Environmental and Human Safety of Major Surfactants

Volume 1. Anionic Surfactants

Part 4. Alpha Olefin Sulfonates

Final Report To:
The Soap and Detergent Association
475 Park Avenue South
New York, New York 10016

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Synopsis

Alpha olefin sulfonates (AOS) are efficient readily biodegradable cleaning agents that possess a high degree of chemical stability and have good water solubility characteristics. AOS usage in consumer products in the U.S. today is mainly in liquid hand soaps with minor use in shampoos. AOS use outside the U.S. has principal consumer applications in household cleaning and personal care products. Current estimates indicate that AOS represents approximately 1% of the total anionic surfactants utilized worldwide.

AOS products are mixtures of two major components: sodium alkene sulfonates and hydroxyalkane sulfonates with the sulfonate group in the terminal position and the double bond or hydroxyl group located at various positions along a linear aliphatic chain. Although actual data are limited, and the biodegradation pathways for AOS are not well defined, these compounds appear to be readily biodegraded under both laboratory and environmental conditions. For example, influent sewage containing about 2% AOS as a fraction of total surfactant content has been shown to be completely cleared of AOS during passage through a sewage treatment plant.

In terms of AOS impact on water quality, there are presently no standards in the U.S. or Europe specifically referring to alpha olefin sulfonates. If present, these anionic surfactants are included among those measured in the environment using the MBAS method. AOS is not specifically monitored in either the U.S. or Europe.

Aquatic toxicity data report LC_{50} values in fish ranging from 0.3mg/L to 21 mg/L, with the harlequin fish being the most sensitive. Longer-chain AOS compounds are consistently more toxic than those with shorter alkyl chains. *Daphnia magna* is the only invertebrate that has been tested, and LC_{50} values range from 7.0 mg/L for $C_{16\text{-}18}$AOS to 18.0 mg/L for $C_{14\text{-}16}$AOS.

Little information is available on the subacute or chronic effects of AOS on aquatic organisms. The gills appear to be the primary site of AOS toxicity. Toxicity is directly related to changes in interfacial tension between the gill and water, since oxygen absorption is thought to be severely hindered when the tension decreases beyond a certain critical point. Protein complex formation between dissolved surfactants and gill surface tissues was thought to be another primary mode of action. ^{14}C-AOS accumulates primarily in the gills and secondarily in the gall bladder.

The only study found concerning the toxicity of AOS to plants showed no significant effect on the germination or growth of tomato, barley and bean plants watered with solutions of 10, 25, or 40 mg/L AOS.

The mammalian toxicity data that are available for AOS at doses far in excess of normal use levels and the relative ease of AOS biodegradation indicate that the use of these surfactants does not pose a significant hazard to human health. Their safety is recognized by the Food and Drug Administration which has approved their use as indirect food additives. The ammonium, calcium, magnesium, potassium and sodium salts of AOS are approved for use with the stipulation that the alkyl group is in the range of C_{10-38} with not less than 50% in the range of C_{14-16}.

The alpha olefin sulfonates exhibit a moderately low order of toxicity in rodents. AOS is slightly to severely irritating to rabbit skin depending on concentration. Skin sensitization observed in guinea pigs has been attributed to the presence of unsaturated 1,3-sultones and chlorosultones. These sultones are not normally present in commercial formulations. AOS concentrations of 1% are not ocular irritants in rabbits, but concentrations greater than 5% are capable of producing severe ocular damage. A single set of mutagenicity experiments showed a positive response in a host-mediated assay with rats; however, the response may be due to minor components in the sample tested. All other mutagenicity studies are negative. With respect to teratogenic effects, an increase in cleft palates was found in offspring of mice given 300 mg/kg of AOS by gavage during gestation as well as an increased incidence of minor skeletal anomalies in both mice and rabbits at this dose level. However, these responses occurred only at doses where the dams exhibited toxic responses and are not considered to be primary effects of AOS. Carcinogenicity studies have yielded negative results.

AOS is readily absorbed after oral administration to rats. It is primarily excreted in the urine. The metabolic fate of orally and intravenously administered AOS was studied in male Wistar rats. After oral administration, about 80% of ^{14}C-AOS was rapidly absorbed from the gastrointestinal tract. The blood level peaked at 3 hours. Within 24 hours of the dose, 72% was excreted in the urine and 22% in the feces, some of which may have resulted from primary biliary excretion. After intravenous injection, 50% of the dose was excreted in the urine within 1 hour and 90% appeared in the urine within 6 hours. These results suggest that no accumulation of ^{14}C-AOS occurs and that it is rapidly absorbed, metabolized and excreted.

The percutaneous absorption of ^{14}C-labeled AOS has also been studied in male Wistar rats under various conditions. Absorption through the intact skin is minimal. Application to the intact dorsal skin resulted in the absorption of 0.6% of the applied dose, while application of the same volume to damaged skin increased absorption 80-fold.

In human volunteers, negligible to mild skin irritation was observed in 24-hour patch tests with 1-2% active AOS samples. Also in humans, increased irritation was noted as the study progressed in a 10 day occlusive patch test with a 0.8% active concentration of AOS. Positive sensitization responses reported in one study have been attributed to the presence of unsaturated 1,3-sultones which are not normally present in commereial formulations.

ALPHA OLEFIN SULFONATES

I. INTRODUCTION

Alpha olefin sulfonates (AOS) have been available since the 1930s. AOS are reportedly efficient, readily biodegradable cleaning agents (Kerfoot and Flammer, 1975; Yamane and Okumura, 1989; Yamane, 1992). They offer detergency and foam properties comparable to those of LAS and possibly possess a slight advantage over LAS in hard water (Marquis, 1968). AOS also possess a high degree of stability and have good water solubility characteristics (Marquis, 1968; Marquis *et al.*, 1966; Tomiyama *et al.*, 1969).

AOS is used extensively in Japan and South Korea in both laundry and liquid dishwashing detergents (Okumura, 1985). In the U.S., AOS was used in liquid dishwashing detergents in the 1970's, in place of some LAS and alcohol ethoxysulfates. AOS usage in the U.S. today in consumer products is mainly in liquid hand soaps with minor use in shampoos. AOS usage has increased significantly outside the U.S., with principal commercial applications including household cleaning and personal care products (Yamane, 1980 and 1992; Okumura, 1985). Worldwide and North American consumption estimates for AOS are 40 thousand metric tons and 10 thousand metric tons, respectively. Current estimates indicate that AOS represents approximately 1% of the total anionic surfactants consumed worldwide (Bryan, 1988).

This review was prepared to evaluate the information currently available on AOS with respect to:

(1) environmental fate and distribution including biodegradation,
(2) effects on wild and domestic flora and fauna,
(3) product use and safety for humans as indicated by tests with laboratory animals and by data on human exposure.

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II. CHEMISTRY

A. Product Chemistry

Commercial AOS are produced by reaction of vaporized sulfur trioxide with linear α-olefins followed by immediate neutralization of sultone intermediates. AOS products are mixtures of two major components: alkene sulfonates and hydroxyalkane sulfonates, where the double bond and hydroxyl groups may be located at various positions along the aliphatic chain. In recent years new processes have been developed for the manufacture of high quality α-olefins.

The development of advanced sulfonation teehnology (improved falling-film type reactors) in conjunetion with improved availability of high quality α-olefin stock has allowed the manufaeture of high quality AOS product without bleaching. There are three major stages in the production of AOS. These are:

(1) Synthesis of n-,α-olefins,
(2) Sulfonation of n-,α-olefins with subsequent aging for a short period, and
(3) Neutralization/hydrolysis of the resultant acid mixture to saponify the sultone by-products to sulfonates.

1. Synthesis of n-,α-olefins

The alkyl chain lengths of the α-olefins utilized in the production of commercial AOS generally range from fourteen to eighteen carbons; α-olefins with eleven through twenty alkyl carbons have also been used (Marquis, 1968). Industrially, α-olefins are synthesized via oligomerization of ethylene using either Ziegler (tricthyl aluminum) or non-Ziegler catalysts. Alpha-olefins can also be produeed via thermal cracking of paraffin wax but, due to limited availability of waxy crudes and other process deficiencies, this method is not being practiced commercially at this time. Even-numbered, ethylene-derived α-olefins (C_4-C_{30}) are purer than paraffin-derived olefins in α-olefin content, and are reported not to contain the diolefinic and naphthenic components found in paraffin-derived α-olefins (Stepan Chemical Company, unpublished data). Minor components found in ethylene-derived α-olefins include branched and internal olefins, as well as traces of

paraffins, diolefins and aromatics (Turner, 1982).

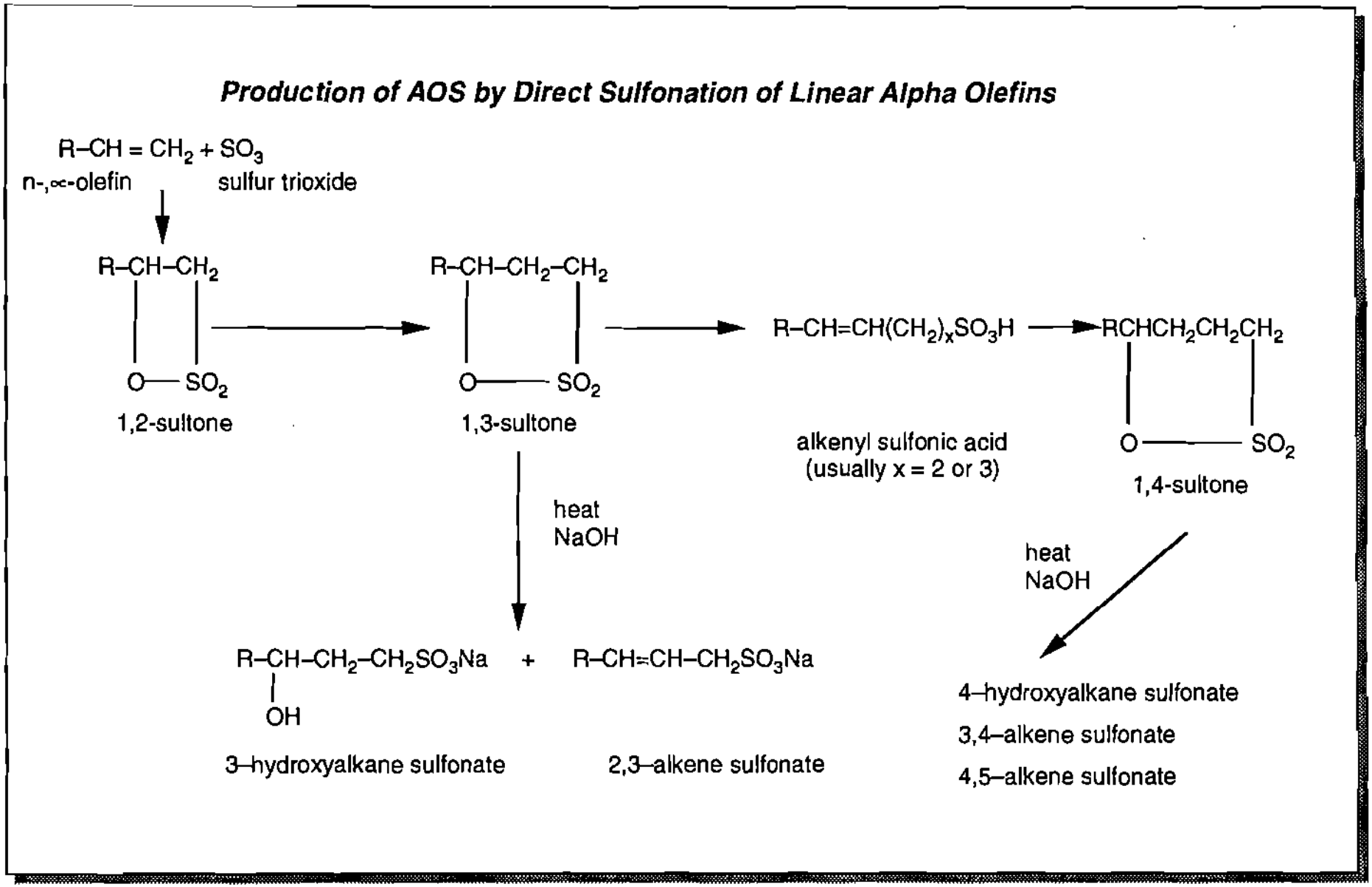
High quality detergent range olefins contain approximately 92-96% (weight) linear α-olefins; branched olefins are present at approximately 1-5% (weight). Branching results when olefins other than ethylene enter the chain and tends to increase with carbon number in some processes more than others. Most of the branching (60-80%) is at the 2-carbon position; the remaining branched components are 1-alkenes where branching is remote from the double bond. Linear internal olefins are generally present at levels of 1-3% (weight), the level increasing slightly with carbon number. Internal olefins arise primarily by isomerization of 1-alkenes during purification. The major internal olefins are linear cis- and trans-2-alkenes. Normal paraffins and other saturated hydrocarbons are present only at trace amounts; diolefins and aromatics are expected at even lower levels (Shell Chemical Co., 1982).

2. Sulfonation

The development of improved continuous SO_3 film sulfonation technology and refinement during the 1970s of the neutralization/hydrolysis step has permitted production of high quality AOS for consumer products. The direct sulfonation of α-olefin with SO_3, via continuous falling film techniques, involves two parallel reactions: the formation of alkenylsulfonic acid with the concomitant migration of the double bond and the production of intermediate sultones, as shown in Figure II-1. Some by-products of the sulfonation reaction are shown in Figure II-2.

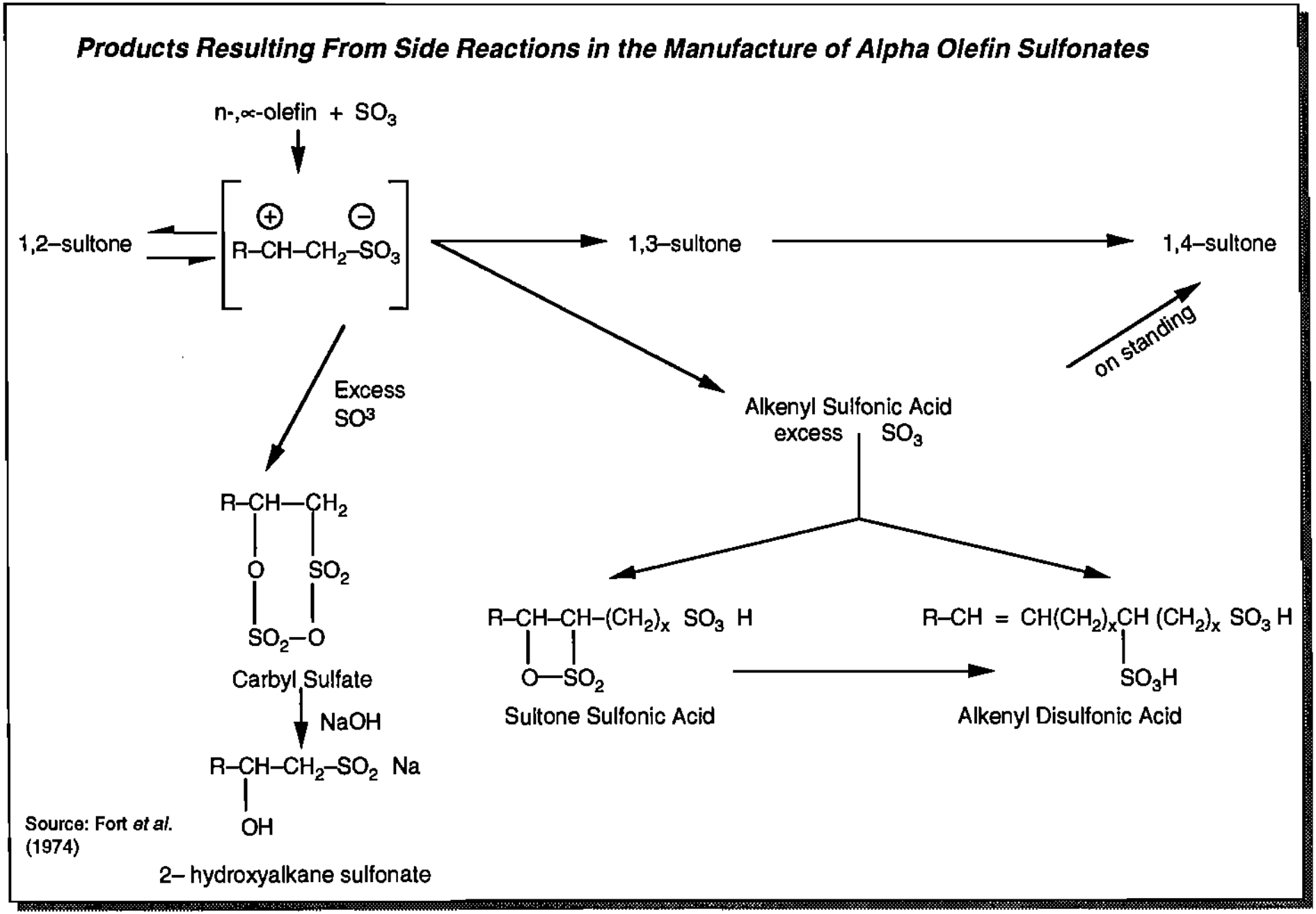
With continuous thin film reactors, SO_3:olefin molar ratios in the range of 1.0 to 1.2 are typically employed (Fort, 1974), and a complex mixture of alkene mono- and disulfonic acids, sultones, and some unreacted olefins is obtained. Variations in the molar ratios of the reactants or slight modifications in the reaction conditions can alter the composition of the reaction products and lead to the formation of a variety of secondary products. In addition, the overall sultone content of the acid reaction mixture may increase on standing, at the expense of the alkylsulfonic acid. Reaction conditions are set to obtain high AOS yield (i.e., low levels of unsulfonated organics) without introducing excessive color or sodium sulfate. Hashimoto *et al.* (1973) analyzed α-olefin sulfonic acids with NMR spectroscopy and found a mixture of alkene sulfonic acids, 1,3-sultones, 1,4-sultones, and olefins. The sultones are converted to alkene sulfonates and hydroxyalkane sulfonates during hydrolysis. Two unknown peaks, believed to be precursors to Δ'-alkene disulfonic acid, were assigned to 1,2-alkane disultones.

Figure II-1



Note: R stands for the remainder of the n-alkyl group not specified explicitly in the chemical formula. The number of carbon atoms in the R-group thus varies through the reaction scheme.

Figure II-2



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It is believed that the metastable 1,2-sultone and/or its zwitterion may also be formed as an intermediate in the sulfonation of α-olefins (Mori *et al.*, 1971). In the presence of excess SO_3, the 1,2-sultone reacts rapidly to form carbylsulfate (cyclic pyrophosphate) which upon hydrolysis yields the 2-hydroxysulfonate, a less desirable component due to its limited solubility. In addition, the 1,2-sultone is inherently unstable and upon standing (2-15 min.) rapidly isomerizes to the 1,3-sultone which upon hydrolysis yields the more soluble 3-hydroxysulfonate. If the mixture is allowed to age too long, the 1,3-sultone isomerizes to the 1,4-sultone, which is much more difficult to hydrolyze (Kirk-Othmer, 1983).

3. Neutralization/Hydrolysis

In order to convert the acid reaction products to water soluble surfactants, the reaction mixture is neutralized and the products are hydrolyzed with a slight excess of sodium hydroxide, thus saponifying the intermediate alkane sultones. Use of appropriate hydrolysis conditions is important to ensure low levels of alkane sultones, some of which may be mild skin sensitizers (Roberts and Williams, 1983). The hydrolysis rate decreases in the order of 1,2-sultone > 1,3-sultone > 1,4-sultone. Mildly alkaline hydrolysis conditions favor the formation of hydroxyalkane sulfonates while elevated temperatures and/or the presence of a limited supply of water favors the formation of alkene sulfonates. If sultone sulfonic acids are present during hydrolysis, they yield hydroxyalkane and alkene disulfonates, while alkylcarbyl sulfates, if present, readily hydrolyze to 2-hydroxyalkane sulfonate (Shell Chemical Co., unpublished data).

This process typically yields AOS mixtures of approximately 60-65% alkene sulfonates, 30-35% hydroxylalkane sulfonates, and 5-10% disulfonates. Various positional isomers of the alkene sulfonates and hydroxyalkane sulfonates have been reported (Gentempo, 1985; Williamson, 1993). Sodium C_{14-16} AOS is typically shipped as a 35-40% active matter solution in water. Sodium C_{16-18} AOS is typically a 28-30% AM slurry in water at ambient temperature.

The formation of certain unsaturated and chloro-sultones as by-products in AOS surfactants has been addressed by Roberts and Williams (1983) and Roberts *et al.* (1987). Roberts and Williams (1983) demonstrated the production of highly potent skin sensitizers, 2-chloroalkane-1,3-sultones and alk-1-ene-1,3-sultones, from the reaction of hypochlorite bleach and alk-2-ene sulfonates, normally contained in AOS at approximately 30% of the active matter. They also demonstrated the production of 3-chloroalkane-1,4-sultones and 4-chloroalkane-1,3-sultones from the reaction

of alk-3-ene sulfonates, minor components of AOS, and hypochlorite bleach. In the more recent paper, Roberts *et al.* (1987) examined the conditions under which alk-1-ene-1,3-sultones are formed and how their formation in AOS can be avoided. The authors determined that alk-1-ene-1,3-sultones are not formed to a detectable extent (0.5 ppm), even under very severe sulfonation conditions, unless the AOS is treated with hypochlorite bleach, emphasizing the importance of avoiding such bleaching in AOS manufacture. Alk-1-ene-1,3-sultones were present at significant levels (up to 80 ppm) in all AOS samples known to have been treated with chlorine bleach.

B. Analytical Methods

Commercial AOS mixtures are composed primarily of alkene sulfonates, hydroxyalkane sulfonates and disulfonates with several positional isomers in each series. Colorimetry methods such as the MBAS method involving reaction of the sulfonate group may be used for non-specific analysis of AOS; however, disulfonate species may interfere with the reaction. NMR spectroscopy has also been reported to be useful in determining alkene sulfonates in AOS mixtures. Chromatographic techniques are becoming more widespread and represent a valuable tool for AOS analysis. Paper chromatography, TLC, GC with derivatization, and HPLC have all been utilized for AOS analysis. Reverse-phase HPLC with methanol:water eluent contoining a salt or acidic modifier has been particularly useful for the separation and determination of the major components of AOS mixtures. Separation of congeners and positional isomers within the component categories has also been achieved with HPLC.

1. Anionic Surfactant Methods

Many articles and reviews have documented the analysis of anionic surfactants. Since LAS are the most widely used of the anionic surfactants, they have also been the most widely studied with respect to analytical methods. There are fewer references to AOS-specific methods in the literature. However, since many of the LAS methods are not specific to LAS analysis, they may be used for the analysis of other anionic surfactants, including AOS.

Most of the physical methods (e.g., determination of foaming potential and measurement of surface tension) are applicable to AOS surfactants; these have been described previously and the

reader is referred to the LAS chapter. Similarly, chemical techniques (colorimetric, volumetric, or potentiometric methods) demonstrated for other anionic surfactants are expected to be applicable to AOS surfactants as well since they usually depend on reaction of the sulfate or sulfonate group. Colorimetric methods in general and the MBAS method in particular (described in the LAS chapter) are useful tools for the analysis of AOS. Oba *et al.* (1968) reported good reproducibility with the MBAS method in the determination of AOS, i.e., the alkene sulfonates and hydroxyalkene sulfonates, at concentrations about 0.8 µg/mL. However, disulfonate salts (impurities present in various proportions in commercial AOS formulations) tend to reduce the methylene blue response.

Newer physicochemical techniques developed for the analysis of LAS or anionic surfactants, in general, may also be applicable to AOS analysis. These techniques include chromatographic techniques such as TLC, GC, GC/MS, and HPLC, as well as NMR, polarography, and MS/MS. The speeific procedures for isolation, derivatization, separation, and detection of AOS may differ from those developed for LAS. 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. AOS-Specific Methods

This section is limited to a discussion of methods that have been demonstrated for the analysis of AOS and which have been reported in the literature.

a. Titration Methods

A simple, rapid method is available for the quantitative determination of alkene-1-sulfonates, principal components of commercial AOS products, without interference from the hydroxyalkane-1-sulfonates which arc also present (McClure, 1978). The analysis involves titrating the sample in 98% acetic acid-2% water solution with a stream of ozone to a photometric endpoint using Rouge Organol BS indicator. The method detects the presence of both unreacted olefins and sodium alkene-1-sulfonates. An extraction-GC process is then utilized to measure the typically small amounts of unreacted olefins; the alkene-1-sulfonate content is determined by difference. Relative percent differences of less than 1% were reported for replicate samples containing 0.5 to 1.5 meq/g of unsaturation. Parallel analyses of a known sample by ozone titration and NMR gave values which were within 2% of each other.

Standard two-phase titration with Hyamine 1622 and a color indicator proved to be a fast and precise method of analysis for low molecular weight AOS, containing low levels of disulfonates (Beranger and Holt, 1986). For heavier AOS, incremental additions of Hyamine over several days was required; in the presence of ~20% disulfonates, titration was not possible. With the addition of Na_2SO_4, as suggested by Wickbold (1971) for mixtures containing disulfonates, AOS were titrated more easily; titration of heavy AOS was faster but still required Hyamine additions over several days. Heating the reaction vessel to 40°C was reported to result in a quicker reaction.

Oba *et al.* (1976) reported the quantitative analysis of AOS in sewage or river water samples by infrared (IR) spectroseopy. Anionic surfactants were extracted with chloroform as methylene blue (MB) complexes. Sulfate-type surfactants were removed by acid hydrolysis; the residual sulfonate-type surfactants, including AOS, were released from the MB complex by acid hydrolysis and converted to sulfonyl chloride derivatives for IR spectroscopy. AOS surfactants were identified by IR bands at 524 cm^{-1}.

Crilly and McGowan (1962) also used IR procedures for sulfonate determinations, while Hashimoto *et al.* (1973) employed NMR spectroscopy. In their review of analytical methods available for surfactants, Llenado and Neubecker (1983) reported that AOS surfactants can also be analyzed by MS techniques. The secondary ion emission patterns of AOS and other anionic surfactants were described by Nelen *et al.* (1981).

^1H NMR spectroscopy was used to determine alkene sulfonates in AOS mixtures by Castro *et al.* (1985); under normal conditions, 1-alkene sulfonates show a signal separated from the other positional isomers. ^{13}C NMR spectroscopy, although not as quantitative, provides valuable information on the cis/trans ratio of the main positional isomer (Boyer *et al.*, 1982). Gentempo *et al.* (1985) also reported the use of ^{13}C NMR spectroscopy to observe the carbon backbone of AOS compounds. Relatively pure fractions of alkene sulfonates and hydroxyalkane sulfonates were obtained by fractional crystallization of AOS; chemical shifts of the functionalized carbons of these fractions were assigned. The authors reported that a routine method for the determination of alkene isomer distribution and alkene sulfonate/hydroxyalkane sulfonate ratios is being developed.

b. Chromatographic Methods

Because commercial AOS products are complex mixtures of positional isomers of alkene sulfonates, hydroxyalkane sulfonates, and disulfonates, various chromatographic methods have been useful in the analysis of AOS. Fudano and Konishi (1971) applied a salting-out chromatographic technique to the determination of mixtures of hydroxyalkane sulfonates and alkene sulfonates, and Puschel and Prescher (1968) used paper chromatography and UV detection procedures to separate and identify the monosulfonates obtained during the sulfonation of α-olefins.

Allen and Martin (1971) developed a TLC technique for the separation of alkene and hydroxyalkane mono- and disulfonates. Following separation, the material was charred with heat and SO_3 fumes, then quantified by photodensitometric determinations. The method was reported to be linear in the 0-5 μg range, with decreased sensitivity at higher concentrations. This procedure can be modified to detect unhydrolyzed sultones. Maruyama *et al.* (1982) also reported the TLC separation of alkene sulfonates and hydroxyalkane sulfonates in AOS mixtures.

AOS, as such, are not sufficiently volatile to be analyzed with gas chromatography. However, the sulfonates can be hydrogenated, converted to their sulfonyl chloride derivatives and subsequently subjected to thermal decomposition gas chromatography (Nagai *et al.*, 1970; Kirkland, 1960).

Separation and determination of the components of AOS mixtures by high performance liquid chromatography (HPLC) has been shown to be rapid, and does not involve derivatization. Improved technology, quality and availability are making HPLC a valuable tool for surfactant analysis. However, the difficulty in preparing pure standard AOS components in addition to the number of components and isomers present in AOS mixtures make quantitative analysis difficult.

Several applications of HPLC in the analysis of AOS surfactants have been summarized by Jandera (1984). Zeman (1981) reported the separation of AOS into components containing various functional groups by HPLC on silica gel columns using n-hexane/isopropanol/water/acetic acid as the mobile phase. The compounds eluted in the following order: alkene sulfonates, hydroxyalkane sulfonates, sulfate sulfonates, and disulfonates. However, the differences in the retention times of homologs were not very significant. The method was tested on α-olefin

sulfonates with 12, 14, 16, and 18 carbon atoms and applied to analysis of commercial AOS samples.

Johannessen *et al.* (1983) described a qualitative reverse-phase HPLC method for separating the various components of AOS mixtures. The method utilized a Dupont Zorbax TMS column and a methanol:water (3:1) mobile phase containing 0.4 M sodium nitrate. Separation of most C_{14} and C_{16} hydroxyalkane sulfonates and alkene sulfonates by carbon number, hydroxy group position, and double bond position was achieved. Overlap of the C_{16} 3-hydroxylakane sulfonate and C_{14} 2-alkene sulfonate peaks was observed. Disulfonate peaks, the most polar components of AOS, eluted early in this reverse-phase HPLC system.

Castro and Canselier (1985) also demonstrated the separation and determination of AOS components (i.e., alkene sulfonates, hydroxyalkane sulfonates, and disulfonates) by reverse-phase HPLC on Altex Ultrasphere C_{18} column) with acidified (10^{-3}M HNO_3) methanol: water (3:1) as eluent. Quantitative determinations were made possible by the use of a moving-wire flame ionization detector (FID). The linear relationship between log k (retention) and carbon number was demonstrated for each class of AOS components. Separation and quantitative determination of the components of commercial C_{14} AOS and C_{14}-C_{16} AOS and a pilot-plant C_{16} AOS mixture were achieved with this method. Separation of alkene sulfonate isomers by double bond position was not demonstrated.

Beranger and Holt (1986) presented an improved HPLC technique for quantitative determination of the mono-sulfonate components in AOS mixtures. The method utilized a Merck CH-18 column with a methanol: water eluent containing 0.2 M $NaNO_3$. A UV detector and a refractive index detector were used. Optimal separation was achieved with 15% water for C_{14}-C_{18} AOS, with 10% water for C_{16}-C_{24} AOS, and with 5% water for C_{20}-C_{30} AOS. The authors report that the problem of unresolved C_{16} 3-hydroxyalkane sulfonate and C_{14} 2-alkene sulfonate peaks was avoided; separation of C_n 3-OH sulfonates and C_{n-2} 2-alkene sulfonates was achieved provided the solvent system contained at least 10% water.

Analytical techniques not involving good separation of AOS components provide only total percentages of the major components (e.g., alkene sulfonates or hydroxyalkane sulfonates) in mixtures containing individual compounds of various carbon chain lengths. For this reason, it is difficult to compare the results of volumetric, spectroscopic, and calorimetric determinations with

the results of chromatographie procedures such as HPLC. Keeping this in mind, however, the relative amounts of alkene sulfonates and hydroxyalkane sulfonates determined by iodinc value and hydroxyl value, in conjunetion with alkene sulfonate isomer determinations by ^1H NMR spectroscopy, were reported to give data consistent with those obtained by HPLC (Castro and Canselier, 1985).

An isotachophoresis method for determining weight percents (%w) of monosulfonates and disulfonates and total AOS (%w monosulfonates and %w disulfonates) has been summarized (Shell Chemical Co., 1984). This method has been used routinely in research laboratories for quantifying AOS components, in conjunction with HPLC methods for measuring hydroxyalkane sulfonates.

3. Methods for Minor Components in AOS

Several papers have reported the use of chromatographic techniques for the analysis of residual amounts of alkane sultones present in AOS surfactants and formulations. Slagt *et al.* (1976) described a HPLC method for the quantitative determination of both 1,3- and 1,4-sultones with a limit of detection of 10 mg/kg. Duplicate determinations of a known sample of 1,4-sultone gave standard deviations of 8-9 mg/kg at a level of 300 mg/kg. The reliability of the method was tested by the addition of known amounts of 1,3- and 1,4-sultones. All of the added 1,4-sultone was recovered quantitatively, even when extracted 11 days after spiking. Eighty-five percent (85%) of the 1,3-sultone, which is far less stable, was recovered one hour after spiking and only 45% of the added amount was detected after 11 days.

Wolf and McPherson (1976) reported the separation and semiquantitative estimation of nine C_{14} sultones by TLC on silica gel. Vapor phase charring with 20% fuming sulfuric acid and measurement of the charred compounds by photodensitometrie determinations gave detection limits of several ppm sultones in olefin sulfonates. This method is limited by the usual quantitative problems of TLC.

Callahan *et al.* (1976) developed a GC method specifically for the analysis of C_{14}-1,4-sultones, unsaturated 1,3-sultones, and 2-chloro 1,3-sultones but the method has also been used for other sultones. The method is reportedly capable of detecting less than 1 ppm of these sultones in a 38% AOS solution. In recovery studies with C_{14}-1,4-sultone, a standard deviation of $\pm$ 2 ppm was

observed at 10-30 ppm levels of sultone.

Brain *et al.* (1984) used tandem mass spectrometry (MS/MS) for the analysis of trace levels of unsaturated 1,3-sultones and their precursors (hydroxy-1,3-sultones and chloro-1,3-sultones) in raw materials, formulated AOS surfactant mixtures, and their solutions. Chromatographic methods (TLC, HPLC, GC, GC/MS) have been used to measure unsaturated sultones but lack the required sensitivity, selectivity and freedom from matrix interferences. The negative ion chemical ionization (CI) MS/MS method presented by Brain *et al.* (1984) provides excellent sensitivity and selectivity free of matrix interferences. Mean precision and recovery values for the unsaturated 1,3-sultones at concentrations ranging from 0.01 ppm to 100 ppm in AOS surfactants are 10% and 85-95%, respectively. Similar values were obtained for unsaturated 1,3-sultone analysis in formulated AOS products and dilute AOS solutions.

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III. BIODEGRADATION

Biodegradation studies with AOS are somewhat limited, but the data available indicate AOS are readily biodegraded under both laboratory and environmental conditions.

A. Laboratory Test Systems

Although scant, the available data indicate AOS are quickly and readily biodegraded as shown by BOD (51.6% at 5 days) and evolved CO_2 data (65.7%) for AOS in the C_{12}-C_{18} range. AOS are completely degraded (100% MBAS) in both fresh and seawater within 3 to 5 days under aerobic conditions. Some anaerobic degradation also occurs, ranging from 19-34% at 14 days to 31-43% at 28 days.

1. Oxygen Uptake - Biochemical Oxygen Demand

The O_2 uptake of C_{14}-C_{18} α-olefin sulfonates has been reported as 85% of ThOD in the closed bottle test (Gerike, 1987). The biochemical oxygen demand for AOS in the C_{12}-C_{18} range and containing up to 40% hydroxyalkane sulfonates averaged 51.6% at 5 days, while under the same conditions, glucose had a BOD_5 of 69.6% (Procter & Gamble Co., unpublished data).

2. CO_2 Evolution

Kravetz *et al.* (1982) determined the primary and ultimate biodegradability of a series of single carbon cut AOS (C_{12}, C_{14}, C_{16}, C_{18}) by CO_2 evolution and MBAS using a modified shake flask procedure with aclimated microorganisms. The tested AOS samples were found to undergo 98-99% primary biodegradation within 3 days. Alkyl chain length was noted to affect the rate of ultimate biodegradation of the samples, however. Although C_{12}AOS and C_{14}AOS degraded similarly (~65% evolved CO_2 by 30 days), increasing the alkyl chain from C_{14} to C_{18} decreased the rate and amount of evolved CO_2. All AOS test samples, however, reached greater than 50% mineralization within two weeks; 75 to 80% of a glucose sample was mineralized in the same time period.

The percent of theoretical CO_2 evolved with the AOS surfactants, cited in the oxygen uptake section above, averaged 65.7% compared to 87.5% for glucose (Procter and Gamble Company, unpublished data).

3. Die-Away Tests

a. River Water Test

The degradation of AOS in river water is rapid. Kikuchi (1985) studied the degradation of C_{15-18}AOS (MBAS) at various temperatures in water from the Tama River, Japan. Degradation was complete within 2-5 days at temperatures ranging from 15-27°C, while at 10°C roughly 75% degradation occurred after 9 days. Using water from the same river, Sekiguchi *et al.* (1975a) found that on the third day of the study, no AOS (20 mg/L added) could be detected (as measured by MBAS) in samples of Tama River water. The extent of degradation of 5 mg/L AOS in seawater was also examined; no MBAS activity could be detected in samples of seawater at 5 days. Similar findings were reported for C_{15-18} AOS by Marquis *et al.* (1966).

b. Fortified and Inoculated Waters

Alpha-olefin sulfonates are readily biodegraded in screening tests that employ fortified and inoculated waters. Gerike (1987) reports 85% removal of C_{14-18}AOS in the Modified OECD screening test (based on COD removal) and 99% MBAS removal in the OECD screening test.

With an activated sludge inoculum, Miura *et al.* (1979) found that the MBAS response to 100 mg/L AOS virtually disappeared by 15 days while removal of TOC approached 90% by 8 days. In another study, Itoh *et al.* (1979) reported complete biodegradation (100% loss of MBAS response) of C_{12}AOS and approximately 30% of theoretical CO_2 evolved by 10 days.

Gafa and Lattanzi (1974) found that three commercial AOS products all degraded (as measured by MBAS) greater than 90% within 4 days in a die-away static type test (Swiss EMPA method, which employs BOD water innoculated with clarified sewage). The products tested were 100% linear C_{14-16}AOS (BIOTERGE AS 35-CL™), and >95% linear C_{15-18}AOS (HOSTAPUR OS™). Similar findings were reported by Lundahl *et al.* (1972).

c. Shake Culture Test

In a shake culture test with Bunch-Chambers media, Sekiguchi *et al.* (1972) noted that C_{15-18}AOS lost more than 99% of its MBAS activity and 90% of its total organic carbon content in one day. By 5 days, 100% of the surfactant (TOC) had been removed. Using the Soap and Detergent Association shake culture test, Marquis *et al.* (1966) reported 96-97% removal of C_{15-18}AOS. The duration of the study was not given.

Oba *et al.* (1968) examined the biodegradability of C_{15} alkenylsulfonate [pentadecene-(2)-sulfonate-(1)], C_{14} hydroxyalkane sulfonate [3-hydroxytetradecane sulfonate-(1)] and three linear C_{15-18}AOS with varying (<4, 15, and 50%) disulfonate content in a shake culture system. All compounds biodegraded (as measured by MBAS) greater than 96% with no significant difference noted due to disulfonate content.

4. Simulated Treatment Processes

a. Activated Sludge

Maag *et al.* (1975) found that 97%, 98% and 94% of a 20 mg/L concentration of C_{14}AOS, C_{16}AOS and C_{14-18}AOS had been removed in 17, 7 and 8 days, respectively, using the OECD confirmatory test (1971).

b. Anaerobic Systems

Oba *et al.* (1967) compared the anaerobic degradation of linear C_{15-18}AOS in a shake culture system in which the inoculum used was either activated sludge obtained from a sewage treatment plant or sludge removed from the bottom of a private cesspool. In the sewage plant sludge system, 19% of the surfactant had degraded (as measured by MBAS) by 14 days and 31% had been degraded at 28 days. Somewhat improved degradation was seen in the system employing sludge from a private cesspool; i.e., 34% degradation at 14 days and 43% by 28 days.

5. Influence of Test System Variables

a. Sorption

Urano *et al.* (1984) studied the sorption of C_{12} AOS to river sediments. The equilibrium quantities sorbed were proportional to the organic carbon content of the sediments, and the sorption isotherms fit the Freundlich equation (quantity sorbed, [mg/g of organic carbon] equals the K_{OC}, [dimensionless], multiplied by the equilibrium aqueous concentration [mg/mL] raised to the 1/n power). For AOS the K_{OC} was 0.65 and n 1.1, which indicated slightly weaker sorption than such surfactants as C_{12} LAS and C_{12} AES.

B. Field Studies

MBAS and IR analyses of raw municipal sewage and effluent from two Japanese sewage treatment plants over a one-year period indicated that the surfactant content of the influent sewage contained approximately 2% AOS which was completely removed during passage through the treatment plant.

Sekiguchi, Oba, and co-workers (Sekiguchi *et al.*, 1975b; Oba, 1974; Oba *et al.*, 1976) analyzed raw municipal sewage and effluent from two Japanese sewage treatment plants for a one-year period. MBAS and IR analyses of influent and effluent sewage revealed that the surfactant content of the influent sewage contained about 2% AOS which was completely removed during passage through the sewage treatment plant.

C. Metabolic Pathways of Biodegradation

Very little information is available on the biodegradation pathways of AOS. Swisher (1987) describes one study of the desulfonation step catalyzed by alkanesulfonate-α-hydroxylate. β-Nonene-1-sulfonate degraded through a monenoic acid, while with α-octenesulfonate α-hydrosoxylation also appeared to be occurring.

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IV. ENVIRONMENTAL LEVELS

A. Water Quality Standards

There are presently no standards in the United States or Europe specifically referring to alpha olefin sulfonate (AOS). If present, these anionic surfactants are included among those measured in the environment using the MBAS method. Standards applying to MBAS levels were discussed in Volume 1, Part 1, LAS (Arthur D. Little, 1991).

B. AOS in Natural Water Bodies

AOS is not presently being monitored in the United States or Europe. In limited monitoring for AOS in Japan, levels have been reported below 0.002 mg/L in rivers and estuaries (Yamane, 1992). MBAS measurements in water bodies include these surfactants as well as other anionics. These levels have been discussed in Volume 1, Part 1, LAS (Arthur D. Little, 1991).

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V. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. Acute Toxicity

Reported LC_{50} values in fish range from 0.3 mg/L to 21 mg/L, with the harlequin fish being most sensitive. In a test with bluegills, the 24-hour LC_{50} values for dimer olefin sulfonate and vinylidine AOS were 97 mg/L and 58 mg/L, respectively. Mortality after the first 24 hours is higher under continuous-flow than static conditions, indicating possible surfactant biodegradation or absorption in static toxicity tests. Longer-chain AOS are consistently more toxic than those with shorter alkyl chains. Daphnia magna was the only invertebrate tested. LC_{50} values range from 7.0 mg/L for C_{16-18}AOS to 18.0 mg/L for C_{14-16}AOS. Vinylidene AOS appears to be the most toxic producing a 24-hour LC_{50} of 2.47 mg/L. Toxicity decreases to negligible levels with biodegradation. The 3-day EC_{50} value for $C_{16.4}$AOS in the green algae, Selenastrum capricornutum, is 45 mg/L. The only information available on AOS toxicity to microorganisms was a study in which 150 mg/L AOS limited Escherichia coli growth to 5 colonies per plate.

a. Fish

Acute toxicity data for fish are summarized in Table V-1. The range oNa-f LC_{50} values from these reports is 0.3 mg/L to 21 mg/L; of the species tested, harlequin fish were the most sensitive. The only toxicity values appearing outside of this range are for non-commercial developmental compounds, the LC_{50} for dimer olefin sulfonate to bluegill (97 mg/L) (Colgate Palmolive Co., unpublished data) and the LC_{50} of vinylidene C_{16} AOS (58 mg/kg) for the same species (Shell Chemical Co., unpublished data). The data of Reiff *et al.* (1979) support the earlier finding that AOS surfactants with longer alkyl chains are more toxic; i.e., for C_{16-18}AOS, the LC_{50} values were generally 4-10 times lower than for C_{14-16}AOS.

Toxicity values do not appear to vary greatly with species or test conditions. Mortality after the first 24 hours is higher under continuous flow than static conditions. These results indicate that degradation or adsorption may be occurring under static conditions.

Table V-1

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Goldfish (*Carassius auratus*)	AOS C_{12-16} 33.3% active*	Static, 20°C, hardness-10°, 10 fish/conc.	LC_{50} 6 hr - 11.2	Gafa (1974)
	AOS C_{14-18} 31.1% active*		LC_{50} 6 hr - 3.0	
	AOS Bioterge As 35 - CL C_{14-16} (MW 205)	Fish - 6-7 cm, static, 20°C, hardness - 10°, 10 fish/conc.	LC_{50} 6 hr - 10.7	Gafa and Lattanzi (1974)
	AOS C_{15}-C_{18} (MW 232)		LC_{50} 6 hr - 3.1	
	AOS Hostapur OS C_{15}-C_{18} (MW 228)		LC_{50} 6 hr - 3.8	
	AOS C_{14-16} 90% active MW 312-316	Fish - 5-6 cm, static, 20°C, hardness - 150 mg $CaCO_3$/L	LC_{50} 24 hr - 7.1 (6.2-8.0) 48 hr - 6.9 (6.0-7.8)	Unilever Research Laboratories, unpublished data

*Percent by weight of the surfactant component of the formulation.

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Goldfish (*Carassius auratus*)	AOS C_{16-18}, 90% active* (MW 340-344)	Fish - 5-6 cm, static, 20°C, hardness - 150 mg $CaCO_3$/L	LC_{0-100} 24 hr - 1.0-3.0	Unilever Research Laboratories, unpublished data
Golden orfe (*Idus idus melanotus*)	AOS C_{12-16}	Fish - 5-7 cm, static, 20°C, tap water	LC_0 48 hr - 3.0	Fischer (personal communication) as cited in Gloxhuber (1974)
			LC_{50} 48 hr - 4.2	
			LC_{100} 48 hr - 6.0	
	C_{14-16}AOS 90% active	hardness 268 mg $CaCO_3$\L 20°C, dynamic	LC_{50} 96 hr - 4.9	Relff *et al.* (1979)
		268 mg$CaCO_3$L hardness, 20°C, static	LC_{50} 96 hr - 3.4	
		150 mg$CaCO_3$L hardness, 20°C, static	LC_{50} 48 hr - 5.7	
	C_{16-18}AOS	268 mg$CaCO_3$L hardness, 20°C, dynamic	LC_{50} 48 hr - 1.0	
		268 mg$CaCO_3$L hardness, 20°C, static	LC_{50} 96 hr - 0.9	
		150 mg$CaCO_3$L hardness, 20°C, static	LC_{50} 48 hr - 1.9	

*Percent by weight of the surfactant component of the formulation.

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Fathead minnow-continued (*Lepomis macrochirus*)	AOS BIOTERGE AS 40™ C_{14-16}	Fish - 1.2 cm, static, 21°C, pH 7.0-7.2, hardness - 100 mg $CaCO_3$/L	LC_{50} 24 hr - 8.2*	Monsanto Co., unpublished data
Japanese killifish (*Oryzias latipes*)	AOS C_{15-18}		LT_0 5 hr - 0.5 LT_{100} 5 hr - 2	Tomiyama (1975)
Japanese *himedaka*	AOS (C# not stated)	0 mg/L $CaCO_3$ hardness 10 " 20 " 50 " 100" 500"	LC_{50} 48 hr -3 -1 -1 -1 -1 -0.5	Tomiyama (1978)
Carp (*Cyprinus carpio*)	AOS - technical grade (C# not stated)	Fish - 3.5-5.5 cm, static, 21°C, pH 7.5-7.8, 10 fish/conc, 4 conc tested	LC_{50} 24 hr - 3.2	Lopez-Zavala et al. (1975)
White tilapia (*Tilapia melanopleura*)	AOS	Fish - 5.0-7.0 cm, static, 21°C, pH 7.5-7.8, 10 fish/conc, 2 conc tested	LC_{50} 24 hr - 2.0	

*Average from three tests

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Golden orfe - continued (*Idus Idus melanotus)*	AOS C_{14-16}, 90% active* (MW 312-316)	Fish 5-5 cm, static 20°C, hardness - 150 $CaCO_3$mg/L	LC_{50} 24 hr - 4.7 (4.5-5.0) 48 hr - 4.1 (3.9-4.3)	Unilever Research Laboratories, unpublished data
	AOS C_{16-18},90% active* (MW 340-344)		LC_{50} 24 hr - 1.2 (1.0-1.4) 48 hr - 1.1 (1.0-1.2)	
Golden orfe (*Leuciscus idus melanotus*)	50-50 mixture of olefin sulfonate and succinic acid mono-sulpho-ester	Experimental conditions unknown	LC_{20} 48 hr - 5	Mann and Stach (1974)
			LC_{100} 48 hr - 6	
			LC_{50} 24 hr - 8.4 (7.2-9.9) 96 hr - >7.5, <8.7	Colgate -Palmolive Co., unpublished data
Bluegill (*Lepomis macrochirus*)	AOS (C# not known)	Fish - 1.2 g, 18°C	no effect level - 7.5	Colgate -Palmolive Co., unpublished data

*Percent by weight of the surfactant component of the formulation.

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Bluegill - continued (*Lepomis macrochirus*)	AOS	Fish - 1.2 g, 18°C	LC_{50} 24 hr - 7.1 (4.8-10.6) 96 hr - 7.1 (4.8-10.4) no effect level - 4.9	Colgate-Palmolive Co., unpublished data
	Dimer olefin sulfonate	Fish - 1.2 g, 18°C	LC_{50} 24 hr - 97.1 (69.1-137.0	
	AOS C_{14-18}, 28.9% active*	Fish - 1.1g, 21°C, pH 7.1	LC_{50} 24 hr - 1.61 (1.27 - 2.04) 96 hr - 1.40 (1.20-1.64) no effect level - 1.2	Shell Chemical Co., unpublished data

*Percent by weight of the surfactant component of the formulation.

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Bluegill - continued (*Lepomis macrochirus*)	Vinylidene AOS C_{16}*** 17.9% active*	Fish - 1.1 g, 21°C, pH 7.1	LC_{50} 24 hr - 58.2 (4.7-71.9) 96 hr - 57.3 (44.5-70.6) no effect level - 37	Shell Chemical Co., unpublished data
Fathead minnow (*Pimephales promelas*)	AOS C_{14-16}	Fish - 1.0 g, 20°C	LC_{50} 24 hr - 8.6 (5.3-14.0)	Colgate-Palmolive Co., unpublished data
	AOS C_{16-18}		LC_{50} 24 hr - 1.8 (1.5-2.2)	
	AOS C_{14}	Fish - 1.2 cm, static, 21°C, pH 7.0-7.2, hardness - 100 mg $CaCO_3$/L	LC_{50} 24 hr - 15-21**	Monsanto Co., unpublished data
	AOS C_{16}		LC_{50} 24 hr - 3.2-6.9**	
	AOS C_{18}		LC_{50} 24 hr - 0.5-0.8**	

*Percent by weight of the surfactant component of the formulation.
**Range from three tests.
***AOS derived from 2-hexyl-1-decene (1-octene dimer).

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Guppy (*Poecilia reticulatus*)	AOS, 90% C_{14-16} (MW 312-316)	Fish - 1.0 cm, static, 20°C, hardness - 20 mg $CaCO_3$/L	LC_{50} 24 hr - 10.1 (9.3-11.0) 48 hr - 10.1 (9.3-11.0) 96 hr - 9.7 (8.9-10.6)	Unilever Research Laboratories, unpublished data
	AOS, 90%, C_{16-18} (MW 340-344)		LC_{0-100} 24 hr - 1.0-2.0	
Harlequin fish (*Rasbora heteromorpha*)	AOS C_{14-16}	Fish - 1.3-3.0 cm, continuous flow, 20°C, hardness - 20 mg $CaCO_3$/L	LC_{50} 24 hr. 6.2 (5.5-7.1) 48 hr - 4.8 (4.2-5.6) 96 hr - 3.3 (2.3 - 4.2)	Unilever Research Laboratories, unpublished data
	AOS C_{16-18}		LC_{50} 24 hr - 1.3 (1.2-1.4) 48 hr - 0.9 (0.7-1.1) 96 hr - 0.5 (0.3-0.7)	

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Harlequin fish-continued (*Rasbora heteromorpha*)	C_{14-16} AOS 90% active*	20 mg $CaCO_3$/L hardness 20°C, dynamic**	LC_{50} 96 hr - 3.3	Reiff et al. (1979)
	C_{16-18} AOS 90% active		LC_{50} 96 hr - 0.5	
Minnow (*Phoximus phoximus*)	AOS C_{14-16}	Fish - 5 cm, static, 10°C, hardness - 210 mg $CaCO_3$/L	LC_{50} 24 hr - 5.3 (5.1-5.5)	Unilever Research Laboratories, unpublished data
	AOS C_{16-18}		LC_{50} 24 hr - 1.4 (1.3-1.5)	
Brown trout (*Salmo trutta*)	AOS C_{14-16}	Fish - 2.0-4.0 cm, continuous flow, 15°C, hardness - 250 mg $CaCO_3$/L	LC_{0-100} 24 hr 0 2.7-4.3	Unilever Research Laboratories unpublished data
			LC_{50} 48 hr - 3.5 (3.2-3.9 96 hr - 3.1 (2.7-3.5)	

*Percent by weight of the surfactant component of the formulation.
**Dynamic refers either to continuous or intermittent replacement of the test solution.

Table V-1 (continued)

Acute Toxicity of AOS to Fish

Species	Surfactant	Experimental Conditions	Test/Duration/Toxicity (mg/L as active surfactant) (95% Confidence Limits)	Source
Brown trout - continued (*Salmo trutta*)	AOS C_{16-18}	Fish - 2.0-4.0 cm, continuous flow, 15°C, hardness - 250 mg $CaCO_3$/L	LC_{0-100} 24 hr - 9.5-1.2	Unilever Research Laboratories, unpublished data
	C_{14-18}AOS 90% active*	26-30 mg $CaCO_3$/L hardness, 15°C, dynamic	LC_{50} 48 hr - <0.3-0.5	Reiff et al (1979)
			LC_{50} 96 hr - 2.5-5.0	
Rainbow trout (*Salmo gairdneri*)	AOS C_{14-16}	Fish - 8-10 cm, static, 15°C, hardness - 20 mg $CaCO_3$/L	LC_{50} 24 hr - 5.1 (3.8-7.2) 48 hr - 3.5 (2.9-4.1)	Unilever Research Laboratories, unpublished data
	AOS C_{16-18}		LC_{50} 24 hr - 0.8 (0.6-0.9) 48 hr - 0.6 (0.5-0.8)	
	50-50 mixture of olefin sulfonate and succinic acid monosulpho-ester		LC_{50} 24 hr - 7.5	Mann and Stach (1974)

*Percent by weight of the surfactant component of the formulation.

b. Invertebrates

Daphnia magna, a cladoceran, is the only invertebrate for which toxicity information was found. A 24-hour LC_{50} of 15.6 mg/L was reported for this species at 20°C for a 34.2% active Na-AOS with an equivalent weight of 299 (Continental Oil Co., unpublished data). In a static bioassay with this species at 20°C, an LC_{50} value of 14 mg/L (expressed in terms of mg/L of sodium dodecyl benzene sulfonate) was observed at 24 hours by Lundahl *et al.* (1972) while a slightly lower LC_{50} value (9.26 mg/L) was reported for a 28.9% active C_{14-18} AOS (Shell Chemical Co., unpublished data). A 24 hr LC_{50} value of 2.47 mg/L was seen with this species with a non-commercial vinylidene C_{16} AOS derived from 1-octene dimer (Shell Chemical Co., unpublished data).

Similar results were found in another study with this species. Under static conditions at 20°C, 24 hr LC_{50} values of 16.6 (95% Confidence Limit: 15.3-18.0) mg/L and 7.7 (95% Confidence Limit: 7.0-8.5) mg/L were found for C_{14-16}AOS and C_{16-18}AOS, respectively (Unilever Research Laboratories, unpublished data).

Lundahl and Cabridenc (1976) found that AOS toxicity decreased steadily over time as a result of biodegradation. After 48 hours of biodegradation, the acute toxicity to *Daphnia* had diminished to negligible levels.

These limited results suggest that at least *Daphnia magna* are more tolerant of AOS than most of the fish species tested.

c. Algae and Microorganisms

Yamane *et al.* (1984) exposed the green algae, *Selenastrum capricornutum*, to AOS (with an average alkyl chain length of 16.4) for 3 days. The EC_{50} value (growth inhibition) was 45 mg/L.

Lundahl *et al.* (1972) examined the effects of various concentrations of AOS on *Escherichia coli* incubated at 37°C on gelatin media. An AOS concentration of 150 g/L was the lowest concentration of AOS which did not allow the development of more than 5 colonies per plate.

No other information on the effects of AOS on algae or microorganisms was found.

2. Sublethal and Chronic Toxicity

Embryonic mortality of loach embryos exposed to 1.7-13.3 mg/L AOS primarily occurs during gastrulation and at the time of organogenesis. Mortality generally occurs at the prehatching stage of rainbow trout embryos exposed to 3.3-30 mg/L AOS. The hatching of fathead minnow eggs was reduced by 60-70% (compared to controls) as a result of exposure to 7.5 mg/L C_{14-16}AOS, while a 3.2 mg/L concentration caused 100% mortality in hatchlings. No mortality was observed at 1.8 mg/L AOS. Continuous exposure of midges to 9.0 mg/L C_{14-16}AOS through two life cycles resulted in a 24-62% survival decrease (compared to controls). A 4.5 mg/L concentration had no effect on either generation.

Little information is available on the subacute or chronic effects of AOS on aquatic organisms. Lesyuk *et al.* (1983) evaluated AOS at different developmental stages in the loach (*Misgusnus fossilis*) and the rainbow trout (*Salmo gairdneri*). Mortality of loach embryos exposed to 1.7-13.3 mg/L AOS primarily occurred during the gastrulation stage (14-15 hours after fertilization) and at the time of organogenesis (38-42 hours after fertilization). Nearly 58% of the prolarvae were deformed following treatment of the embryos in 10 mg/L. At concentrations of 11.7-13.3 mg/L, all prolarvae were deformed. A significant reduction in loach prolarvae survival was also observed following a 12-day treatment of 10.0 mg/L AOS. Rainbow trout embryos were exposed to 3.3-30 mg/L AOS. Mortality occurred at the prehatching stage and, to a lesser extent, before the formation of the vascular system. At high concentrations, embryonic mortality was observed at the time of organogenesis and gastrulation. The periods of embryo incubation and larval hatching were also extended. At the end of the study, a 50% mortality was reported in fingerlings exposed to 2.5 mg/L AOS.

Hatching of fathead minnow (*Pimephales promelas*) eggs was 12-24% at 7.5 mg/L C_{14-16} AOS, while lower concentrations and the control showed 72-100% hatchability. No survival of hatched eggs was seen at AOS concentrations of 3.2 mg/L and greater. No effect on survival was observed at 1.8 mg/L. For comparison, the 24 hr LC_{50} for adults of this species was found to be 1.78 mg/L AOS (Colgate Palmolive Co., unpublished data).

In a long-term study (Colgate Palmolive Co., unpublished data), midges (*Chironomns tentans*) were exposed to C_{14-16} AOS continuously through two generations. Survival in the first generation was reduced to 25% and 66% in two tests at 9.0 mg/L (compared to 87-90% in the control group). Survival of the

second generation was also reduced (66% and 0%, respectively) at 9.0 mg/L. No effect on either generation was found at 4.5 mg/L.

No further information on this aspect of AOS toxicity was found.

3. Mode of Action

The gills appear to be the primary site of AOS toxicity. Toxicity is directly related to changes in interfacial tension between the gill and water since oxygen absorption is thought to be severely hindered when the tension decreases beyond a certain critical point. Protein complexing between dissolved surfactants and gill surface tissues was thought to be another primary mode of action. ^{14}C-AOS accumulates primarily in the gills followed by the gall bladder.

The mode of action of AOS has been examined to some extent, mostly in relation to its effects on fish gills. Gafa (1974) found that toxicity could be correlated with surface tension only within classes of chemicals. However, in an examination of AOS and other surfactants, he felt that toxicity could be directly related to changes in interfacial tension. He stated that, "it may be postulated that in the presence of surfactants, a critical gills-solution interfacial tension exists for each type of fish below which adsorption of oxygen by the gills is greatly hindered."

Tomiyama (1975) felt that the biological effects of sulfonate-type surfactants were related to surfactant bonding with gill protein. In an examination of this question, he found that the addition of egg albumen to AOS minimized the toxic effect to Japanese killifish. Although he concluded "that the biological effect of sulfonate-type surfactants is due to the formation of surfactant-fish protein complexes", there are no additional data to support this view.

Tomiyama (1974) observed significant absorption of AOS by the gills in goldfish, while almost none of the surfactant was found in the alimentary canal. In water containing 10 mg/L AOS, the rate of accumulation in the gills increased rapidly over a short period: after half an hour, gill levels of AOS were 0.3 µg/g; after 1 hour, 2.5 µg/g; and after 3 hours, 48 µg/g.

In a separate experiment with ^{14}C-AOS, Tomiyama (1978) found that ^{14}C accumulated most strongly in the gills, and next in the gallbladder. The author did not describe the effects or mode of action in either tissue.

4. Interaction with Other Chemicals

Addition of 2100 or 4200 mg/L egg albumin decreases the toxicity of 5 to 10 mg/L AOS in goldfish. A slight decrease in AOS toxicity was also reported in Japanese himedaka as water hardness increased. No conclusive evidence of synergism between linear tridecyl benzene sulfonate and C_{14-16}AOS or C_{16-18}AOS was found when tested in bluegills.

Tomiyama (1974) reported that the addition of 2100 or 4200 mg/L egg albumin to 5 or 10 mg/L AOS solutions decreased the toxicity of the surfactant to goldfish (*Carassius auratus*). The decrease was most pronounced with 4200 mg/L egg albumin at the lower AOS concentration.

The data of Tomiyama (1978) indicate that the 48-hour LC_{50} value for Japanese *himedaka* decreases slightly, though the effect may be insignificant, as water hardness increases (see Table V-1). The author attributed this trend to increased uptake rates and complex formation in harder water.

One additional study which explored the toxic effect of AOS in combination with other chemicals found no conclusive evidenee of synergistic effects between linear tridecyl benzene sulfonate and C_{14-16}AOS or C_{16-18}AOS when tested on bluegill (Colgate Palmolive Co., unpublished data).

B. Effects of AOS on Terrestrial Plants

The only study found concerning the toxicity of AOS to plants shows no significant effect on the germination or growth of tomato, barley and bean plants watered with solutions of 10, 25 or 40 mg/L AOS.

Lopez-Zavala *et al.* (1975) studied the effect of technical grade AOS on tomato, barley, and bean plants. Seeds in pots or plots of land were watered daily with 10, 25, or 40 mg/L solutions of AOS. The authors found no significant difference between the germination and growth of the plants exposed to the surfactant and the control group.

No additional studies on AOS toxicity to plants were found.

C. Effects of AOS on Birds and Wildlife

No information was found on the effects of AOS exposure to birds and wildlife.

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VI. HUMAN SAFETY

The data available on AOS toxicity at doses far in excess of normal use levels and the relative ease of AOS biodegradation indicate that the use of these surfactants does not pose a significant hazard to human health. Their safety is recognized by the Food and Drug Administration who have approved their use as indirect food additives. The ammonium, calcium, magnesium, potassium and sodium salts of AOS are approved for use with the stipulation that the alkyl group is in the range of C_{10-38} with not less than 50% in the range of C_{14-16} (Code of Federal Regulations, 1987).

A. Animal Studies

The alpha olefin sulfonates exhibit a moderately low order of toxicity in rodents. Acute oral LD_{50} values in mice are larger (2500 to >4000 mg/kg) than intravenous, intraperitoneal and subcutaneous values (≤1600 mg/kg) indicating either a low rate or incomplete AOS absorption or rapid metabolism and elimination. AOS is slightly to severely irritating to rabbit skin. Skin sensitization observed in guinea pigs has been attributed to the presence of unsaturated 1,3-sultones and chlorosultones; however, these are not normally present in commercial formulations. AOS concentrations of 1% are not ocular irritants in rabbits but concentrations greater than 5% are capable of producing severe ocular damage. A single set of mutagenicity experiments showed a positive response in a host-mediated assay with rats; however, the response may be due to materials having no direct relationship to the surfactant. All other mutagenicity studies are negative. With respect to teratogenic effects, an increase in cleft palates was found in offspring of mice given 300 mg/kg of AOS by gavage during gestation as well as an increased incidence of minor skeletal anomalies in both mice and rabbits at this dosage level. However these responses occurred only in groups in which the dams exhibited toxic responses. Carcinogenicity studies have given negative responses.

1. Acute Toxicity

a. Oral

Alpha olefin sulfonates have been shown to have a moderately low oral toxicity in rats and mice (Oba and Tamura, 1967; Oba *et al.*, 1968; Webb, 1966; Ogura and Tamura, 1967; Tomiyama *et al.*, 1969; TerHaar, 1983; unpublished data: American Cyanamid Co., Arco Chemical Co., Colgate Palmolive Co., Continental

Oil Co., Ethyl Corp., Procter & Gamble Co., Shell Chemical Co., Stepan Chemical Co., Witco Chemical Corp.).

LD_{50} values ranged from 1300-2400 mg/kg in rats and 2500-4300 mg/kg in mice. Due to variations in AOS activity, purity, formulations, etc., correlations between α-olefin chain length and toxicity cannot be readily made. However, chain length does not appear to be a major determinant of acute oral toxicity in rodents.

Signs of acute oral toxicity in rodents include vocal crying, crouching, malaise, ataxia, diarrhea, weakness, anemia and inhibited respiration prior to death. These are typical signs of surfactant toxicity. Gross necropsy revealed gastrointestinal tracts filled with a bloody fluid, congestion of lungs, kidneys, liver and adrenal glands and an opaque stomach (Oba and Tamura, 1967; Oba *et al.*, 1968; Continental Oil Co., unpublished data).

b. Inhalation

Groups of ten rats were exposed for one hour to a powdered aerosol of either C_{14-16}AOS flake (90% active) at a concentration of 229 mg/L of air or a spray-dried formulation containing 17% C_{14-16}AOS at a concentration of 221 mg/L. Information on particle size for either treatment was unavailable. All rats in both treatment groups survived the one-hour exposure period, and except for an increase in preening behavior, appeared normal throughout the experiment. Immediately after exposure, five rats from each group were killed and examined for any gross pathological changes. Mild petechial hemorrhage of the lungs was noted in two animals exposed to the AOS flake and one animal exposed to the spray-dried formulation. The remaining animals were killed after a 14-day observation period and were indistinguishable from controls upon gross pathological examination (Witco Chemical Corp., unpublished data).

c. Percutaneous

i. Acute Dermal Lethality

The acute dermal toxicity (LD_{50}) of 2 different commercial samples C_{14-16}AOS (with 95% confidence limits) in rabbits was reported to be 1,130 (520-2,460) mg/kg and 2,150 (1,630-2,850) mg/kg, respectively

(American Cyanamid Co., Ethyl Corp., unpublished data). In another study, the dermal LD_{50} values for undiluted C_{14-18}AOS and a 36.8% solution of C_{16} vinylidene AOS in rabbits were estimated to be 578 mg/kg and between 90 and 358 mg/kg, respectively (Shell Chemical Co., unpublished data).

ii. Skin Sensitization

The studies summarized below show that AOS itself is not a skin sensitizer. However, sultones, espeeially unsaturated 1,3-sultones and chloro-sultones, which are possible contaminants of AOS, are very strong allergens. Although the occurrence is relatively rare, clinical dermatitis from materials contaminated with unsaturated sultones and chloro-sultones has been reported and demonstrates the need for strict process specification and control to minimize contamination. The long term use of AOS containing less than 0.05 ppm unsaturated sultone in Japanese light duty liquids without outbreaks of clinical dermatitis supports the efficacy of production quality control (Oba *et al.* 1985). Roberts and Williams (1983) concluded that sensitizing sultones would not be formed at levels sufficient to constitute a hazard under normal use conditions.

A batch of lauryl ether sulfate produced in the 1960's sensitized consumers who used dish washing liquids containing the surfactant (Magnusson and Gilje, 1973). Initial observations implieated contaminants as thc cause of the outbreak of dermatitis and further investigation identified these as strongly sensitizing sultone species (Lindup and Nowell, 1978). Subsequently, it was recognized that certain unsaturated and chloro-sultones are potential contaminants of AOS (Connor *et al.* 1975, Ritz *et al.* 1975, Goodwin *et al.* 1983). As a consequence, the following manufacturing process specification has been recommended:

1. No bleaching with hypochlorite during manufacture
2. Total saturated sultone internal plus terminal <200 ppm on 100% active detergent basis
3. Terminal saturated sultone <100 ppm on 100% active detergent basis
4. No detectable unsaturated sultone

AOS produced under these conditions does not have a significant scnsitization potential in the guinea pig maximization test (Lever Brothers Company, unpublished data) and is, therefore, highly unlikcly to give rise to allergic contact dcrmatitis when used as a surfactant.

In sensitization tests conductcd in U.S. consumer volunteers, a sodium C_{14-16}AOS paste and an AOS-containing light duty liquid dishwashing detergent failed to induce sensitization but did elicit strong dermal

responses in one subject which was interpreted to be pre-existing sensitization. AOS was used in concentrations of up to 0.06% with unsaturated sultones present at up to 0.002 ppm. The number of subjects used in the test was not reported (Bay and Danneman, 1985).

In patch tests conducted in 542 subjects who used AOS-containing consumer products, 15 exhibited positive sensitization responses to 1.3 ppm unsaturated sultones in 0.046% sodium lauryl sulfate. In a product use test, 2 of 264 subjects using an AOS-containing light duty liquid laundry detergent developed hand dermatitis and had positive reactions to patch tests with AOS paste and/or unsaturated sultones in sodium lauryl sulfate. None of the 248 control subjects using a non-AOS containing detergent experienced hand dermatitis (Bay and Dannenan, 1985).

Following the demonstration that clinical dermatitis in the 1960's batch of sodium lauryl ether sulfate discussed earlier was limited to a particular batch of AOS, LES 13-2035 (Walker *et al.* 1973), contaminants which were sensitizers were isolated and characterized. Sensitizers were found in the petroleum ether extract of SLES (Magnusson & Gilje, 1973) and were polar and stable. Using bioassays of fractional extracts of the batch of LES 13-2035, it was determined that the sensitizing components were 1-dodecene-1,3 sultone (C_{12}), 1-tetradecene-1,3-sultone (C_{14}), 2-chloro-1,3 dodecane sultone and 2-chloro-1,3-tetradecane sultone (Connor *et al.*, 1975, 1976). These chemicals and related halogenated sultones were synthesized and tested in guinea pigs.

Those studies established a rank order of potency for induction of sensitization of $C_{16} > C_{14} > C_{12} > C_{10}$. Extensive cross reactivity between the sultones was observed (Ritz, Connor and Sauter, 1975). Goodwin *et al.* (1983) evaluated the sensitization potential of $C_{16}\gamma$, γ and δ, $C_6\gamma$ and δ, and $C_{14}\gamma$. Their studies identified all the sultones studied as sensitizers with the unsaturated sultones being more potent than their saturated counterparts. The C_6 compounds were much less potent than the C_{16} analogues (Goodwin *et al.*, 1983. The potency of hexadec-1-ene 1,3 sultone was investigated by Basketter and Roberts (1990). Using a constant challenge concentration of 50,000 ppm, they were able to demonstrate that as little as 0.1 part per trillion could induce sensitization in an adjuvant based assay with the optimal range being 1-10,000 ppm. Induction doses outside this optimal range required higher challenge doses to elicit a response.

This conclusion has been generally supported by studies in man and by surveillance of the marketplace in Japan (Oba *et al.* 1985), although Bay and Danneman (1985) and Robinson *et al.* (1989) concluded that use in a light duty liquid in the USA might pose a small sensitization risk. In this respect, the details of

types and levels of sultones present in the samples of AOS investigated is critical. This was not given for the latter studies, which are discussed in more detail below.

A sodium C_{14-16}AOS paste contaminated with 3.3 ppm unsaturated 1,3-sultone and light duty liquid dishwashing detergents containing contaminated AOS did not induce sensitization when tested in human repeat insult patch studies. Two batches of AOS were tested at 0.06% on 374 subjects and 5 prototype detergents containing 7-10% AOS at 0.15-2% w/v aqueous (0.01-0.02% AOS) on 534 subjects. One subject developed strong reactions to a light duty liquid early in the induction period suggesting presensitization; none of the other 907 developed allergic responses. To determine the prevalence of presensitization in U.S. consumers, 542 subjects who routinely reported using products containing AOS were patch tested with 1.3 ppm unsaturated sultones in a 0.046% sodium lauryl sulfate solution. Fifteen subjects exhibited positive patch responses, but all used AOS containing products without adverse skin effects. In a 9 month product use test of non-sensitized subjects (based on patch response), 2/264 subjects using AOS-containing light duty liquids developed hand dermatitis and later responded to patch tests with AOS paste and/or unsaturated sultones in sodium lauryl sulfate. None of 248 subjects using a non-AOS detergent developed hand dermatitis. The level of sultone present in the dishwashing detergent tested in the use study was not reported although it was noted that the level was increased by the co-use of bleach, a common "misuse situation" in the U.S. (Bay and Danneman, 1985; Robinson *et al.*, 1989).

Studies on older AOS samples are largely of historical interest but are presented for completeness and to emphasize the importance of monitoring the quality of AOS incorporated in consumer products. These studies were carried out before the role of unsaturated and chloro-sultones in sensitization had been elucidated.

Guinea pig sensitization studies have been conducted on major and minor ingredients of current production commercial AOS. Hydroxyalkane sulfonate C_{12-18} (21% active) and alkene sulfonate C_{12-18} (21% active) were both found to be non-sensitizers in the guinea pig (Shell Chemical Co., unpublished data). Current commercial AOS should not contain the unsaturated and chloro 1,3-sultones which have been shown to be potent sensitizers (see below) but may contain small amounts of slower hydrolysing alkane 1,4-sultones which are a normal intermediate in AOS maufacture.

When guinea pigs were administered small amounts of pure C_{14} or C_{16} alkane 1,4 sultone, no sensitization occurred. However, C_{14} 1,4-sultone containing 2% C_{14} 1,3-sultone produced sensitization in 50-60% of the animals tested. When the C_{14} 1,3-sultone was removed, no sensitization occurred (TerHaar, 1983).

In a separate series of experiments, it was shown that alkane 1,4-sultone materials ($C_{12,14,16}$ and C_{18}-1,4-sultone) did not elicit sensitization nor cross-reactions in guinea pigs sensitized to 1-tetradecene-1,3-sultone (Colgate Palmolive Co., unpublished data)

Eleven studies reported on the skin sensitization potential in the guinea pig of 64 AOS samples mostly derived from C_{14-18} α-olefins (unpublished data: Colgate Palmolive Co., Ethyl Corp., Gulf Research and Development Co., Lever Brothers Co., Procter & Gamble Co., Shell Chemical Co., Stepan Chemical Co.). Sample characteristics were deliberately induced by varying manufacturing conditions to yield a wide range of product types: dcoiled and hypochlorite bleached, hypochlorite bleached only, spray-dried, paste, sodium salts of AOS, and over- and under-saponified at high (169°C) and low (95-100°C) temperature and elevated and normal atmospheric pressure.

Of the 64 test samples, fifty-five of the test samples were reported as non-sensitizers in the guinea pig. Of the nine samples classified as sensitizers, two were photosensitizers by the Vinson-Borselli technique (1966); namely, a 44% active AOS paste and an 80.7% active spray-dried AOS powder. Six of the six guinea pigs tested with the AOS paste and four of the six animals treated with the spray-dried AOS were sensitized (Lever Brothers Co., unpublished data). Two aged samples (possibly several years old) were also found to be sensitizers. Unbleached, 10% active C_{14-16} (2:1) AOS saponified at ~100°C and bleached 10% active C_{14-16}AOS (2:1) AOS saponified at 100°C were sensitizers in 5/10 and 6/10 guinea pigs tested, respectively. Sensitization was initially attributed to incomplete hydrolysis, but results from follow-up studies with similar AOS samples (C_{14-16}AOS, 1:1 ratio) ruled out over- and under-saponification, the presence of saturated sultones and residual oil as causes of sensitization (Gulf Research and Development Co., unpublished data). Two other bleached C_{14-16}AOS samples were also unexplained sensitizers. One sample sensitized 7/10 guinea pigs the first time it was tested, but results could not be duplicated (no positive response in 20 animals). Initial sensitization results were explained as a possible mix-up in the material actually tested or perhaps aging of the sample prior to the second test had resulted in reduced activity. A second C_{14-16}AOS sample was tested and also gave a positive sensitization response in 3 of 10 guinea pigs tested which was believed to be due to incomplete hydrolysis. The frequency of positive response was reduced (1/10) but not eliminated when the latter sample was rehydrolyzed (Ethyl Corp., unpublished data).

A C_{14-16} (3:2) AOS paste (29.4% active) was found to be a skin sensitizer in 10/19 guinea pigs challenged with a 10% aqueous solution of the product; there were 5/10 positive responses with a 5% challenge concentration. Similar findings were reported for a C_{16-18} (55:45) AOS paste (25.7% active); positive

responses were noted in 10/20, 8/20, and 2/20 animals challenged with 20%, 15% and 7.5% aqueous solutions, respectively, of the product (Procter & Gamble Co., unpublished data).

In another study, repeated topical application of undiluted C_{16-18}AOS resulted in a 50% incidence of positive responses (10/20) in guinea pigs challenged with a 20% aqueous solution of the surfactant. When these animals were tested for cross-reactivity to other AOS materials, positive reactions were observed in 4/19 animals with a 10% aqueous solution of C_{14-16}AOS and in 4/19 animals challenged with a 10% aqueous solution of C_{16} vinylidene olefin sulfonate. No reactions (0/19 animals) were observed when these animals were cross-challenged with pure C_{16}AOS (20% aqueous solution). It should be noted that the interpretation of cross-reactivity was equivocal due to the presence of reactive control animals (Procter & Gamble Co., unpublished data).

In order to clarify these findings, the primary sensitization study was repeated in a second laboratory, the only difference between the two tests being that a 15% aqueous solution was used as the challenge concentration. In this study a positive response was noted in 40% of the animals (8/20). These animals were then cross-challenged with a 10% aqueous solution of C_{14-16}AOS, a 20% aqueous solution of pure C_{16}AOS and a 10% aqueous solution of C_{16} vinylidene olefin sulfonate. The results indicated 3/20 animals cross-reacted to the C_{14-16}AOS material and 4/20 animals cross-reacted to the C_{16} vinylidene olefin sulfonate. These responses were consistent with those observed in the first study. However, in contrast to the results obtained in the first study, 7/20 animals cross-reacted to the pure C_{16}AOS material. It should be noted that in this study the assignation of cross-reactivity to C_{14-16}AOS was equivocal since 1/6 control animals yielded reactivity. No further attempt was made to investigate the discrepancy in cross-challenge data from the two laboratories (Procter & Gamble Co., unpublished data).

A specially prepared AOS slurry which was bleached at pH 9.5 induced sensitization (10/10) when the challenge concentrations were 1% or 3% solutions of the slurry. The reactions were attributed to the presence of 1-tetradecene-1,3-sultone and the 2-chloro derivative. Rechallenge experiments with 3 µg of purified C_{14} and C_{16} sultones (either the 1-alkene or the 2-chloro) reaffirmed sensitization effects. Quantitatively, the lower thresholds of tolerance to induction of sensitization by 1-tetradecene-1,3-sultone and/or the 2-chloro derivative in a specially prepared AOS slurry was 9 ppm or less (Colgate-Palmolive Co., unpublished data). However, guinea pigs already sensitized to the specially bleached AOS slurry reacted upon challenge to nanogram quantities of the sultone materials.

Table VI-1

Acute Dermal Irritation of AOS to Rabbits

Carbon Chain	% AOS	Primary Irritation Score
C_{14}	10	0.6[g]
C_{14-16}	10	3.6[c]
C_{14-16}	1	0.2[d]
C_{14-16}	1	0.29[a]
C_{14-16}	10	1.0[g]
C_{14-16}	10	*6.2[a]
C_{14-16}	-18	4.8[b]
C_{14-16}	20	2.9[d]
C_{14-16}AOS slurry	40	4.8[h]
$C_{14-16-18}$	10	1.4[g]
C_{16}	10	1.1[g]
C_{16-18}	25.7	**6.6[f]
C_{16-18}	10	**8.0[f]
C_{17}	10	1.3[g]
C_{18}	27.5	2.3[f]
C_{18}	10	2.9[f]
NH_4C_{15-18}	30	3.0[e]
AOS paste	44	3.9[e]
AOS spray-dried	80.5	3.1[e]
C_{17-20}	35	**4.6[g]

* Primary irritant according to Federal Hazardous Substances Act.

** Primary irritant according to Draize procedure.

Key: unpublished data:

[a]American Cyanamid Co.	[b]Arco Chemical Co.	[c]Continental Oil Co.
[d]Ethyl Corporation	[e]Lever Brothers Co.	[f]Procter & Gamble Co.
[g]Stepan Chemical Co.	[h]Witco Chemical Corp.	[g]Shell Chemical Co.

iii. **Skin Irritation**

The majority of data (unpublished data; American Cyanamid Co., Arco Chemical Co., Continental Oil Co., Ethyl Corp., Lever Brothers Co., Procter & Gamble Co., Stepan Chemical Co., Witeo Chemical Corp.) concerned with the dermal irritation of AOS show it to be slightly to severely irritating to rabbit skin (see Table VI-1). Three of the AOS products tested were classified as primary irritants. The data, however, are inconsistent. In one study C_{14-16}AOS (10% active) was evaluated as a primary irritant (American Cyanamid Co., unpublished data), and only as a slight irritant in another study (Stepan Chemical Co., unpublished data). Sueh factors as AOS purity, method of production and/or variations in experimental technique may account for this inconsistency.

In a vaginal mucosal irritation study, 1 ml of a 1% aqueous solution of AOS (derived from C_{14-16} α-olefins) did not cause vaginal mucosal irritation in dogs examined at 24 hours (Ethyl Corp., unpublished data).

d. Ocular

Data from numerous studies show that 1% concentrations of AOS are not ocular irritants in rabbits (Iimori *et al.*, 1972a,b; unpublished data: Ameriean Cyanamid Co., Ethyl Corp., Stepan Chemical Co.). Furthermore, Iimori *et al.* (1972a,b) working with C_{10}AOS, C_{12}AOS, C_{16}AOS, C_{18}AOS, and C_{14-19}AOS found that the chain length of the α-olefins used in AOS production had no influence on ocular irritancy in the rabbit at 1% concentrations. However, at concentrations of 5%, Iimori and co-workers (1972a,b) reported C_{14-19}AOS to be mildly irritating. Other investigators have found 5% concentrations of AOS to be mildly to severely irritating to rabbit eyes and capable of producing corneal necrosis (unpublished data: American Cyanamid Co., Stepan Chemical Co.).

There is general agreement that higher concentrations (10-40% of AOS) are moderately to severely irritating to rabbit eyes (unpublished data: Continental Oil Co., Ethyl Corp., Shell Chemical Co., Procter & Gamble Co., Stepan Chemical Co., Witco Chemical Corp.). In a typical example, 0.1 ml of 10% C_{14-16}AOS instilled in the conjunetival sac of rabbits produced slight to moderate erythema of the conjunctiva, moderate chemosis of the lids, slight discharge and discrete corneal opacity within 24 hours. Although

some improvement was seen by 72 hours, corneal opacity remained (Continental Oil Co., unpublished data). At variance with the majority of data, 6%, 12%, and 20% C_{16-18} AOS active solutions (BIO-TERGE AS-30™ formulation) were reported to be essentially non-irritating to rabbit eyes (Stepan Chemical Co., unpublished data).

e. Other Routes of Exposure

The acute toxicity of C_{15-18}AOS in mice by a variety of routes of administration was examined by Oba and Tamura (1967). They found:

LD_{50} Value (mg/kg of C_{15-18}AOS) in Mice		
Method of Administration	LD_{50} Values	
	24 hours	7 days
Intravenous	90	90
Intraperitoneal	300	170
Subcutaneous	1,660	-----

It is interesting to note that when administered intraperitoneally, the LD_{50} value for AOS decreased with time, suggesting a possible delayed toxic effect. In addition to the signs of toxicity cited under acute oral administration, Ogura and Tamura (1967) observed a writhing reflex following intraperitoneal administration and, when given intravenously, AOS resulted in clonic convulsions and necrosis in the injected portion of the tail.

2. Subacute Toxicity

a. Oral

AOS (70% C_{14}:30% C_{16}) was incorporated into the diet of rats on an active ingredient basis at levels of 2.5%, 1.25% and 0.625% for seven days. The "no effect" dosage level was between 0.625% and 1.25% dietary AOS. AOS concentrations of 2.5% and 1.25% caused a slight increase in the liver to total body weight ratio in male rats and the 2.5% dietary level also correlated with a significant body weight depression for a duration of 2 and 7 days, respectively, in male and female rats (American Cyanamid Co., unpublished data).

In another study, rats were fed a diet containing 0, 40, 200 or 1000 mg/kg/day AOS (bleached, dried; 89.7% active; 0.15% sultone content) for 90 days. No statistically significant differences from controls were noted in hematological or biochemical parameters, growth, or food consumption but a slight increase in the liver to total body weight ratio was recorded for both sexes at the 1000 mg/kg treatment level. No gross microscopic anatomical changes in the liver or other organs and tissues were reported (Chevron Chemical Co., unpublished data).

Incorporation of 50, 150 or 500 mg/kg of C_{14-16} (65:35) AOS (34% active) in the diet of rats for 91 days were reported to produce no treatment-related toxic or histopathological changes. Apparent anomalies were observed in the hematologic parameters, possibly related to hemoglobin synthesis. Organ-to-body weight ratios were within established ranges. No other details were available (Procter & Gamble Co., unpublished data). Similar findings were noted in rats given 0, 50, 150 or 500 mg/kg C_{16-18}AOS (34% active) in their diet for 91 days. Red blood cell counts for females in the 500 mg/kg treatment group were significantly higher than controls, but corresponding hematocrit and hemoglobin values were not. High hematocrit and hemoglobin values were also seen in females on the 150 mg/kg diet, and significantly higher hematocrit levels were noted in male rats given 50 mg/kg AOS (Procter & Gamble Co., unpublished data). In both this and the study previously cited, some relationship appears to exist between dietary intake of AOS and hemoglobin synthesis in the rat.

b. Inhalation

No deaths were reported in rats (forty per group) following 20, six hour exposures to either 0.9% or 10% concentrations of 90% active C_{14-16}AOS flake over a 30 day period.

In the low level treatment group, no changes from control values were seen with respect to body weight, food intake, blood chemistry (examined at 2-week intervals) and gross pathology was also normal. At the higher exposure level, histopathological evaluations revealed a significant increase in stomach lesions: 19/40 rats showed edema and acute inflammation cell infiltration and 13/40 rats had ulceration of the squamous lining of the stomach. These lesions were attributed to stress factors in the treated population (Witco Chemical Corp., unpublished data).

c. Dermal

The subacute dermal irritancy of AOS and AOS-containing formulations has been evaluated by several

investigators (Sadai *et al.*, 1972; unpublished data: Ethyl Corp., Lever Brothers Co., Stepan Chemical Co.). In three separate studies, 10 applications (within 14 days) with either 0.5% or 1.0% AOS produced no skin irritancy or skin fatigue in rabbits (Stepan Chemical Co., unpublished data).

In another study, two milliliters/kg/day of a 5% aqueous solution of AOS (34% active) were applied to the backs of six rabbits for 91 days. At necropsy, hematology, organ weights and organ-to-body weight data were all normal. Skin irritation was rated mild to moderate (non-suppurative dermatitis, parakeratosis, hyperkeratosis). One of the six rabbits had a firm, swollen salivary gland which upon microscopic examination exhibited inflammation and hyperplastic changes (Procter & Gamble Co., unpublished data).

Cumulative, open-patch test application of 0.1 mL of a 2% aqueous solution of either C_{16-18}AOS (57.6% C_{16}: 40.8% C_{18}) or C_{12}AOS to the shaved backs of guinea pigs twice daily for a total of nine treatments resulted in nil-to-slight and slight-to-moderate cumulative skin irritation, respectively. Skin irritation scores of 0.42 and 1.67, respectively, of a possible 4 points were recorded for these two samples (Imokawa, 1979).

In a 28-day study, 20 applications (0.2 ml, 5 days/week for 4 weeks) with either a 1% aqueous solution of C_{14-16}AOS or a 1% AOS formulation to rabbits produced no effect on intact skin, and only questionable exfoliation and hyperemia on abraded skin (Ethyl Corp., unpublished data). In rats, 20% and 30% aqueous solutions of AOS (99.5% C_{16}) caused no visible skin changes after 15 daily applications. Histological examination of tissues from the 30% AOS group indicated some withering of the horny skin layer of the back and pronounced withering of the oral mucosa (the animals were not restrained after surfactant application); the tongue was essentially normal in appearance (Sadai *et al.*, 1972).

In an epilated guinea pig test, 8% aqueous solutions of either C_{15-18}AOS or a liquid detergent formulation containing 30% AOS were found to be mildly irritating, and a 12% concentration of the detergent formulation was moderately irritating following two 4-hour applications, 24 hours apart (Lever Brothers Co., unpublished data).

3. Chronic Toxicity

a. Oral

In a two-year study, rats (50 male and 50 female/group) were fed 97.9% active C_{14-16}AOS at dietary levels of 1000, 2500, and 5000 ppm (see below). The only adverse effects recorded were a significant reduction in body weight gain between weeks 14 and 26 of the study for both males and females receiving 5000 ppm AOS and a marginally lower food intake during the first year in females receiving the 5000 ppm diet. Blood chemistries, urinalyses, and histopathological findings were all comparable to control values. The authors calculated that the highest level of AOS in the 2-year feeding study (representing about 0.5% of the diet) was at least 1000 times the estimated maximum daily exposure to humans using AOS-containing products and therefore, AOS would not appear to represent a hazard to human health (Hunter and Benson, 1976).

Dietary Level (ppm)	Mean Daily Uptake (mg/kg/day)	
	Male	Female
1000	39	57
2500	96	132
5000	195	259

(Hunter and Benson, 1976)

4. Carcinogenicity

Carcinogenicity testing of AOS has been reviewed by Oba (1992). Four carcinogenicity studies evaluated AOS and indicated that AOS is not carcinogenic in rodents when administered either percutaneously or orally. In one study, no changes in skin or organ structures related to treatment, other than occasional dermatitis, were noted in Swiss-Webster mice (21/group) following 2 years of twice-weekly dermal applications of 5% aqueous solutions of either C_{15-18}AOS (90% active), hexadecane 1,4-sultone, or a sultone concentrate (64% active) extracted from the process stream during the sulfonation of C_{15-18} α-olefin (Chevron Chemical Co., unpublished data). In another investigation, Hunter and Benson (1976) observed no increased incidence of tumors in CFX rats fed up to 5000 ppm of C_{14-18}AOS for a period of two years.

The carcinogenic potential of several AOS samples was assessed in 400 Long-Evans rats divided into four treatment groups (50/sex/group) as follows:

(1) deionized water (vehicle controls)

(2) essentially hydrolyzed, composite sample of C_{14-16}AOS and C_{16-18}AOS (30.0% active)

(3) partially hydrolyzed sample of AOS (30.9%) identical to (2) but containing a residual level of sultone

(4) commercial C_{14-16}AOS (38.9% active)

Each preparation, as a 10% active (v/v) aqueous solution, was applied twice weekly to the clipped dorsal surface at a dose level of 1 mL/kg for 24 months. Mean body weights, food consumption, hematology, urinalysis, mortality, and gross post-mortem observations were comparable for all four groups. A higher incidence of yellow staining of the anogenital fur (a common observation in aging rats) was seen in treated rats compared to controls but could not be attributed to urinary excretion of sultones. Group 2 males displayed a slightly lower mean kidney weight and significantly lower mean kidney to body weight ratio than did control animals but all other organ and organ to body weight ratios were comparable to control values. Gross and histopathological examinations revealed a similar incidence of neoplastic lesions in test and control rats, predominantly of the type commonly found in aging rats, with no carcinogenic effect attributable to the percutaneous application of the AOS test materials (Soap and Detergent Association, Colgate Palmolive Co., unpublished data).

A dermal carcinogenicity study has also been conducted with Swiss Webster mice. Groups of mice (40/sex/group) were treated three times a week for 92 weeks with 0.02 mL of test materials applied to the shaved interscapular surface. The study contained six treatment groups: 20% C_{14-18}AOS, 25% C_{14-18}AOS, 20% C_{14-16}AOS, 25% C_{14-16}AOS, 6.7% C_{16}-1,4-sultone, 8.3% C_{16}-1,4-sultone. There were also untreated, water and acetone control groups. A brief summary of the study (no data reviewed) stated that no significant toxicity or histopathology attributable to treatment was found (International Alpha Olefin Sulfonate Group, unpublished data).

Although there have been several reports that the lower molecular weight alkane sultones, particularly 1,3-propane sultone, are potent carcinogens in mice and rats (Druckrey *et al.*, 1968, 1970; Van Duuren *et al.*, 1971; Ulland *et al.*, 1971; Slaga *et al.*, 1973; Hooson *et al.*, 1971), there is no indication at present that either AOS or the higher molecular weight sultones (in the surfactant range) resulting from the synthesis of AOS are carcinogenic.

5. Teratogenicity

Teratogenicity testing of AOS has been reviewed by Oba (1992). A single study was found which assessed the possible teratogenic and embryopathic potential of AOS. Palmer *et al.* (1975) administered C_{14-18}AOS by gavage to pregnant rats (20/dose), mice (20/dose) and rabbits (13/dose) at dosage levels of 0.2, 2, 300 and 600 mg/kg/day. Mice and rats were treated on days 6-15 of pregnancy, rabbits on days 6-18 of pregnancy. The sultone content of the AOS was not determined.

None of the rats showed any manifestations of maternal toxicity regardless of dose. On the other hand, all of the rabbits administered 600 mg/kg AOS died; anorexia, diarrhea, and body weight loss were observed prior to death. Only one dam died at 300 mg/kg. Survivors showed initial anorexia and body weight loss.

Similarly, six of the maternal mice treated with 600 mg/kg AOS died, 5 dams lost their entire litters and survivors exhibited reduced activity, piloerection and retarded body weight gain. No mice died at 300 mg/kg, but a similar pattern of toxicity was seen and 5 dams lost their litters. Both maternal mice and rabbits showed initial retardation in body weight gain at 0.2 and 2.0 mg/kg of AOS.

Litter parameters (e.g., litter size, embryonic deaths, litter weight, mean pup weight) were unaffected in rats at all treatment levels and in mice and rabbits at 0.2 and 2.0 mg/kg AOS. Fetal abnormalities were noted in mice and rabbits only at the two dosage levels in which maternal toxicity occurred (i.e., 300 and 600 mg/kg AOS).

In rabbits, slightly lower, but not significant, mean pup weight was seen for the 300 mg/kg treatment group. The incidence of minor skeletal anomalies in this group was higher (23% vs 7% for controls) with a significantly higher proportion of pups (87% vs 59% for controls) having an extra rib.

Due to the high incidence of total litter loss seen in the dams, a high mean embryonic loss and low mean litter size were evident in groups of mice given either 300 or 600 mg/kg AOS. If total litter loss data were excluded, however, litter size and embryonic loss were comparable to control values. Lower litter weights and significantly lower mean pup weights (compared to control values) were observed for all levels of AOS studies, but the authors noted that control values (litter weight 14 g, mean pup weight 1.23 g) were higher than normally seen in their laboratories (11.5 g and 1.04 g, respectively) with this particular species.

With respect to anomalies, four pups (3 litters) at 600 mg/kg and 2 pups (1 litter) at 300 mg/kg had cleft palates. There was also a significantly higher incidence (98.5% vs 1% for controls) of skeletal anomalies (generally retarded ossification) seen at 600 mg/kg; a higher incidence (22-39%) of skeletal anomalies was also observed at the lower treatment levels.

6. Mutagenicity

Mutagenicity testing of AOS has been reviewed by Oba (1992). Concentrations of 5000 mg/kg of four AOS products (21-38% active) were not mutagenic when tested with *Salmonella typhimurium* TA 1535 or *Saccharomyces cerevisiae* D3 in a host-mediated assay with mice. Propane sultone (460 mg/kg), however, was mutagenic in both systems, while 1,4-butane sultone was found to be mutagenic for *S. typhimurium* but not mutagenic for *S. cerevisiae* (Colgate Palmolive Co., unpublished data).

Similarly, *in vitro* studies (Ames assay, 1973) with *S. typhimurium* TA 1535 indicated that none of the above four AOS products (2 mg/plate) was mutagenic. Both propane sultone (25 µg/plate) and 1,4-butane sultone, (125 µg/plate) were mutagenie in this test system. Additionally, none of the four AOS products increased the mitotic recombination frequency in *S. cerevisiae* D 3 (Colgate Palmolive Co., unpublished data).

Seven olefin sulfonate preparations tested at concentrations up to 2 mg/plate were found to be non-mutagenic in assays with *Salmonella typhimurium* bacterial strains TA 1535, TA 1536, TA 1537, or TA 1538. The sodium salt of a C_{17-20} olefin sulfonate was tested in strains TA98, 100, 1535, 1537 with and without activation at concentrations ranging from 10^{-8} to 10^{-10} mg/plate. Negative results were obtained in all cases (Shell Chemical Co., unpublished data). Five of these preparations tested at concentrations of 0.01, 0.1 and 1.0% were also non-mutagenic in the yeast assay with *Sacharomyces cervesiae* D 3.

The responses of acid or alkaline-bleached olefin sulfonate were confounded by poor survival of the yeast cells. When concentrations of test materials which did not decrease survival were used, there were no differences between control and treated groups. On the other hand, the alkaline-bleached slurry gave only a 3% average survival. Without metabolic activation, the alkaline-bleached sample did not elicit positive responses at 0.01% and showed 88% average survival. The test was repeated at 0.02, 0.04, 0.06, 0.08, and 1% concentrations and no evidence of mutagenic effects was obtained. Survival of the cells was 12% and 21% for the acid-bleached and alkaline-bleached samples tested at 1% respectively. Both 1,3-propane sultone and 1,4-butane sultone served as the positive controls and were mutagenic in these systems

(Colgate Palmolive Co., unpublished data).

In another study, a concentration of 283 mg/kg of a particular batch of C_{14-16}AOS (28.4% active) was found to be mutagenic in a host-mediated assay with rats when tested with *Salmonella typhimurium* TA 1530 (a point mutant), but not mutagenic with *S. typhimurium* TA 1534 (a frameshift mutant). *In vitro* plate assays with AOS concentrations up to 1% were negative for both strains. Host-mediated assays with equivalent AOS products from other suppliers were also negative. Since the pH of the original AOS sample was extremely high (11.3), the possibility that alkalinity could be a factor in the mutation frequency was explored. The original AOS sample was neutralized to a pH of 8.5 with sulfuric acid and subsequently readjusted to a pH of 11.3 with sodium hydroxide. Both products gave negative responses in the host-mediated assay. The original sample was next extracted with ether in an attempt to remove the causative agent. The ether extract was not tested, but the remaining aqueous fraction was mutagenic with TA 1530. However, the average number of histidine reversions (His+) was reduced from 1202 and >10,000 (two experiments) reversions seen with the original AOS sample (283 mg/kg) to 477 reversion with the aqueous fraction (210 mg/kg). The investigators concluded that the causative agent(s) in this batch of C_{14-16}AOS could be: (1) partially, but not completely removed by ether extraction; (2) could apparently be destroyed by reducing the pH to 8.5 with sulfuric acid; (3) did not result from high alkalinity; and (4) were generated *in vivo* because all *in vitro* studies with these compounds were negative (Procter & Gamble Co., unpublished data). Since this finding of mutagenicity with a single commercial AOS sample is unique, the results may be due to substances having no direct relationship to this surfactant.

At a concentration of 1000 µg/ml AOS (36% active) was found to be 100% cytotoxic against actively dividing human diploid fibroblasts (WI38 cells) *in vitro*. Reduction in cell viability of 80 and 85% were noted for AOS which were 38% and 21% active, respectively. However, there are no indications that AOS produce DNA damage in these mammalian cells *in vitro*. Although a generally low level of DNA repair, which in some cases exceeded control values, was seen in human diploid fibroblasts (WI38 cells) exposed to 0.1, 1, 10, 100 or 1000 µg/ml AOS in an unscheduled DNA synthesis assay, there was no indication that AOS induced unscheduled DNA synthesis (Colgate Palmolive Co., unpublished data).

7. Pharmacology

a. Absorption and Metabolism

AOS is readily absorbed after oral administration to rats. It is primarily excreted in the urine. Absorption through the skin is minimal. The metabolic fate of orally and intravenously administered AOS was studied in male Wistar rats by Inoue *et al.* (1982). The AOS was a 55:45 mixture of sodium 3-hydroxy alkane sulfonate and sodium alkenyl sulfonate which was labeled with ^{14}C. The animals received either a single oral dose of 100 mg/kg or an intravenous dose of 10 mg/kg. After oral administration, about 80% of ^{14}C-AOS was rapidly absorbed from the gastrointestinal tract. The blood level peaked at 3 hours. Within 24 hours of the dose, 72% was excreted in the urine and 22% in the feces. Within 12 hours, 4.3% was excreted in the bile. After intravenous injection, 50% of the dose was excreted within 1 hour. Within 6 hours, 90% was excreted in the urine. These results suggest that no accumulation of ^{14}C-AOS occurs and that it is rapidly absorbed, metabolized and excreted.

Minegishi *et al.* (1977) investigated the percutaneous absorption of ^{14}C-labeled AOS in male Wistar rats under various conditions. Application of 0.5 mL of a 0.2% solution of ^{14}C-AOS to the intact dorsal skin, which was allowed to dry naturally, resulted in the absorption of 0.6% of the applied dose; after 24 hours, 0.33% of the applied radioactivity was found in urine, 0.08% in bile and 0.21% in body organs. Similar experiments in which the skin was wiped off 0.5 or 1.5 hours after application indicated that no further absorption occurred after 1.5 hours. The excretion of radioactivity in urine and bile peaked between 3 and 6 hours, then gradually decreased thereafter up through 90 hours post-exposure. Application of the same volume to damaged skin (no stratum corneum) increased absorption 80-fold. Approximately 50% of the applied radioactivity was absorbed and subsequently excreted in urine (36.26%) and bile (1.83%); an additional 12.28% was present in body organs 30 hours post-dosing.

No methemoglobin-forming activity was seen in mice following either oral or intraperitoneal administration of near LD_{50} levels of AOS (Tamura and Ogura, 1969a).

b. Cellular Effects

Tamura and Ogura (1969b) reported that stabilization of erythrocyte membranes by AOS decreased in the order of: goat > human > rabbit > rat. This order of stabilization correlated with the lecithin content of the erythrocyte membrane which also decreased in the same order.

c. Isolated Muscle Preparations

Ogura and Tamura (1968) reported on the effect of AOS exposure (10^{-5}- 10^{-4}M) in an isolated frog muscle preparation and in an isolated clam heart preparation. When tested on the abdominal rectal muscle of the frog, *Rana nigromasculata*, AOS was found to be a non-competitive antagonist of acetylcholine; i.e., acetylcholine-induced contractions were suppressed. AOS exposure, however, had no influence on KC1-induced contractions.

In the isolated clam heart preparation, AOS was seen to exert a reversible negative inotropic action; i.e., weakening the force of muscular contraction, but pretreatment with AOS did not inhibit the cardiac action (i.e., positive inotropic action) of either 5-hydroxytryptamine or norepinephrine.

d. Antibody Responses

Rabbits were immunized over a 2½ month period with either C_{15-18}AOS, human serum albumin (HSA) or with a lipid-free, HSA-AOS complex. Serum from immunized rabbits was then tested via a precipitin reaction for the presence of antibodies for any of the above three antigens. AOS was found to possess no antigenic properties in the rabbit and immunization with the HSA-AOS complex did not provoke the formation of AOS antibodies in rabbit serum (Iimori and Ushiyama, 1971).

B. Human Studies

Negligible to mild skin irritation was observed in human volunteers in 24-hour patch tests with 1-2% active AOS samples. In another study, increased irritation was noted as the study progressed in a 10 day occlusive patch test with a 0.8% active concentration of AOS. Positive sensitization responses have been attributed to the presence of unsaturated 1,3-sultones.

1. Skin Irritation and Sensitization

Low concentrations of AOS cause negligible to mild irritation, even after repeated exposures. Rare reports of skin sensitization in sodium lauryl ether sulfate have been linked to the presence of unsaturated 1,3-sultones, a potential contaminant in AOS.

In 24-hr human patch tests using 1% and 2% concentrations of AOS, irritancy was reported by both Webb (1966) and Witco Chemical (unpublished data) to be negligible while Oba *et al.*, (1968) and Procter & Gamble Co. (unpublished data) found AOS concentrations of 1% and 1.5%, respectively, to be mild irritants with reactions ranging form erythema to fissure accompanied by scaling. Further, in a ten-day occlusive patch test, an 0.8% active concentration of AOS caused increasing irritation as the study progressed (Witco Chemical Corp., unpublished data).

In immersion studies, a 0.3% AOS concentration caused negligible irritation after 30 one-minute hand immersions in a 1-hour period (Tomiyama *et al.*, 1969) and a 0.25% concentration of a liquid detergent formulation containing 17% AOS was classified as a mild irritant in a test involving 15-minute immersions, three times daily for up to a maximum of 15 days. Fifty percent of the test subjects were able to complete 12 immersions before reaching a predetermined irritation level (a score of "2") at which point treatment was discontinued (Lever Brothers Co., unpublished data).

In a study using a standard Draize test, no evidence of contact sensitization was found in 88 men following application of an occlusive patch containing an induction dose of 8% aqueous solution of AOS 3 times per week for a total of 10 applications. Because of severe irritation, the final challenge was made with a 4% AOS solution two weeks after the last application (TerHaar, 1983).

A sensitization study was carried out in 195 subjects who received approximately 0.2 ml of a 1% AOS solution under an occlusive patch. The 1% AOS solution contained 28 ppm 1,3-sultones (saturation unspecified). The patches were applied 3 times per week during the first 3 weeks. A challenge application was applied after a 1 week rest period. Eight of the 195 subjects were sensitized. Five of the sensitized subjects were then challenged with AOS containing 1 ppm of 1,3-sultone. Positive results were seen in 3 out of 5 subjects (TerHaar, 1983).

C. Epidemiology

No reports of injury resulting from human exposure to alpha olefin sulfonates have been found.

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