to 2 μg/L in the Savannah River, less than 10 km downstream from the point of discharge (mainly dilution).

$APE_{1\&2}$ that sorb and settle with solid organic material may be transformed to AP under anaerobic conditions in sediment. Partitioning is greater in mud, with a higher fraction of organic matter, than in sand. The lipophilicity and a correspondingly high distribution coefficient of AP favor its persistence and accumulation in sediments that are not disturbed. APE metabolite concentrations in sediment are more widespread, with measurements for NPE_{0-2} varying from 18 μg/kg (ppb) in a marine lagoon in the Adriatic Sea to 24,350 mg/kg in the Glatt River.

The approximate order of elimination efficiency in groundwater, from greatest to least is: APE_2, APE_1, AP, APE_2C, APE_1C. The greatest reduction in concentration in the aquifer infiltrated by the Glatt River is observed within the first 2.5 m of the aquifer. Overall elimination efficiencies are greater during the warmer summer season, pointing to the importance of temperature, ranging between 4 and 20°C, in controlling biodegradation rates.

Temperature ceases to be an issue at a well located 7 m from the river bed, where a constant temperature between 10 and 12°C is maintained. Though high readings are occasionally seen for NPE_2, NPE_1, and NP, their concentrations tend to be below 10 μg/L, and are usually less than 1 μg/L. Fluctuations in concentration measurements may be due to sudden packets of pollution that overburden the system, or to depletion of dissolved oxygen. Sorption of APE_2, APE_1, and AP retards the movement of these compounds through aquifer material, allowing more time for biotransformation to occur. $APE_{1\&2}C$ are the most soluble of the metabolites, and therefore most mobile and least likely to sorb or biodegrade. $NPE_{1,2}C$ concentrations measured in the aquifer underlying the Glatt River are as high as 29 μg/L. This concentration is reduced to approximately 5 μg/L or less within the first 15 m of the river bed. Both biodegradation and dilution contribute to the decrease in $APE_{1\&2}C$ concentrations.

Halogenated Derivatives

One of the final steps in water treatment, particularly in the United States, is disinfection through the addition of chlorine. While destroying waterborne pathogens, the introduction of chlorine and other halogens, that are present with the chlorine as impurities, leads to undesirable reactions with organics that persist in the treated water, or that are present in the receiving waters as humus material. Trihalomethanes -- such as $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$ -- are the predominant organic halogens formed, and are the most studied (*204-206*); chloroform ($CHCl_3$), believed to be carcinogenic, is most prevalent. For this reason, studies that investigate the formation of trihalomethanes far outnumber studies that investigate the formation of other classes of organic halogens, such as the halogenated phenols and the halogenated derivatives of alkylphenol ethoxylates. Yet worldwide concerns about the persistence, accumulation, and toxicity of halogenated phenols and halogenated APE metabolites warrant investigations into their formation, behavior and fate.

Halogenated phenols and halogenated derivatives of alkylphenol ethoxylates have been found in secondary effluents (*18, 190*). Halogenated phenols have been found in the Llobregat River, near Barcelona, Spain (*207*), and halogenated derivatives of APEs have been found in tap water from a water treatment plant in Barcelona (*19, 20, 22*). The Llobregat River is heavily polluted, serving a population of 3.2 million (*21*), in addition to many industries -- textile, dyeing, tanning, leather, and surfactant manufacturers (*21, 22*) -- that discharge their effluents into it. Salt mines located upstream are the source of high levels of bromide that are found in the river (*21*). Chlorination is the means of disinfection in the Barcelona water treatment plant, as well as in the U. S. plants studied by Reinhard (*190*) and Ball (*18*). In

Figure 25, Figure 9 has been adjusted to show the effect that chlorination in a water treatment plant has on APE products. A summary of the major findings from halogenated APE research follows.

- Aerobic transformation of APE metabolites proceeds more quickly and more completely than anaerobic transformation. Under both conditions, APE_2 and APE_2C accumulate as precursors to the halogenated forms (*18, 23*).

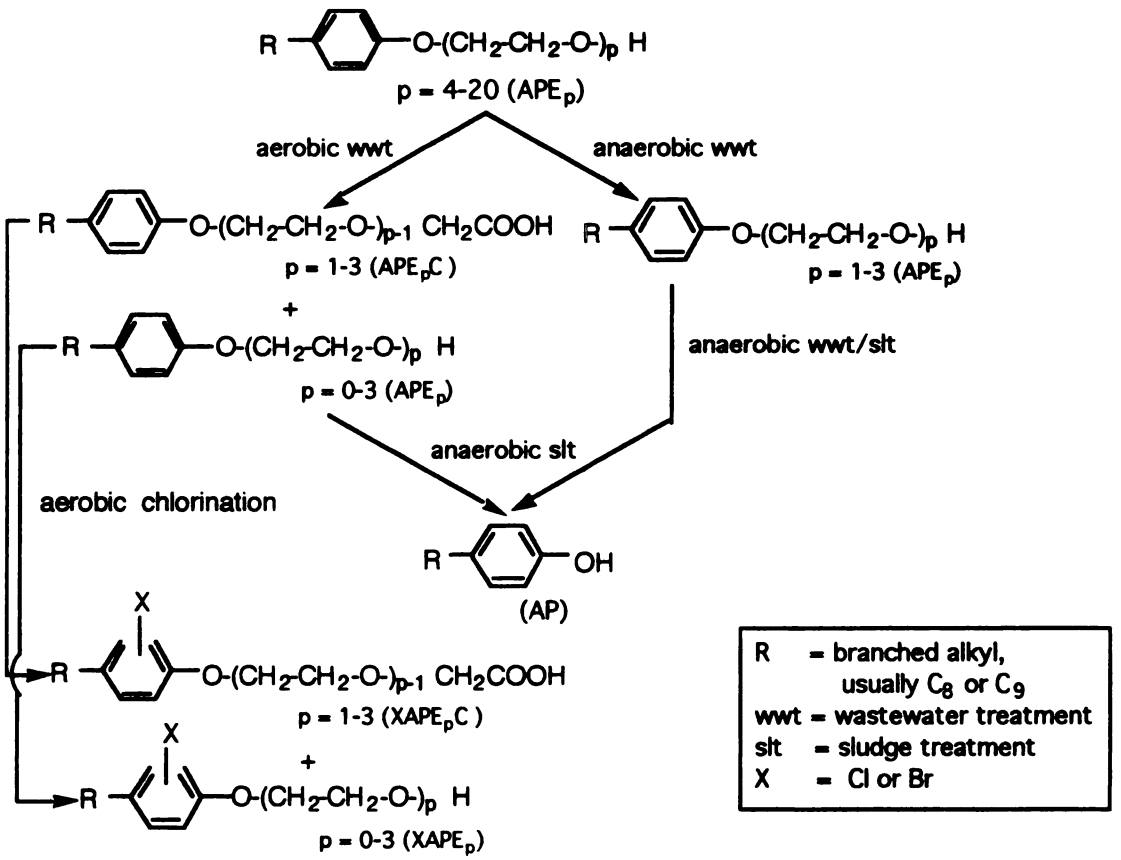


Figure 25. Proposed APE pathways in an STP with chlorination. Adapted from (*15*).

- Bromide and bromide derivatives are highly reactive. The presence of bromide speeds the rate of transformation to halogenated derivatives (*18, 190, 204*). Formation of brominated products is significantly faster than formation of chlorinated products (*18, 20-22*).

- The aromatic structure is preferentially brominated during wastewater chlorination (*18, 20, 21, 190*). This is depicted in the results summarized in Table 28; concentrations of halogenated OPE_ps and OPE_pCs that were measured in secondary effluents in a water treatment plant in Palo Alto, California (*18*) are listed. Grab samples were collected three times a day -- 9 am, 2pm, and 7 pm -- for five consecutive days, and mixed into daily composite samples on a flow-weighted basis. The second column summarizes the results of sample measurements on days 3 and 4, the two days with the highest load. The information in column 4 of Table 28 denotes the number of days for which the compound of interest could be quantified, not quantified, and not detected.

Table 28. Concentrations of Halogenated OPEs and OPECs Measured in Secondary Effluents in Palo Alto, California [1]

Compound	Concentration (≤ 2 day avg) (μg/L)	Concentration (≤ 5 day avg) (μg/L)	Number of days with data; days Not Quantified; days not detected [2]
$BrOPE_1C$	NQ; NQ	NQ	0; 5 days NQ
$BrOPE_2C$	18 ± 1.4	8.1 ± 9.1	5
$BrOPE_3C$	10.7 ± 6.1	10.7 ± 6.1	2; 1 day NQ; 2 days nd
$BrOPE_4C$	nd; NQ	nd	1 day NQ; 4 days nd
$ClOPE_2C$	5.9 ± 4.0	4.0 ± 3.1	4; 1 day nd
$ClOPE_3C$	nd; 6.1	6.1	1; 1 day NQ; 3 days nd
$ClOPE_4C$	nd; 3.9	3.9	1; 4 days nd
$BrOPE_1$	0.9; NQ	0.9	1; 1 day NQ; 3 days nd
$BrOPE_2$	1.5; nd	1.5	1; 4 days nd
$ClOPE_2$	nd; 0.5	0.5	1; 4 days nd

[1] Ref. (*18*). [2] Detection limit 0.5 μg/L.

- The biotransformation of halogenated APE_p and APE_pC ($p > 2$) leads to the production and accumulation of $XAPE_2C$ (X = halogen group), which resists further biodegradation under both aerobic and anaerobic conditions (*23*).
- Halogenated APs accumulate as a result of anaerobic biodegradation of APE and APEC (*23*).

- Dechlorination does not reverse halogenation. Halogenated derivatives remain in treated water even after dechlorination .
- High concentrations of XAPEC (60 ppm) are inhibitory to an anaerobic methanogenic consortium (*23*).

Many questions about halogenated organics remain unanswered. The exact mechanism by which halogenation occurs is not known. The factors that influence the extent of halogenation (*18*) and reaction kinetics (*204*) are not understood. Toxicity data specific to halogenated APE derivatives were not found. The extent to which these compounds persist, accumulate, and are present in various environmental compartments needs to be investigated. Additional research is required to determine the answers.

Toxicity

As is the case with determining environmental levels of surfactants, several approaches have evolved to determine toxicity, the factor that is used to satisfy the HAZARD part of the risk assessment equation (RISK = HAZARD * EXPOSURE). Acute toxicity is measured in terms of LC_{50} for organisms, or EC_{50} for plants. Chronic toxicity is measured in terms of no observed effect concentration (NOEC), or lowest observed effect concentration (LOEC). Further, chronic toxicity is determined in relation to some observable, measurable effect, such as growth (length or weight), maturity, spawning, fertilization, reproduction, development, hatch rate, or survival rate. Theoretically the bioconcentration factor (BCF) is the ratio of the concentration of a test compound that partitions to a particular animal or plant organism to the concentration of the test compound that remains dissolved in the water phase. Definitions of these terms are provided in

Appendix B. Determinations of chronic toxicity are preferred over acute toxicity when assessing environmental safety (*208*); values for both are provided in Appendix E (Table 32). Table 29 summarizes the data in Appendix E by providing ranges (from low to high) of estimated toxicity values according to environmental conditions -- fresh water, sediment, or marine water. This facilitates the process of comparing toxicity data to the environmental levels found in Tables 10 and 22 - 24. Bioconcentration factors are provided in Tables 30 and 31. Highlights of the findings about AE and APE toxicity follow.

- The toxicity ranking of linear and branched AEs, APEs, and recalcitrant degradation products (APs), from most to least, is according to:

  AP > AE (lin) > AE (br) > APE_{3-20}.

  This relationship is true for both aquatic animals and plants (algae) that have been tested in fresh water and marine water environments (Appendix E). The toxicity of AE and APE nonionic surfactants increases with increasing hydrophobicity and decreasing hydrophilicity. The larger the hydrophobic group, and the shorter the ethoxy chain, the more toxic is the compound (*142*). Linear AEs are more toxic than branched AEs and APEs, probably because cell membranes are impermeable to the bulky branched or aromatic structures. However, linear AEs degrade rapidly and are present in environmental samples at concentrations well below toxicity thresholds. Branched AEs and APEs are an environmental concern because they degrade slowly, and thus are released with their metabolic products to surface waters in secondary effluents (*17, 142, 181*). Branched AEs are used in lesser quantities and are not considered to be a major problem. APs accumulate as biodegradation products of APEs. They are the most toxic and are the greatest concern environmentally.

Table 29. Toxicity of Nonionic Surfactants to Aquatic Organisms - Summary (μg/L)

Compound Environment	Ranges-Acute LC_{50}	Ranges-Chronic NOEC	Ranges-Chronic LOEC
NP			
Fresh water	79[1] - 920[2]	7.4[9] - 24[10]	14[9] - 39[10]
Sediment (μg/kg)	26,100[3] - 260,000[4]	--	--
Marine water	43[5] - 300[6]	3.9[11] - 6.7[12]	6.7[11] - 56[15]
AE - C_mE_p (l)			
Fresh water	460[2,16] - 2920[7,16]	400[13,20] - 2000[9,16]	1000[13,14,20]
Marine water	710[8,17] - 5600[8,18]	--	--
AE - C_mE_p (b)			
Fresh water	6100[7,19] - 11,600[1,19]	1000[13,19] - 4000[14,19]	2000[13,19] - 4000[14,19]
NPE_9 [22]			
Fresh water	4600[7] - 14,000[1]	1000[13] - 10,000[14]	2000[13] - 10,000[14]
Marine water	1230[8] - 29,600[8,21]	--	--

[1] Water fleas, 48 hrs. [2] Rainbow trout juveniles, 96 hrs. [3] Midge larvae, 14 days. [4] Tadpoles, 10 days. [5] Shrimp, 96 hrs. [6] Shrimp, 96 hrs; this is 2nd highest value for marine water organisms; see Appendix E for further explanation. [7] Fathead minnows, 96 hrs. [8] Shrimp, 48 hrs. [9] Fathead minnows; survival. [10] Water fleas; reproduction rate. [11] Shrimp; length at 28 days. [12] Shrimp; survival and reproduction. [13] Fathead minnows; growth. [14] Water fleas; growth. [15] Mussels; growth and strength at 32 days. [16] $C_{14\text{-}15}E_7$. [17] $C_{13}E_{10}Cl$. [18] $C_{10}E_4$. [19] $C_{13}E_7$(b). [20] $C_{12\text{-}15}E_9$. [21] $NPE_9OSO_3^-$. [22] Avg. EO units/molecule = 9; EO chain varies between 1 and 20 EO units/APE molecule.

- Linear AEs: Both acute (*17, 142, 188, 209*) and chronic (*17, 209*) toxicities of primary linear alcohol ethoxylates are eliminated during secondary wastewater treatment. Maki *et al.* (*209*) evaluated toxicities in three different solutions -- carbon-filtered tap water, stream water, and secondary effluent -- and observed that 99% of all mortalities occurred during the first 24 hours, indicating that AE degradation products are not lethal. Further, mortalities were too low to determine LC_{50} values for secondary effluents. Their findings corroborate either central fission or ω-oxidation of the alkyl chain, rather than step-by-step elimination of the ethoxy units, as the probable pathway for biodegradation (*17*).

- Branched AEs and APEs: Kravetz *et al.* (*17*) found that chronic toxicity is not eliminated during secondary treatment of branched AEs and APEs. Step-by-step elimination of the ethoxy units is the preferred pathway when the hydrophobe is a highly branched aliphatic or an aromatic associated with a highly branched alkyl chain. This creates products with shorter ethoxy chains and greater hydrophobicity. Not only are these products more likely to sorb to particles that settle with sludge or sediment, but they also degrade more slowly, are more likely to exhibit aquatic toxicity (*17, 142, 209*), and, in the case of APEs, are precursors to APs.
- APs in fresh water: Measured concentrations of nonylphenols in fresh water occasionally exceed acute and chronic toxicity thresholds. Acute LC_{50} and chronic NOEC and LOEC toxicity values in Table 29 range from 79 to 920, 7.4 to 24, and 14 to 39 μg/L, respectively.
  - -- From the Glatt River study (*133*), NP concentrations in three out of 110 samples were 8 μg/L or higher, Table 22. Two samples measured at 8 μg/L, and one sample measured at 45 μg/L.
  - -- NP concentrations measured in receiving waters of the discharge from a textile mill's waste stream exceed toxicity thresholds in a small creek and in small rivers within 7.2 km of the point of discharge; NP concentrations were 3000, 200, and 30 μg/L at respective test points 1.6, 2.4, and 7.2 km downstream. The final NP concentration (2 μg/L) in the Savannah River, 9.6 km downstream from the source, was below toxicity thresholds (*136*).
  - -- NP concentrations in Lake Geneva, Switzerland (*14*), and the thirty rivers study in the United States (*54*), were below all toxicity thresholds (Table 23).

- APs in marine water: From the data available, it is uncertain whether concentrations of nonylphenol derivatives of NPE exceed acute and chronic toxicity thresholds in marine water. Acute LC_{50} and chronic NOEC and LOEC toxicity values for marine water range from 43 to 300, 3.9 to 6.7, and 6.7 to 56 μg/L, respectively (Table 29).
  - Kvestak *et al.* measured NPE_p concentrations in sewer lines that transport untreated wastewater, at the sewer outlets, and in Sibenik Harbor, Croatia (Table 24). The highest risk of contamination from NP is near the sewer outlets, where dilution, sorption and sedimentation play a role in NPE_p removal from the water column. A measurement of the NP concentration at the sewer outlet is not provided. The NP concentration at the sewer outlet following dilution was estimated roughly to be 2.6 μg/L by examining the values provided in Table 24, calculating a ratio of total NPE_p concentration at the outlet to NPE_p concentration in the sewer line (302/580 = 0.52), and applying the ratio to a sewer line NP concentration value (5 μg/L) that was obtained from Figure 3c in (*137*). From this estimation, the concentration of nonylphenols is below toxicity thresholds, but is close enough to suggest a need for additional research. Given the high degree of partitioning to solid fractions and subsequent settling, already discussed, an examination of sediment concentrations of NPE derivatives, especially NP, may be more meaningful. Given the rate at which NPE_p is introduced into the harbor, it is likely that NP concentrations are accumulating to toxic levels in the sediments nearest to the sewer outlets.
  - NP concentrations in the Venice Lagoon, Italy (*138*), were below toxicity thresholds (Table 24).

- APs in sediment: Acute LC_{50} toxicity values for sediment range from 26,100 to 260,000 μg/kg (Table 29). Determinations of chronic toxicity thresholds in sediments were not found in the literature.
  - -- Environmental levels measured in research conducted in the United States (Table 23) and Italy (Table 24) are well below determined acute toxicity thresholds.
  - -- NP levels as high as 13,100 μg/kg have been measured in Glatt River sediments (Table 22). This is below the acute toxicity threshold, but should be monitored.
  - -- The minimum chronic toxicity value for NP in both fresh water and marine water environments in Table 29 is about an order of magnitude less than the minimum acute toxicity value. If this relationship holds for sediments, then NP concentrations in sediments could exceed chronic toxicity thresholds.
- A higher concentration of suspended solids or colloidal material can decrease the toxicity of a surfactant to some organisms (*209*) while increasing toxicity to others (*25*). This is because a surfactant's sorption to suspended solids decreases its bioavailability (*209*) in the water column, but increases its bioavailability to bottom dwelling organisms that may feed upon particles associated with surfactants (*25*).
- Of the organisms tested, the fathead minnow is the most sensitive of the fresh water organisms (*17, 54*), and the shrimp is the most sensitive of the marine water organisms (*54*) (Table 29).
- Patoczka and Pulliam (*142*) determined the acute toxicity of AEs and APEs in continuous-flow activated sludge reactors to *Mysidopsis bahia*, a marine organism. They observed that toxicity increases when the EO chain of a linear AE molecule is capped with a chlorine group (Appendix E).

Contrary to this, polarity increases and toxicity decreases when a phosphate or sulfate group is added to the end of the EO chain of an aromatic based surfactant (Appendix E).

Determination of bioconcentration factors provides another means to evaluate the environmental safety of a compound that appears to be safe for a particular organism based on acute and chronic toxicity tests. It is used to assess potential harm to consumers of that organism, to consumers at higher trophic levels, or long term deleterious effects to the organism itself (*210*).

Ahel *et al.* (*26*) estimated BCFs of fresh water organisms under field conditions. They measured NP, NPE_1, and NPE_2 concentrations in predominant algae, three species of fish, and a wild duck species found in and along Chriesbach Creek, a Glatt River tributary that also receives secondary effluents. Only the most abundant alga, *Cladophora glomerata*, was sampled from the Glatt River; algal BCFs were estimated for the most polluted Glatt River locations, 15 and 30 km downstream from the river's inlet at Greifensee. Fish and algal BCFs are summarized in Table 30. Concentrations varied widely according to compound, organism, time of year, and location (*26*). High concentrations of NP and the lower oligomers, NPE_1 and NPE_2, occurred in the algae examined. Mechanical removal of the algae that covers the river at summer's end removed a substantial amount of surfactant derivatives that were sorbed to the algae; concentrations measured in algal samples collected during autumn were five to twelve times lower than concentrations measured in samples drawn during the summer (data not included). Ahel *et al.* attribute this to lower exposure times for the fall samples. The BCFs estimated for algae collected from Criesbach creek were generally lower than BCFs in samples taken from the Glatt River (Table 30). Lower BCFs were estimated for the fishes than the algae (Table 30), indicating that biomagnification due to ingestion of

algae was not occurring in these organisms. Concentrations in the duck were comparable to concentrations measured in the fishes (data not included). Although ecological risk cannot be determined based on this information alone, bioconcentration is sufficient to warrant concern about the potential harm of these compounds to man (*26*).

Table 30. Bioconcentration Factors of Nonylphenol Ethoxylate Derivatives in Fresh Water Animals and Plants [1]

Species	Tissue	BCF NP	BCF NPE_1	BCF NPE_2
Chriesbach creek [2]				
Fish				
Squalius cephalus Heck. [3]	muscle	46	8	14
Squalius cephalus Heck. [4]	gut, liver, gills	118-359	15-78	3-149
Barbus barbus L. [5]	muscle	97	135	245
Barbus barbus L. [5*]	gut, liver, gills	8-251	3-38	3-15
Salmo gairdneri [6]	muscle	38	18	5
Salmo gairdneri [6]	gut	410	304	319
Algae				
Cladophora glomerata		9750	205	460
Fontinalis antipyretica		1080	40	65
Potamogeton crispus		640	50	200
Glatt River [7]	Downstream Location			
Alga				
Cladophora glomerata [8]	15 km	7700	5000	1800
Cladophora glomerata [8]	30 km	6600	3500	1000

[1] Ref. (*26*). [2] To determine BCFs: Concentrations of NP, NPE_1 and NPE_2 in water from Chriesbach creek are 3.9, 23, and 9.4 μg/L, respectively. [3] Sample size, n = 4. [4] Range of BCFs determined from two composite samples where n = 4 and n = 3; high value is estimated BCF in liver. [5] n = 2; [5*] range of BCFs determined for n = 2; high value is estimated BCF in liver. [6] n = 1. [7] To determine BCFs: Concentrations of NP, NPE_1 and NPE_2 in water from the Glatt River at 15 and 30 km are 3.3, 16, and 16 μg/L and 1.1, 4.0. and 4.7 μg/L, respectively. [8] August data.

Ekelund *et al.* (*25*) designed laboratory experiments with running seawater to determine bioconcentration factors for ^{14}C labeled 4-nonylphenol in three marine organisms -- *Mytilus edulis* L. (common mussel), *Crangon crangon* L. (common shrimp), and *Gasterosteus aculeatus* L. (stickleback). They measured NP concentration in the total organism, rather than in

individual tissues. They conducted two experiments, and for each experiment estimated two bioconcentration factors for each organism, based on tissue weight and fat weight. Their results are provided in Table 31. Except for shrimp, their BCF estimates are considerably higher than those provided for fresh water organisms (Table 30).

Table 31. Bioconcentration Factors of Nonylphenol in Marine Organisms [1,2]

Organism	Compound	BCF Test 1/Test 2; in tissue (t) or fat (f)
Crangon grangon L. shrimp	labeled NP	110/90; (t) 7500/5500; (f)
Gasterosteus aculeatus L. stickleback	labeled NP	1200/1300; (t) 17,800/16,700; (f)
Mytilus edulis L. mussel	labeled NP	2740/4120; (t) 169,300/216,600; (f)

[1] Ref. (*25*). [2] Test conditions: dosing = 16 days; elimination = 32 days.

Beyond concerns about toxicity and bioconcentration of APE metabolites is an ever-growing body of evidence that some alkylphenols, as persistent metabolites of APE surfactants, may contribute to more deleterious ecotoxicological damage. Within the last 20 years, a variety of anthropogenic chemicals that may disrupt biological processes by mimicking or blocking the processes of naturally produced hormones, such as estrogen, have been identified. Several pesticides, polychlorinated biphenyls, combustion pollutants, and plastics ingredients, as well as alkylphenols (penta- to nonylphenol) have been named as contributors to this growing concern (*30-32*). Though research related to the estrogenic effects of alkylphenolic compounds is in its infancy, recent findings provide evidence that these compounds are at least weakly estrogenic (*28, 33, 34*).

White *et al.* tested 4-octylphenol, 4-nonylphenol, 4-nonylphenoxy-carboxylic acid, and 4-nonylphenol diethoxylate to determine the strength of estrogenicity (*33*). They found that the order of estrogenicity is:

$OP > NPE_1C > NP = NPE_2$.

Ethoxylated alkylphenols with more than three ethoxy units did not display estrogenic behavior. An assay test for evaluating estrogenic potency provides evidence that OP is less potent than 17β-estradiol, a natural estrogen; OP stimulates vitellogenin secretion from hepatocytes of male trout to about the same extent as 17β-estradiol when its concentration is 1000 times greater -- 10^{-5} M for OP vs. 10^{-8} M for 17β-estradiol (*28*). NP is between 10 and 20 times less potent than OP. These compounds also stimulate breast cancer cell growth and transcriptional activity in the estrogen receptor; they are mediated by the estrogen receptor and compete with 17β-estradiol for binding to the estrogen receptor (*33*). Concentrations much higher than those found in the environment are required to initiate a response in *in vitro* estrogen assay systems. The effect of chronic exposure to these compounds as they accumulate over long periods of time is unknown (*34*). To date there is no evidence that the endocrine disrupting behavior of the alkylphenolic compounds has caused irreversible damage to any species (*34*), but their widespread use in large quantities is reason for concern.

Conclusions and Recommendations

Biodegradation and sorption are the major mechanisms that determine the environmental fate of alcohol ethoxylates and alkylphenol polyethoxylates. Alcohol ethoxylates are removed readily from wastewaters, biodegrading by central fission or ω-oxidation. Although results are not consistent across all studies, relatively recent data suggest that sorption plays an important role in removing long chain AEs from the aqueous phase. Conflicting results from partitioning studies, the detection of AEs in surface water samples, and higher toxicity levels of parent AE surfactants dictate the need for additional research in this area.

The behavior of the alkylphenol polyethoxylates (APE_{3-20}) and their metabolic products ($APE_{1\&2}$, AP, $APE_{1\&2}C$) varies with environmental conditions. The major pathway of biodegradation is step-by-step elimination of the ethoxy units. Widely divergent conclusions regarding the extent of APE elimination, the percentages of APc released to the environment, the effects of sorption, and the extent to which toxic metabolites (AP, $APE_{1\&2}$) accumulate point to the complexity of APE environmental behavior and fate. Though a plethora of data has been collected, knowledge about partitioning behavior, interactions with colloidal material, and the behavior, fate, and environmental effects of carboxylated APEs and halogenated derivatives of APEs is lacking.

The distribution, persistence and accumulation of alkylphenol ethoxylate products, especially the octyl- and nonylphenol ethoxylates, is cause for concern in consideration of: $APE_{1\&2}C$ concentrations found in river water and AP concentrations found in digested sludge, algae and sediments; the partitioning characteristics of the lipophilic alkylphenol and alkylphenol mono- and diethoxylate derivatives; the likelihood that anaerobic conditions will prevail in sediments, thus favoring biotransformation of $APE_{1\&2}$ to AP

after settling; the likelihood that resuspension under fast flow conditions will reintroduce metabolites to the water column; evidence that $APE_{1\&2}C$ are not readily removed from groundwater; evidence that persistent, toxic halogenated derivatives form during chlorination of secondary effluents and ensuing river transport; evidence that halogenated derivatives are present in drinking water; the possibility that the persistence and accumulation of OP and NP, halogenated and nonhalogenated, could lead to levels that exceed toxicity thresholds determined for algae and aquatic organisms; and evidence that OPE and NPE metabolites may disrupt endocrine behavior.

In order to alleviate existing deficiencies in knowledge about the interrelationships of biotransformation and physical-chemical processes of alcohol ethoxylates and metabolites of alkylphenol ethoxylates, future research should focus on the following areas:

1) Further development of analysis techniques for extracting, separating, and identifying nonionic surfactants and their metabolites, including halogenated derivatives, at trace concentrations in complex environmental matrices;
2) Effect of colloidal material on biotransformation, transport, and fate of AEs, and derivative products of APEs, halogenated and nonhalogenated;
3) Partitioning/sorption behavior of AEs, to ascertain that the alcohol ethoxylate parent surfactants are not accumulating to toxic levels in environments with high concentrations of suspended solids or colloids;
4) Determination of the extent to which alkylphenols and carboxylated alkylphenol ethoxylates, halogenated and nonhalogenated, are accumulating in fresh water and marine water sediments;

5) Better definition of the behavior and fate of carboxylated APEs, halogenated and nonhalogenated, through determination of: partitioning behavior in fresh and marine waters; the effect of the concentration of suspended solids and colloidal material; removal efficiency in surface waters and groundwater under warm and cold temperature conditions; extent of accumulation in surface water and groundwater environments;
6) Determination of the toxicity thresholds for carboxylated APEs, halogenated and nonhalogenated;
7) Determination of the fate of APs and the lower oligomers when land applied in digested sludge or algae that has been mechanically removed from natural waters;
8) Determination of the mechanisms in effect when volatilization and photolysis remove APs from surface waters; determination of disposition of APs if volatilization is the removal mechanism;
9) Determination of the estrogenic effects of APE metabolites to aquatic organisms and ultimately, to man;
10) Selection of an alternative surfactant for future replacement of the alkylphenol polyethoxylates.

APPENDICES

APPENDIX A

APPENDIX A

SEWAGE TREATMENT PLANTS

After cleaning is complete the wash water is flushed to a distribution system that transports the water to an industrial or municipal wastewater treatment plant. A discussion about the flow of sewage through a wastewater treatment plant provides an understanding of how surface waters, groundwaters, and soils are also affected. Most sewage treatment facilities are designed to provide secondary as well as primary treatment. Some also offer tertiary treatment. Figure 4 is a generalized flow chart of the activated sludge sewage treatment plant seen commonly in the United States (*56*). Biofilters would replace the aeration tank in a flow chart for a trickling filter plant. An important aspect of the sewage treatment process is sludge handling (dewatering) and disposal. Approximate times for the various waste processing segments are provided in Table 3. Typical suspended solids concentrations are in Table 4.

Primary treatment involves the physical removal of solids by screening, skimming, and/or settling. Screening removes the largest solids, and occurs before the influent enters the primary settling tank. Skimming removes floating solids; settling removes settleable solids. The purpose of the primary sedimentation tank, or clarifier, is to reduce the load going into the secondary treatment stage. Primary settling can remove up to 70% and 40% of the suspended solids and organic matter (as measured by BOD_5), respectively, from influent wastewater. This occurs because low solubility organic compounds are more likely to sorb to the solid organic material that tends to settle in the primary clarifier. The settled material is removed periodically from the bottom of the primary settling tank, thickened, and placed in a

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digester, where biodegradation continues. Supernatant from the thickened sludge can be cycled back to the primary clarifier for additional treatment.

Effluent from the primary clarifier is routed to secondary treatment, which removes organic matter biologically or chemically. Conventionally, secondary treatment is a two step process that involves biological treatment and sedimentation. Suspended growth (e.g., activated sludge), or attached growth (e.g., trickling filter) systems, or a combination of the two, are used for biological removal of organics. The activated sludge process is chosen most frequently worldwide for biological treatment of municipal wastewater. Biological removal occurs in the aeration tank; settling occurs in the secondary clarifier (Figure 4). Mean cell-residence time and food to microorganism ratio are used initially to design the activated sludge system, and later to control the activated sludge process. All of the aeration tank's contents are referred to as the "mixed liquor". The most critical factor in successful biological treatment is maintaining the aeration tank environment to optimize microorganism growth, by monitoring the food-to-microorganism ratio (F/M) and the mixed liquor volatile suspended solids (MLVSS). The microorganisms convert organic waste to CO_2, NH_3, new cells, and other end products. Aeration (by diffusion or mixing) provides enough oxygen and mixing to ensure continued successful operation. Additionally, pH, alkalinity and temperature must be monitored closely.

Effluent from the aeration tank includes suspended solids and organic material that are settled in the secondary clarifier. This forms a sludge that is removed periodically. Part of the sludge is recycled to the aeration tank, thus maintaining a constant number of microorganisms, or MLVSS, in the aeration

APPENDIX A

tank. The remainder is wasted; this portion is thickened before placement in a digester for further stabilization prior to disposal. The supernatant from the thickening process is recycled back to the primary clarifier for additional treatment. The effluent from the secondary clarifier is disinfected prior to release to surface waters to ensure against contamination by pathogenic organisms. Once in the surface waters, components or their metabolites may biodegrade further; or they may sorb to particles and settle to the river or lake bed. Depending on the level of the water table, an underlying aquifer may also be affected (Figure 3).

APPENDIX B

APPENDIX B

GLOSSARY OF TERMS

acclimation/adaptation - process by which an organism is able to revise its metabolic capabilities so as to live and propagate on an organic compound to which it has not been previously exposed (*6*);

advection - the process by which solutes are transported by the bulk motion of the flow of groundwater (*59*);

bioaccumulation - uptake of compound by an organism from its environment; dependent on bioavailability, concentration in organism is approximately equal to concentration in environment (*210*);

bioconcentration - uptake of compound by an organism across its membranes from its environment; concentration in organism will be greater than concentration in environment (*210*);

bioconcentration factor - the quotient of the concentration of a compound at equilibrium in organism divided by the concentration of compound in surrounding aquatic environment (*210, 211*);

biodegradation - destruction of chemical compounds by the biological action of microorganisms -- bacteria, fungi, or yeasts; bacteria are the predominant microorganisms found in the environmental compartments where surfactants and builders are most likely to be found (*6, p. 7*);

biotransformation - change in chemical structure of chemical compounds brought about by microbial action (*35*);

colloids - filterable solids (*56*); microparticles or macromolecules that are small enough (1 nm - 1μm) to move primarily by Brownian motion, rather than by settling due to gravitational forces; include humic substances, proteins, viruses, cell fragments, and some bacteria in natural waters; provide another organic micro-environment to which organics can absorb, thus affecting environmental behavior and fate (*35*);

dilution factor - the sum of stream flow and effluent flow divided by effluent flow (*49*);

dispersion - the tendency of the solute to spread as it moves along its path (*59*);

EC_{50} - used to measure toxicity of a test compound to plant life, usually algae; for any given test compound, it is the effect concentration at which the specific growth rate for a culture medium with the test compound is 50% of the specific growth rate for the culture medium without the test compound (*212*);

food-to-microorganism ratio (F/M) - BOD concentration divided by the product of the hydraulic retention time and the concentration of mixed liquor volatile suspended solids (*56*);

homolog - refers to variations in the number of carbon atoms in the hydrophobic (alkyl) moiety of a surfactant;

hydraulic retention time - as related to activated sludge systems, the volume of the aeration tank divided by the daily influent flow (*56*);

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inherent biodegradability - a classification assigned to chemicals which may biodegrade, though not within the standardized test periods set for ready biodegradability, indicating need for a period of acclimation (*6, 83*); the tests: more closely represent environmental conditions, with higher biomass and lower test compound concentrations; allow for the occurrence of cometabolism by providing longer exposure times and multiple carbon sources; measure DOC die away or CO_2 evolution (aerobically) or CO_2/CH_4 (anaerobically) (*83*);

isomer - refers to structural variations possible in the hydrophobic moiety of a surfactant (i.e., branching configurations, aromatic ring placement on an alkyl chain, or *para-*, *ortho-*, or *meta-* arrangement on an aromatic ring);

LC_{50} - used to measure acute toxicity of a test compound to an organism; the lethal concentration of test compound is the concentration at which 50% of the population of organisms of interest is killed after exposure during a set period of time; tests are set up to run for 24, 48, 96, or more hours, depending on the sensitivity of the organism;

LOEC - lowest observed effect concentration; used to measure chronic toxicity; the lowest concentration at which an effect (such as to growth, development, time to maturity, extent of maturity, spawning, fertilization, reproduction, hatch rate, or survival rate) is observed;

mean cell-residence time (θ_C) - as related to activated sludge systems, the mass of organisms in the aeration tank divided by the mass of organisms removed from the entire system per day (*56*);

mineralization - the complete conversion of an organic chemical to carbon dioxide, water and other stable inorganic forms of the elements, primarily nitrogen, sulfur, and phosphorus (*35, p. 485, 79*);

mixed liquor volatile suspended solids (MLVSS) - the mass concentration of active microorganisms in an aeration tank (*56*);

NOEC - no observed effect concentration; used to measure chronic toxicity; the highest concentration, detected through tests, at which no effect (such as to growth, development, time to maturity, extent of maturity, spawning, fertilization, reproduction, hatch rate, or survival rate) is observed;

oligomer - refers to variations possible in the size of the hydrophilic moiety of a nonionic surfactant (usually relates to the ethoxy units, although propoxy units may also be involved); may also refer to polyethylene glycol molecules that possess between 2 and 8 ethoxy units; *oligo-* and *mer* meaning "few parts" (Greek) (*213*);

partition coefficient - a ratio that describes the relative abundance of a compound between two phases, usually the concentration of a compound associated with an organic phase to the concentration of the same compound associated with aqueous phase -- e.g., octanol:water (K_{ow}), hexane:water (K_{hw}), sediment:water (K_{sw}) (*35*);

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primary biodegradation - the alteration in the chemical structure of a molecule by biological action so that characteristic properties (e.g., foaming or surface tension for surfactants) are no longer evident (*6, 79*); analytical procedures designed to test for primary biodegradability were the original means to determine the acceptability of surfactants based on biodegradability (*2, p. 329, 46, p. 375*);

ready biodegradability - a classification assigned to chemicals which undergo immediate and rapid mineralization according to any of several standardized preliminary screening tests (*84, 186*); the tests: are 28 days duration; are run at low biomass, yet high test compound concentrations; are carried out aerobically in aqueous solution under unacclimated conditions; measure dissolved organic carbon (DOC) die away, CO_2 evolution, or oxygen uptake (BOD) (*83, 84*);

recalcitrance - resistance to degradation by microbial action (*96*);

suspended solids - non-filterable solids, usually greater than 1.2 μm in diameter; (*56*); subject to gravitational settling; usually separated into volatile and non-volatile fractions during analysis of wastewater and surface waters;

ultimate biodegradation - the complete conversion of a molecule to carbon dioxide, water, inorganic salts, and cellular material (*6, 79*); currently the accepted measurement (*46*);

APPENDIX C

APPENDIX C

ESTIMATION OF K_{ow} USING FRAGMENT CONSTANTS

The octanol-water partition coefficient (log K_{ow}) included in Table 6 for $NPE_{1-3}C$ was calculated from structural group contributions as indicated below. In Table 6, log K_{ow} and log K_{hw} values are about equal as the number of ethoxy units increases incrementally for the lower oligomers: log K_{ow} for NPE_1, NPE_2, and NPE_3 is 4.17, 4.21, and 4.20, respectively; log K_{hw} for NPE_1 and NPE_2 is 3.4 and 3.3, respectively. From these experimentally obtained values the ethoxy groups appear to have little effect on partitioning. Therefore log Kow = 4.20 was used for the Table 6 calculation, and provides a reasonable approximation for $NPE_{1-3}C$.

NPE_{1-3} log K_{ow}:	4.20	Approximated in (*100*).
	0.98	Removal of terminal alcohol group as estimated in (*35*).
	- 1.11	Addition of carboxylic acid group as estimated in (*35*).
$NPE_{1-3}C$ log Kow:	4.07	

More discussion about this estimation process is found in Schwarzenbach (*35*).

APPENDIX D

APPENDIX D

NONIONIC SURFACTANT BIODEGRADATION PATHWAYS

A series of laboratory experiments with thin-layer chromatography (TLC), and infrared (IR) and nuclear magnetic resonance (NMR) spectrometry led Patterson *et al.* to conclude in 1970 that central fission and ω-oxidation of the hydrophobe operate simultaneously, but as two independent mechanisms, to degrade a variety of commercial surfactant AE molecules (*139, 141*).

On the other hand, Tobin *et al.*, in 1976, (*74*) and Vashon and Schwab, in 1982, (*86*) found that hydrolytic cleavage of the ether bond joining alkyl and ethoxy chains (central fission) was most likely to occur in their systems. Tobin *et al.* (*74*) degraded $C_{12\text{-}15}E_9$ in shaker flask cultures, field experiments in a lake environment, and a bench scale activated sludge system. They applied a combination of the Wickbold procedure and gas chromatography (GC) to HBr cleavage products to observe rapid loss of the starting surfactant and disappearance of the entire alkyl moiety, respectively. This, together with the identification of ethoxylate material in the lake experiment and low CO_2 evolution in the shake flask experiment, was the basis for their conclusion that central fission followed by oxidation of the alkyl chain was the mechanism at work. Vashon and Schwab (*86*) measured the rate and extent of mineralization (via CO_2 production), in estuarine water, of trace concentrations of both nonionic and anionic ^{14}C-labeled surfactants: $*C_{16}E_3$, $C_{12}*E_9$, $*C_{16}E_9S$, and $C_{16}*E_3S$. The 'S' designates presence of a sulfate group on the terminal ethoxy unit. $*C_{16}E_3$ and $*C_{16}E_9S$ were labeled on the α-alkyl C; $C_{12}*E_9$ and $C_{16}*E_3S$ were labeled uniformly in the ethoxy chain. Vashon and Schwab observed rapid mineralization of the α-alkyl carbon but very slow

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mineralization of the ethoxy chain. They likewise concluded that hydrolytic central fission initiates biodegradation in the AEs.

In 1985, Steber and Wierich examined alkyl or ethoxy radiolabeled metabolites (^{14}C) from the biodegradation of stearyl alcohol ethoxylates ($C_{18}E_7$) in a model continuous flow sewage treatment plant. Their results support Patterson's conclusions that central fission and hydrophobic ω-oxidation occur simultaneously to degrade AE molecules (*140*) . Steber and Wierich, 1987, took their work a step further when they ran similar experiments (*110*) in an anaerobic sludge digester. They observed that only the hydrolytic pathway was effected anaerobically (*110*), and that hydrolysis occurred either directly or oxidatively. Swisher (*6*) suggests several alternative oxidative hydrolytic pathways, which are presented in Figure 7 and discussed in more detail in the narrative.

Related to the third possible point of attack, Steber and Wierich, 1985, (*140*) concluded that the presence of dicarboxylated alkyl-^{14}C polygycols and monocarboxylated alkyl-labeled *p*EO intermediates provides evidence for microbial degradation of the ethoxy chain, although at a much slower, perhaps even time-delayed, rate. Steber and Wierich are joined by others (*6, 113, 117, 118*) in suggesting that degradation of the ethoxy chain occurs in a stepwise fashion, beginning with the terminal monomer unit, similarly to the degradation of polyethylene glycols (PEG's). More details are provided in Figure 8 and the narrative.

A pathway that will not be discussed in detail, but deserving mention, is one reviewed by Swisher (*6*). Swisher cites the works of Zahn, 1980, (*214*) who deduced carboxylation of the terminal alcohol group of the EO chain followed

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by two one carbon scissions (first decarboxylation, then release of formate), and Schöberl, 1983, (*215*) whose results support Zahn's deductions.

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TOXICITY OF NONIONIC SURFACTANTS TO AQUATIC ORGANISMS

Table 32. Toxicity of Nonionic Surfactants to Aquatic Organisms

Organism	Compound	Measure of toxicity (µg/L) [1] A: Acute - LC_{50} or EC_{50} (alga); duration N: NOEC; chronic effect L: LOEC; chronic effect	Test conditions	Ref.
Fresh water				
Pimephales promelas fathead minnow	$C_{12-15}E_9$ (linear)	A: 1600; 96 hrs. N: 400; growth L: 1000; growth	25°C/intact surf't in effluent from continuous activ'd sludge biotreater/ EPA procedures	(*17*)
	$C_{13}E_7$ (branched)	A: 6100; 96 hrs. N: 1000; growth L: 2000; growth	same as above	(*17*)
	$C_{14-15}E_7$	A[2]: 1130 - 1260; 96 hrs. N: 800; survival	20°C/carbon-filtered tap water/ static toxicity	(*209*)
	$C_{14-15}E_7$	A[2]: 1290 - 1500; 96 hrs. N: 800; survival	20°C/ stream water/ static toxicity	(*209*)
	$C_{14-15}E_7$	A[2]: 2090 - 2920; 96 hrs. N: 2000; survival	20°C/2° effluent/ static toxicity	(*209*)
	NPE_9(b)	A: 4600; 96 hrs. N: 1000; growth L: 2000; growth	25°C/intact surf't in effluent from continuous activ'd sludge biotreater/ EPA procedures	(*17*)
	NP	A: 300 & 135; 96 hrs. N: 23; hatch rate L: >23; hatch rate N: 23; length, 28 days L: >23; length, 28 days N: 7.4; survival, 33 days L: 14, survival, 33 days	EPA Good Laboratory Practices protocols	(*54*)
Salmo gairdneri rainbow trout-juveniles	$C_{14-15}E_7$ $C_{14-15}E_{11}$	A[2]: 460 - 1210; 96 hrs.[3] A[2]: 970 - 1240; 96 hrs.[3]	15°C/in effluents from biofilters/ continuous flow	(*188*)
	NP	A: 230; 96 hrs A: 480; 24 hrs	EPA Good Laboratory Practices protocols	(*54*)
rainbow trout - juveniles	NP	A: 920 & 560; 96 hrs.	15-16°C/static toxicity in fresh water aquarium	(*202*)

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Table 32. (cont'd)

rainbow trout - embryos	NP	A^2: 364 - 604; 24 hrs.	9.5-11°C during exposure; then 9.5 - 28.5°C/fresh water aquarium/ exposure followed by 48 hrs in clean water	(*202*)
Daphnia magna water flea	$C_{12-15}E_9$ (linear)	A: 1300; 48 hrs. N: 1000; growth L: 1000; growth	25°C/intact surf't in effluent from continuous activ'd sludge biotreater/ EPA procedures	(*17*)
	$C_{13}E_7$ (branched)	A: 11,600; 48 hrs N: 4000; growth L: 4000; growth	same as above	(*17*)
	NPE_9 (branched)	A: 14,000; 48 hrs N: 10,000; growth L: 10,000; growth	same as above	(*17*)
	NP	A: 190 & 440; 48 hrs N: 24; reproduction rate L: 39; reproduction rate	EPA Good Laboratory Practices protocols	(*54*)
Daphnia pulex water flea	NP	A^2: 79 - 201; 48 hrs. A^2: 114 - 238; 48 hrs.	23-27°C/fresh water/ incubator	(*202*)
Selenastrum capricornutum green alga	$C_{12-14}E_4$ $C_{12-14}E_9$ $C_{12-14}E_{13}$ $NPE_{8.1}$ $NPE_{8.9}$	A: 2000-4000; 48 hrs. A: 4000-8000; 48 hrs. A: 10,000; 48 hrs. A: 20,000; 48 hrs. A: 50,000; 48 hrs.	EC_{50} criteria: retardation of specific growth rate to 50% of that in culture medium	(*212*)
	NP	A: 410; 96 hrs.	EPA Good Laboratory Practices protocols	(*54*)
Nitzschia fonticola blue-green alga	$C_{12-14}E_9$	A: 5-10,000; 48 hrs.	EC_{50} criteria: retardation of specific growth rate to 50% of that in culture medium	(*212*)
Microcystis aeruginosa diatom	$C_{12-14}E_9$	A: 10-50,000; 72 hrs.	same as above	(*212*)
Fresh water sediment				
Rana catesbiana tadpole	NP	A: 260,000 ppb (µg/kg); 10 days	EPA Good Laboratory Practices protocols	(*54*)
Chironomus tentans midge larva	NP	Sub-A: 26,100 ppb (ug/kg); 14 days	MATC - Maximum acceptable toxicant concentration/ EPA Good Laboratory Practices protocols	(*54*)

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Table 32. (cont'd)

Marine water				
Mysidopsis bahia shrimp	$C_{13}E_{10}Cl$ $C_{13}E_{9.75}$ $C_{10}E_4$ NPE_9 $NPE_9OSO_3^-$ $NPE_9OPO_3^{-2}$	A: 710; 48 hrs. A: 2200; 48 hrs. A: 5600; 48 hrs. A: 1230; 48 hrs. A: 29,600; 48 hrs. A: 4600; 48 hrs.	23-25°C/continuous flow act'd sl rctrs/ static-renewal / N. J. Admin. Code, 7:18 (amended 7-84)	(*142*)
	NP	A: 43; 96 hrs. N: 3.9; length @ 28 days N: 6.7; reproduction N: 6.7; survival L: 6.7; length @ 28 days L: 9.1; reproduction L: 9.1; survival	EPA Good Laboratory Practices protocols	(*54*)
Crangon septemspinosa shrimp	NP	A: 300; 96 hrs.	10°C/static/HPLC	(*24*)
Salmo salar Atlantic salmon	NP	A: 190; 96 hrs. A: 160 & 130; 96 hrs.	10°C/static/HPLC 10°C/flow-through/ HPLC	(*24*)
Mytilus edulis mussel	NP	A: 2600[4]; 96 hrs. L: 56; growth and strength @ 32 days	EPA Good Laboratory Practices protocols	(*54*)
Skelatonema costatum marine alga	NP	A: 27; 96 hrs.	EPA Good Laboratory Practices protocols	(*54*)

[1] Concentration is µg/L, unless designated otherwise. [2] 95% confidence interval. [3] Toxicity based on 4 days is reported, rather than study duration time of 7 days, to facilitate comparisons. [4] This acute LC_{50} value appears high relative to other values in this table for acute and chronic toxicity. Therefore the next highest acute toxicity value, 300 µg/L, was used in Table 29 as the upper bound for marine organisms exposed to NP.

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