Environmental and Human Safety of Major Surfactants

Volume I. Anionic Surfactants

Part 2. Alcohol Ethoxy Sulfates

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Synopsis

Alcohol ethoxy sulfates (AES) are used in hand dishwashing liquids, laundry detergents, shampoos and miscellaneous specialty industrial applications. They have low water hardness sensitivity and are high foamers while being relatively mild to the skin. About 500 million pounds were produced in 1988, approximately 90% of which went into dish and laundry uses. Typieal AES used in consumer formulations have linear or essentially linear alkyl chains in the range of C_{12-15} with 2 to 3 units of ethylene oxide, on average, per mole of alcohol and are sodium or ammonium salts.

No U.S. or European standards exist relating to AES concentrations in surface or ground water. Traditional analytical methods for monitoring trace anionic surfactant levels in the environment do not distinguish AES from other widely used anionics. Laboratory and field tests demonstrate that AES are readily biodegradable both aerobically and anaerobically, generally within several days. Removal of 98 to 100% has been measured in municipal activated sludge sewage treatment plants. Over the range of AES chemical structures found in commercial products, neither the alkyl chain length nor the ethoxylate size appear to affect the rate of biodegradation.

Intact AES, like other surfactants, are acutely toxic to aquatic organisms. The majority of available data suggests that the degree of toxicity dependa on a combination of the alkyl and ethoxylate chain lengths. Toxicity appears to be greatest at C_{16} and decreasing in potency at longer and shorter alkyl chain lengths. Shorter ethoxylate chains tend to have more toxicity potential than longer ones. Due to rapid rate and completeness of biodegradation, though, environmental impact is minimal under normal conditions of use.

AES have a low order of acute oral and dermal toxicity in mammals; oral LD50 values range from 1.7 to greater than 5 gm/kg, and dermal values range from 4 to 13 gm/kg. The skin irritancy of AES solutions varies

depending on the concentration of active matter, but they can generally be considered mildly irritating. Eye irritation also depends on concentration. Undiluted solutions with active matter of about 35% usually were severely irritating under the conditions of the rabbit assay. AES are not regarded as being skin sensitizers.

Subchronic animal studies show that AES are not cumulatively toxic even when administered in the diet at concentrations as high as 1% w/w [10,000 ppm]. Similarly, no toxic effects or evidence of tumorigenicity was reported in chronic studies at doses up to 1% in the diet, or when AES solutions were repeatedly applied to the skin. 1,4-Dioxane is a minor contaminant of AES, but no toxic effects have been attributed to dioxane in AES at these low levels.

AES and detergent formulations containing AES have been examined in two-generation studies. There was no evidence of reproductive toxicity or developmental toxicity. There was no increase in fetal malformation in a Segment II developmental toxicity assay.

AES have not been shown to be mutagenic or clastogenic in a series of assays including the Ames bacterial assay, morphological transformation of CHO cells, male mouse dominant lethality assay, chromosomal aberration in human cells in culture or in mouse or rat bone marrow <u>in vivo</u>.

The extent of absorption after oral ingestion in rats is dependent on the EO/alkyl chain mole ratio. AES with a ratio of 3:1 were readily absorbed, metabolized and excreted, whereas an AES with mole ratio of 9:1 was not readily absorbed. In contrast, dermal absorption for the 3:1 AES was minimal. Absorbed AES are metabolized at the alkyl chain. The ethoxysulfate moiety appears to be metabolically stable.

Few studies have been reported in humans other than to indicate skin and eye irritation, again depending on the concentration of active matter. One outbreak in Norway of skin sensitization to a dishwashing liquid containing AES was reported. This was shown to be due to a sultone and/or chlorosultone contaminant in one batch of AES. Modern AES manufacturing methods preclude the formation of these impurities.

ALCOHOL ETHOXY SULFATES

I. INTRODUCTION

The alcohol ethoxy sulfates (AES) are known for their reduced sensitivity to water hardness, their high foaming capabilities and their "softness" to the skin (Kerfoot and Flammer, 1975). AES have principally been used as components in light duty liquid dishwashing products and laundry detergent formulations, but are also utilized in shampoos and other household specialty products. Their use has grown rapidly. In 1978 production volume was 128 million pounds. This increased to over 500 million pounds in 1984. Approximately 90% of this volume goes into the household applications of powder and liquid laundry and liquid hand dishwashing products (U.S. International Trade Commission, 1979; Dean, 1985).

This review was prepared to evaluate information on AES with respect to:

(1) environmental fate and distribution, including biodegradation,

(2) effects on wild and domestic flora and fauna,

(3) product use and environmental safety for humans as indicated by tests with laboratory animals and by data on human exposure.

A list of chemical designations used in this chapter can be found in Appendix A.

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II. CHEMISTRY

A. Product Chemistry

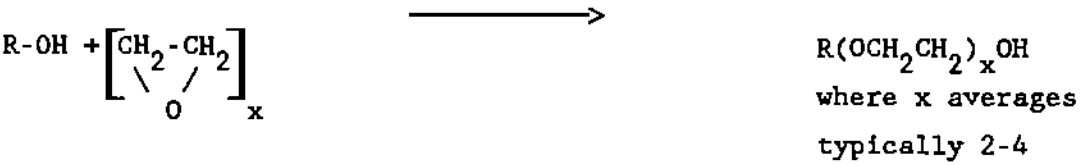
AES are produced by ethoxylation of fatty alcohols, followed by sulfation and neutralization to form the sodium or ammonium salt. AES in commercial formulations generally have up to four ethoxy groups per mole.

Alcohol ethoxy sulfates (AES) are produced by ethoxylation of the parent alcohols with ethylene oxide, followed by sulfation and neutralization generally to the sodium or ammonium salts. The commercial AES product is typically a mixture of homologs; some concentrated AES also contain ethanol as a solubilizer.

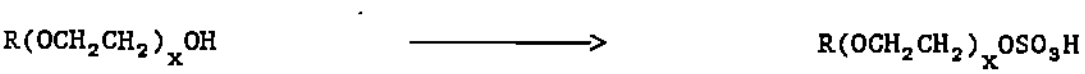
Ethoxysulfates derived from linear random secondary alcohols were introduced in the US in the 1960's, but have not been in significant use in the US in recent years. The parent alcohol is currently produced solely in Japan.

The three major steps in AES production are outlined below (Walker et al., 1967).

(1) Ethoxylation of a fatty alcohol (prepared from either vegetable, animal or petroleum sources), typically using KOH as catalyst



(2) Sulfation of the product with either sulfur trioxide (SO_3) or chlorosulfonic acid ($ClSO_3H$)



(3) Neutralization with base to form the sodium or ammonium salt

$$R(OCH_2CH_2)_xOSO_3H \longrightarrow R(OCH_2CH_2)_xOSO_3^- Na^+ \text{ (or } NH_4^+)$$

Precursor alcohols are typically primary, and may be either linear or branched. However, linear alcohols are the preferred raw material for the production of AES used in detergent formulations because of their uniformity, high purity and biodegradability. The feed typically contains a mix of alcohols of varying lengths, and addition of ethylene oxide produces a distribution of ethoxylated alcohols. The alkyl chain length of commercial AES is usually in the range of C_{12}-C_{15}; a molar ethylene oxide:alcohol ratio of 3:1 is also typical (Kirk-Othmer, 1983).

Commercial AES is typically shipped as a high active aqueous solution at about 55-60% concentration with 8-14% ethanol as a solubilizer, or as a low active aqueous solution at about 25-28% actives concentration without solubilizer.

Commercial AES may also contain minor quantities of unsulfated ethoxylated alcohols or sulfated polyethylene glycols, some sodium or ammonium sulfate, and some sodium or ammonium chloride if prepared using chlorosulfonic aeid. Irgolic and Hobill (1987) reported that chromatograms of two commercial AES indicate the presence of ethoxy sulfate as a major component (>50%) and at least six other components.

AES also typically contains traces of 1,4-dioxane. This cyclic diether is formed when polyoxyethylene chains are exposed to certain acidic reaction conditions. Such conditions exist temporarily during sulfonation of ethoxylated alcohols. Typical 1,4-dioxane levels are 500 ppm on actives or lower. (Shell Oil Company, 1979, 1980).

B. Analytical Methods

AES comprise one of several classes of anionic surfactants, and as such can be measured with many of the same procedures used in the detection of LAS. MBAS analytical methods, for example, measure AES along with other anionic surfactants. There are a few specific references to AES analytical methods, including derivatization-GC and HPLC methods.

1. Anionic Surfactant Methods

Since linear alkylbenzene sulfonates (LAS) are the most widely used of the anionic surfactants, they have also been the most widely studied with respect to analytical methods. Although there are few references to AES-specific methods in the literature, many of the LAS methods are not specific to LAS analysis and may be used for the analysis of other anionic surfactants, including AES. Most of the physical methods such as determination of foaming potential and measurement of surface tension are applicable to AES surfactants; these have been described previously and the reader is referred to Part 1 of Volume I in LAS. Similarly, chemical techniques (spectrophotometric, volumetric, or potentiometric methods) demonstrated for other anionic surfactants are expected to be applicable to AES surfactants since they are based on reaction of the sulfate or sulfonate group.

Some differentiation among the anionic surfactants can be achieved with non-specific methods such as the MBAS colorimetric methods. For example, the susceptibility of anionic sulfate surfactants to hydrolysis in acidic media ean be used to distinguish them from anionic sulfonate surfactants which exhibit excellent hydrolytic stability under similar conditions. Total anionic sulfates (including AES) can be estimated as the difference in MBAS values before and after hydrolysis.

Newer physicochemical techniques developed for the analysis of LAS or anionic surfactants, in general, may also be applicable to AES analysis. These techniques may include chromatographic techniques such as TLC, GC, GC/MS, and HPLC, as well as polarography and MS/MS. NMR techniques may be applicable to analysis of bulk samples. The specific procedures for isolation and derivatization of AES may differ slightly from those developed for LAS; the chromatographic conditions and analytical detector appropriate for AES may also require some adjustment or substitution. However, the basic analytical principles are the same and the reader is referred to the LAS section for a description of the available methods.

2. AES-Specific Methods

The discussion in this section is limited to methods that have been demonstrated for the analysis of AES and which have been reported in the literature.

Wickbold (1976) developed an ion chromatography method utilizing a macroporous anion exchange to separate aqueous AES solution into various components (alcohol ethoxylate, alcohol ethoxysulfate, polyglycol sulfate, sodium sulfate, sodium chloride). The procedure involved passing the ethanolic surfactant solution through a strong acid cation exchanger and a macroporous weakly basic anion exchanger. The nonionic components are contained in the eluent. Fractional elution of the anion exchanger with aqueous ammonium hydrogen carbonate solution and an isopropanol-aqueous ammonium hydrogen carbonate mixture selectively released (1) inorganic chloride, inorganic sulfate and polyglycol sulfate, and (2) AES, respectively. This eluate was evaporated to dryness, converted to the sodium salts and weighed. The procedure is suitable for the investigation of AES with ethoxy chain lengths up to 4 units/mole (i.e., AES in the commercial range). Above that length, the hydrophilic nature of the surfactant is such that it is eluted along with polyglycol sulfate and the inorganic components. This method was used to characterize commercial AES solutions. No data

were provided on the elution patterns of other anionic surfactants which may represent potential interferences to the determination of AES components in environmental samples.

Terweij-Groen et al. (1981) reported an HPLC method for the analysis of different classes of anionic surfactants, including AES. The stationary phase was silica SI-60 and the mobile phase was a mixture of $CHCl_3$-EtOH-crown ether. The column effluent was mixed with the fluorogenic acridinium cation; the ion pair formed by the anionic surfactant and the cationic acridinium was extracted into the organic phase and monitored fluorometrically.

Bear (1986) developed a reversed-phase ion pair HPLC procedure for the characterization of alkylbenzenesulfonates. Optimization of pH, counter ion concentration, and mobile phase polarity provided a linear relationship between retention time and alkyl chain number. The same chromatographic conditions were also used for the analysis of an ethoxyalkylbenzene sulfate surfactant; a UV spectrophotometric detector was used for detection.

Irgolic and Hobill (1987) used HPLC for the separation of sulfur-containing surfactants, including AES; an inductively coupled argon plasma vacuum emission spectrometer (ICP) monitoring the 180.7 nm sulfur line served as the sulfur-specific detector. The method is well-suited for fingerprinting commercial surfactants. In the reported method, AES surfactants were not co-analyzed with other anionic surfactants (e.g., AS and LAS) to determine whether these might interfere with the AES analysis. The 2-σ detection limit for the HPLC-ICP system was determined to be approximately 15 ng sulfur, corresponding to a 50-μL injection of a surfactant solution with sulfur concentration of 0.3 mg/L.

Sones et al. (1979) reported the analysis of alcohol ether sulfates by GC. The relative amounts of AES in mixtures separated from detergent formulations were determined by a procedure involving acid hydrolysis

to give ethoxylated alcohols which were then converted to alkyliodides for GC analysis.

Neubecker (1985) developed a method for determination of AES in wastewater and surface waters at ppb levels. The AES compounds were concentrated on an anion-exchange resin, eluted with methanolic HCl, and hydrolyzed to the alkylethoxylate (AE). The AE compounds were extracted from the remaining ionic species, derivatized to the alkyl bromides and analyzed by GC. Recovery of AES homologs was 68-78% at a sensitivity of approximately 1 ppb. Results indicate that only about 10% of the total MBAS response on the same samples was attributable to AES surfactants.

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III. BIODEGRADATION

This section will consider the information available on the biodegradability of alcohol ethoxy sulfates (AES). The procedures employed in the study of the biodegradation of anionic surfactants under both laboratory and field conditions were examined in detail in Part 1 (LAS, Section III).

A. Laboratory Test Systems

Essentially linear alcohol ethoxy sulfates are readily biodegraded in laboratory test systems, generally within several days. This is true of tests that use synthetic media or natural fresh water or seawater as well as in tests that simulate treatment processes. Biodegradation also occurs under anaerobic conditions.

1. Oxygen Uptake - Biochemical Oxygen Demand

Alcohol ethoxy sulfates, as measured by their biochemical oxygen demand at 5 (BOD_5) or 20 (BOD_{20}) days, appear to be substantially biodegraded. Neither the length of the alkyl chain (see Table III-1) nor the length of the ethoxylate portion of the molecule, at least within the range normally used in detergent formulations (i.e., 2 to 4 EO units/mole), appear to significantly influence the rate of degradation. By 20 days, all AES tested had achieved a BOD greater than 70% of the theoretical oxygen demand (ThOD).

2. CO_2 Evolution

The degradation of the AES surfactants cited above was also monitored (Table III-1) using Sturm's evolved CO_2 procedure (1973) which lasts for 26 days. All were readily biodegraded; the percentage of evolved CO_2 ranged from 71 to 100 percent of theoretical (Procter & Gamble Co., unpublished data).

TABLE III-1

AES BIODEGRADABILITY SUMMARY

Surfactant	%BOD_5*	%BOD_{20}**	%CO_2†
$NaC_{10}AE_{2.1}S$	64	TD‡	93
$NaC_{12}AE_{2.1}S$	58	TD	81
$NH_4C_{12}AE_3S$	51 & 55°	78 & 90	74 & 76
$NH_4C_{13}AE_3S$	49	73	74
$NH_4C_{14}AE_3S$	51	70	71
$NH_4C_{15}AE_{2.6}S$	55	71	71
$NaC_{15.9}AE_{2.1}S$	56	TD	75 & 81
$NaC_{12-14}AE_6S$	68	100	78
$NaC_{12-14}AE_{12}S$	35	75	100
$NH_4C_{12-14}AE_{12}S$	40 & 42	59 & 77	79 & 81
$NaC_{16-18}AE_6S$	44	88	89
Glucose	70°°	98°°	87-93***

*Percent biochemical oxygen demand at 5 days.

**Percent biochemical oxygen demand at 20 days.

†Percent of theoretical CO_2 production, using Sturm's evolved CO_2 procedure (1973).

‡Total depletion of oxygen.

°Results from two separate experiments.

°°Swisher, 1987.

***Sturm, 1973.

(Procter & Gamble Co., unpublished data, except as indicated)

In other studies, linear $C_{12-14}AE_1S$ evolved 79.6% of the theoretical CO_2 (TCO_2) over 25 days when present initially at 20 mg/L (D-glucose control evolved 71.7% TCO_2); when present at 10 mg/L 66.1% TCO_2 was

evolved (Procter & Gamble Co., unpublished data). In both cases, an inoculum of 1% (by volume) acclimated sludge was used, and the test temperature was 22-24°C. Linear $C_{12-14}AE_{12}S$ evolved 41.5% TCO_2 when initially present at 20 mg/L (D-glucose control: 87.4% TCO_2); when initially present at 10 mg/L, 52.2% of TCO_2 was evolved (Procter & Gamble Co., unpublished data). For this compound, test conditions were 1% unacclimated sludge as an inoculum, 25°C, and 25 day duration.

$C_{14-16-18}$ (14:32:54) AE_3S was found to be readily degraded (based on evolved CO_2) over a 26-day study. Biodegradation occurred even at reduced temperatures, although at a reduced rate as would be expected. Degradation values of 75%, 70%, 52% and 41% of theoretical CO_2 were recorded for this surfactant at temperatures of 20°, 15°, 10° and 5°C, respectively (Procter & Gamble Co., unpublished data).

Gilbert and Pettigrew (1984) reported similar results for $C_{12-15}AE_3S$ (Dobanol 25-3S) in the modified Sturm test over 28 days; 82.6% was degraded. In another study, Itoh et al. (1979) examined the biodegradability (evolved CO_2 production) of 20 mg/L of coconut alcohol-derived AE_3S, oxo-alcohol $C_{12-15}AE_3S$, and sec-alcohol $C_{12-15}AE_3S$. All 3 surfactants evolved approximately 40-50% of the theoretical CO_2 by 5-10 days, and primary biodegradation was complete (100% loss of MBAS) by 10 days.

3. Die-Away Tests

a. River Water Test

AES appear to be readily degraded in river water die-away tests. Using water from the Tama River in Tokyo, Yoshimura and Masuda (1982) found 100% loss of MBAS activity of $C_{12-14}AE_3S$, n-$C_{12}AE_3S$, oxo-$C_{11-15}AE_3S$, sec-$C_{12}AE_3S$, branched-$C_{12}AE_3S$, and branched-$C_{18}AE_3S$ within ten days. When measured as TOC, however, only about 1% of branched-$C_{18}AE_3S$, 15% of sec-$C_{12}AE_3S$, 65% of branched-$C_{12}AE_3S$ and oxo-$C_{11-15}AE_3S$, were degraded within 10 days; $C_{12-14}AE_3S$ and n-$C_{12}AE_3S$ were 100% degraded.

Kikuchi (1985) performed die-away tests at various temperatures using water from the same river. He reported 95-100% degradation (as measured by MBAS) within three days for $C_{12}AE_1S$, $C_{12}AE_3S$, and $C_{12}AE_5S$ at 10°C. Similar results were reported at the same temperature for C_{14}AES (after almost 2 days), C_{12}AES and $C_{16\text{-}18}$AES (after almost 3 days and C_{12}AES (after nearly 6 days), all of which contained an average of three ethoxy groups. At 17°C, these same 7 surfactants (except for C_{12}AES with an average of 3 ethoxy groups, which was not reported) were 95-100% degraded in 1-1/2 to <3 days. Results of temperature effects on the degradation rate of this latter compound are given in Section 5.b.

Allred and Huddleston (1967) reported that n-$C_{12}AE_{4.0}S$ (equivalent to an average of 4 EO units/mole) had been degraded (as measured by MBAS) 45% at 5 days, 98% at 10 days and 100% by day 20. Similarly, n-$C_{12\text{-}14}$ (40:60) AE_3S was found to be degraded 100% (by MBAS) in 2 to 5 days (Continental Oil Co., unpublished data), and $C_{14\text{-}16\text{-}18}$ (4:30:66) AE_3S lost 95% of its methylene blue activity within 3 days at 22°C and within 12 days at 4°C (Procter & Gamble Co., unpublished data).

b. Seawater Test

Sales et al. (1987) found that branched C_{13}AES (3EO) was rapidly degraded (as measured by MBAS) in natural seawater in a modification of the river water die-away test (1 mg of surfactant and 25 g of wet sediment were added to 100 mL of seawater). Roughly 80% was degraded within 5 days and 90% within 20 days at 25°C. Degradation kinetics were best fit by a second degree polynomial.

Vashon and Schwab (1982) studied the mineralization of radiolabeled AES at µg/L concentrations in estuarine water from Escambia Bay, Florida. The degradation rate was rapid: $C_{16}AE_9S$ initially present at concentrations of 1.43, 13.6, and 140 µg/L exhibited first order kinetics with rate constants varying from 0.31-0.39 $days^{-1}$ (half life of ~2 days) and corresponding asymptote of 96.7-82.4% of theoretical

CO_2 evolved. $C_{16}AE_3S$ initially present at concentrations of 1.21, 10.9, and 113 µg/L displayed first order kinetics at only its lowest concentration, with a rate constant of 0.10 $days^{-1}$ (half life of ~7 days) and an asymptote of 74.5% of theoretical CO_2 evolved.

c. Fortified and Inoculated Waters

The degradation of AES in tests using fortified and inoculated waters is rapid. Gilbert and Pettigrew (1984) reported 100% degradation of $C_{12-15}AE_3S$ (Dobanol 25-3S) in the modified OECD screening test. Yoshimura and Masuda (1982) found that $C_{12-14}AE_3S$, n-$C_{12}AE_3S$, and oxo-$C_{11-15}AE_3S$ derived from linear primary alcohol were degraded within 5 days (as measured by MBAS) when present at 30 mg/L in an artificial medium inoculated with activated sewage sludge. However, 75% of the branched $C_{12}AE_3S$ remained after 10 days under the same conditions.

Utilizing an activated sludge inoculum, Miura et al. (1979) found that 100 mg/L $C_{12}AES$ disappeared completely (as measured by MBAS) in less than 5 days, while TOC removal and BOD/TOD values were between 50-70% of theoretical at 5-10 days.

d. Shake Culture Test

Degradation of AES has also been found to be rapid by the shake culture test. Kravetz et al. (1982) observed $C_{12-15}AES$ ($C_{13.2}$ average, 3EO average) to be nearly completely degraded (as measured by MBAS) after 4 days (initial concentration of 30 mg/L, acclimated inoculum). Degradation as measured by CO_2 evaluation was nearly 80% after 32 days.

Linear C_{12-14} (40:60) AE_3S and n-$C_{12}AE_{40\%}S$ (equivalent to an average of 4 EO units/mole) were both reported to be degraded (as measured by MBAS) 100% within 2-3 days (Continental Oil Co., unpublished data; Allred and Huddleston, 1967, respectively).

Heinz and Fischer (1967; cited in Swisher, p 375, 1970) reported that $C_{12}AE_3S$ had biodegraded (as measured by MBAS) 96% after 15 days in an open shake flask test.

e. Bunch - Chambers Test

Bunch and Chambers (1967; cited in Swisher, p. 375, 1970) employing their own die-away test found that n-pri-$C_{12}AE_3S$ and n-sec $C_{11-15}AE_3S$ had degraded (as measured by MBAS) 100% and 96-98%, respectively, after one week.

4. Simulated Treatment Processes

a. Activated Sludge

Gilbert and Pettigrew (1984) reported that, in the OECD semi-continuous activated sludge test, 95.8% of $C_{12-15}AE_3S$ (Dobanol 25-3S) was degraded.

In semi-continuous activated sludge processes, n-$C_{12-14}AE_{40\%}S$ (equivalent to an average of 4 EO units/mole), n-C_{12-14} (40:60) AE_3S and three samples of $C_{14-16-18}AE_3S$ with different alkyl chain length ratios (i.e., 14:32:54; 4:30:66; 38:36:26) all were degraded (as measured by MBAS) 98 to 100% in a single 24-hour cycle (Allred and Huddleston, 1967; unpublished data: Continental Oil Co. and Procter & Gamble Co.).

b. Trickling Filters

Removal of $C_{14-16-18}$ (14:32:54) AE_3S from a trickling filter sewage treatment plant averaged 73% over an eight-week period. The detection method was MBAS (Procter & Gamble Co., unpublished data).

c. Anaerobic Systems

Little published information is available on the anaerobic degradation of AES.

$C_{14\text{-}16\text{-}18}$ (14:32:54) AE_3S at levels of 26 and 52 mg/L were fed into two laboratory-scale septic tanks for eight months. AES removals (as measured by MBAS) of 81% and 72%, respectively, were reported. The effluent from each tank was then passed through an aerobic seepage bed; overall removal was 98.5% and 99%, respectively (Procter & Gamble Co., unpublished data). Removal of $C_{14\text{-}16\text{-}18}$ (14:32:54) AE_3S added at levels of 20, 50, and 100 mg/L was greater than 95% (method of detection unspecified) in a six-month anaerobic sludge digester study (Procter & Gamble Co., unpublished data). Gilbert and Pettigrew (1984) report that 3-7% AES (by weight on dry solids basis) significantly inhibits the production of gas during the anaerobic digestion of sewage solids.

5. Influence of Test System Variables

a. Inoculum

Goodnow and Harrison (1972) studied the ability of 45 strains of 34 species in 19 genera of aerobic bacteria commonly found in water or sewage to degrade $C_{12}AE_3S$. All bacteria tested except Azotobacter beijerinckii ATCC 19360 and Mima polymorpha ATCC 9957 degraded (as measured by MBAS) the surfactant a minimum of 41% up to a maximum of 100% within 72 hours. The A. beijerinckii inoculum was killed at an AES concentration of 0.1 g/L, while M. polymorpha had degraded only 15% of the AES during 72 hours of incubation.

b. Temperature

One published study of temperature effects on AES biodegradation in the laboratory was found. Kikuchi (1985) observed the degradation (as measured by MBAS) of $C_{12}AE_3S$ in a river water die-away test at 27, 21, 15°C, and 10°C. In all cases, over 90% degradation occurred; within 2 days at 27 and 21°C, within 5 days at 15°C, and within 9 days at 10°C.

Procter & Gamble Co. (unpublished data) examined the rate of AES degradation as a function of temperature. Although the rate was reduced at lower temperatures (e.g., 5°C), as might be expected, biodegradation did occur (see III.A.2. of this chapter for details.)

c. Sorption

Urano et al. (1984) studied the sorption of $C_{15}AE_5S$ on seven sediments. The amount sorbed was correlated with the organic carbon content of the sediments, and the sorption isotherm was nearly linear, yielding a K_{oc} of 1.1.

B. Field Studies

The few field studies that have been conducted on AES degradation demonstrate its removal by sewage treatment processes. Very high (98-100%) removal has been measured in municipal activated sludge sewage treatment plants. Removal by on-site anaerobic and aerobic sewage treatment processes ranged from 44-66% in one study.

Ninety-eight percent removal of $C_{12-15}AE_3S$ (Dobanol 25-3S) has been reported for activated sludge treatment at 15°C and 99% removal at 8°C (Gilbert and Pettigrew, 1984). Household detergent products containing 10-13% AE_3S were exclusively used for laundering purposes in 14 homes for approximately a one-year period. Ten homes had septic tank-type sewage treatment, while the remaining four homes had aerobic cavitette-type sewage treatment systems. No adverse effects were observed with respect to operation of the units, and AE_3S removal (as measured by MBAS) ranged from 46-66 percent (Procter & Gamble Co., unpublished data).

Utilizing their far infrared method, Oba et al. (1976) found 16% of the surfactant content present in raw municipal sewage entering two

Japanese sewage treatment plants consisted of AES plus alkyl sulfates. These surfactants were 100% removed during passage through the two treatment plants.

C. Effect of Chemical Structure

The effects of chemical structure on the biodegradation rate of AES are generally considered to be analogous to those for alkyl sulfates. Conflicting results on the effect of the number of ethoxy groups have been reported. However, over the range of structures employed in commercial AES products, structural effects are minor, and biodegradation occurs rapidly for essentially linear AES.

Swisher (1987) has stated that structural effects on AES biodegradation generally are the same as those for the alkyl sulfates. Therefore, the biodegradability of C_{6-14} linear alkyl chains increases with chain length although at chain lengths of C_{18} and above degradability decreases due to low solubility in water (Fisher et al., 1983).

Yoshimura and Masuda (1982) report increasing biodegradability of singly or doubly branched AES as the number of ethoxy groups increased from one to five. This finding is unusual (Swisher, 1987), and inconsistent with the data preaented in Table III-1. Vashon and Schwab (1982) found that the sulfate moiety had no effect on the mineralization rate of the alkyl or ethoxylate groups of $C_{16}AE_9S$ or $C_{16}AE_3S$, when they compared the degradation of these compounds and their corresponding alcohols.

D. Metabolic Pathways of Biodegradation

The principal degradation pathway of AES is the etherase cleavage of the C-O bonds forming mono-, di,- and tri (ethylene glycol) sulfates, which

subsequently undergo ω-oxidation. The alkyl chain formed by this cleavage undergoes ω/β-oxidation. Other degradative pathways include desulfation and ω/β-oxidation of the alkyl chain.

Cain (1987) identified three possible routes for the biodegradation of the AES model compound dodecyltriethoxy sulfate. They are: hydrolytic desulfation, etherase cleavage of the ethoxylate moieties, and ω/β oxidation of the alkyl chain. The dominant pathway is etherase cleavage of the C-O bonds to form mono-, di-, and tri(ethylene glycol) sulfates which subsequently undergo ω-oxidation. Gilbert and Pettigrew (1984) have stated that the long chain alcohol formed upon the cleavage of the alkyl moiety is oxidized to a fatty acid which then undergoes β-oxidation.

Yoshimura and Masuda (1982) also mention the enzymatic hydrolysis of the sulfate group to give an alcohol ethoxylate as a degradation pathway, as well as the initial attack of the terminal methyl group followed by β-oxidation. Vashon and Schwab (1982), however, found that, in radio-tracer studies of linear AES degradation, hydrolysis of the ether linkage occurred prior to mineralization of either the alkyl or ethoxylate chain. This was true for alcohol ethoxylates as well as AES.

Hales et al. (1982) report that Pseudomonas sp., strain DES1 was able to degrade sodium dodecyltriethoxy sulfate by either of the first two methods described above by Cain. Both of these mechanisms leave sulfated glycol residues, which under environmental conditions do not persist.

E. Summary

The data available on biodegradation indicate that linear AES surfactants as a class readily undergo primary biodegradation in the laboratory and under field conditions in both aerobic and anaerobic

systems. Within the range utilized in detergent formulations, neither increments in length of the alkyl chain nor the length of the ethoxylate portion of the molecule appear to significantly influence the rate of biodegradation. Based upon BOD and evolved CO_2 data, AES also undergo rapid ultimate biodegradation to CO_2 and H_2O.

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IV. ENVIRONMENTAL LEVELS

A. Water Quality Standards

There are presently no standards in the United States or Europe specifically restricting alcohol ethoxy sulfates. These anionic surfactants are included among those measured in the environment using the MBAS method. The regulations which apply to anionic surfactants were discussed in Part 1, LAS.

B. AES in Natural Water Bodies

AES are not presently being monitored, as such in the United States or Europe. MBAS measurements in water bodies can include AES surfactants as well as other anionics though AES are not measured specifically. Levels of anionic surfactants detected in natural water bodies were discussed in Part 1, LAS.

V. ENVIRONMENTAL SAFETY

The information available on the environmental toxicity of AES is limited. No data were found concerning the susceptibility of wildlife or plants. Some new studies have been conducted to determine the toxicity of AES to fish. These are discussed below.

A. Aquatic Toxicity

The LC_{50} values for the bluegill sunfish (Lepomis macrochirus) range from 0.3 mg/L for $C_{15.9}AE_{2.1}S$ to 375 mg/L for $C_{10}AE_{2.1}S$. The majority of LC_{50} values, however, are between 1 and 10 mg/L. Toxicity appears to increase with increasing alkyl chain length to a maximum at C_{16}, beyond which toxicity decreases. Conversely, AES compounds with alkyl chains of less than 16 carbons have decreasing toxieity with each increment in the ethoxylate chain from 2 to 6 EO units. Longer-chain AES compounds ($>C_{16}$) increase in toxicity as the ethoxylate chain is lengthened from 2 to 6. The 24-hour LC_{50} values for AES range from 5 to 37 mg/L in Daphnia magna. The sodium salt of $C_{12-14}AE_3S$ was found to be slightly less toxic than the ammonium salt of the same compound. The only other species for which an acute toxicity value was found were the mosquito larvae (Aedes aegypti), with a 24-hour LC_{50} of 11 mg/L; the pink shrimp (Penaeus duorarum) with a 96-hour LC_{50} of 350 mg/L; and the Daphnia pulex with a 48-hour LC_{50} of 20.2 ppm. Three AES compounds appear to have inhibitory effects on the growth of E. coli in culture plates. The lowest concentrations of $C_{12}AE_3S$, $C_{12}AE_3S$ (Ziegler-derived), and $C_{12-14}AE_{2.1}S$ (natural alcohol-derived) which prohibited the development of more than 5 colonies per plate (over 5 days at

37°) are 18, 4, and 2 g/L, respectively. MAC (minimum algistatic concentrations) values are reported for three species of freshwater algae, and range between 10 and 1000 mg/L AES.

1. Fish

Data on the toxicity of AES to fish are summarized in Table V-1; included are two studies which reported sublethal effects in the fathead minnow and the bluegill. Acute toxicity levels, as measured by LC_{50} values, ranged between 1 and 450 mg/L in the data surveyed. In general, the 24 hr LC_{50} values range from 1-10 mg/L. Other AES compounds, however, are less toxic to fish; e.g., Gafa (1974) reported an LC_{50} value of 55 mg/L for $C_{12}AE_{2.6}S$ and Procter & Gamble Co. (unpublished data) recorded a value of 375 mg/L for $C_{10}AE_{2.1}S$.

Newsome (1982) reported LC_{50} values for various species of fish at different ages (Table V-2). With the exception of the guppy, fry were more susceptible than adults. This trend was most evident for the fathead minnow and convict cichlid and less so for the zebra fish. Overall, however, the difference between fry and adults is quite small.

In a comparison of sensitivities of fry and juvenile life stages of fathead minnow, no significant differences in measured LC_{50} values were observed after 4, 7, 14, 28 or 45 days' continuous flow exposure to $C_{14-16}AE_{2.25}S$. The 45 day LC_{50} values for fry and juveniles were 0.63 (95% CL: 0.45-0.88) mg/L and 0.94 (95% CL: 0.61-1.98) mg/L, respectively. In addition, no significant effects on length and weight of either life stage were noted (Procter and Gamble Company, unpublished data).

TABLE V-1

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L)	Source
Goldfish (Carrassius auratus)	$C_{12}AE_{2.6}S$ MW-300	20°C, static, hardness - 10°	LC_{50} 96 hr - 55.0	Gafa (1974)
	$C_{14}AE_{3.0}S$ MW-346		- 6.0	
	$C_{16}AE_{3.4}S$ MW-392		- 41.0	
	$C_{12}AE_{2.6}S$ MW-300, 5% branched		- 66.5	
	$C_{14}AE_{3.0}S$ MW-346, 5% branched		- 8.1	
	$C_{15}AE_{3.2}S$ MW-368.5, 5% branched		- 3.7	
	$C_{11-16}AE_{3}S$	fish length - 6 cm, 20°C static, hardness - 200 mg/L $CaCO_3$	LC_{50}-LC_{100} 24 hr - 10.0-15.0 48 hr - 10.0-15.0	Unilever Research Laboratories, unpublished data

TABLE V-1 · Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L)	Source
Fathead minnow (*Pimephales promelas*)	$C_{11}AE_4S$	Static, 21°C, pH - 7.0-7.2, hardness - 100 mg/L $CaCO_3$	LC_{50} 24 hr - 17.0 48 hr - 8.0	Monsanto Co., unpublished data
	$C_{12}AE_2S$		LC_{50} 24 hr - 1.5 48 hr - 1.5	
	$C_{14}AE_2S$		LC_{50} 24 hr - 1.8 48 hr - 1.3	
	$C_{14}AE_4S$		LC_{50} 24 hr - 4.0	
	$C_{14}AE_6S$		LC_{50} 24 hr - 9.3	
	$C_{16}AE_2S$		LC_{50} 24 hr - 1.0	
	$C_{16}AE_4S$		LC_{50} 24 hr - 0.9	
	$C_{16}AE_6S$		LC_{50} 24 hr - 0.8	

TABLE V-1 - Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limits)	Source
Fathead minnow (*Pimephales promelas*) - continued	$C_{18}AE_2S$	Static, 21°C, pH - 7.0-7.2, hardness - 100 mg/L $CaCO_3$	LC_{50} 24 hr - 80	Monsanto Co., unpublished data
	$C_{18}AE_4S$		LC_{50} 24 hr - 15	
	$C_{18}AE_6S$		LC_{50} 24 hr - 2.1	
	$C_{13.7}AE_{2.25}S$	1 yr, flow-thru, 21°, 120 mg/L hardness, pH 7.4	Growth inhib. - 0.22 NOEC - 0.1	Naki (1979a)
	C_{12-14} AES (ammonium salt)	Static, 22°C, pH - 7.3, hardness - 50-52 mg/L $CaCO_3$	LC_{50} 96 hr - 13 (10-18)	Procter and Gamble Co., unpublished data
Fathead minnow fry (*Pimephales promelas*)	$C_{14-16}AE_{2.25}S$	45 day, flow-thru	LC_{50} - 0.63 (0.45-0.88)	

TABLE V-1 - Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limits)	Source
Fathead minnow fry (*Pimephales promelas*) (continued)	$C_{14\text{-}15}AE_{2.25}S$	Continuous flow, 19.4°C pH = 6.9 - 8.4 hardness - 145 mg/L $CaCO_3$	LC_{50} 96 hr - 0.95 7 day - 0.85 14 day - 0.73 (0.55 - 0.98) 28 day - 0.63 (0.45 - 0.88) 45 day - 0.63 (0.45 - 0.88)	Procter and Gamble Co., unpublished date
Fathead minnow juvenile (*Pimephales promelas*)	$C_{14\text{-}16}AE_{2.25}S$	45 day, flow-thru	LC_{50} - 0.94 (0.61-1.98)	Procter and Gamble Co., unpublished data
	$C_{14\text{-}15}AE_{2.25}S$	Continuous flow, 19-4°C pH = 7.7 - 7.9, hardness - 150 mg/L as $CaCO_3$	LC_{50} 96 hr - 1.2 7 day - 1.2 14 day - 0.93 (0.66 - 1.42) 28 day - 0.93 (0.66 - 1.42) 45 day - 0.44 (0.61 - 1.98)	Procter and Gamble Co., unpublished data
Guppy (*Lebistes reticulatus*)	A sulfated polyglycol ether of a primary alcohol with 3 EO groups/molecule, 59.5% active material	Static, 25°C, males - 0.05 g - 0.08 g females - 0.12 g - 0.38 g young (14 days old) - 0.007 g	LC_{50} 24 hr: male - 8 female - 5 young - 4	Van Emden *et al.* (1974)

TABLE V-1 · Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limits)	Source
Guppy (*Poecilia reticulatus*)	$C_{11-16}AE_3S$	Static, 20°C, fish length - 1.0 cm, hardness - 150 mg/L $CaCO_3$	LC_{50} 24 hr - 4.7 (2.7-5.8)	Unilever Research Laboratories, unpublished data
Golden orfe (*Idus melanotus*)	$C_{11-16}AE_3S$	Static, 20°C, Fish length - 7 cm, herdness - 150 mg/L $CaCO_3$	LC_{50} 24 hr - 4.3 (4.0-4.6)	Unilever Research Laboratories, unpublished data
	$C_{12-15}AE_3S$	268 mg/L hardneas as as $CaCO_3$, 20°C	LC_{50} 96 hr - 3.3-6.2	Reiff *et al.*, (1979)
	$C_{12-15}AE_3S$	static, 150 mg/L hardness as $CaCO_3$, 20°C	48 hr - 5.7	Reiff *et al.*, (1979)
Harlequin (*Rasbora heteromorpha*)	$C_{11-16}AE_3S$	Continuous flow, 20°C, Fish - 1.3-3.0 cm, hardness - 20 mg/L $CaCO_3$	LC_{50} 24 hr - 4.4 (3.9-5.0) 48 hr - 3.9 (3.4-4.5)	Unilever Research Laboratories, unpublished data
	$C_{12-15}AE_3S$	20 mg/L hardness	96 hr - 3.9	Reiff *et al.*, (1979)

TABLE V-1 - Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limit)	Source
Minnow (*Phoxinus phoxinus*)	$C_{11-16}AE_3S$	Static, 10°C, fish length - 5 cm, hardness - 210 mg/L $CaCO_3$	LC_{50} 24 hr - 5.8 (5.5-6.2)	Unilever Research Laboratories, unpublished data
Brown trout (*Salmo trutta*)		Continuous flow, 15°C, fish length - 2.8 or 5.8 cm, hardness - 26-36 mg/L $CaCO_3$	LC_{50} 24 hr - 5.7 (4.3-7.8) 48 hr - 2.6 (1.7-3.7)	Unilever Research Labortories, unpublished data
			LC_{0-100} 96 hr - 1.0-2.5	
	$C_{12-15}AE_3S$	flow-thru, 26-30 mg/L hardness	LC_{50} 96 hr - 1.0-2.5	Reiff *et al.*, (1979)
		250 mg/L hardness	96 hr - 1.5	
Rainbow trout (*Salmo gairdneri*)	$C_{11-16}AE_3S$	Static, 15°C, fish length - 8-10 cm, hardness - 20 mg/L $CaCO_3$	LC_{50} 24 hr - 2.4 (2.0-2.9) 48 hr - 1.9 (1.5-2.3)	Unilever Research Laboratories, unpublished data

TABLE V-1 - Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limits)	Source
Rainbow trout (*Salmo gairdneri*) - continued	$C_{11-16}AE_3S$	Continuous flow, 15°C, fish length - 3.0 cm, hardness -300 mg/L $CaCO_3$	LC_{0-100} 24 hr - 3.0->4.6	Unilever Research Laboratories, unpublished data
			LC_{50} 48 hr - 2.8 (2.3-3.4) 96 hr - 2.2 (1.8-2.2)	
	$C_{12-15}AE_3S$ (Dobanol 25-3S/27)	static, 15°C, 260 mg/L hardness, pH 8.2-8.6	96 hr - 8.9 (7.3-10.3)	Shell Chemical Co., unpublished data
	$C_{12-13}AE_2S$ (Dobanol 23-2S/28)		96 hr - 28 (23-35)	
	$C_{9-11}AE_{2.5}S$ (Dobanol 91-2.5S)		96 hr - 400-450	
Bluegill (*Lepomis macrochirus*)	$C_{14.7}AE_1S$	Static, 20°C, fish length 1.0 g, pH - 7.1, hardness -35 mg/L $CaCO_3$	LC_{50} 24 hr - 2.79 (2.22-3.52) 48 hr - 2.14 (1.78-2.58) 96 hr - 1.90 (1.55-2.31)	Procter and Gamble Co., unpublished data

TABLE V-1 · Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L)	Source
Bluegill · continued	C_8AE_3S	Static, 21°C, pH 7.1, hardness · 35 mg/L $CaCO_3$	LC_{50} 24 hr · >250	Procter & Gamble Co., unpublished data
	$C_{10}AE_{2.1}S$		375	
	$C_{12}AE_{2.1}S$		87	
	$C_{12}AE_{3.0}S$**		73**	
	$C_{12}AE_{3.0}S$**		37**	
	$C_{13}AE_{3.0}S$		24	
	$C_{14}AE_{1.9}S$		4.3	
	$C_{14}AE_{2.6}S$		>5.7<7.5	
	$C_{14}AE_{3.0}S$		7.1	
	$C_{14.7}AE_1S$		1.9	
	$C_{15}AE_{2.6}S$		>2.1<2.4	
	$C_{15.9}AE_{2.1}S$		0.3	

** There is a difference in the branching on the two $C_{12}AE_xS$ samples.

TABLE V-1 · Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limit)	Source
Bluegill · continued	$C_{17.9}AE_{1.9}S$	Static, 21°C, pH 7.1, hardness · 35 mg/L $CaCO_3$	LC_{50} 24 hr · 10.8	Procter & Gamble Co., unpublished data
	$C_{19.6}AE_{1.1}S$		24 hr · 15	
	$C_{12-14}AES$ (ammonium salt)	Static, 22°C, pH 7.2, hardness · 42 · 44 mg/L as $CaCO_3$	LC_{50} 96 hr · 24 (18-32)	
	$C_{12-14}AE_{12}S$ (ammonium salt)	Static, 20-21°C, pH 7.4, hardness · 36 mg/L as $CaCO_3$	LC_{50} 96 hr · 74.5 (67.7 · 82.0)	
	$C_{16}AE_3S$	flow-thru, 13°C, 120 mg/L hard., pH 7.4	Incr. ventilation rate · 48 hr · 0.39	Maki (1979b)

TABLE V-1 · Continued

ACUTE TOXICITY OF AES TO FISH

Species	Surfactant	Experimental Conditions	Toxicity (mg/L) (95% Confidence Limit)	Source
Sheepshead minnow*** (Cyprinodon variegatus)	$C_{14-16}AE_{2.25}S$	static, 21°C, pH 7.9, salinity 30°C	LC_{50} 96 hr - 0.39 (0.3 - 0.53)	Procter and Gamble Co., unpublished data
	$C_{12-14}AES$ (Ammonium salt)	static, 22°C, pH - 8.0, salinity = 32 parts per thousand	LC_{50} 96 hr - 2.3 (1.3 - 3.7)	Procter end Gamble Co., unpublished data
	$C_{14-15}AE_{2.25}S$	static, 21°C, pH 7.9 salinity = 30 parts per thousand	LC_{50} 96 hr - 0.39 (0.3 - 0.53)	Procter and Gamble Co., unpublished data
	$C_{12-14}AE_{12}S$ (Ammonium salts)	Static, 21°C, pH 7.9 salinity 24 parts per thousand	LC_{50} 96 hr - 25 (19 - 32)	Procter and Gamble Co., unpublished data
Japanese killifish (Oryzias latipes)	AES	Test conditions unknown	LC_{50} 48 hr - 10	Tomiyama (1974)

*** Marine species

TABLE V-2

96-HOUR AES* LC_{50} VALUES FOR FRESHWATER FISH

Species	Common Name	Fry (3-weeks old) mg/L	Juvenile (12-weeks old) mg/L	Adult (20-weeks old) mg/L
Pimephales promelas	Fathead Minnow	1.5	2.5	2.2
Cichlasoma nigrofasciatum	Convict Cichlid	2.5	2.5	3.1
Brachydanio rerio	Zebra Danio	2.2	1.8	2.4
Lebistes reticulatus	Guppy	2.4	2.4	2.1

*AES derived from C_{12}-C_{15} alcohol ethoxylate with an average of 3 ethylene oxide units per mole.

Source: Newsome, C.S. (1982)

The lowest level at which a sublethal effect was observed was 0.22 mg/L $C_{13.7}AE_{2.25}S$, which caused growth inhibition in the fathead minnow during a one-year chronic exposure test. A NOEC of 0.1 mg/L was also reported for this species (Maki, 1979a). In a test of 0.39 mg/L $C_{16}AE_3S$, the ventilation rate of bluegills increased significantly during 48 hours of exposure. The NOEC for ventilation rate was between 0.24 and 0.39 mg/L of exposure (Maki, 1979b).

There is some evidence to suggest that toxicity tends to increase with increasing alkyl chain length (see Procter & Gamble Co. and Monsanto Co., unpublished data, Table V-1). Testing with 14 different AES compounds with carbon chains ranging from 8 to 19.6 carbons and 1 to 3 EO units (Procter & Gamble Co., unpublished data), showed that $C_{15.9}AE_{2.1}S$ was the most toxic to bluegill with a 96 hr LC_{50} of 0.3 mg/L. Toxicity generally increased with increments in the carbon

chain. Other data (Shell Chemical Company, unpublished) also show that toxicity increases with increasing alkyl chain length. In toxicity tests with rainbow trout, the 96-hour LC_{50} value decreased from 400-450 mg/L for $C_{9-11}AE_{2.5}S$ to 8.9 mg/L for $C_{12-15}AE_3S$.

Several conclusions were made as a result of work with the fathead minnow relating alkyl chain length, EO number, and toxicity of linear primary AES (Monsanto Co., unpublished data):

1. The AES surfactants show a somewhat different relationship between carbon chain length and fish toxicity than other anionic surfactants. The toxicity of AES was greatly affected by changes in the EO numbers with carbon length being of lesser importance.

2. The toxicity of AES with an alkyl chain of less than 16 carbons was greatest with 2EO. This toxicity decreased with increased EO number when the size of the carbon chain was kept constant.

3. With an alkyl chain length equal to or greater than 16 carbons, the EO-toxicity relationship was reversed; that is, the toxicity decreased drastically with decreasing EO numbers from 6 to 4 to 2 units/mole.

4. The toxicity of surfactant samples peaked at an alkyl chain length of 16 carbons (24 hr. LC_{50} - 0.8-1.0 mg/L) and for this particular alkyl chain length (i.e., C_{16}), toxicity was not substantially affected by the number of EO units/mole.

In contrast, Gafa's (1974) tests with goldfish showed $C_{16}AE_{3.4}S$ to be one of the less toxic AES compounds. Therefore, the above generalizations cannot be confirmed with the limited information available.

The whole body concentration factors after 72 hours exposure to AES, the uptake rates, and the elimination rates were greatly affected by

the number of oxyethylene units on the surfactant molecule. Kikuchi et al. (1980) showed that when three oxyethylene units were introduced to the C_{12}-AS molecule (i.e., $C_{12}AE_3S$ sodium dodecyltri(oxyethylene)-sulfate), the concentration factor in carp (Cyprinus carpio) increased to 18 while the elimination rate was unchanged. As the number of oxyethylene units increased to five (i.e., $C_{12}AE_3S$ sodium dodecylpenta(oxyethylene)sulfate), the concentration factor was only 4.7 and the uptake and elimination decreased in comparison to the $C_{12}AE_3S$ values. In both cases, elimination from the gills and hepatopancreas was rapid, while elimination from the gallbladder was slow.

2. Invertebrates

Several studies have been conducted to determine the toxicity of AES to the water flea (Daphnia magna). A 24 hr LC_{50} value was reported for $C_{12-14}AE_3S$ (ammonium salt) of 16.3 mg/L. The sodium salt of this compound showed a LC_{50} value of 18.9 mg/L (Continental Oil Co., unpublished data). Another study found that the 24 hr LC_{50} for the same species was 19.6 mg/L using $C_{11-16}AE_3S$ (Unilever Research Laboratories, unpublished data).

Lundahl et al. (1972) reported 24 hr LC_{50} values for Daphnia magna with $C_{12}AE_3S$, $C_{12}AE_3S$ (Ziegler-derived), and $C_{12-14}AE_{2.2}S$ (natural-alcohol derived). The respective toxicity values were 5.0, 37, and 21 mg/L expressed as sodium dodecyl benzene sulfonate.

The 48 hr LC_{50} range for AES in Daphnia pulex was 15.8 to 24.6 ppm with an average value of 20.2 ppm (Moore et al., 1986).

The 96-hour LC_{50} value for ammonium $C_{12-14}AES$ under nominal concentrations of the active ingredient was 5.7 mg/L in D. magna (Procter and Gamble, unpublished data).

Further information on aquatic invertebrate toxicity was provided in a chronic toxicity test with Daphnia magna (Maki, 1979a). The 21-day EC_{50} for inhibition of reproduction (with respect to total young produced) by $C_{13.67}AE_{2.25}S$ was 0.37 mg/L in continuous-flow conditions and 120 mg/L $CaCO_3$ water hardness. The 96-hr and 21-day LC_{50} values for the same surfactant were 1.17 mg/L and 0.74 mg/L, respectively. A chronic NOEC of 0.27 mg/L was established for this species.

In a test with Daphnia, Lundahl and Cabridenc (1976) found that the toxicity of C_{12ave}AES (lauryl ether sulfate) decreased steadily with time as a result of biodegradation. After 30 hours in static conditions, the solution was virtually non-toxic. No toxicity values were reported.

Other species of invertebrates were tested by Van Emden et al. (1974). The 24 hr LC_{50} to Aedes aegypti for AE_3S was found to be 11 mg/L, and the LC_{100} was 12 mg/L. These authors also reported that the 24 hr LC_{100} for snails was 12 mg/L AE_3S.

Pink shrimp (Penaeus duorarum) have also been tested for their susceptibility to AES. The 96 hr LC_{50} for $C_{14-16}AE_{2.25}S$ was 350 (95% CL: 220-590) mg/L; the no observed effect level for this acute exposure was less than 120 mg/L $C_{12-14}S$ (Procter and Gamble Co., unpublished data). The 96 hour LC_{50} value for $C_{12-14}AE_{12}S$ (ammonium salt) and $C_{14-15}AE_{2.25}S$ (sodium salt) for this species was >1000 mg/L and 350 mg/L, respectively based on nominal concentrations. The 48 hour EC_{50} value in the Eastern oyster (Crassostrea virginica) was 9 mg/L (95% C.L.: 3-30 mg/L) based on nominal concentrations (Procter and Gamble, unpublished data).

The limited results discussed above suggest that at least those few invertebrates that have been tested may be slightly less susceptible to AES than are fish.

3. Toxicity of AES to Microorganisms and Algae

The effect of AES on microorganisms in relation to biodegradation was discussed in Section III.A.5.a. Lundahl et al. (1972) examined the bactericidal effect of $C_{12}AE_3S$, $C_{12}AE_3S$ (Ziegler-derived) and $C_{12-14}AE_{2.2}S$ (natural-alcohol-derived) on E. coli. The lowest concentrations which prohibited the development of more than 5 colonies per plate (for 5 days at 37°C) were 18, 4, and 2 g/L, respectively.

Yamane et al. (1984) studied AES-induced growth inhibition in the green algae, Selenastrum capricornutum. An EC_{50} value of 65 mg/L was reported for sodium polyoxyethylene alkyl ether sulfate with an average chain length of 12.8 and an average number of 3 oxyethylene units.

The MAC_5 (minimum algistatic concentration for a 5-day exposure) of AES (formulation not provided) in three species of fresh water algae is as follows:

	MAC_5
Selenastrum capricornutum	>10 and <100 mg/L
Navicula seminulum	>10 and <100 mg/L
Microcystis aeruginosa	>100 and <1000 mg/L

(Procter & Gamble Co., unpublished data)

The 5-day algistatic concentrations for $C_{14-15}AE_{2.25}S$ and $C_{12-14}AES$ in S. capricornutum were 42 mg/L (95% C.L. = 20-93 mg/L) and 101 mg/L (95% C.L. = 42-312 mg/L), respectively while the 5-day algisideal concentrations were >320 mg/L and >1000 mg/L, respectively (Procter and Gamble, unpublished data).

Kutt and Martin (1974) exposed a red tide dinoflagellate (Gymnodinium breve) to various concentrations of a coconut-alcohol-derived ethoxy sulfate (containing 19% active material) for 48 hours. A concentration of 2.5 μg/L caused 87% mortality, 12.5 μg/L resulted in 63% mortality,

and only 44% perished in 50 μg/L. No explanation was offered for the abnormal inverse relationship between toxicity and surfactant concentration.

In a toxicity test with the alga, Laminaria saccharina, Pybus (1973) used concentrations between 5 x 10^{-5} mg/L and 5 x 10^{4} mg/L of a detergent containing $C_{12(ave)}$AES (sodium lauryl ether sulfate), sodium dodecyl benzene sulfonate, and lauric diethanolamide. In 50 mg/L, zoospores of L. saccharina were inhibited from swimming in 7 minutes, and in 500 mg/L, swimming ceased in 15 seconds. A concentration of 0.1 mg/L prevented the zoospores from settling (an action which normally precedes development into sporophytes). The author hypothesized that the detergent mixture attacked the proteinaceous flagella on the zoospores; this would account for the loss of mobility.

B. Toxicity of AES to Terrestrial Plants

No data were found.

C. Toxicity of AES to Wildlife

No data were found.

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VI. HUMAN SAFETY

The data reviewed below on AES surfactants indicate a low order of toxicity in experimental animals following either acute or chronic oral and dermal exposure. Furthermore, there are no indications that AES surfactants are mutagenic, carcinogenic, teratogenic, or detrimental to reproductive parameters in laboratory animals. The use of detergent formulations containing AES, therefore, would not appear to pose a hazard to human safety.

An expert panel has reviewed the safety of sodium lauryl ether sulfate and ammonium lauryl ether sulfate as part of the Cosmetics, Toiletries and Fragrances Association's continuing evaluation of ingredients used in cosmetics (Cosmetics, Toiletries, and Frangrances Association, 1983).

A. Animal Studies

> *The alcohol ethoxy sulfates exhibit a low order of oral and dermal toxicity in test animals. Acute ocular and skin irritancy responses in rabbits to aqueous solutions of AES ranged from slight to moderate. Undiluted AES, however, are moderate to severe dermal irritants and severe eye irritants in rabbits. No significant adverse effects were noted in rats administered AES concentrations up to 0.5% of the diet for periods of up to two years, nor were there any indications of a direct carcinogenic effect resulting from the ingestion of AES surfactants in feed or drinking water. Detergents containing AES gave no indications of embryotoxic or teratogenic effects in mice, rats or rabbits. AES gave no mutagenic results in either in vitro or in vivo tests.*

1. Acute Toxicity

a. Oral

Alcohol ethoxy sulfates have been shown to have a low order of toxicity in the rat following oral administration. LD_{50} values ranged from 1700 to greater than 5000 mg/kg on an active ingredient basis (Brown and Muir, 1970; Tusing et al., 1962; Walker et al., 1967; unpublished data: Continental Oil Co., Ethyl Corp., Procter & Gamble Co., Stepan Chemical Co.).

Signs of the acute oral toxicity noted in rats following AES administration at concentrations approaching LD_{50} values include an immediate decrease in motor activity, a decreased respiratory rate, ruffed fur and abdominal griping and diarrhea which often persists up to 6 days post-dosing. Nearly all deaths occur within 24-48 hours of dosing with loss of coordination, prostration, and deep breathing evident prior to death (unpublished data: Ethyl Corp.; Procter & Gamble Co.).

Zerkle et al. (1987) evaluated the neuropharmacologic effects of alkyl ethoxylates when used in combination with other surfactants, including $NaAE_3S$. Rats were given single oral doses (10 ml/kg) of an aqueous solution containing 25% alcohol ethoxylate (nonaethylene glycol mono-n-tridecyl ether), 30% $NaAE_3S$ and 15% ethyl alcohol. The animals were observed for up to 2.5 hours after administration. Ataxia was observed in 1 of 3 animals 2 hours after administration. Since neurologic effects were also observed in animals receiving only 25% aqueous alcohol ethoxylate, these effects cannot be attributed to $NaAE_3S$.

b. Inhalation

Rats (number unspecified) survived a one-hour inhalation exposure to a 60 mg/L concentration of a 59% active solution of n-NH_4C_{12-14} (40:60)

AE_3S delivered at a flow rate of 7 L/min. (Continental Oil Co., unpublished data). No additional information was available.

c. Percutaneous

i. Acute Toxicity

In the rabbit, the dermal LD_{50} values reported for AES on both intact and abraded skin ranged from 4100 to 12,900 mg/kg (Shell Chemical Company, 1984; unpublished data: Continental Oil Co.; Ethyl Corp.; Procter & Gamble Co.). With concentrations approaching LD_{50} values, moderate to severe erythema and edema are generally noted at 24 hours with severe desquamation and fissuring evident at the end of one week. Death generally occurred 3-4 days after treatment (unpublished data: Ethyl Corp.).

ii. Skin Irritation

AE_3S (28% active) was classified as a primary skin irritant according to the Draize procedure when applied undiluted to the intact and abraded skin of rabbits. This undiluted material produced moderate to severe erythema with eschar formation but no dermal injury in depth. Edema ranged from barely perceptible to slight for intact skin and from barely perceptible to moderate with abraded skin (Ethyl Corp., unpublished data). In other studies, undiluted AE_6S (26% active) (Procter & Gamble Co., unpublished data), $C_{10-12}AE_9S$ and $C_{14-18}AE_{11}S$ (Ethyl Corp., unpublished data) were classified as moderate skin irritants according to the Draize procedure.

Occluded, 24-hr exposure of rabbits to 2, 10, or 20% aqueous solutions of $C_{12ave}AES$ produced minimal to no skin irritation. No graded response was seen; primary skin irritation scores were ~1 (of a possible 8 points) for all three test concentrations (Ciuchta and Dodd, 1978).

A single 24-hr occluded application of undiluted n-pri-$NH_4C_{12-15}AE_3S$ (Dobanol™ 25-3A/60) or n-pri-$NaC_{12-15}AE_3S$ (Dobanol™ 25-3S/27) to rabbit skin resulted in moderate or mild skin irritation, respectively. Tests were also undertaken to determine the acute skin handling hazards associated with the use of the surfactants per se and after they had been subjected to hypochlorite bleaching; no differences in skin irritancy were detected for these products in rabbits after a single 24-hr occluded application (Shell Research Limited, unpublished data).

In a comparison of the skin irritancy of natural-alcohol-derived versus synthetic-alcohol-derived AES, mild to moderate erythema with slight scaliness was seen in rabbits 14 days after a single 24-hour occluded application of undiluted synthetic-alcohol-derived $NaC_{16-18}AE_3S$ (54.4% active). The undiluted natural-alcohol-derived $NaC_{16-18}AE_3S$ (40.6% active) produced mild erythema and spotted necrosis leading to permanent scar tissue in two of three rabbits and involved approximately 25% of the test site (Procter & Gamble Co., unpublished data). The natural-alcohol-derived product appears to be more acutely irritating to rabbit skin than the synthetic product.

Dilute solutions of AES appear to be non-irritating to the skin. Only slight erythema and edema were observed on the skin of weanling rats after 3 days of twice daily applications of either 5% or 10% (w/v) solutions of $NaAE_3S$ (Prottey and Ferguson, 1975). Similarly, in a rabbit patch test with a 1% aqueous solution of AE_3S (28% active), no erythema or edema was seen on intact skin. Barely perceptible erythema and no edema were noted with abraded skin (Ethyl Corp., unpublished data). A 1% aqueous solution of $C_{14}AE_{11}S$ was also reported to be non-irritating to the skin (Ethyl Corp., unpublished data). However a 1% active solution of natural-alcohol-derived $NaTE_3S$ was mildly irritating and 1% active synthetic-alcohol-derived $NaC_{14-16}AE_3S$ moderately irritating to rabbit skin when applied with occlusion for 24 hours (Procter & Gamble Co., unpublished data).

ii. Skin Sensitization

A 1% aqueous solution of AE_3S was applied dermally to guinea pigs 3 times/week for 9 applications. When challenged two weeks later, no reaction occurred nor was there any edema or erythema seen, indicating no skin sensitization had occurred (Ethyl Corp., unpublished data).

Topical application of a 0.1% active aqueous solution of $NaC_{12}AE_2S$ 3 times per week for 3 weeks elicited no skin sensitization in guinea pigs when topically challenged 10 days after the final application. In animals induced intradermally at the same dose, a blistering effect was seen at one hour after the challenge injection, and by 24 hours, very strong (++) positive responses were seen in 3/10 guinea pigs and a definite positive reaction (+) in the remaining 7 animals. Some 48 hours after the challenge injection, 6 animals still had a definite positive reaction and 4 had a slight positive response (Brown and Muir, 1970).

Stemming from an outbreak of severe allergic contact dermatitis in Norway in 1966, Walker et al. (1973) carried out a series of investigations to determine the source of this response. Working with guinea pigs, they found that following petroleum ether extraction of $C_{12}AES$ (LES 13-2035 i.e., a batch of AES shown to be sensitizing or known to contain sensitizing material) the residual was non-allergenic. In contrast, the petroleum ether extract did contain a sensitizer(s). A contaminant in this particular batch of AES, not AES itself, was the sensitizing agent. Furthermore, it is interesting to note that the particular batch in question was seven years old at the time of these tests indicating that the sensitizing agent was chemically stable or had been formed during the seven years of storage.

Connor et al. (1975, 1976) eventually identified the sensitizers in AES (LES 13-2035) to be 1-dodecene-1,3-sultone, 1-tetradecene-1,3 sultone, 2-chloro-1,3 dodecene sultone and 2-chloro-1,3-tetradecene sultone. The authors speculated that perhaps small quantities of dodecene and tetradecene were present during the sulfonation reaction and were

carried throughout the manufacturing process. Ritz et al. (1975) have tested the above sultones in guinea pigs and found them to be very potent sensitizers.

Conner et al. demonstrated that these sultones could be formed from 1-alkenes by sulfonation with sulfur trioxide, neutralization, then reaction of the alk-2-ene 1-sulfonate components with sodium hypochlorite bleach under low pH conditions. Roberts et al. (1983, 1987) have discussed this reaction further. It is evident that the unsaturated- and the chloro-sultones were the result of conditions not normally present and readily avoidable in AES manufacture. An extremely sensitive analytical method for 1-alkene 1,3-sultones using tandem mass spectrometry has been presented by Brain et al. (1984).

Morikawa et al. (1978) tested the skin sensitizing potential of a series of AES surfactants in guinea pigs. All animals were induced by intradermal injection (8% aqueous solution) and topical application (20% aqueous solution, 48 hr. occluded) and challenged two weeks later by topical application (8% aqueous solution). The surfactants tested included: $C_{11-15}AE_1S$, $C_{12}AES$, $C_{12}AE_1S$, $C_{12}AE_5S$, $C_{12-16}AES$, $C_{12-16}AE_1S$, $C_{12-16}AE_3S$, $C_{12-16}AE_5S$, $C_{14}AE_1S$, $C_{14}AE_3S$, $C_{16}AE_3S$. None induced skin sensitization in guinea pigs. In addition, Morikawa tested two samples of $C_{12}AE_3S$ sulfated either with sulfur trioxide or chlorosulfonic acid. The chlorosulfonic acid preparation was not a skin sensitizer (0/10) in guinea pigs while 15/15 animals gave positive reactions with the sulfur trioxide preparation. Allergenic activity was found by chromatographic separation techniques to be concentrated in one fraction of the sulfur trioxide preparation. This allergenic material was isolated and identified as 1-dodecene-1,3-sultone, presumably formed as a by-product of the sulfur trioxide reaction conditions.

d. Ocular

Undiluted AES were determined to be severe eye irritants and were classified as corrosive in tests done according to the Draize procedure. Eye irritation studies in rabbits with several undiluted

AES (e.g., $C_{10-12}AE_3S$, $C_{12-13}AE_3S$, $C_{12-13}AE_6S$, $C_{14-16}AE_{11}S$) resulted in extensive corneal damage, inflammation and hemorrhage of the iris and maximal conjunctival irritation with no significant improvement seen over a 7-day period (unpublished data: Continental Oil Co.; Ethyl Corp.; Procter & Gamble Co.). Undiluted n-pri-$C_{12-13}AE_2S$ produced severe eye irritation in rabbits; moderate eye irritancy was noted with a 10% aqueous solution of this surfactant, while a 1% concentration was practically non-irritating. No irritation was seen with a 0.1% solution (Shell Research Limited, unpublished data).

A 10% aqueous solution of $NaC_{12-14-16}AES$ (STEOL-CS-125™) was found to be moderately irritating according to the Draize procedure (Stepan Chemical Co., unpublished data). In other studies, 2-10% aqueous solutions of AES produced iritis and slight to moderate conjunctivitis which generally cleared by 2 days. Rinsing after installation of the AES greatly reduced the severity of eye effects (Procter & Gamble Co., unpublished data). One to two percent aqueous AES solutions produced only minimal conjunctival irritation (Brown and Muir, 1970; Ethyl Corp., unpublished data; Procter & Gamble Co.).

A study was conducted to determine the effect of AES alkyl chainlength on eye irritation potential. AES with chainlengths ranging from 8-20 were instilled into rabbit eyes (0.1 ml of a 15% aqueous solution). AES in the C_{12-16} range produced more severe effects than AES with longer or shorter chains. This was primarily manifested by longer clearing times >7 days vs. 1-7 days) (Procter & Gamble Co., unpublished data).

Instillation of 0.1 mL of 2, 10 or 20% aqueous solutions of $C_{12ave}AES$ into rabbits' eyes produced minimal eye irritation. Irritancy scores were similar in magnitude for all three test concentrations (≤15 of a possible 110 points) with no signs of irritation evident by 72 hr (Ciuchta and Dodd, 1978).

In a study of surfactant penetration into rabbit eyes, $C_{18}AES$ labeled with tritium was found to penetrate the cornea. Within 0.5 hours of a

single application, the surfactant was found in all ocular tissues. Thc highest levels were seen in the cornea followed by the choroid, retina and iris. The rate of loss was slow with the label remaining after 48 hours. Repeated applications led to increased binding. Permeability, ocular and systemic uptake were all greater in juvenile rabbits than in adult rabbits (Clayton et al., 1985).

e. Other Routes of Administration

A 1% aqueous solution of $NaAE_3S$ (28% active) was not irritating to the vaginal mucosal tissue of 3 dogs examined 24 hours after treatment. Applied undiluted, this product produced a slight redness in 2 of 3 dogs and a deeper, more diffuse irritation of the tissue in the third test animal (Ethyl Corp., unpublished data).

Ciuchta and Dodd (1978) also examined the irritation induced by the above surfactant in a mouse writhing test. Mice were injected intraperitoneally with 0.2 mL of various concentrations of an aqueous solution of $C_{12ave}AES$ and observed until a positive response was elicited. A concentration of 0.23% was calculated to produce writhing in 50% of the animals.

2. Subacute Toxicity

a. Oral

No adverse effects were observed in rats (12/sex/group) fed diets containing 40, 200 or 1000 ppm of either $C_{12-15}AE_3S$ or $C_{12}AE_3S$ for 91 days. At a 5000 ppm level, however, increased organ weights were seen in both treatment groups. Both male and female rats fed 5000 ppm $C_{12-15}AE_3S$ had increased liver weights. In the 5000 ppm $C_{12}AE_3S$ group, increased kidney weights were noted in males and increased kidney, liver and heart weights in females. Histopathological examinations revealed no tissue abnormalities. The 5000 ppm $C_{12-15}AE_3S$-treated males also exhibited a slight, but statistically significant increase

in serum total protein concentration (6.2 g/100 mL vs 5.9 for controls) but the ratios of serum protein fractions were unaffected (Walker et al., 1967).

Leuschner et al. (1969) studied the subacute toxicity of a mixture of 13% NH_4AES and 15% LAS in rats and dogs. The details of this study are described in Chapter 1. Doses of 0.5 mL/kg/day in rats were without effect. At 10 mg/kg/day in dogs, intestinal necrosis and infiltration with chronic inflammatory cells were noted.

No adverse effects on histopathology were noted in Sprague-Dawley rats administered dietary levels of 0, 0.025, 0.25 or 2.5% w/w of a liquid dishwashing detergent for 13 weeks. The detergent contained 15% NH_4 C_{10-14}LAS, 13% Mg C_{12-16} alkyl sulfate and 13% NH_4 AE_3S. The only effect was an increase in the relative liver weight at the 2.5% level (Scailteur et al., 1986).

In another study, 0.25, 0.5 or 1.0% $C_{12-13}AE_6S$ (on active ingredient basis) was added to the diet of rats (20/sex/group) for 3 months. Five animals/sex/group were killed after one month. All treated animals survived; behavior, food consumption, hematology, clinical chemistries and urinalyses were all comparable to control values. Slightly lower mean body weights (generally less than 5% of control weight) were recorded at 3 months for the 0.5% males and both sexes at the 1% dietary level. Significantly lower ($p < 0.05$) values were noted in the absolute mean adrenal weight and in the mean adrenal to body weight ratio for females after one month on the 1% $C_{12-13}AE_6S$ diet. At 3 months, the absolute mean heart weight was lower in females on the 0.5% diet and significantly lower ($p < 0.05$) in females given 1% $C_{12-13}AE_6S$ in their diets. No compound-related changes were observed upon histopathological examination (Procter & Gamble Co., unpublished data).

In a similar study, rats (20/sex/group/treatment) were given 0.01, 0.1 or 1% of either natural- or synthetic-alcohol-derived $NaC_{16-18}AE_3S$ in the diet for 91 days. The only parameters which were significantly different from control values were red blood cell counts in females and food consumption and body weight gain in males on the 1% natural-

alcohol-derived diets and the liver to body weight ratios for females given either the 0.1% natural- or 1% synthetic-alcohol-derived diets.

The red blood cell counts in females on the 1% natural-alcohol-derived diet were significantly lower (although within established ranges) than controls (7.31 vs 7.79 million/mm^3 for controls). The males in this group showed significantly lower total food consumption (1902 g vs 2051 g for controls) and body weight gain (364 g vs 393 g for controls) when compared to control values. The liver to body weight ratios for females on the 0.1% natural- and 1% synthetic-alcohol-derived diets were significantly higher (3.63×10^{-2} and 3.61×10^{-2}, respectively) than control values (3.15×10^{-2}) The increases in liver weights were considered an adaptive response to an increased metabolic demand. (Procter & Gamble Co., unpublished data).

Similar studies were conducted with $C_{12-13}AE_3S$ and $C_{12-13}AE_1S$ (20/sex/group). AE_3S was administered in the diet of 0.1, 0.5 and 1.0% (active ingredient) and AE_1S was administered in the diet at 0.1, 1.0, 1.5, 2.0, and 2.5%. Again, the only treatment-related effects observed were increased liver weights and liver to body weight ratios (doses >0.1%) and slight body weight decreases (1.5% or greater) (Procter & Gamble Co., unpublished data).

b. Percutaneous

i. Skin Irritation

Sixty-five percutaneous applications of a 5% active aqueous solution of $C_{12-13}AE_6S$ to the intact skin of 6 restrained rabbits (2 mL/kg) over a 91-day period produced minimal to pronounced dermal irritation (erythema, drying, fissuring, desquamation). Histopathological examination of other tissues was unremarkable (Procter & Gamble Co., unpublished data). Similarly, 20 applications of this surfactant at the same concentration to the abraded skin of 6 restrained rabbits in a 28-day period resulted in localized dermal irritation (erythema, drying and desquamation) with papular eruptions seen by week 2 through week 4. Histopathology was normal (Procter & Gamble Co., unpublished data).

In two similar 28-day skin irritation studies, unrestrained rabbits with abraded skin were treated with aqueous solutions of either $NaC_{16-18}AE_3S$ or $NH_4C_{12-13}AE_3S$ at 2 ml/kg. The first topical application contained 200 mg/kg of active ingredient; all applications thereafter contained 50 mg/kg of active ingredient. Mild skin irritation was observed with both treatments. Histological examination of the tissues revealed mild skin irritation with $NaC_{16-18}AE_3S$ and moderate to severe skin irritation with the $NH_4C_{12-13}AE_3S$ preparation. One of 12 rabbits treated with the latter preparation died from unknown causes on day 5 of the study; the tissues were too autolyzed to examine (Procter & Gamble Co., unpublished data).

Twenty applications of 0.2 mL of a 1% aqueous solution of AE_3S (28% active) to the abraded and intact skin of rabbits over a 28-day period produced negligible hyperemia and slight exfoliation of the abraded skin and negligible exfoliation of intact skin (Ethyl Corp., unpublished data).

In another study, a 10% solution of a hand dishwashing liquid (19% TES and 19% LAS) gave a moderate skin irritation during 91 days exposure of intact rabbit skin or 27 days exposure of abraded rat skin (Procter & Gamble Co., unpublished data).

Cumulative, open-patch test application of 0.1 mL of a 2% aqueous solution of C_{12-13}(52:48) AE_3S (99.8% pure) to the shaved backs of guinea pigs twice daily for a total of nine treatments resulted in slight to moderate skin irritation. A skin irritation score of 1.25 of a possible 4 points was recorded (Imokawa, 1979).

Three 6 hr occluded skin applications of 10% aqueous dilutions of n-pri-$NH_4C_{12-15}AE_3S$, n-pri-$NaC_{12-15}AE_3S$, or n-pri-$C_{12-13}AE_2S$ produced moderate to severe skin irritation in rabbits. However, only slight irritation was noted with 1% concentrations and minimal to no irritation at concentrations of 0.1% (Shell Research Limited, unpublished data).

In another experiment, application of a 10% aqueous solution of n-pri-$C_{12-13}AE_2S$ daily, five days per week, for 4½ weeks to the skin of guinea pigs and rabbits (unoccluded) produced severe skin irritation; no irritation was noted with either 1% or 0.1% concentrations. Intradermal and topical induction of guinea pigs with hypochlorite bleached and unbleached samples of n-pri-$NH_4C_{12-15}AE_3S$, n-pri-$NaC_{12-15}AE_3S$, or n-pri-$C_{12-13}AE_2S$ followed by topical challenge produced no skin sensitization (Shell Research Limited, unpublished data). Similar skin sensitization studies with 12 separate batches of n-pri-$C_{12-15}AE_3S$ also resulted in no skin sensitization in guinea pigs (Shell Research Limited, unpublished data).

Mice (25/sex/group) were treated dermally with $C_{12-13}AE_3S$ (0.1 ml of 2.4 or 6.9% aqueous solutions) five times per week for four or thirteen weeks. No mortality and no significant differences in body weight were observed. Gross necropsy of animals revealed no effects other than skin irritation at the treatment site. Skin tissues were examined histopathologically. The only effect observed was minimal to slight acanthosis in mice treated with the high dose for 13 weeks (Procter & Gamble Co., unpublished data).

3. Chronic Toxicity

a. Oral

No deleterious effects with respect to survival, growth rate, food consumption, or clinical laboratory findings were noted in rats (30/sex/group) given 0, 0.1 or 0.5% $C_{12}AE_3S$ in the diet for two years. Individual liver and kidney to body weight ratios deviated from control values but no abnormalities were found upon histopathological examination of these or other organs and tissues. An occasional tumor (type and incidence unspecified) was found in various groups. The tumors were characterized as "typical" of those commonly found in aged rats and did not appear to be associated with the ingestion of AES (Tusing et al., 1962).

In another two-year study, rats were administered $C_{12}AE_3S$ (25% active) in the drinking water (20/sex/group) at a concentration of 0.1 percent. At termination, survival, growth, food consumption, body weights, clinical laboratory findings, hamatology and urinalyses were all comparable in control and traated animals. The only unusual findings were a slight, but consistently higher water consumption by all rats receiving the test compound in their drinking water and a significant difference in the empty cecum to body weight ratio of females. Absolute organ weights were all comparable to controls and no consistent gross or histopathology was found. Generally, pathological findings for controls and treated rats after two years on test were varied and consisted predominantly of incidental findings attributable to advanced age. Various types of benign and malignant tumors were found in both groups; the frequency of tumors in the treated group was not significantly different from that of control animals (Procter & Gamble Co., unpublished data).

A two-year oral toxicity study was conducted with groups of albino rats fed a mixture of LAS/TE_3S at dietary levels of 0.0, 0.1, 0.5 or 1.0%. The results obtained during the investigation revealed a reduction in body weights and weight gain for males fed 0.5% and males and females fed 1.0%. Females fed 0.5% exhibited body weight and weight gain reduction during the first 14 months of the study. However, the females fed 0.5% exhibited body weight and weight gain which compared favorably with those of control by the conclusion of the investigation. All animals fed 0.1% exhibited normal body weight and weight gains. The results obtained from all groups for the following parameters were all within normal ranges for albino rats of this age and strain: mortality, reactions, hematologic studies, organ weights and ratios, gross and microscopic pathologic findings and tumor findings (Procter & Gamble Co., unpublished data).

4. Carcinogenicity

No papillomas or other skin tumors were observed in 30 female Swiss mice following twice weekly percutaneous applications with a 5% aqueous

solution (0.1 mL) of $C_{12}AE_3S$ for two years (Tusing et al., 1962). Further, thrice weekly percutaneous applications of a 10% aqueous solution of an 18.6% C_{16-18}AES (tallow alcohol ethoxy sulfate) and 15.6% LAS formulation to 50 Swiss ICR mice for 18 months did not induce any carcinogenic response either on the skin or systemically (Procter & Gamble Co., unpublished data).

No indications of an increased incidence of tumors were noted in two chronic feading studies with rats given AES at levels up to 0.5% of the diet or 0.1% in drinking water for two years (Tusing et al., 1962; Procter & Gamble Co., unpublished data).

Trace levels of 1,4-dioxane, an animal carcinogen, have been reported to be present in formulated AES products (Shell Oil Co., 1979). This material is a trace by-product formed during sulfation of alcohol ethoxylates to alcohol ethoxy sulfates.

Concentrated sodium and ammonium salts of AES were reported to contain between 250 and 600 ppm 1,4-dioxane (Shell Oil Co., 1980). The actual concentration of 1,4-dioxane in formulated household dishwashing products would appear to be so low as to pose no substantial risk to users; <1 to 45 ppb assuming an ~2000-fold consumer dilution of a commercial formulation containing 15% AES concentrate. Shampoos present a different picture since the product is at least momentarily handled undiluted just prior to lathering the hair. 1,4-Dioxane values of 100 ppm or less can be calculated for auch exposures. However, this kind of exposure is very short, and any 1,4-dioxane will be greatly diluted and removed with water after a few minutes.

Appel (1988) has concluded based on an exposure and risk assessment that "In view of this safety margin, a consumer health risk is not to be expected from products containing dioxane-contaminated alkyl ether sulfates as the active substances, that AES-containing 1000 ppm 1,4-dioxane does not pose a significant risk to consumers in a shampoo."

High concentrations of 1,4-dioxane (5000 or 10,000 ppm) added to the drinking water of rats and mice for two years have been reported to produce liver and nasal carcinoma (NCI, 1978; Kociba et al., 1974). At 0.1% (1000 ppm), some renal and hepatic degenerative changes were noted in rats (mice were not tested below 5000 ppm), however, rats given 0.01% (100 ppm) 1,4-dioxane in drinking water for two years exhibited no toxic effects or elevated incidence of tumors (Kociba et al., 1974).

Metabolic studies of 1,4-dioxane in rats suggest that there is a threshold for the toxic effects of dioxane and that this coincides with saturation of the metabolic detoxification pathway (Young et al., 1978). Animal carcinogenicity is only seen at high doses which saturate metabolic detoxification. No toxicity is observed at doses that are eliminated by first order kinetics that are not saturated.

Also, there is inconclusive evidence of skin tumor induction when mice were topically treated with dioxane and of cancer promotion when the topical application was preceded by 1 week with application of DMBA (NIOSH, 1977).

Air monitoring to measure worker exposure to 1,4-dioxane indicated that atmospheric levels of no more than 0.1 ppm on an 8 hr time weighted average (TWA) basis, with most analyses indicating less than 0.1 ppm, were present in the workplace atmosphere, even during open drumming procedures (Shell Oil Company, 1980; Conoco Chemicals, 1980). Point source monitoring of some key equipment areas indicated less than 1 ppm 1,4-dioxane in all instances (Conoco Chemieals, 1980). These values are all far below the current OHSA Standard of 100 ppm (TWA) and the ACGIH TLV of 25 ppm (TWA) for 1,4-dioxane. It has been shown that human volunteers exposed to 50 ppm 1,4-dioxane in air for 8 hours were able to completely metabolize (99.3% of total dose) or excrete (0.7% of total dose) unchanged this amount in the urine, and concluded that 1,4-dioxane would not accumulate in the body upon repeated exposure to levels of 50 ppm or less (Young et al. (1977).

5. Teratogenesis and Reproductive Effects

Pregnant rats were administered 50, 100, and 500 mg/kg/day of $C_{12-13}AE_1S$ by oral gavage on days 6-15 of gestation. Effects observed were a decrease in maternal body weight gain and food consumption. There were no treatment-related maternal effects noted at necropsy or following a uterine examination of day 13 of gestation. The incidence of fetal malformations in AES-treated groups was not different from the control group (Procter & Gamble Co., unpublished data).

Several investigators have studied the effects of administering a commercial liquid detergent formulation containing both AES and LAS to pregnant mice, rats and rabbits (Iimori et al., 1973; Iseki, 1972; Nolen et al., 1975; Palmer et al., 1975). Except at dosage levels which were toxic to the dams, no significant differences in the litter parameters of laboratory animals compared to control values were noted in these studies. Levels up to 300 mg/kg of a mixture containing 55% TE_3S and 45% LAS given orally to rabbits on days 2 through 16 of gestation up to 800 mg/kg given to rats on days 6 through 15 of gestation gave no indications of any embryotoxic or teratogenic effects attributable to AES (Nolen et al., 1975). Details of the studies cited above have besn previously reported (Part 1, LAS). There are no indications from the available data that detergent formulations containing AES at doses which are several orders of magnitude above possible human exposure levels posed any teratogenic hazard to laboratory test animals.

As part of a chronic feeding study cited previously, 10 rats/sex/group fed diets containing 0.1% of $C_{12}AE_3S$ (25% active) were mated after 14 weeks on test. The F_1 generation was maintained on the parental diet and mated at 100 days of age. The F_2 generation was fed the same diet for 5 weeks, then killed. No adverse effects on fertility, lactation, litter size or survival and growth of the offspring were seen. Hematological, biochemical and histopathological findings were all comparable to controls (Procter & Gamble, Co., unpublished data).

Similarly, no adverse parental toxicity or significant differences in either litter parameters or viability of offspring were noted in two generations of rats fed diets containing either 0.1%, $C_{12}AE_3S$ (Tusing et at., 1962) or 1% (800 mg/kg/day) of a detergent formulation containing 55% TE_3S and 45% LAS (Nolen et al., 1975).

6. Mutagenicity

Exposure in culture to n-pri-$C_{12-15}AE_3S$ did not increase the incidence of mutations in the bacteria, Salmonella typhimurium (strains TA 98, TA 100, TA 1535, TA 1537 or TA 1538; 2000 μg/plate) and Escherichia coli (WP2 and WP2 uvrA; 500 μg/plate), and did not induce mitotic gene conversion in the yeast, Saccharomyces cerevisiae JD1, (5 mg/mL) with or without liver microsomal activation. The frequency of chromatid and chromosome aberrations in rat liver cells exposed in culture to 100 μg/mL of surfactant for 24 hours did not differ significantly from that of control cultures (Shell Research Limited, unpublished data; Yam et al., 1984).

No morphological cell transformations were observed in Syrian golden hamster embryo cells exposed in culture to concentrations up to 50 mg/mL $C_{12-13}AE_{2.5}S$ (53:43) (Inoue et al., 1980).

In a series of studies with a 55% AES:45% LAS mixture, no significant differences from control values were noted in a dominant lethal study or in vivo or in vitro cytogenetic studies. In the dominant lethal assay, male mice were orally administered either 100, 150 or 200 mg/kg subacutely or 500, 750, or 1000 mg/kg acutely of the surfactant mixture. No significant differences from water-dosed controls were observed in the mutagenic index. Similarly, no significant differences in chromosomal anomalies were found in bone marrow cells of male rats given 40, 500 or 1000 mg/kg of the surfactant mixture orally, then killed 18, 24 or 48 hours post-dosing. Likewise, human leukocytes

incubated for 18, 24 or 48 hours with 4, 40 or 200 μg/L of the surfactant mixture exhibited no increased incidence of chromosomal anomalies above the water control group (Procter & Gamble Co., unpublished data).

An in vivo study indicated that AES alone is not clastogenic. Hope (1977) reported that the incorporation of $C_{12-15}AES$ into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosomes of rat bone marrow cells.

7. Pharmacology

a. Metsbolism

In both man and rat, an oral dose of $C_{16}AE_3S$ (labeled with ^{14}C in the 1-position of alkyl group) was readily absorbed from the gastrointestinal tract and excreted principally via the urine (see Table VI-1) with lesser amounts found in feces and expired air. Conversely, an oral dose of $C_{16}AE_9S$ (also labeled the ^{14}C in the 1-position of the alkyl group) was poorly absorbed by both species. Most of the recovered radioactivity was in the feces; less than 2% of the label appeared in rat bile within 72 hours. Small amounts of radioactivity were found in urine and expired air with less than 2% of the radioactivity remaining in the carcass, tissues and organs at 72 hours (McDermott et al., 1975).

TABLE VI-1

EXCRETION OF RADIOLABELED AES IN RAT AND MAN

% of Dosed Activity

(Recovered 72 hours after administration)

Compound	$^{14}C_{16}AE_3S$		$^{14}C_{16}AE_9S$		$C_{16}AE_3{}^{35}S$	$C_{16}A^{14}E_3S$
Species	Rat	Man	Rat	Man	Rat	Rat
Urine	50	80	0.6	4	62	66
Feces	26	9	82	75	26	19
Expired Air	12	7	4	6	--	0
Bile	--	--	1.8	--	--	--
% Recovery	93	96	93	85	90	91

McDermott et al., 1975

Oral doses of either $C_{16}AE_3S$ (labeled with ^{35}S in the sulfate group) or $C_{16}AE_3S$ (labeled with ^{14}C in the 1-position of the oxyethylene chain) were readily absorbed from the gastrointestinal tract of the rat and excreted primarily in urine. There was no evidence of hydrolysis of the sulfate group or of metabolism of the ethoxylate portion of the molecule (McDermott et al., 1975).

The length of the ethoxylate portion of an AES molecule appears to determine the metabolic fate of that compound following oral administration in both man and rat. $C_{16}AE_3S$ was readily absorbed, metabolized and excreted principally in the urine while $C_{16}AE_9S$ was poorly absorbed and excreted primarily unchanged in the feces. The major metabolite found in the urine of both man and rat following a dose of either $^{14}C_{16}AE_3S$ or $^{14}C_{16}AE_9S$ was isolated and identified. It had the following structure: $-OOCCH_2(OCH_2CH_2)_xOSO_3^-$ where x equals either 3 or 9, respectively (McDermott et al., 1975).

Taylor et al. (1978) studied the metabolic fate of orally, intraperitoneally or intravenously administered [1-^{14}C]-$C_{11}AE_3S$ or [1-^{14}C]-$C_{12}AE_3S$ in the rat. Both compounds were extensively metabolized (ω,β-oxidation) with the proportion of radioactivity appearing in urine and respired air generally independent of the route of administration. Some sex differences in the proportions of radioactivity excreted in urine and respired air were seen but total recoveries for $C_{11}AE_3S$ and $C_{12}AE_3S$ were comparable. The majority of radioactivity was excreted in urine but expired air was a significant route of elimination for the C_{11} derivative. By the oral route, 67% of the administered radioactivity with $C_{11}AE_3S$ appeared in urine of male rats compared to 45% in females; expired air contained 19% and 35% of administered radioactivity, respectively; 4-5% was present in feces for both sexes. With $C_{12}AE_3S$, only 2% of administered radioactivity was eliminated in expired air, 8-11% in feces with 86% (females) to 95% (males) excreted in urine. The major urinary metabolite of $C_{12}AE_3S$ was identified as 2-(triethoxy sulfate) acetic acid; with $C_{11}AE_3S$, the major urinary metabolite was tentatively identified as 3-(triethoxy sulfate) propionic acid.

Black and Howes (1979), measured the percutaneous absorption of ^{14}C labelled $NaC_{12}AE_3S$ and $NaC_{15}AE_3SO_4$. The $NaC_{12}AE_3S$ was applied to rats as 150 μl of a 1% w/v solution and the $NaC_{15}AE_3SO_4$ was applied as 200 μl of 1% w/v surfactant dissolved in 1% w/v LAS. The ^{14}C levels were measured in urine collected over 48 hours. Penetration of $NaC_{12}AE_3S$ was 0.39 $\pm$ 0.12 $\mu g/cm^2$ while that of $NaC_{15}AE_3SO_4$ was 0.26 $\pm$ 0.19 $\mu g/cm^2$. In experiments in which application was continued for up to 20 minutes, skin penetration was proportional to the duration of contact. It was also proportional to the number of applications.

In another study, an aqueous solution (0.6 mL of $C_{12}AE_3S$ labeled with ^{14}C at the 1-position of the alkyl chain) was rubbed in the skin of guinea pigs for 10 minutes. The test area was then washed and covered with a patch for 24 hours. Of the total radioactivity recovered (122%), 2.4% had penetrated the skin; 1.4% was excreted in urine, feces, and expired air; 57% remained at the site of application; and 62% was recovered in the wash rinsings (Prottey and Ferguson, 1975).

B. Human Studies

Instillation of 10-20% concentrations of a liquid formulation containing 9% AES into the eyes of human volunteers was nonirritating. Patch tests with 10% AES produced no skin irritation, while moderate irritation was noted with a 25% concentration in a 10-day occlusive patch test. Clinical trials with more than 1500 batches of AES in 70,000 women gave no evidence of allergic response.

1. Skin Irritation and Sensitization

It has been demonstrated that surfactants in solution induce the swelling of isolated human stratum corneum. The highest levels of swelling were induced by anionic surfactants. Increasing the degree of ethoxylation of AES while holding the alkyl chain constant at C_{12} to C_{14}, significantly lowers the level of swelling. However, when the degree of ethoxylation exceeds 6 moles of ethylene oxide, no additional reductions in the swelling response occurs (Rhein et al., 1986; Blake-Haskins et al., 1986).

Occluded patch tests with a single 24-hour application of 0.25 mL of a 1% aqueous solution of $NaC_{12}AES$ in 50 human volunteers produced no reaction in 45 individuals and only a slight irritation in five subjects (Smeenk, 1969).

No irritation was observed in a 24 hr patch test on humans (average of 10 subjects) with 10% concentrations of 20% active $C_{12-14}AE_3S$ (ALFONIC 1412-S™), $C_{12-15}AE_3S$ and $C_{12-15}AE_3S$ derived from NEODOL®25 alcohol. Moderate irritation was seen, however, with 25% concentrations of these products in a 10-day occlusive patch test with ten test subjects. The completely linear alcohol-based material was somewhat less irritating than the two slightly branched derivatives; i.e., irritation scores at the end of 10 days were 0.15, 1.1, and 1.0, respectively, of a possible

maximum score of 4 (Witco Chemical Corp., unpublished data).

A study by Tavss et al. (1985) demonstrated a correlation between surfactant-induced in vitro epidermis curling and in vivo skin irritation. A 2.4% solution of NH_4AE_3S caused strips of epidermis to twist and curl while a 10% solution applied to the forearm of subjects caused mild to moderate skin irritation by the fifth day of application.

A 25% concentration of a product containing 9% active NH_4AES was reported to be non-irritating to male and female genitalia when applied once daily for a two week period (Witco Chemical Corp., unpublished data).

Walker et al. (1973) have reported that clinical trials with more than 1500 batches of AES in 70,000 women gave no evidence of allergic response. The skin sensitization potential of AES in humans was evaluated using the Human Repeat Insult Patch Test (Stotts, 1980). AES concentrations from 0.14 to 0.03% were evaluated. There was no evidence of sensitization in the 581 panelists patch tested with AES. The AES evaluated has an alkyl carbon length from 12-18 and a molar ethoxylate ratio of 3 or 6 (Procter & Gamble Co., unpublished data).

2. Ocular Irritation

Ten and 20% concentrations of a liquid formulation containing 9% active NH_4AES as the only surfactant were found to be non-irritating following instillation into the eyes of 20 human volunteers (Witco Chemical Corp., unpublished data).

3. Epidemiology

a. Accidental Exposure

A unique outbreak of severe allergic contact dermatitis occurred in Norway in 1966. Associated with the use of a liquid dishwashing

product containing 18.7% C_{12}AES, this outbreak was eventually traced to a particular batch of C_{12}AES (LES 13-2035) (See discussion on page VI-5). Patch tests with a 30% concentration of the dishwashing product (6.5% AES) were performed on individuals who had developed dermatitis after use of the detergent. Positive responses occurred in all 23 individuals tested as well as in 3 of 29 controls. Similar tests with C_{12} AES (LES 13-2035) produced 18 of 18 possible positive responses and 5 positive responses in 29 control subjects (Magnusson and Gilje, 1973).

Walker et al. (1973) attempted to determine whether the outbreak was due to AES itself or to a contaminant present in this particular batch of AES. Sensitization studies in guinea pigs revealed that the sensitizing materials were, in fact, impurities and not AES.

A 45-year-old factory worker employed in a plant which manufactured AES (LES 13-2035) developed dermatitis of the hands and other parts of the body some weeks after emptying this material from barrels into a mixer. Several of the barrels contained the AES batch (LES 13-2035) discussed on page VI-5, and subsequently shown to be contaminated with certain sultone sensitizers. In pateh tests with 1% aqueous solutions of batch LES 13-2035 of AES, another batch of AES, the extracted unsulfated matter present in bateh LES 13-2035 and a distillate of the C_{12} alcohol raw material, the subject responded positively to batch LES 13-2035 which contained sultone sensitizers, to AES, and to the extracted unsulfated material in LES 13-2035, but not to the lauryl alcohol extract (Magnusson and Gilje, 1973).

No further reports of injury resulting from human exposure to AES in use or manufacturing situations have been found.

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Appendix A

AES Nomenclature and Abbreviations

APPENDIX A - AES NOMENCLATURE AND ABBREVIATIONS

Throughout this chapter the designation AES has been used to indicate alcohol ethoxy sulfates. The number of carbon atoms in the alkyl chain is numerically designated with a subscript. Mixtures of various alkyl chain lengths are indicated by a numerical range and, if available, the ratio of each carbon chain length is given in parentheses immediately thereafter.

The degree of ethylene oxide polymerization is given by a subscript which indicates either the average number of ethylene oxide units, if the designation is a single number, or a range. For example:

> n-NaC_{12-14} (40:60) AE_3S - the linear, sodium salt of alcohol ethoxy sulfate consisting of 40% C_{12} and 60% C_{14} and possessing an average of three ethylene oxide units.

Occasionally, the abbreviation TES has been used to indicate tallow alcohol ethoxy sulfates which are derived from natural alcohols and usually contain 16-18 carbon units.

All concentrations of AES surfactants named in this chapter are expressed as "active" unless otherwise specified.