Environmental and Human Safety of Major Surfactants

Volume I. Anionic Surfactants

Part 3. Alkyl Sulfates

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The Soap and Detergent Association
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Synopsis

Alkyl sulfates (AS) are used in consumer laundry powders and are widely used in specialty products such as hair shampoos, cosmetics, toothpastes, etc. They are also extensively utilized as wool-washing agents.

There are presently no environmental standards of water quality with respect to alkyl sulfates. Levels of AS, as such, in streams and waterways are not presently being monitored, but MBAS levels would include AS, if present. Biodegradation studies indicate linear primary AS readily undergo primary biodegradation under both laboratory and field conditions. Slightly branched and secondary AS are also readily biodegraded. Highly branched AS biodegrades at a considerably slower rate.

Concentration of LC50 values determined in acute toxicity tests for AS exposed to aquatic fauna range from 0.35 mg/L to 1000 mg/L, depending on the individual species' sensitivity and the life stage of the test organisms. The toxicity of AS increases with increases in alkyl chain length and water hardness, and also as the experimental water temperature increases from acclimation temperature. Whole-body bioconcentration factors are generally low (<10x), but the radiolabel from alkyl sulfates accumulates to higher levels (5-80x) in the gall bladder and hepatopancreas. Hard water is also more conducive to uptake of AS than soft water. The toxic effect of AS is observed primarily in the gills, and is thought to result from the formation of a complex between the surfactant and the surface-bound proteins.

Aquatic algae have exhibited toxicosis at AS concentrations from 10 mg/l to 1000 mg/L, and terrestrial plants are adversely affected by concentrations as low as 1 mg/L in applied water. One study indicated that waterfowl may be subject to increased risk of hypothermia in detergent-polluted waters; AS (19 mg/L) was found to dissolve the waterproofing oils in feathers of exposed ducks. Bacteria and other
microorganisms may autolyze when exposed to concentrations of 0.1 mg AS/L; immobilization and growth inhibition have been observed at concentrations of 10 to 1000 mg AS/L.

With respect to human safety, AS can be generally classified as non-toxic. They exhibit a low order of acute mammalian toxicity and are rapidly metabolized and excreted in urine. Daily ingestion of 250 mg AS/kg for two months reduced cholesterol induced aortic atherosclerotic lesions in rabbits. No deleterious effects were produced in rats fed 1% AS in the diet for one year. There are no indications from long-term feeding or skin-painting studies that AS exhibit any carcinogenic activity.

Ingestion of AS by laboratory animals during gestation produced no detrimental effects on litter parameters except at doses that were severely toxic to the dams. Percutaneous applications of some concentrated AS samples (10-20%) to mice during early gestation were embryotoxic, but skin application of these concentrated samples during later stages of pregnancy were neither embryotoxic nor teratogenic. A decreased number of implantations was noted following applications of lower concentration of AS (2%) during early gestation, but the number of animals examined was too small to allow definitive conclusions to be drawn. Application of 10% AS twice daily to the backs of pregnant mice prior to implantation interrupted cleavage of eggs, retarded fetal development and elevated the incidence of deformed embryos. However, application of 2% or 20% AS to mice during late pregnancy did not interrupt gestation.

Although concentrated AS samples are primary skin and eye irritants in laboratory animals, repeated occluded skin exposure to 0.1% AS is non-irritating. Similar results are noted in humans. Little or no ocular irritation is observed in rabbits at concentrations of 1% AS or less. Ocular irritation is observed in mice at concentrations of 0.2% AS and greater.
ALKYL SULFATES

I. INTRODUCTION

Until the mid-1960's, anionic alkyl sulfate (AS) surfactants were predominantly used in household and industrial wool-washing applications (Tomiyama et al., 1969).

Although AS are still widely used in specialty products such as hair shampoos, cosmetics, toothpaste, and carpet cleansers, laundry applications of AS have dropped considerably in the United States, being practically replaced by an alkyl sulfate-alkyl ethoxy sulfate blend (Matson, 1978).

Production of alkyl sulfates in the U.S., Japan and Western Europe totalled 220,000 metric tons in 1982 making up 6% of the anionic market (Jakobi et al., 1987). Since 1980, production of AS for all market segments has increased about 15%, however, industrial usage is expected to decrease by 1% per year through 1995 (Sherman et al., 1987).

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

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

A list of chemical designations used in Volume 1, Part 3 can be found in Appendix A.
BIBLIOGRAPHY


II. CHEMISTRY

A. Product Chemistry

*Commercial alkyl sulfates (AS) are manufactured primarily by reacting the parent alcohol with sulfur trioxide or chlorosulfonic acid.*

Commercial alkyl sulfates are usually manufactured from primary alcohols by conventional sulfation with either sulfur trioxide ($SO_3$) or chlorosulfonic acid ($CISO_2H$), followed by neutralization with an appropriate base (Kirk Othmer, 1983). AS prepared in this manner (reaction below) are generally referred to as primary AS.

$$
SO_3 \quad \text{or} \quad NaOH, \ e.g. \\
R-OH \quad \rightarrow \quad R-OSO_2NH^+ \quad \rightarrow \quad R-OSO_2Na^+
$$

The parent alcohols for these sulfates range in carbon chain length from $C_8$ to $C_{18}$, and are predominantly linear. Precursor alcohols include those which are completely linear as well as those which are derived from linear olefins via oxo-type chemistry and which contain some methyl-branched components. Small quantities of primary AS are also produced from alcohols derived from highly branched olefins via oxo-type chemistry. See Appendix A for nomenclature and abbreviations.

Commercial AS is typically shipped as a low active matter aqueous solution (25-40% AM) or a high active paste (90+% AM). AS is most usually supplied in the sodium salt form. Other common neutralizing cations are ammonium, triethanolamine, and magnesium. Unless they are specifically removed, AS will also typically contain minor amounts of the unsalted parent alcohol, some sodium (or other cation) sulfate, and if manufactured by the chlorosulfonic acid route, some sodium (or other cation) chloride.
Secondary AS, popular in Europe at one time, have never been used extensively in the U.S. and are no longer of commercial significance. They were prepared by sulfation of linear C₈-C₁₈ alphaolefins with sulfuric acid (Kirk-Othmer, 1983) as shown below.

\[
\begin{align*}
\text{R-C-C} & \xrightarrow{\text{H}_2\text{SO}_4} \text{R-C-C-R (isomer mix)} \\
\text{R-C-C} & \xrightarrow{\text{NaOH}} \text{R-C-C-R}
\end{align*}
\]

The sulfate ester group does not necessarily add at the double bond position, but rather at any position along the chain except the terminal carbon atoms, distinguishing the product from the primary AS. A complex mixture of secondary AS isomers thus occurs (Higgins and Burns, 1975; Kerfoot and Flammer, 1975; Swisher, 1970).

B. Analytical Methods

AS compounds can be determined by volumetric, spectrophotometric, chromatographic or ion-selective electrode methods. In the spectrophotometric methods, the cationic dyes methylene blue, Remacryl Blue B, Remacryl Red 2BL and ethyl violet were found to be useful but are not generally specific to AS. Chromatographic techniques are the most selective analytical methods and interferences can be diminished by careful selection of detectors.

1. Anionic Surfactant Methods

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 few specific references to AS analytical methods. However, many of the LAS methods are not specific to LAS analysis and may be used for the analysis of other anionic surfactants. For example, colorimetric methods such as the Azure A and MBAS methods and titration and volumetric techniques rely on the reaction of the sulfate...
or sulfonate group with a specific reagent; these methods are applicable to AS surfactants as well as LAS surfactants.

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

Newer techniques developed for analysis of LAS or anionic surfactants, in general, may also be applicable to AS analysis. These techniques may include GC, HPLC, GC/MS, TLC, and, to a lesser extent, polarography and ion-sensitive membrane electrode potentiometry. NMR techniques may be applicable to analysis of bulk samples. Therefore, the reader is referred to the LAS discussion. This section will be limited to a discussion of reports of AS-specific analyses.

2. AS-Specific Methods

a. Volumetric Methods

Concentrations of AS and LAS compounds were determined volumetrically by Ivanov (1979) at a detection limit of ≥25 mg/L and typical relative standard deviation of ≤5%. Samples were titrated against a standard acid with a Bromophenol Blue-Acid Chrome Dark Blue indicator, hydrolyzed in concentrated HCl under reflux, and titrated again in the presence of the same indicator.

b. Spectrophotometric Methods

Several spectrophotometric methods for the determination of specific AS compounds have been reported. In their review, Llenado and Jamieson (1981) summarize a method based on development of a blue chloroform
phase when alkyl sulfates are extracted with methylene blue (Perov et al., 1979), as well as the use of cationic dyes Remacryl Blue B and Remacryl Red 2BL for the determination of lauryl sulfate (Yaneva and Borisova-Pangarova, 1978).

Motomizu et al. (1982) evaluated several cationic dyes as reagents for the spectrophotometric determination of anionic surfactants, including dodecylsulfate. Of the dyes examined, ethyl violet was the most useful. Trace amounts of dodecylsulfate and other anionic surfactants were reacted with ethyl violet to form ion associates which were subsequently extracted into toluene (or benzene) in a single extraction. Absorbance was determined spectrophotometrically at 615 nm, the maximum absorbance of the dodecylsulfate ion associate. This method is not specific for dodecylsulfate since the absorption maxima of other anionic surfactants tested were almost the same. The effect of coexisting inorganic ions was also examined; interfering absorbance was diminished by washing the organic extract with aqueous solution. The method was applied to the determination of ppb levels of anionic surfactants in water; anionic surfactant recovery values were 97-107%.

c. Chromatographic Methods

Williams (1982) reported the development of ion chromatographic (IC) methods for the separation of ionic organosulfur compounds, including AS. Observing that the HPLC determination of AS is hampered by lack of suitable chromophores for UV detection, Williams (1982) demonstrated the usefulness of IC and a conductivity detector for the separation of 9 ppm methane sulfonate and 20 ppm methylsulfate, as well as the separation of other ionic surfactants ranging in concentration from 10 ppm to 50 ppm. The methane sulfonate/methyl sulfate separation was achieved with 0.001 M NaHCO₃ eluent; interferences were noted with a 0.003 M NaHCO₃/0.0024 M Na₂CO₃ eluent. In general, retention time of the ion increases with carbon length; however, resolution of methane and ethane sulfonates and disulfonates was not completely achieved. As
expected, higher-valence ionic species were observed to be more strongly retained on the ion-exchange resins and substitution of halogen atoms on ethane sulfonate was observed to produce longer retention times, directly related to the size of the halogen atom. A UV detector was also used in conjunction with the normal conductivity detector for certain compounds amenable to UV detection, eliminating potential interferences. Since many common inorganic anions exhibit UV absorption below 220 nm, these interferences are best eliminated by using a wavelength above 220 nm.

Irgolic and Hobill (1987) used HPLC for the separation of sulfur-containing surfactants, including AS; an inductively coupled argon plasma vacuum emission spectrometer (ICP) monitoring the 180.7 nm sulfur line served as the sulfur-specific detector. AS surfactants analyzed included dodecylsulfate and tetradecylsulfate. The method is well-suited for fingerprinting commercial surfactants; however, many of the isomeric and homologous components of the anionic surfactants were not completely resolved. The analysis of Henkel alkyl sulfates (50 µL of 400 mg/L) produced 3 peaks attributable to sulfate, dodecylsulfate and tetradecylsulfate. Injection of Kodak dodecylsulfate (10 µL of 700 mg/L) produced a single peak; larger injection volumes failed to produce additional peaks. The chromatogram of raw wastewater spiked with small amounts of shampoo and dishwashing liquid exhibited sulfate as the major peak with prominent peaks for dodecylsulfate and tetradecylsulfate. The wastewater matrix did not interfere with the HPLC chromatography or the sulfur-specific detector. The 2-σ detection limit for the HPLC-ICP system was determined to be approximately 15 ng sulfur, corresponding to a 50 µL injection of a surfactant solution with sulfur concentration of 0.3 mg/L.

d. Ion-Selective Electrode Methods

Llenado and Jamieson (1981) summarized three ion-selective electrode methods that have been reported for the analysis of AS compounds.
- A liquid membrane surfactant-sensitive electrode capable of detecting $10^{-6}$ M sodium dodecylsulfate was reported by Anghel et al. (1976).

- An ion-specific electrode sensitive to $C_8-C_{10}$ alkyl sulfates was described for monitoring AS surfactants in process solutions and wastewaters by Rakhamaniko et al. (1978).

- The preparation of a dodecylsulfate electrode using a solution of cetylhexyltrimethylammonium dodecylsulfate in nitrobenzene was described by Popa et al. (1974).

More recently, Kresheck and Constantinidis (1984) undertook a study of the behavior of octyl sulfate and decyl sulfate electrodes and the utility of ion-selective electrodes for rapid determination of surfactant ion concentrations. The electrodes functioned properly from 20°C to 35°C in aqueous solutions and at 25°C in various aqueous mixtures. Calibrations were shown to be necessary for each solvent system used as the electrode response was characteristic of the aqueous solution and the individual electrode and showed some drift with time. The useful lifetime of an individual electrode could not be predicted. The authors indicated that the results support cautious use of these ion-specific electrodes in certain bioanalytical applications. The major limitation to use in the presence of polymers (including proteins) is their effect on the electrode response.


III. BIODEGRADATION

As a class, alkyl sulfates are biodegraded quite readily. Swisher (1987) lists the results of numerous biodegradation tests on various alkyl sulfates which show this. Linear, primary AS generally undergo complete primary biodegradation within a few days and secondary and slightly branched AS surfactants are also biodegraded quite readily. In contrast, some highly branched AS have been observed to degrade at a considerably slower rate, showing no degradation after 18 days.

A discussion of the procedures utilized to investigate the biodegradation of anionic surfactants, including the alkyl sulfates, can be found in Part 1 of Volume I, (LAS, Section III).

A. Laboratory Test Systems

Alkyl sulfates are readily biodegraded in laboratory test systems. This is true of screening tests using an artificial, inoculated medium, die-away tests with natural river water or seawater, as well as tests that simulate treatment processes. Biodegradation also occurs in anaerobic test systems. These generalizations apply to linear or slightly branched alkyl sulfates, on which most testing has been done. Some highly branched types degrade more slowly.

1. Oxygen Uptake - Biochemical Oxygen Demand

Alkyl sulfates are readily biodegraded in standard 5- or 20-day BOD tests. Swisher (1987) reports 20-day BOD values of 96%, 72%, and 75% of theoretical for a primary linear alkyl sulfate, primary 2-ethyl-hexyl sulfate, and secondary, linear C_{10-13} alkyl sulfates, respectively. Neither slight branching nor increments in the length of the carbon chain appear to exert a significant effect on the rate of degradation.
(see Table III-1). For example, after 5 days, slightly branched C₁₁AS had utilized 73% and 67%, respectively, of their theoretical oxygen demand (Procter & Gamble Co., unpublished data). Glucose generally utilizes about 70% of its theoretical oxygen demand in this time period (Swisher, 1970).

TABLE III-1
BIODEGRADABILITY OF ALKYL SULFATES

<table>
<thead>
<tr>
<th>Carbon Chain Length</th>
<th>% BOD₅*</th>
<th>% BOD₂₀**</th>
<th>% CO₂†</th>
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<tbody>
<tr>
<td>C₁₁</td>
<td>72.9</td>
<td>T.D. ††</td>
<td>93.2</td>
</tr>
<tr>
<td>C₁₁₅</td>
<td>72.9</td>
<td>T.D.</td>
<td>89.1</td>
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<tr>
<td>C₁₂</td>
<td>63.1</td>
<td>T.D.</td>
<td>85.0</td>
</tr>
<tr>
<td>C₁₃₅</td>
<td>65.7</td>
<td>93.9</td>
<td>85.8</td>
</tr>
<tr>
<td>C₁₂₃</td>
<td>51.0</td>
<td>96.2</td>
<td>74.4</td>
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<td>C₁₂₁₄</td>
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<td>70.1</td>
<td>95.0</td>
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<td>C₁₅</td>
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<td>T.D.</td>
<td>94.7</td>
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<td>C₁₆</td>
<td>62.5</td>
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<tr>
<td>C₁₆₃₈</td>
<td>60.3</td>
<td>T.D.</td>
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</tr>
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</table>

* Percent biochemical oxygen demand at 5 days.
** Percent biochemical oxygen demand at 20 days.
† Percent of theoretical CO₂ production.
†† Total depletion of oxygen
= Slight methyl branching.

(Procter & Gamble Co., unpublished data)

2. CO₂ Evolution

Alkyl sulfates are also found to be readily biodegraded in screening tests that are based on CO₂ evolution. Gilbert and Pettigrew (1984) report 95.7% of the theoretical CO₂ production for DUBANOL 25 sulfate (C₁₂-C₁₅ primary alcohol ~75% normal, 25% isomeric) using the modified
Sturm test. Itoh et al. (1985) report that roughly 80% of an initial sodium dodecyl sulfate solution (6.2 mg/L TOC in BOD diluent water inoculated with a municipal sewage culture) was degraded within 5 days at 27°C. Little further degradation occurred during the succeeding five days; no explanation was given for this phenomenon, which was also observed for glucose.

The AS surfactants cited above for BOD testing were examined using Sturm's evolved CO₂ procedure (1973). All were readily degraded (See Table III-1) with the percent of evolved CO₂ ranging between 74% and 95%. Under these conditions, glucose evolved 80-85% CO₂ (Procter & Gamble Co., unpublished data).

3. Die-Away Tests

a. River Water Test

Maurer et al. (1971) reported that 5 mg/L of C₁₆ AS underwent complete primary biodegradation (as measured by MBAS) after one day in a river water die-away test. Similarly, greater than 95% of n-C₁₂₋₁₄₋₁₆ AS had been biodegraded (MBAS) in one day (Huddleston and Allred, 1967) and complete removal (MBAS) of 10 mg/L of n-pri-C₁₂₋₁₄₋₁₆ (64:25:11) AS was achieved by 2 days (Continental Oil Company, unpublished data).

After 3 days, Sekiguchi et al. (1975) could detect (as MBAS) none of the 20 mg/L C₁₂ AS added to samples to Tama River water. However, it took 20 days for the total organic carbon (TOC) to dissipate completely as compared to 13 days for the same concentration of glucose.

b. Sea Water Test

Sales et al. (1987) found that linear C₁₂ alkyl sulfate was rapidly degraded in natural seawater in a modification of the river water die-away test (1 mg of surfactant and 25 g of wet sediment were added to 100 ml of the seawater). Roughly 90% was degraded within five days.
at 25°C. No lag period took place, and kinetics were observed to be first order. Counts of microorganism colonies in the water showed a rapid rise from a negligible number at day 0 (compared to the number in the sediment) to a maximum at day 3, falling off to near 0 by day 21. Counts in sediments followed a similar pattern of growth and decline, except that compared to the water much greater numbers were present initially and at the end of the test period.

Vives-Rego et al. (1987) observed rapid degradation of sodium dodecyl sulfate at 20 mg/L in uninoculated seawater at 22°C. Degradation was essentially complete within three days and followed first order kinetics, with a half-life ranging from 0.26-0.34 days. The extent of degradation of 5 mg/L of C_{12}AS in seawater was also examined by Sekiguchi et al. (1975); no MBAS activity could be detected at 5 days. In 4 separate tests with Chesapeake Bay water, Cook and Goldman (1974) found that it took an average of 1 to 3 days to achieve a 75% decrease in azure-A-reactive substances for C_{12}aveAS.

### c. Fortified and Inoculated Waters

Gilbert and Pettigrew (1984) reported 95% loss of DOC in 28 days when DOBANOL™-25 sulfate was tested using the modified OECD screening test. Gordon et al. (1972) found that 40 mg/L C_{16}AS was completely degraded (as measured by MBAS) after 2 days. Crauland et al. (1967) noted that it took unacclimated bacteria 3 days to completely biodegrade (MBAS) C_{14}AS, but acclimated bacteria required only one day to achieve the same results.

The work of Miura et al. (1979), referred to in Part 1, also involved biodegradation of 100 mg/L sample of sodium dodecylsulfate. With activated sludge inoculum, MBAS disappeared completely in less than 5 days, while removal of TOC and BOD/TOD (total oxygen demand) approached 100% between 10 and 15 days. Similar results were reported by Itoh et al. (1979). The two alkyl sulfates tested, C_{12}aveAS and a coconut-alcohol-derived AS, were the most readily biodegraded of all the surfactants tested.
In a static die-away test using the Swiss EMPA method, Gafa and Lattanzi (1974) found that 3 commercial AS were all biodegraded (MBAS) greater than 95% within 3 days. The materials tested included: 100% linear C_{12-14-16}AS (based on ALFOL 1216/P\textsuperscript{m}, mol. wt. 206); 85% linear C_{12-16}AS (based on DOBANOL 25\textsuperscript{m}, mol. wt. 206); and 55% linear C_{11-13-15}AS (based on DIADOL HA 115\textsuperscript{m}, mol. wt. 203). Similar findings were reported for n-C_{12-ave}AS by Arpino (1969) and Lundahl et al. (1972).

d. Shake Culture Test

In a shake culture test with Bunch-Chambers media, Sekiguchi et al. (1972) noted that C_{12-ave}AS and C_{12-13}AS (DOBANOL\textsuperscript{m}-23) sulfate lost between 95-100% of their MBAS activity and greater than 85% TOC in one day. By 5 days, 100% of the TOC activity was gone. Oba et al. (1967) also reported 100% MBAS removal after 24 hours with C_{12-ave}AS but found that only 47% COD removal had been achieved in this time. Ripin et al. (1970), on the other hand, found that 8 days were required to achieve 90% biodegradation (MBAS) of C_{12}AS while Allred and Huddleston (1967) found 3 days sufficient time to completely remove (MBAS) 30 mg/L C_{12-ave}AS. In another study, 30 mg/L of n-pri-C_{12-14-16} (64:25:11) AS also was completely biodegraded (MBAS) in 2 days (Continental Oil Co., unpublished data).

4. Simulated Treatment Processes

a. Activated Sludge

Gilbert and Pettigrew (1984) report 95.8% removal of DOBANOL\textsuperscript{m} 25 sulfate in the OECD semi-continuous activated sludge test.

Janicke (1971) observed that n-C_{12}AS was completely biodegraded (>99% MBAS and >94% TOC removals) up to a surfactant loading level of 100 mg/L in a laboratory-scale, activated sludge unit. At concentrations from 100 to 200 mg/L degradation was nearly 100%, but at concentrations of 100 mg/L and higher, the following signs of surfactant overloading were evident: reduction in nitrification; a 20-25% reduction in the
degradation of organic nitrogen; high turbidity of the effluent and the disappearance of protozoa from the sludge. These observations were not reported for lower concentrations.

Fisher and Gerike (1975) reported 99% removal (MBAS) of n-C₁₂AS in the OECD confirmatory test (1971) after 1 day. Similar results were reported by Sakaguchi et al. (1975) and Allred and Huddleston (1967) in a semicontinuous activated sludge unit. Linear pri-C₁₂₋₁₄₋₁₆ (64:25:11) AS was also completely biodegraded (MBAS) in a single 24-hour cycle in a semicontinuous activated sludge unit (Continental Oil Co., unpublished data). The removal of 20 mg/L sodium C₁₄₋₁₅ alkyl sulfate in a 7-day, semicontinuous activated sludge test was 101.7% (± 1.3%) (Procter & Gamble Company, unpublished data). The test was conducted at a temperature of 22-24°C, and results were based on soluble organic carbon removal.

Borstlap (1967) examined the intermediates obtained in batch activated sludge units containing 7.5 liters of unacclimated activated sludge and 50 liters of 500 mg/L AS surfactant as the sole carbon source. After one week, no MBAS activity could be detected for n-C₁₄AS but intermediates amounting to 7-10% of the mass of the original substance were found. After 3 weeks, MBAS activity for n-oxo-C₁₄AS and a highly branched tetrapropylene-derived oxo-C₁₃AS was zero and 18%, respectively, with 13-18% and 51%, respectively, of the mass of the original substrates present as intermediate products or active material. Although the intermediates could not be identified precisely, the presence of sulfonate and carboxylate groups in the intermediates was established by a potentiometric titration. The presence of hydroxylic groups could be established, and an overall formula of the intermediate mixture was derived from an elemental analysis.

b. Trickling Filters

The only available data on the extent of AS degradation in a trickling filter process are the field trial findings of Mann and Reid (1971)
which indicated a high order of AS biodegradability. See Section III.C of this report for details.

c. Anaerobic Systems

Wagener and Schink (1987) found sodium dodecyl sulfate to be completely degradable under anaerobic conditions in waters inoculated with sediment from a polluted creek or anoxic sludge from a sewage treatment plant digestor. Hydrogen sulfide was produced in the process, and methanogenesis was inhibited at surfactant concentrations ≥100 mg/L.

Oba et al. (1967) examined the anaerobic degradation of \( \text{C}_{12} \) AS in a shake culture system using an inoculum of activated sludge taken from a sewage treatment plant. MBAS removal was quite rapid: 66% the first day, 98% after 3 days and 100% by 7 days. A 39% reduction in COD was also recorded at 7 days. The same test procedure was repeated with an inoculum consisting of sludge taken from the bottom of a private cesspool. Analysis on the 14th day of the test indicated a 98% MBAS removal.

In another study, 25 mg/L of a coconut-alcohol-derived AS were fed into an anaerobic digester tank system over a 3½ month period. The tank had a capacity of 336 gallons of sewage per day. Average retention time was 68.5 hours. Surfactant removal averaged 66% (measured as the number of ³⁶S counts in a chloroform extract) with BOD and COD removals of 36% and 41%, respectively (Procter & Gamble Co., unpublished data).

Under microaerophilic conditions (≤1 ppm \( \text{O}_2 \) level), Maurer et al. (1971) found that 5 mg/L \( \text{C}_{18} \) AS completely biodegraded (MBAS) within 3-6 days at 25°C in a river water test. At a concentration of 10 mg/L, it took 9-10 days to completely biodegrade at 25°C and 34 days at 35°C. In another study conducted under microaerophilic conditions, Gordon et al. (1972) found that 95% of \( \text{C}_{18} \) AS had biodegraded (MBAS) in a static die-away test at 35°C after 7 days. However, after 17 days, when data collection ceased, roughly 30% of the TOC remained.
B. Influence of Test System Variables

The limited data available suggest that test system variables have only small effects on the degradation of alkyl sulfates, probably because of their ready biodegradability. A wide variety of bacteria are able to degrade alkyl sulfates, minimizing the effects of different inoculums. Temperature effects were not observed in two studies of treatment processes; very high removal was achieved at both winter and summer temperatures.

1. Inoculum

Daubner et al. (1981) tested the ability of various individual microorganisms to degrade a C_{12-14} alkyl sulfate and sodium lauryl sulfate at concentrations up to 100 mg/L. The survival periods in water were assessed by colony counts using a plate method; detergent biodegradation was measured with MBAS (test method not specified); and respiration activity was determined by the Warburg method. At concentrations of less than 0.5 mg/L the surfactants had no observable effect on pure cultures of either E. coli, P. aeruginosa, or S. typhimurium; at concentrations of 0.5 - 100 mg/L they had a stimulating effect. E. coli and P. aeruginosa showed similar abilities to degrade the two surfactants. Both microorganism degraded sodium lauryl sulfate to less than 10% of its initial concentration within 168 hours, while under the same conditions and after the same time, the C_{12-14} alkyl sulfate was degraded to 25-35% of the initial amount.

Goodnow and Harrison (1972) examined the degradation (MBAS) of a tallow alkyl sulfate (C_{16-18}) at concentrations of 10, 50 and 500 mg/L by 45 strains of 34 species of bacteria representing 19 genera found in water, soil and sewage. The bacterial strains came from a number of sources, including a commercial supplier and sewage sludge. No further
acclimation was performed. Of the bacteria tested, all except two degraded the surfactant between 19 and 100% within 72 hours; no degradation (0%) of the surfactant had occurred with either Acetobacter peroxydans ATCC838 or Escherichia coli B/r at 72 hours.

2. Temperature

In a laboratory-scale activated sludge plant, Gilbert and Pettigrew (1984) observed 98% removal of Dobanal 56 sulfate at 15°C and 99% removal at 8°C. Similarly, Mann and Reid (1971) found that DOBANOLm-25 (C_{12-15} primary alcohol, ~75% normal, 25% isomeric) and a coconut-alcohol-derived AS biodegraded 97-98% (MBAS) during the winter and spring months in a trickling filter sewage treatment plant.

C. Field Studies

Field studies indicate that alkyl sulfates are readily biodegraded in the natural environment and during sewage treatment. Sodium dodecyl sulfate was observed to degrade in groundwater, and alkyl sulfates have been found to be effectively removed by trickling filters.

Thurman et al. (1986) studied the fate and transport of several detergents in groundwater of a sand and gravel aquifer in Massachusetts. Sodium dodecyl sulfate (MBAS) appeared to be degraded within 300 m down gradient of the point of infiltration, with groundwater flow of 0.25-0.7 m/day. However, the presence of this compound was inferred from historical usage and not direct measurement. Further study is being conducted to identify and determine the behavior of specific surfactants.

Oba et al. (1967) analyzed raw municipal sewage entering two Japanese sewage treatment plants over a one-year period. Of the total surfactant content entering the sewage treatment plants, 16% consisted of AS and AES surfactants which were completely removed during passage.
through the plants. The sum of AS and AES concentrations was determined by subtracting LAS, ABS and AOS concentrations from the concentration of MBAS. (The sulfonates were converted to their sulfonyl chloride derivatives and quantified using an infrared method; no detection limits were specified.)

In field trials in a trickling filter sewage treatment plant, Mann and Reid (1971) found that pri-AS derived from either coconut alcohols or DOBANOL®-25 (C₁₂₋₁₅ primary alcohol, -75% normal, 25% isomeric) displayed a high order of biodegradability (96-98% removal of MBAS). All households served by the plant used only the surfactant under study for a two month period, resulting in influent concentrations of MBAS in the settled sewage of 35.6–53.1 mg/L.

D. Metabolic Pathways of Biodegradation

The metabolism of linear primary alkyl sulfates is most often described by the following process: hydrolysis of the sulfate ester yielding the corresponding alcohol, oxidation of this alcohol to an aldehyde and subsequently to a carboxylic acid, β-oxidation of the carboxylic acid. The degradation of secondary and branched alkyl sulfates is generally much slower, in part, because many of the microbial enzymes responsible for removal of the sulfate group of n-pri-AS are less effective with secondary and branched analogues.

Linear primary alkyl sulfates readily undergo primary biodegradation via sulfatase enzymes, which split off the sulfate ester group forming inorganic sulfate and the corresponding alcohol. The alcohol is then oxidized to the corresponding aldehyde, and subsequently to the carboxylic acid, which is degraded by β-oxidation (Gilbert and Pettigrew, 1984; Fisher et al., 1983; Higgins and Burns, 1975; Swisher, 1987).
Alkyl chains with simple methyl branching are reported to undergo β-oxidation as well, and some strains of *Pseudomonas* are able to degrade the surfactant totally without prior desulfonation (Fisher et al., 1983).

The enzymes responsible for removal of the sulfate group from secondary alkyl sulfates (sulfohydrolases) were studied by Matcham et al. (1977). They found differences in specificity for chain-length and position of the sulfate group, among enzymes from two microbial species, *Comamonas terrigena* and *Pseudomonas C₁₂B*.

In the U.S.S.R., Stafskaya et al. (1976, 1979) have identified several bacteria capable of degrading C₁₂AS, with loss of MBAS and hydrolysis to sulfate and dodecanol. The bacteria involved include *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Flavobacterium devorans* and *Achromobacter guttatus*. *Aerobacter aerogenes*, isolated with *C. freundii*, is apparently able to grow only on the products of the latter organism's attack on C₁₂AS.

Some secondary or branched AS degrade less rapidly than linear primary alkyl sulfates. The work of Huyser (1961) and Hammerton (1955, 1956) indicated that several branched primary and secondary AS were resistant to biodegradation. For example, during an 18-day river water test, the following compound did not degrade (MBAS) at all:

$$
\text{R-CH}_2\text{OSO}_3^-\text{Na}^+ \xrightarrow{\text{enz}} \text{R-CH}_2\text{OH} + \text{Na}_2\text{SO}_4
$$
while branched chain primary alkyl sulfates such as:

\[
\begin{align*}
\text{CH}_3 \\
\text{CH}_3 \text{-} \text{C-CH}_2 \text{-} \text{CH}_2 \text{-} \text{OSO}_3^{-} \text{Na}^+ \\
\text{CH}_3 \quad \text{CH}_3
\end{align*}
\]

and cyclic alkyl sulfates such as:

\[
\begin{align*}
\text{CH}_2 \text{-} \text{CH}_2 \\
\text{CH}_2 \text{CH}_2 \text{-} \text{CH}_2 \text{-} \text{CH}_2 \text{-} \text{CH}_2 \text{-} \text{CH}_2 \text{-} \text{CH}_2 \text{-} \text{CH}_2 \text{-} \text{OSO}_3^{-} \text{Na}^+ \\
\text{CH}_2 \text{CH}_2
\end{align*}
\]

were completely degraded in 14 and 7 days, respectively (Huyser, 1961). The microbial sulfatase enzymes that hydrolyze n-pri-AS appear to be inactive against secondary and branched analogues, although some microbes are able to produce sulfatases that are specific for these compounds (Higgins and Burns, 1975). For example, Fitzgerald and Payne (1972) found that \textit{Pseudomonas C}_{12}B was induced by secondary AS to form pri- and sec-alkylsulfatases.

Furthermore, they found that alkyl sulfatase synthesis was essentially unaffected by the presence in the culture medium of sulfate or cysteine but sulfatase synthesis was repressed by the presence of a number of carbon sources including some primary and secondary alcohols, acetate, propionate, etc.

Additional work in this area was reviewed extensively by Swisher (1987).
It thus appears that linear primary alkyl sulfates and secondary and slightly branched AS are readily biodegraded, but that the biodegradability of certain highly branched compounds cannot be predicted with any great assurance from the information that is presently available.


CONTINENTAL OIL COMPANY, unpublished data.


III-15

PROCTER & GAMBLE COMPANY, unpublished data.


IV. ENVIRONMENTAL LEVELS

A. Water Quality Standards

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

B. AS in Natural Water Bodies

AS are not presently being monitored, as such in the United States or Europe. MBAS measurements in water bodies include AS surfactants as well as other anionics. Levels of anionic surfactants detected in natural water bodies were discussed in Volume I, Part 1, LAS. As the previous section (III - BIODEGRADATION) has shown, AS degrade rapidly and completely in most environmental situations and the contribution of AS to environmental anionic surfactant levels is certainly minimal.
V. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. AS Structure-Activity Relationship

The majority of studies in fish and invertebrates show AS toxicity, bioaccumulation and residual properties to increase as alkyl chain length increases. One study reported toxic effects of AS on Hydra attenuata reproduction to decrease as alkyl chain length increases. Similar conflicting data are also reported for the goldfish and the bluegill.

The majority of studies show AS toxicity to increase as the alkyl chain length increases. In 24 hr toxicity tests with Daphnia magna, EC50 (immobilization) values decreased by approximately one-half for every additional carbon in the alkyl chain, resulting in a range of 8200 to 42 mg/L for C5 to C13AS (Lundahl and Cabridenc, 1978). Wright (1976) found C10AS approximately ten times as toxic to barnacle larvae (Elminius modestus) as C6AS. Pronounced effects of alkyl chain length were observed in tests by Kikuchi et al. (1976), in which LC50 values for the Japanese killifish (Oryzias latipes) decreased by a factor of ten for every two-carbon increase for C12 to C18AS (see Table V-1). Kikuchi and Wakabayashi (1984) later found a 75-fold difference in sensitivity to the Japanese killifish with C18 AS being most toxic and C12 being least toxic in a 48-hour LC50 study.

Increased toxicity with increasing alkyl chain length was also observed to some extent by Tukmachev and co-workers (1977) in their work with yeast protoplasts (species unknown). C12AS had the highest lytic activity for plasma membranes as compared to C8, C10, C11, C13 and C14AS.
<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L) (95% Confidence Limit)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese killifish (Oryzias latipes)</td>
<td>NaC₁₂₈ AS</td>
<td>LC₅₀ - 70 5.9 0.78 10</td>
<td>24 hr., distilled water</td>
<td>Kikuchi et al. (1976)</td>
</tr>
<tr>
<td></td>
<td>NaC₁₄₈ AS</td>
<td></td>
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<tr>
<td></td>
<td>NaC₁₆₈ AS</td>
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<tr>
<td></td>
<td>NaC₈₈ AS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Zebrafish (Brachydanio rerio)</td>
<td>NaC₁₂ AS</td>
<td>7.79</td>
<td>24 hr., 350-375 mg/L hardness (as CaCO₃) pH 8.2, flow-through, nominal</td>
<td>Fogels and Sprague (1977)</td>
</tr>
<tr>
<td></td>
<td>NaC₁₂₈ AS</td>
<td>3-week old fry 9.9</td>
<td>96 hr., static, 280-320 mg/L hardness (as CaCO₃), 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-week old juvenile 20.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-week old adult 12.8</td>
<td></td>
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</tr>
<tr>
<td>Flagfish (Jordanella floridae)</td>
<td>NaC₁₂ AS</td>
<td>8.10</td>
<td>24 hr., 350-375 mg/L hardness (as CaCO₃), pH 8.2, flow-through, nominal</td>
<td>Fogels and Sprague (1977)</td>
</tr>
<tr>
<td>Goldfish (Carassius auratus)</td>
<td>n-C₁₂ AS 93% Al, MW-186</td>
<td>LC₅₀ - 50.0</td>
<td>6 hr., static, 20°C, hardness 10⁰ based on 400 g/L CaCl₂, 6H₂O and 181 MgSO₄ 7H₂O, 10⁰ fish/conc.</td>
<td>Gafa (1974)</td>
</tr>
</tbody>
</table>

*Active Ingredient
<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L) (95% Confidence Limit)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish-continued (Carassius auratus)</td>
<td>n-C_{14} AS, 92.4% AI*, MW-214</td>
<td>LC_{50} - 5.0</td>
<td>6 hr., static, 20°C, hardness - 10° based on 400 g/L CaCl_{2}·6H_2O and 181 g/L MgSO_{4}·7H_2O, 10 fish/conc.</td>
<td>Gafa (1974)</td>
</tr>
<tr>
<td></td>
<td>n-C_{16} AS, 95.3% AI, MW-242</td>
<td>&gt;300</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C_{12·16} AS, 94.3% AI, MW-206</td>
<td>&lt; 12.0</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>n-C_{13} AS, 94.8% AI, MW-200</td>
<td>&lt; 18.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C_{14} AS, 94.3% AI, MW-214</td>
<td>&lt; 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-C_{12·15} AS, 95.8% AI, MW-206</td>
<td>&lt; 7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{14} AS, branched, 98% AI, MW-214</td>
<td>&lt; 49.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C_{16} AS, branched, 94% AI, MW-242</td>
<td>&lt; 7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALFOL 1216-P(n-C_{12·16} AS), MW-206</td>
<td>&lt; 12.0</td>
<td>6 hr., static, fish - 6-7 cm, hardness - 10° based on 27.5 g/L CaCl_{2} and 22.5 g/L MgSO_{4}·7H_2O, 10 fish/conc.</td>
<td>Gafa and Lattanzii (1974)</td>
</tr>
<tr>
<td></td>
<td>DOBANOL 25 (C_{12·15} AS), MW-203</td>
<td>&lt; 7.8</td>
<td></td>
<td></td>
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</tbody>
</table>

* Active Ingredient
<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish—continued</td>
<td>DIADOL HA 115 (C11-15 AS), MW: 203</td>
<td>LC50 8 hr - 8.1</td>
<td>Static, fish - 6-7 cm, hardness - 10°C as CaCO₃, 10 fish/conc.</td>
<td>Gafa and Lettanz (1974)</td>
</tr>
<tr>
<td>(Carassius auratus)</td>
<td>Na-C12 ave</td>
<td>LT 100 at 70 mg/L AS 90-110 minutes &gt;24 hrs</td>
<td>Static, fish - 6 cm, pH - 6.8-7.2 hardness - 300 mg/L CaCO₃, distilled water</td>
<td>Tovell et al. (1974)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Na-C12 ave</td>
<td>LT 100 at 70 mg/L AS 40-45 minutes</td>
<td>Static, fish 10 cm, pH - 6.8-7.2 hardness - 300 mg/L CaCO₃</td>
<td>Tovell et al. (1974)</td>
</tr>
<tr>
<td>(Salmo gairdneri)</td>
<td>Na-C12 ave</td>
<td>LT 100 at 100 mg/L AS 4.9 hrs (3.9-6.1)</td>
<td>Static, fish - 27 g, hardness - 25 mg/L CaCO₃</td>
<td>Abel and Skidmore (1975)</td>
</tr>
</tbody>
</table>

* Lethal time for 100% of the population
### TABLE V-1 - Continued

**ACUTE TOXICITY OF ALKYL SULFATES TO FISH**

<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L) (95% Confidence Limit)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow Trout-continued</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt;AS</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; : 4.62</td>
<td>24 hr., 350-375 mg/L hardness (as CaCO&lt;sub&gt;3&lt;/sub&gt;) pH 8.2, flow-through, nominal</td>
<td>Fogels and Sprague (1977)</td>
</tr>
<tr>
<td>(Salmo gairdneri)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bluegill</td>
<td>Na-C&lt;sub&gt;1&lt;/sub&gt;AS</td>
<td>- 1000</td>
<td>96 hr, static, 18°C, pH 7.1 hardness - 35 mg/L CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Procter and Gamble Company (unpublished data)</td>
</tr>
<tr>
<td>(Lepomis macrochirus)</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;11&lt;/sub&gt;AS</td>
<td>- 26.0 (19.0-35.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-C&lt;sub&gt;11&lt;/sub&gt;AS, branched</td>
<td>- 16.5 (13.1-21.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na-C&lt;sub&gt;12&lt;/sub&gt;AS</td>
<td>- 6.83 (4.06-5.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;-C&lt;sub&gt;12&lt;/sub&gt;AS</td>
<td>- 20.3 (16.0-25.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Surfactant</td>
<td>Toxicity (mg/L)</td>
<td>Experimental Conditions</td>
<td>Reference</td>
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<tr>
<td>Bluegill-continued (Lepomis macrochirus)</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;13&lt;/sub&gt; As, branched</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; 18.4 (15.2-22.2)</td>
<td>96 hr., Static, 18°C, pH 7.1, hardness - 35 mg/L CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Procter and Gamble Co. (unpublished data)</td>
</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;15&lt;/sub&gt; As</td>
<td>5.19 (3.97-6.77)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;15&lt;/sub&gt; As (not branched)</td>
<td>3.39 (2.59-4.43)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;15&lt;/sub&gt; A9, branched</td>
<td>2.13 (1.37-3.31)</td>
<td></td>
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<tr>
<td></td>
<td>Na-C&lt;sub&gt;14&lt;/sub&gt;-C&lt;sub&gt;15&lt;/sub&gt; As (not branched)</td>
<td>4.2 (3.2-5.6)</td>
<td>96 hr., Static, 22°C, pH 7.2, hardness - 42 mg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;16&lt;/sub&gt; As</td>
<td>21.7 (16.7-28.1)</td>
<td>96 hr., Static, 18°C, pH 7.1, hardness - 35 mg/L CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;-C&lt;sub&gt;12&lt;/sub&gt;-C&lt;sub&gt;14&lt;/sub&gt; As</td>
<td>3.2 (2.8-3.7)</td>
<td></td>
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<tr>
<td></td>
<td>C&lt;sub&gt;16&lt;/sub&gt;-C&lt;sub&gt;18&lt;/sub&gt; As</td>
<td>76.0 (50-116)</td>
<td></td>
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<tr>
<td></td>
<td>Na-C&lt;sub&gt;12&lt;/sub&gt; As</td>
<td>4.5</td>
<td>96 hr., 125 mg/L hardness (as CaCO&lt;sub&gt;3&lt;/sub&gt;) pH 7.4, flow-through, nominal</td>
<td>Bishop and Perry (1979)</td>
</tr>
</tbody>
</table>
### ACUTE TOXICITY OF ALKYL SULFATES TO FISH

<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L) (95% Confidence Limit)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Guppy</strong> <em>(Lebistes reticulatus)</em></td>
<td>Na(_{12ave}) AS</td>
<td>Minimum concentration with lethal effect: 10 mg/L</td>
<td>ASTM D 1345-59</td>
<td>Borstlap (1967)</td>
</tr>
<tr>
<td></td>
<td>Na(_{12ave}) AS</td>
<td>(\text{LC}_{50}) 3-week old fry: 18.3</td>
<td>96 hr., static, 280-320 mg/L hardness (as (\text{CaCO}_3)), pH 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
<tr>
<td></td>
<td>Na(_{12ave}) AS</td>
<td>12-week old juvenile: 16.2</td>
<td>96 hr., static, 280-320 mg/L hardness (as (\text{CaCO}_3)), pH 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
<tr>
<td></td>
<td>Na-C(_{12ave}) AS (not branched)</td>
<td>20-week old adult: 13.5</td>
<td>96 hr., 22°C, pH 7.3, 50 mg/L hardness</td>
<td>Procter and Gamble, unpublished data</td>
</tr>
<tr>
<td><strong>Fathead Minnow</strong> <em>(Pimephales promelas)</em></td>
<td>Na(_{12ave}) AS</td>
<td>(\text{LC}_{50}) 3-week old fry: 10.2</td>
<td>96 hr., static, 280-320 mg/L hardness (as (\text{CaCO}_3)), pH 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
<tr>
<td></td>
<td>Na(_{12ave}) AS</td>
<td>12-week old juvenile: 17.0</td>
<td>96 hr., static, 280-320 mg/L hardness (as (\text{CaCO}_3)), pH 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
<tr>
<td></td>
<td>Na(_{12ave}) AS</td>
<td>20-week old adult: 22.5</td>
<td>96 hr., static, 280-320 mg/L hardness (as (\text{CaCO}_3)), pH 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
</tbody>
</table>
TABLE V-1 - Continued

ACUTE TOXICITY OF ALKYL SULFATES TO FISH

<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheepshad Minnow</td>
<td>Na-C_{12-14} AS (not branched)</td>
<td>LC_{50} 0.46</td>
<td>96 hr., 22°C, pH 7.9, salinity = 32°/oo</td>
<td>Procter and Gamble, (unpublished data)</td>
</tr>
<tr>
<td>(Cyprinodon variegatus)</td>
<td>NaC_{12ave} AS</td>
<td>(0.36-0.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minnow</td>
<td>C_{12} AS</td>
<td>-30.5</td>
<td>flow-through, 96 hr., 8-15°/oo salinity, 22°C</td>
<td>Lundahl and Cabridenc (1978)</td>
</tr>
<tr>
<td>(Phoxinus phoxinus)</td>
<td>C_{12 ave} AS</td>
<td>-1.39</td>
<td>96 hr., 60-70 mg/l hardness (as CaCO_{3}, pH 7.3, static</td>
<td>Verma et al. (1978)</td>
</tr>
<tr>
<td>Minnow</td>
<td>C_{12 ave} AS, triethanolamine salt</td>
<td>-1.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Macrones vittatus)</td>
<td>Convict cichlid</td>
<td>NaC_{12ave} AS</td>
<td>3-week old fry - 16.1 96 hr., static, 280-320 mg/l hardness (as CaCO_{3}, pH 7.8-8.2, 25°C</td>
<td>Newsome (1982)</td>
</tr>
<tr>
<td>(Cichlasoma nigrofasciatum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic Silverside</td>
<td>NaC_{12ave} AS</td>
<td>-2.8</td>
<td>96 hr., continuous flow, 8-15°/oo salinity, 22°C</td>
<td>Roberts et al., (1982)</td>
</tr>
<tr>
<td>(Menidia menidia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The carbon number of the alkyl chain also has an important effect on the uptake, distribution and excretion of AS. Wakabayashi et al. (1985) reported that AS bioaccumulation increased while the rate of excretion decreased with increasing alkyl chain length.

Bode et al. (1978) examined the effects of a series of AS with varying alkyl chain lengths (C_{10}, C_{12}, C_{14}, C_{16}) on budding (reproduction) in Hydra attenuata. Concentrations of 2 \times 10^{-2} \text{ mM}, 2 \times 10^{-1} \text{ mM} and 2 \text{ mM} were tested at 20°C. Contrary to most correlations of toxicity and alkyl chain length, toxicity was found to decrease with increasing alkyl chain length. C_{16}AS had no significant effect at 2 \text{ mM}; C_{14}AS exerted no significant effect at 2 \times 10^{-1} \text{ mM}. The decrease in toxicity with alkyl chain length was attributed to reduced water solubility and resulting loss of surfactant activity at the assay temperature. Concentrations of 2 \times 10^{-1} \text{ mM} C_{10}AS and C_{12}AS produced lethality within 24 hour and 10 days, respectively.

Data reported in Table V-I also shows some conflicting data on the effect of chain length in AS toxicity for the goldfish and the bluegill. In these species, no correlation is shown between toxicity and alkyl chain length. Nonetheless, the majority of the studies support the view that toxicity increases as alkyl chain length increases.

2. Acute Toxicity

AS LC_{60} values vary greatly in fish and invertebrates with a range of 1 to >300 and 1 to >200 mg/L, respectively. Early growth stages are generally more sensitive to AS than adults. Growth of marine phytoplankton and algae are inhibited by 10 mg/L AS or greater while soil bacteria are inhibited at concentrations of 100 ppm.
a. Fish

The available acute toxicity data for fish are summarized in Table V-1. The LC₅₀ values range from about 1 to >300 mg/L, although most values range from 5-20 mg/L. These values show no particular correlation with carbon chain length or molecular weight among these surfactants for certain species of fish, particularly the goldfish and the bluegill. Most of the toxicity tests were conducted with the sodium salt of C₁₂AS.

The range of reported LC₅₀ values for alkyl sulfates, both for the Japanese killifish (Oryzias latipes), was 0.78 to 70 mg/L. The variation was attributed to the difference in alkyl chain length used, with NaC₁₆AS being apparently much more toxic than NaC₁₂₄ve AS. Five other species were also tested for sensitivity to C₁₂AS, for which the range of LC₅₀ values was 4.5 to 30.5 mg/L.

Abel (1978) determined mean survival periods (LT₅₀) for two fish species at constant concentrations of NaC₁₂₄ve AS. The LT₅₀ value in these static tests for rainbow trout (Salmo gairdneri) in 42 mg/L was 45 hours; for brown trout (Salmo trutta) in 18 mg/L, the LT₅₀ was 24.5 hours. Kikuchi et al. (1976) reported that earlier growth stages in goldfish (Carassius auratus) are the most sensitive.

With the exception of the guppy, Newsome (1982) also found fry to be more susceptible to NaC₁₂₄ve AS than adults. The trend was most evident for the fathead minnow and the convict cichlid, and less so for the zebra danio (see Table V-1).
b. Invertebrates

The available acute toxicity studies for invertebrates are summarized in Table V-2. The range of toxicity for these limited data is 0.35 to >200 mg/L. In a static test, the 48 hr LC₅₀ for larvae of the horse clam (*Tresus capax*) was 0.35 mg/L NaC₁₂AS (Cardwell *et al.*, 1978). In another experiment (Tatem *et al.*, 1976), the grass shrimp (*Palaemonetes pugio*) was reported to have a maximum 96 hr LC₅₀ of 162 mg/L NaC₁₂AS. Tatem *et al.* (1976) have hypothesized that grass shrimp are less susceptible to C₁₂AS because they are inactive, bottom-dwelling organisms.

The 48-hr LC₅₀ of NaC₁₂AS in the lungworm, *Arenicola marina* L., was 15.2 mg/L (Conti, 1987). Microscopic examination revealed the thoracic epidermis to be the most resistant region of the lungworm and the gills and caudal epidermic receptors were the most sensitive.

Lundahl and Cabrieden (1976) conducted experiments on *Daphnia* to determine the toxicity of the degradation products of C₁₂AS. In this instance, the toxicity of the surfactant, as measured by immobility, increased to a maximum at 30 hours of exposure, then rapidly dropped off to almost negligible toxicity. The authors cited previous work which indicated "that alkyl sulfates degrade by the hydrolysis of the sulfonic ester function followed by the oxidation in acid of the formed alcohol." The authors suggested that this hypothesis would explain the results of the experiment, since dodecanoic acid (from degraded C₁₂AS) is much more toxic than C₁₂ AS, but is very short-lived in solution.

c. Algae and Microorganisms

Ukeles (1965) studied the effect of Mg-C₁₂ave AS (CONCO SULFATE M™) on 12 species of marine phytoplankton (Chlorophyceae) at 1, 10, 100, and 1,000 mg/L. No growth of any species occurred at the two highest
TABLE V.2
THE ACUTE TOXICITY OF ALKYL SULFATES TO INVERTEBRATES

<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brine shrimp</td>
<td>Na-C(_{12a}) AS</td>
<td>LC(_{50}) 3.6</td>
<td>24 hr., Static, 24.5°C, artificial seawater</td>
<td>Price et al. (1974)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Mysid Shrimp</td>
<td>Na-C(_{12a}) AS</td>
<td>- 7.24</td>
<td>96 hr., static, salinity 20.0%/oo, 22°C</td>
<td>Roberts et al., (1982)</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Mysid Shrimp</td>
<td>Na-C(_{12a}) AS</td>
<td>- 6.62</td>
<td>96 hr., static, 22°C salinity - 20.0%/oo</td>
<td>Roberts et al., (1982)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Daphnia magna</td>
<td>Na-C(_{12a}) AS (Ziegler derivative)</td>
<td>LC(_{50}) 13.5</td>
<td>Static, 20°C ± 1°C Daphnia 72 hrs. old, synthetic river water</td>
<td>Lundahl et al. (1972)</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Na-C(_{12-14}) AS (natural alcohol derivative)</td>
<td>24 hr - 6.3</td>
<td>48 hr - 2.8</td>
<td></td>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Na-C(_{12-14}) AS (not branched)</td>
<td>- 2.7</td>
<td>48 hr., 250 mg/L hardness 22°C, pH 8.1</td>
<td>Procter and GemLe, (unpublished data)</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Na-C(_{12}) AS</td>
<td>- 1.8</td>
<td>48 hr., 125 mg/L hardness (as CaCO(_3)), pH 7.4, flow-through, nominal</td>
<td>Bishop and Perry (1979)</td>
</tr>
<tr>
<td>Species</td>
<td>Surfactant</td>
<td>Toxicity (mg/L)</td>
<td>Experimental Conditions</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Mosquito</td>
<td>Na-C_{12} Na-C_{12}</td>
<td>LC_50: 50-78</td>
<td>Static, 24 hr. old pupae</td>
<td>Piper and Maxwell (1971)</td>
</tr>
<tr>
<td></td>
<td>NH-C_{10} AS</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triethanolamine - C_{12}</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nac AS</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na-2 ethylhexyl sulfate</td>
<td>&gt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacific oyster larvae</td>
<td>NaC_{12}, 100%</td>
<td>LC_50: 0.58-1.16</td>
<td>48 hr., 29%/oo salinity</td>
<td>Cardwell et al. (1977)</td>
</tr>
<tr>
<td>(Crassostrea gigas)**</td>
<td></td>
<td>(0.91 avg.)</td>
<td>pH 7.8, static, nominal</td>
<td></td>
</tr>
<tr>
<td>Pacific oyster larvae</td>
<td>NaC_{12}, 100%</td>
<td>1.0</td>
<td>48 hr., 29%/oo salinity</td>
<td>Cardwell et al. (1978)</td>
</tr>
<tr>
<td>(Crassostrea gigas)**</td>
<td></td>
<td></td>
<td>pH 7.8, static, nominal</td>
<td></td>
</tr>
<tr>
<td>Horse clam larvae</td>
<td>NaC_{12}, 100%</td>
<td>0.35</td>
<td>96 hr., 15%/oo salinity</td>
<td>Tatem et al. (1976)</td>
</tr>
<tr>
<td>(Tresus capax)**</td>
<td></td>
<td></td>
<td>20°C, static, nominal</td>
<td></td>
</tr>
<tr>
<td>Grass shrimp, adults</td>
<td>NaC_{12}</td>
<td>52.0-162.0</td>
<td>96 hr., 15%/oo salinity</td>
<td>Bluzat et al. (1976)</td>
</tr>
<tr>
<td>(Pilaeomona gigas)**</td>
<td></td>
<td></td>
<td>20°C, static, nominal</td>
<td></td>
</tr>
<tr>
<td>Scud</td>
<td>NaC_{12}</td>
<td>14.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Gemmarus sp.) **</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snail</td>
<td>NaC_{12}</td>
<td>24.4</td>
<td>96 hr.</td>
<td></td>
</tr>
<tr>
<td>(Lymnea sp.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Time not given
** Marine species
## Table V.2 - Continued

### Acute Toxicity of Alkyl Sulfates to Invertebrates

<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Toxicity (mg/L)</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnat larvae (Chaoborus sp.)</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt;AS</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; - 50</td>
<td>96 hr.</td>
<td>Bluzat et al. (1976)</td>
</tr>
</tbody>
</table>

**Marine species
concentrations. In addition, *Nannochloris* sp. and *Stichococcus* sp. were completely inhibited at 10 mg/L Mg-**C**_{12}AS. The author felt that the morphological appearance of the cell, as well as its composition, were important in determining the effect of surfactants on these unicellular algae.

The aquatic macrophyte, duckweed (*Lemma minor*), was exposed for 7 days to C_{12}AS in a flow-through toxicity test by Bishop and Perry (1979). The resultant EC_{50} values for various parameters were reported as follows: frond count: 43 mg/L; dry weight: 29 mg/L; root length: 18 mg/L; and growth rate: 44 mg/L.

The no observable effect concentration for a marine flagellate (*Dunaliella* sp.) was found to be >1 but <10.0 mg/L (Procter & Gamble Company, unpublished data).

Rockstroh (1967) examined by light and electron microscopy the effect of Na-**C**_{12}AS on ciliates (*Cyrtolophosis*) at concentrations of 0.02-0.2 mg/mL for 4 or 15 minutes. At concentrations of 0.1 and 0.2 mg/mL (4 and 15 min. exposures) autolysis of cytoplasm occurred, releasing the granular component and nuclear matrix. These exposures also led to fissures in the mitochondrial membrane and to the formation of a diffuse mitochondrial edema. Effects at lower concentrations were less severe and included slowing and arrest of ciliary function with later shedding of cilia, deformation of cell shape and loss of cytoplasmic refractility.

The effect of AS on bacteria has been studied by several authors. Kopp and Müller (1965) found that the motility of *Proteus mirabilis* (bacteria isolated from human urine) was completely inhibited by Na-AS at varying concentrations, depending on the carbon chain length. No motility was found at the following concentrations: C_{6}-50 mmoles/L, C_{8}-20 mmoles/L, C_{10}-5 mmoles/L, C_{12}-0.5 mmoles/L, and C_{14}-0.2 mmoles/L. In this case, toxicity seems to increase with increasing carbon chain length.
length. Growth was also impaired at similar concentrations. At 10 mmoles/L, C₆AS, C₁₀AS, and C₁₂AS were bacteriostatic, but C₈AS had no effect at this concentration.

Bernheim (1975) exposed a pigmentless strain of the bacterium, *Pseudomonas aeruginosa*, to concentrations of 0.5 x 10⁻⁴ M to 3.0 x 10⁻⁴ M NaC₁₂AS. Higher concentrations of AS disrupted both inner and outer membranes which resulted in potassium efflux and lysis. At lower concentrations, only the outer membrane was affected, as shown by loss of Alcian Blue staining, minor potassium efflux, and increased swelling of cells after incubation in LiCl solution. The latter effect was attributed to a loss of cell support normally provided by the outer membrane.

Lundahl et al. (1972) studied the growth of *E. coli* on a gelatin medium containing Na-C₁₂AS (Ziegler derived) or Na-C₁₂₋₁₄AS (natural alcohol derived). The concentrations which did not allow the development of more than 5 colonies per plate were 50 g/L and >200 g/L, respectively.

The natural composition of the bacteria population in soils can be affected by AS. Hartman (1966) examined the growth of soil bacteria from an oak forest and a grassy field, and from two samples of surface water. The results are shown in Table V-3. The reduction of colonies counted compared to control was the measure of inhibition. The soil bacteria were more sensitive to AS than the water bacteria, and the percent of surfactant-sensitive bacteria increased with soil depth.

The toxicity of various compounds can be assessed in certain bacteria to estimate a toxic effect in other aquatic organisms. The Microtox® test uses luminescent bacteria such as *Photobacterium phosphoreum* to assess the toxicity of various compounds including AS. The inhibition of light production in these bacteria is the effect measured in the determination of the EC₅₀ values. Various studies have compared the EC₅₀ values in luminescent bacteria to the median lethal concentration (LC₅₀) in fish (Bulich et al., 1981; Dutka and Kwan, 1981, 1982).
TABLE V-3

THE EFFECT OF 100 PPM AS ON SOIL BACTERIA

<table>
<thead>
<tr>
<th>Source of Inoculum</th>
<th>Depth</th>
<th>% Reduction of Bacterial Colonies (compared to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil forest</td>
<td>Surface 14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4&quot;</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>8&quot;</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>15&quot;</td>
<td>67</td>
</tr>
<tr>
<td>Grassy field</td>
<td>Surface 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24&quot;</td>
<td>11</td>
</tr>
<tr>
<td>Brook</td>
<td>Surface 11</td>
<td>11</td>
</tr>
<tr>
<td>Well</td>
<td>Surface 7</td>
<td>7</td>
</tr>
</tbody>
</table>

Source: Hartman (1966)

In general, a good correlation exists between the bacterial luminescence assay and conventional testing procedures. Drawbacks of the Microtox test system include an inability to test some samples at their natural pH (Dutka and Kwan, 1981) and differences in test conditions between laboratories (such as temperature, water hardness etc.) (Bulich et al., 1981) may affect the reproducibility of test results.

3. Sublethal Effects

Sublethal effects of 0.1 to 1 mg/L AS include immobilization, reduced olfactory sensitivity which may impair feeding and migratory behavior, reduced fertilized egg development and abnormal shell development in oysters and clams. Cellular studies in
the sea urchin reveal that AS (20 to 25 mg/L) exerts a major effect in fertilized eggs before the 8-cell stage; however, cellular division continued to be synchronous in all blastomeres after the 16-cell stage.

Various studies have reported numerous sublethal effects (see Table V-4), including immobilization (Wright, 1976) and abnormal development in a variety of aquatic species (Cardwell et al., 1977, 1978). The lowest concentration at which sublethal effects appeared was 0.1 mg/L NaCl, which depressed the olfactory bulb electric response in whitefish (Coregonus clupeaformis) (Hara and Thompson, 1978). The authors considered this a deleterious effect, because reduced olfactory sensitivity could impair feeding and migrating behavior. In a companion experiment, whitefish were found to be attracted to NaCl concentrations of 0.1, 0.5, and 1.0 mg/L; the fish exhibited neither attraction to nor avoidance of 0.01 and 10 mg/L concentrations of NaCl (Hara and Thompson, 1978).

Hidu (1965) studied the effects of AS on the development of fertilized eggs of clams (Mercenaria mercenaria) and oysters (Crassotrea virginica) into free swimming larvae. There was a significant reduction of fertilized egg development (62% and 61% of control) for clams and oysters at 1 mg/L. No development occurred at 2.5 mg/L. Larval survival was not reduced significantly at test concentrations less than 5 mg/L. Clam survival was 68% of the control after a 10 day exposure; oyster survival was 19% of the control after a 12 day exposure. Larval growth was also reduced after these exposures (clams, 70% of control; oyster, 17% of control).

Bozhkova and Isaeva (1984) found that embryos of sea urchins (Scaphechinus mirabilis) treated with 20 μg/mL AS had no micromere formation between the 4th-6th cleavage division. The absence of micromeres in the 4th to 6th cleavage divisions was shown not to affect the capacity of the embryos for spiculogenesis. Further investigation in the sea urchin (Paracentrotus lividus) by Filoso et al. (1985)
revealed the major effect of 20 μg/mL NaC_{12}AS before the 8-cell stage was that the vegetal blastomeres failed to move toward the vegetal poles, which prevented them from falling under the influence of the vegetal pole cytoplasm.

Overall, NaC_{12}AS is not considered toxic to the egg since neither the cleavage rate nor embryo viability is impaired. In fact, in embryos in which detergent treatment prevented micromere segregation, cellular division continued to be synchronous in all blastomeres after the 16-cell stage (Filoso et al., 1985).

Other effects have been observed at AS concentrations ranging from 0.4 to 8200 mg/L, primarily in marine species. The available data are summarized in Table V-4.

Fogels and Sprague (1977) exposed three species of fish to NaC_{12}AS in long-term tests in an effort to determine threshold LC_{50} values. Threshold LC_{50} values were judged to have been attained when a 48 hour period elapsed without mortality. The threshold LC_{50} values for zebrafish and flagfish in these long-term tests were 7.97 and 6.90 mg/L, respectively. No threshold of lethality for rainbow trout was evident; the reported 10-day LC_{50} was 2.85 mg/L.

4. Chronic Toxicity

Few studies on the chronic toxicity of AS to aquatic organisms are reported. One study reports an LC_{0} of 0.2 mg/L C_{12}AS in the flat worm. Toxicity at this level was manifested as a reduced regenerative capacity. A multigeneration study in Daphia revealed a cumulative toxicity for each generation exposed to AS.
Limited data have been reported on the chronic toxicity of AS. In a 30-day test with the flatworm (Dugesia gonocephala), Patzner and Adam (1979) calculated an LC₀ (the highest concentration at which no lethal effect was observed) of 0.5 mg/L C₁₂AS. However, the regenerative capacity of worms was reduced at concentrations as low as one-half the LC₀.

Daphnids were exposed to 0, 2, 4, 6 or 8 mg/L NaC₁₂AS for 10 days (LeBlanc, 1982). After 10 days, offspring were exposed to the same level for 10 days. This procedure was followed for a minimum of 3 generations. Daphnids exposed to nominal concentrations of NaC₁₂AS for four consecutive generations exhibited an increased sensitivity of each successive generation.

5. Mode of Action

The mode of action of AS toxicity has been extensively studied by numerous investigators. One theory is that the concentration of AS influences its mode of action. At concentrations below 120 mg/L, the external membrane of the gill cells are affected causing cell leakage and autolysis. At concentrations above 120 mg/L, chemical denaturation of the cell constituents occurs resulting in rapid cell death. Toxicity may also be due to complex formations between the surfactant and proteins on the gill surface via electrostatic bonding between sulfate and amino groups. Other possible mechanisms include an AS-induced alteration of the interface tension between the gill and water; an increase in cell permeability; or lysis by the action of the surfactant on the protein component of the membrane.
### TABLE V-4

**SUBLETHAL EFFECTS OF ALKYL SULFATES ON AQUATIC ORGANISMS**

<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Concentration (mg/l)</th>
<th>Effects</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitefish (Coregonus clupeaformis)</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt; AS</td>
<td>0.1</td>
<td>Depression of olfactory bulbular elec. response</td>
<td>15 min., 78.4 mg/L CaCO₃ hardness, pH 7.5, 10.5°C, flow-through</td>
<td>Hara and Thoms (1978)</td>
</tr>
<tr>
<td>Japanese ayu (Plecoglossus altivelis)</td>
<td>&quot;formulation AS&quot;</td>
<td>4.0</td>
<td>Est. threshold concentration for avoidance</td>
<td>Tatsukawa and Hidaka (1978)</td>
<td></td>
</tr>
<tr>
<td>Pacific oyster larvae (Crassostrea gigas)*</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt; AS</td>
<td>0.67-1.04 (0.84 avg)</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; abnormal shell development</td>
<td>48 hr., 29°/oo salinity, pH 7.8, static, nominal</td>
<td>Cardwell et al. (1977)</td>
</tr>
<tr>
<td>Pacific oyster larvae (Crassostrea gigas)*</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt; AS</td>
<td>0.95</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; abnormal development</td>
<td>48 hr., 29°/oo salinity, pH 7.8, static, nominal</td>
<td>Cardwell et al. (1977)</td>
</tr>
<tr>
<td>Horse clam larvae (Tresus capax)*</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt; AS</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barnacle nauplii (Elminius modestus)*</td>
<td>C&lt;sub&gt;10&lt;/sub&gt; AS</td>
<td>1.8 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; immobility</td>
<td>30 min., 15°C</td>
<td>Wright (1975)</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;8&lt;/sub&gt; AS</td>
<td>1.7 x 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Marine Species
<table>
<thead>
<tr>
<th>Species</th>
<th>Surfactant</th>
<th>Concentration (mg/L)</th>
<th>Effects</th>
<th>Experimental Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea urchin embryos</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt;AS</td>
<td>20 ug/ml</td>
<td>Prevention of embryo micromere segregation at the 4th cleavage</td>
<td>--</td>
<td>Filosa et al. (1985)</td>
</tr>
<tr>
<td>(Paracentrotus lividus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea urchin embryos</td>
<td>NaC&lt;sub&gt;12&lt;/sub&gt;AS</td>
<td>20 ug/ml</td>
<td>Absences of micromere formation during 4-6th cleavage in embryos</td>
<td>--</td>
<td>Bozhkova and Isaeva (1984)</td>
</tr>
<tr>
<td>(Scaphechinus mirabilis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea urchin embryos</td>
<td>NaC&lt;sub&gt;12ave&lt;/sub&gt;AS</td>
<td>20</td>
<td>Inhib. of micromere formation in eggs</td>
<td>--</td>
<td>Tanake (1976)</td>
</tr>
<tr>
<td>(Hemicentrotus pulcher-rimua)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Temnopleurus toreumaticus)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pseudocentrotus depressus)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Marine species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia magna</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;AS</td>
<td>8200</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; Immobilization</td>
<td>24 hr., pH 7.7, 13°C, flow-through</td>
<td>Lundahl and Cabrindenc (1978)</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;6&lt;/sub&gt;AS</td>
<td>4350</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;7&lt;/sub&gt;AS</td>
<td>2300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;8&lt;/sub&gt;AS</td>
<td>800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;9&lt;/sub&gt;AS</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;10&lt;/sub&gt;AS</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mode of action of AS has been most commonly investigated in fish. The behavioral response involves an increase in swimming activity and an increase in respiratory activity. Later signs of poisoning include surfacing, loss of balance, overturn with loss of mobility, and death (Abel and Skidmore, 1975; Lang, 1967). These symptoms are accompanied by progressive gill damage. Abel and Skidmore found that after a 3 hr exposure (60% LT₅₀) to 100 mg/L Na₂C₁₂₄₈₉₆ AS, 6.1% of the secondary lamellae were affected in rainbow trout, while at death 86.9% were affected.

Tomiyama (1978) has hypothesized that surfactant toxicosis occurs as a result of complex formation between the surfactant and proteins on the gill surface via electrostatic bonding between sulfate and amino groups. On the cellular level, AS has been shown to increase the diameter of nuclei and number of nucleoli in interrenal cells of the head kidney in goldfish (Bromage and Fuchs, 1976). These changes were purported to indicate increased production of corticosteroids; it is not known whether this cellular action is a response to AS-induced stress, or a result of the involvement of the interrenal cells in other homeostatic mechanisms.

Abel (1978) has reported a different mode of action of NaC₁₂₄₈₉₆ AS on rainbow trout above and below 120 mg/L. At lower concentrations, AS "appears to act at a site in the external membrane of the gill cells. Consequent leakage of metabolites renders the cell non-viable and autolysis occurs, i.e., the cell is destroyed by the action of its own lytic enzymes." This was termed the slow type of toxic action. At concentrations exceeding 120 mg/L, AS appeared to act by chemical denaturation of the cell constituents, causing very rapid cell death. High concentrations were also more conducive to rapid absorption of the surfactant by the fish. The author stated that the two modes of action may occur simultaneously at higher concentrations, but since the macroscopic damage caused by both modes is the same, this hypothesis is not verifiable. Toxicity tests on the brown trout provided no evidence for a dual mode of action in this species.
Although damage is primarily observed in the gills, studies by Tovell et al. (1975) showed that after a 24 hr exposure of goldfish (Carassius auratus) to 50 mg/L $^{14}$C or $^{35}$S Na-C$_{12}$AS, the radioactivity was most concentrated in the gall bladder. If the surfactant was administered internally, about 50% of the radioactivity was found in the gall bladder. The gut and the liver concentrated the surfactant to a lesser extent. The principal route of entry was across the skin surface. Excretion was fairly rapid, with levels of radioactivity in unfed fish falling 38%, compared with a reduction of 68% in fed fish in 24 hrs. An examination of the metabolites revealed one principal metabolite, identified as butric acid 4-sulfate, and four minor ones. The authors suggested that the surfactant was absorbed and metabolized by the liver, and the metabolites were returned to circulation or secreted into the gallbladder.

Although the above studies explain the pathways and effects of AS, they do not identify the direct cause of death. Piper and Maxwell (1971) and Gafa (1974) showed that the critical tension for the LC$_{50}$ to mosquito and fish varies from 38-53 dynes/cm. Gafa (1974) suggests that the critical interfacial tension (gills-water) could be equal for all classes of anionic surfactants and may be closely related to toxicity.

Tovell et al. (1974) related toxicity to cell permeability. These authors observed that the presence of bivalent ions greatly increased the toxicity of Na-C$_{12}$AS to goldfish and rainbow trout (see Table V-I). These authors proposed that the different rate of absorption could be due to a change in permeability of certain tissues, or to a change in the availability of the surfactant.

Abel (1976) found that brown trout (Salmo trutta) exposed to Na-C$_{12}$AS at concentrations of 18-100 mg/L showed gill damage including nuclear pyknosis, the formation of lysosomes and eventual dissolution of cell contents. This action was attributed to a disorganization of the cell's permeability barrier. At higher
concentrations, Abel felt that lysis by direct action of the surfactant on the protein component of membranes and cell walls was the mode of action.

6. Effects of Environmental Variables on AS Toxicity

AS toxicity increases in the winter months, most likely due to decreased food supply and a reduced nutritive status at that time of the year. AS toxicity also increases in hard water due to a change in the permeability of the gill and an increased uptake of water. Conversely, turbidity decreases AS toxicity, which may be due to the adsorption of AS by clay particles.

Tatem et al. (1976) found that grass shrimp collected in the spring and summer months tolerated relatively high levels of NaC_{12}AS compared to winter shrimp. For example, two batches of shrimp collected in July and January had respective LC_{50} values of 160 and 77 mg/L. The LC_{50} values also appeared to decrease in relation to holding time in the laboratory. The increase in sensitivity during the winter was attributed to decreased food supply, which resulted in a reduction in nutritive status.

Tovell et al. (1974) showed that Na-C_{12}AS was more toxic to goldfish and rainbow trout in hard water (300 mg/L CaCO_3) than in soft water (60 mg/L CaCO_3) or distilled water. It was observed that uptake increased with hardness as shown in Table V-5. These authors also showed that absorption of alkyl sulfate by fish is a "function of the hardness of the water in which the fish have become acclimatized as well as the water in which the fish are treated." Toxicity increased with the hardness of the acclimatization water in treatment waters of the same hardness.
### TABLE V-5

EFFECT OF WATER HARDNESS ON UPTAKE OF Na-C<sub>12</sub>AS BY FISH*

<table>
<thead>
<tr>
<th>Water Hardness (mg/L as CaCO&lt;sub&gt;3&lt;/sub&gt;)</th>
<th>Rainbow Trout Concentration in Tissue (µg/g ± SE)</th>
<th>Goldfish Concentration in Tissue (µg/g ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (distilled)</td>
<td>--</td>
<td>9.6 ± 0.4</td>
</tr>
<tr>
<td>60</td>
<td>6.8 ± 1.2</td>
<td>24.2 ± 1.9</td>
</tr>
<tr>
<td>200</td>
<td>--</td>
<td>73.9 ± 5.2</td>
</tr>
<tr>
<td>300</td>
<td>42.0 ± 4.8</td>
<td>85.7 ± 7.1</td>
</tr>
</tbody>
</table>

Source: Tovell et al. (1974).

*Fish exposed to 70 mg/L Na-C<sub>12</sub>AS. Rainbow trout died after 35 minutes exposure, goldfish after 112 minutes.

In a toxicity test with carp, Kikuchi et al. (1976) observed increasing toxicity of linear AS compounds as water hardness increased. Another study showed that alkylbenzene sulphonate was more toxic to the fathead minnow in hard water, but sodium alkyl sulphate was more toxic in soft water (Henderson et al., 1959). Despite the conflicting results, the more recent data supports a direct correlation between toxicity and water hardness.

Eyanoer et al. (1985) found that fish in hard water absorb more anionic detergent due to an acute change in the permeability of certain tissue, especially the gill. A change in the availability of detergent may also occur due to an interaction with other dissolved constituents, such as Ca<sup>2+</sup>. Such an interaction could modify the solubility and/or diffusion characteristics of the detergent itself.
Umezawa and Komatsu (1980) showed that Na$_{12}$AS toxicity is affected by the presence of clay particles with a decrease in toxicity as the turbidity increases (see Table V-6). This effect is possibly due to an adsorption of the AS by the clay particles.

**TABLE V-6**

**EFFECT OF TURBIDITY ON AS TOXICITY (PERCENT MORTALITY)**

<table>
<thead>
<tr>
<th>Concentration of NaC$_{12}$AS (mg/L)</th>
<th>0 ppm</th>
<th>100 ppm</th>
<th>200 ppm</th>
<th>400 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>turbidity</td>
<td>turbidity</td>
<td>turbidity</td>
<td>turbidity</td>
</tr>
<tr>
<td>25.0</td>
<td>7%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>27.0</td>
<td>10%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>29.0</td>
<td>36%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>31.0</td>
<td>33%</td>
<td>0%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>32.0</td>
<td>--</td>
<td>10%</td>
<td>0%</td>
<td>--</td>
</tr>
<tr>
<td>33.0</td>
<td>60%</td>
<td>40%</td>
<td>13%</td>
<td>--</td>
</tr>
<tr>
<td>34.0</td>
<td>--</td>
<td>57%</td>
<td>16%</td>
<td>7%</td>
</tr>
<tr>
<td>35.0</td>
<td>87%</td>
<td>65%</td>
<td>34%</td>
<td>24%</td>
</tr>
<tr>
<td>36.0</td>
<td>--</td>
<td>100%</td>
<td>60%</td>
<td>49%</td>
</tr>
<tr>
<td>37.0</td>
<td>100%</td>
<td>--</td>
<td>73%</td>
<td>72%</td>
</tr>
<tr>
<td>38.0</td>
<td>--</td>
<td>--</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Source: Umezawa and Komatsu (1980)

The biodegradation of alkyl sulfates appears to decrease their toxicity to aquatic organisms. Fogels and Sprague (1977) exposed three species of freshwater fish to one-year-old and five-year-old lots of NaC$_{12}$AS in flow-through toxicity tests. The 10-day LC$_{50}$ values were found to be 2.4-3.5 times as high for the five-year-old AS as for the one-year-old surfactant. The reason for this difference is unknown. Even if the variation in toxicity is attributable to decomposition products formed during storage, it does not necessarily follow that these same decomposition products would be formed in the environment.
7. Bioaccumulation of AS

AS is absorbed through the gills then distributed to internal organs by blood and finally concentrated in the gallbladder. In carp continuously exposed to \(^{35}\text{S}\)-labeled AS, all concentration factors gradually decrease indicating an induction of metabolizing enzymes. The \(^{35}\text{S}\) body burden (in carp exposed to 0.5 mg/L \(^{35}\text{S}\)-labeled NaC\text{\_ave}\text{AS for 72 hours and then placed in fresh water}) is reduced by one-half in three days. Another investigator reports the bioconcentration factor in the gallbladder of carp exposed to 1.1 mg/L \(^{35}\text{S}\)-NaC\text{\_ave}\text{AS for 24 hours as 80x the normal value after fish were transferred to freshwater for 48 hours.}

Wakabayashi et al. (1978) exposed carp (Cyprinus carpio) to a 0.5 mg/L solution of \(^{35}\text{S}\)-labeled NaC\text{\_ave}\text{AS for 72 hours in a flow-through aquarium. Maximum levels of \(^{35}\text{S}\) were observed after 24 hours; thereafter, residues in the hepatopancreas decreased, while whole body and gallbladder residues were stable. The maximum \(^{35}\text{S}\) bioconcentration factors observed were 50 for the hepatopancreas, 700 for the gallbladder, and 4 for the whole body. Upon transfer to fresh water, the \(^{35}\text{S}\) body burden was reduced by one-half in three days.

Wakabayashi et al. (1980) later the gills to have the highest amount of radioactivity following exposure of \(^{35}\text{S}\)-C\text{\_ave}\text{AS to carp. However, after a transfer to freshwater, the highest amount of radioactivity was observed in the gallbladder. C\text{\_ave}\text{AS was absorbed through the gills then distributed to internal organs by blood and finally concentrated in the gallbladder. Concentrations reached a maximum value within 2 hours for the gills and within 24 hours for the hepatopancreas and the whole body. All concentration factors gradually decreased despite continuous exposure, which may have been possibly due to the induction of metabolizing enzymes.}
Carp were also used by Kikuchi et al. (1978) in a 24-hour uptake test in 1.1 mg/L $^{35}$S-Na$_{12}$ave AS (water hardness was 24 mg/L as CaCO$_3$). After two hours, the bile bioconcentration factor of 3 was the highest of any measured body part. At the end of 24 hours, bioconcentration factors in the gallbladder and hepatopancreas were 5-7x, 1-2x in the skin surface and kidney, and less than 1x in gills, brain, and muscle tissue. After 48 hours in fresh water, the bioconcentration factor in the gallbladder was 80x, while bioconcentration factors in other tissues and organs were less than one.

Regenerating cubes of the sea sponge (Geodia cydonium) "weakly accumulated" Na$_{12}$AS when placed in solutions of 1 μg/L to 10 mg/L. The surfactant was primarily associated with the protein fractions of cells (Zahn et al., 1977).

8. Interactions with Other Chemicals

No effect is observed on mercury uptake by phytoplankton or mussels with concurrent AS treatment. Also, no effect is reported in goldfish exposed to DDT following 2 months of AS exposure. A direct additive effect did occur when phenol and sodium lauryl sulfate were tested in bacteria; at lower levels, a synergistic or magnified toxic effect was seen. The same investigators reported no change in effects of sodium cyanide to bacteria with AS exposure.

Dugan (1967) exposed goldfish to 4.0 mg/L Na-C$_{12}$ave AS for 2 months, and then tested for susceptibility to DDT. Although some increase in the toxicity of DDT was observed, the results were not statistically significant.

Laumond et al. (1973) reported that the presence of 1 mg/L AS (unspecified) had no significant effect on mercury uptake by phytoplankton (Diogenes sp.) or mussels.
The strongly cationic compound N-alkyl dimethyl benzyl ammonium chloride mixed with an equal molar ratio of NaC_{12}AS induced a 48-hr LC_{50} of 230 ppm, whereas each compound alone had a 48-hr LC_{50} of 0.5 and 27.83 ppm, respectively. The anionic and cationic compounds are thought to react to form a nonsurface active complex that is very biodegradable and much less toxic than either of the compounds by themselves (Moore et al., 1986).

When 0.5 ppm NaC_{12}AS was tested with 20 ppm phenol in the Microtox™ system with the luminescent bacterium, *Photobacterium phosphoreum*, and a battery of other bacterial tests using *Spirillum volutans*, *Pseudomonas fluorescens*, and *Aeromonas hydrophila*, a direct additive effect was seen. A combination of 2.5 ppm phenol plus 0.5 ppm NaC_{12}AS produced a synergistic or magnified toxic effect in the bacteria. No enhancement or synergism of the toxic effects were seen in bacteria treated with 2.5 ppm sodium cyanide and 0.5 ppm NaC_{12}AS (Dutka and Kwan, 1982).

### 8. Effects of AS on Terrestrial Plants

One study reports a stimulatory effect on corn seeds treated with AS, while another reports an inhibition of viral infection in bean plants by sodium decyl sulfate. All other studies report deleterious effects following AS treatment, such as growth inhibition, decreased production and numerous cytological abnormalities.

Nadasy et al. (1972) examined the effects of AS on higher plants and found a stimulatory effect. Corn seeds that had been watered with 0.01, 0.1, and 1.0 g/L C_{12}AS weighed 97, 130, and 136% of the control, respectively. Since the length and dry weight of corn plants were also stimulated at these concentrations the effects were due to actual increases in growth rather than imbibition of water.
Ko et al. (1980) studied the antiviral activities of various detergents in the disease development caused by Cucumber Mosaic Virus (CMV), Cucumber Green Mottle Mosaic Virus (CGMMV) and the Tobacco Mosaic Virus (TMV) in the french bean, Phaseolus vulgaris var. pinto. Sodium n-decyl sulfate (C₁₀) was specifically effective in inhibiting the disease development caused by CMV. Decyl and dodecyl derivatives were effective inhibitors of local lesion formation of TMV on the French bean. Sodium salt of alkyl sulfates were slightly more effective than calcium salts.

Concentrations of 0.02, 0.2 and 2.0 mM NaC₁₂AS severely inhibited Dimorphotheca Sinuata (Cape marigold) callus growth, most likely due to its ability to denature proteins and to solubilize membranes (Ernst et al., 1982).

A reduction in paddy rice production was seen in plants watered with 50 mg/L AS. The reduced grain yield was due to a reduced number of grains per panicle (25 g vs. 30 g for untreated plants). In separate experiments with potted rice plants, AS exposure (50 mg/L watering solutions) produced no effect on plant height, number of tillers (shoots) or dry matter production, but was found to markedly inhibit water absorption by the roots, to inhibit photosynthesis and to result in considerable yellowing of the leaf blade (Taniyama and Nomura, 1978).

Antonielli and Lupattelli (1977) steeped barley seeds (Hordeum vulgare L.) in NaC₁₂ave AS concentrations of 10⁻⁶ M to 10⁻² M for 24 hours, and then allowed them to germinate. The lowest concentration of AS causing significant growth inhibition (11%, as determined by shoot length) was 10⁻³ M.

Dutta et al. (1985) treated germinating seeds of Vigna radiata with 0.1 to 1.0% NaC₁₂ave AS. Numerous cytological abnormalities were observed in the exposed cells and included clumped metaphase, chromosome bridge formation, fragmentation, pulverization, nuclear wall invagination and

V-31
nuclear dissolution. Abnormalities increased as the concentration of the detergent and time of exposure increased.

C. Effects of AS on Birds and Wildlife

Exposure of ducks to AS at temperature(s) as low as 0°C results in a loss of insulation and rapidly falling cloacal temperatures. The current understanding of the cause of this effect is that detergent lessens the surface tension of water thus allowing its penetration through normally-spaced feather barbules.

Choules et al. (1978) placed three ducks in a solution of 19 mg/L C\textsubscript{12}AS in distilled water at 0°C. After 30 minutes, the ducks became wet which was attributed to the dissolution of feather oils. Cloacal temperatures dropped to less than 30°C after 90 minutes of exposure, while control specimens maintained normal (−40°C) temperatures.

Within one hour of being placed in detergent-containing water (19 mg/L AS) at −2°C mallard ducks became wet to the skin, hypothermic, completely paralyzed and unable to hold their heads above water (Russell et al., 1981). Necropsy after death revealed the birds had drowned. Ducks placed in 30°C detergent-containing water (19 mg/L AS) lost body heat to the surrounding water over a 20 hour period. Death in these birds was attributed to exposure. Russell found that feather structure rather than the presence of body oils determines the degree of insulation. Feathers wiped clean of all oils with solvent remained completely waterproof as long as the normal feather architecture remained intact. The barbules of well-groomed feathers fit tightly together to prevent penetration by fluids. Detergents lessen the surface tension of water and allow its penetration through normally-spaced barbules.


PROCTER & GAMBLE COMPANY, unpublished data.


VI. HUMAN SAFETY

The safety of the alkyl sulfates to humans is well recognized by the Food and Drug Administration who have approved the use of broad-cut sodium dodecyl sulfate for use in food as (1) an emulsifier in or with egg whites provided it does not exceed 1000 ppm in egg white solids and 125 ppm in frozen or liquid egg whites; (2) as a whipping agent at the level not to exceed 5000 ppm by weight of gelatin used in the preparation of marshmallows; (3) a surfactant in fruit drinks up to a level of 25 ppm; and (4) a wetting agent in crude vegetable oils and animal fats up to a level of 10 ppm. (Code of Federal Regulations, Title 21, Part 178.822). The Cosmetics, Toiletries and Fragrances Association has also reviewed the safety of sodium lauryl sulfate and ammonium lauryl sulfate as part of the Associations continuing evaluation of ingredients used in cosmetics (Cosmetic Ingredient Reviews Expert Panel, 1983).

Broadcut dodecyl sulfate salts (NH₄, Mg, K, Na) have also been cleared for use in adhesives, cellophane and in paper and paper board for use with dry, aqueous and fatty foods, coatings, closures, rubber articles and textiles. (Code of Federal Regulations, Title 21, Parts 175-177).

In comparison to these food uses, the amounts of exposure to humans resulting from use of these surfactants taken with their facile biodegradability and generally low order of toxicity indicates that the use of alkyl sulfates does not pose a significant hazard to human health.

A. Animal Studies

The acute oral toxicity of AS for rats is generally in the range of 1000 to 4000 mg/kg and thus, these surfactants can be considered to be relatively non-toxic. With respect to their effects on skin, concentrations of 1 percent in occluded exposures
give rise to dermatitis and histological changes in the skin of rabbits and guinea pigs. At 0.1 percent, no effect from AS exposure has been reported. Concentrations of AS above 10 percent result in primary irritation to rabbit eyes tested according to the Draize procedure, while little or no irritation was found with 1 percent solutions. The subacute and chronic oral toxicity studies indicate that the inclusion of sodium \( C_{12}^{\text{ave}} \) AS in the diet of rats at 1 percent for up to one year produces no significant toxic effects. Alkyl sulfates have given negative results in mutagenicity and teratogenicity tests. The available evidence indicates that they are not carcinogenic.

1. Acute Toxicity

a. Oral

The acute oral toxicity of AS to rats generally ranges from above 1000 mg/kg to 4000 mg/kg depending on the nature of the material tested (Table VI-1). For AS used commercially, the LD\(_{50}\) values are 5000 to 15,000 mg/kg. The only comparison of identical compounds in two species revealed that certain slightly branched chain AS were about 3 times as toxic to guinea pigs as to rats (Smyth et al., 1941).

b. Inhalation

Ciuchta and Dodd (1978) exposed Swiss albino mice, in body plethysmographs (heads only), to various concentrations of aerosolized solutions of the sodium, ammonium or triethanolamine salts of \( C_{12}^{\text{ave}} \) AS for 2 min. The percent change from the average control respiratory rate was determined for each concentration at peak inhibition. (Stimulation of the upper respiratory tract by irritants produces reflex inhibition of the respiratory rate.) A 50% reduction in respiratory rate occurred at chamber concentrations (± 95% confidence limits) of 88 (37-259) \( \mu \)g/L, 114 (59-214) \( \mu \)g/L and 135 (81-224) \( \mu \)g/L for the sodium, ammonium and triethanolamine salts, respectively.

VI-2
Ciuchta (1976) reported on a method to determine the effect of surfactants on the upper respiratory tract of guinea pigs, mice and rabbits following inhalation exposure. Aerosolized solutions of 15 and 25 percent aqueous solutions yielded chamber concentrations from 73 to 175 μL. In rabbits exposed to sodium, ammonium or triethanolamine salts of C_{12}ave AS, a 50 to 60 percent inhibition of respiration was found. Similar reductions were found in mice. Guinea pigs proved unsuitable for this test.

When guinea pigs were exposed for 30 minutes to a particulate aerosol of sodium C_{12}ave AS at concentrations of 17.3, 28.9 or 48.6 mg/m³ the severity of coughing increased with the concentration. A decreased response was observed with exposure duration (Zelenak et al., 1982).

c. Percutaneous

The treatment of guinea pigs with a dose of slightly branched C₆-, C₁₄-, or C₁₇AS equivalent to the oral LD₅₀ (see Table VI-1) by holding the surfactant in contact with the skin of the test animals for 4 days resulted in some deaths. The authors indicated that quantitative statements were not justifiable because of uncertainties in the procedures (Smyth et al., 1941).

Guinea pigs exposed for 24 hours to NaC_{12}ave AS at 1 and 5 percent by an occluded patch test responded with reactions of the type observed in toxic dermatitis. The epidermis exhibited areas of necrosis in the upper part of the stratum, while a marked inflammatory response was present in the dermis. With treatment at 0.1 percent, the skin remained normal (Gisslen and Magnusson, 1966).

Occluded, 24-hr exposure of the rabbits to 2, 10 or 20% aqueous solutions of the sodium, ammonium or triethanolamine salts of C_{12}ave AS produced moderate to severe skin irritation. The sodium salt was the
most irritating, particularly at the lowest concentration. Primary irritation scores were as follows:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Sodium Salt</th>
<th>Ammonium Salt</th>
<th>Triethanolamine Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>&gt;5&lt;5.5</td>
<td>&gt;5&lt;5.5</td>
<td>3.5</td>
</tr>
<tr>
<td>10%</td>
<td>6</td>
<td>&gt;5&lt;6</td>
<td>5</td>
</tr>
<tr>
<td>20%</td>
<td>6</td>
<td>6</td>
<td>5.2</td>
</tr>
</tbody>
</table>

(Ciuchta and Dodd, 1978)

In another investigation, single application of NaC\textsubscript{12}AS to mouse skin at a 10 percent concentration gave rise to tissue edema and nuclear pyknosis of epidermal cells within one hour. At two hours, the changes were more advanced with edema and inflammation of the dermis. Repeated applications of 1 and 2.5 percent solutions gave rise to similar effects after 5 to 9 days treatment (Lansdown and Grasso, 1972).

At concentrations of 25 to 30 percent, AS of all carbon chain lengths tested (C\textsubscript{10}-C\textsubscript{16}) were primary irritants for rabbit skin. At concentrations of 1 percent, little or no skin irritation occurred (Brown and Muir, 1970; Olson et al. 1962; Continental Oil Company, Ethyl Corporation, Procter and Gamble Company, unpublished data).

The application of linear and 30% branched chain AS (>90 percent C\textsubscript{12}) to skin of adult rats as 20 and 30 percent solutions produced scabs in 2 to 3 days. At the 20 percent concentration, repeated painting for 16 days resulted in skin ulcers that persisted for the entire test period. At the 30 percent level, the ulcers worsened with time, and 5 of 6 animals died between 8 and 15 days. The rats also exhibited tissue damage on the tongue and oral mucosa as a consequence of licking surfactant from their own backs (Sadai and Mizuno, 1972).

In another histological study with C\textsubscript{12}-C\textsubscript{16} AS which was 70 percent linear and 30 percent branched, Iimori et al. (1971) found hypertrophy and edema of the epidermis in guinea pigs following a 2 hr exposure.
<table>
<thead>
<tr>
<th>Carbon Chain</th>
<th>LD$_{50}$(mg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RATS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{12}$ ave (linear)</td>
<td>1288</td>
<td>Walker et al. (1967)</td>
</tr>
<tr>
<td>$C_8$ (slightly branched)</td>
<td>4120</td>
<td>Smyth et al. (1941)</td>
</tr>
<tr>
<td>$C_{14}$ (slightly branched)</td>
<td>1250</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{17}$ (slightly branched)</td>
<td>1425</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{12}$ (linear)</td>
<td>2730</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{12}$, $C_{18}$</td>
<td>1000-2000</td>
<td>Brown &amp; Muir (1970)</td>
</tr>
<tr>
<td>$C_{12}$, $C_{14}$ (coconut oil)</td>
<td>1000-2000</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{12}$, $C_{14}$, $C_{16}$ (64% $C_{12}$)</td>
<td>2330</td>
<td>Continental Oil Company</td>
</tr>
<tr>
<td>$C_{16}$ (66%)+$C_{18}$ (32%)</td>
<td>13100</td>
<td>Procter &amp; Gamble Company</td>
</tr>
<tr>
<td>$C_{16}$ (27%)+$C_{18}$ (62%)</td>
<td>7640</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{16}$ (57%)+$C_{18}$ (33%)+$C_{20}$ (9%)</td>
<td>-19600</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_8$</td>
<td>3200</td>
<td>Gale and Scott (1953)</td>
</tr>
<tr>
<td>$C_{10}$</td>
<td>1950</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{12}$</td>
<td>2640</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{14}$</td>
<td>&gt;3500</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{16}$</td>
<td>&gt;3000</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{18}$</td>
<td>&gt;3000</td>
<td>&quot;</td>
</tr>
<tr>
<td><strong>MICE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{12}$ ave (linear)</td>
<td>1500</td>
<td>Olson et al. (1962)</td>
</tr>
<tr>
<td>$C_{12}$ ave (linear)</td>
<td>1460</td>
<td>Tomiyama et al. (1969)</td>
</tr>
<tr>
<td><strong>GUINEA PIGS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_8$ (slightly branched)</td>
<td>1520</td>
<td>Smyth et al. (1941)</td>
</tr>
<tr>
<td>$C_{14}$ (slightly branched)</td>
<td>650</td>
<td>&quot;</td>
</tr>
<tr>
<td>$C_{17}$ (slightly branched)</td>
<td>425</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
(unoccluded, but restrained) with 10 and 30 percent solutions of the surfactant. Recovery was not complete in 168 hours with either concentration of test solution.

Guinea pig skin washed with a 25 mM (0.7 percent) solution of broadcut sodium dodecyl sulfate for 5 minutes at 22°C caused a 75 percent increase in extraction of amino acids as compared to water and in addition, resulted in the extraction of 120 mg of protein as compared to none with water. In a series of alkyl sulfates, the maximum extraction of amino acids and proteins occurred with carbon chain length of 12 (Table VI-2) (Prottey and Ferguson, 1975).

**TABLE VI-2**

**EXTRACTION OF PROTEINS AND AMINO ACIDS FROM GUINEA PIG SKIN**

**BY ALKYL SULFATES**

<table>
<thead>
<tr>
<th>Carbon Chain Length</th>
<th>Soluble Protein</th>
<th>Total Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>50.8</td>
<td>62.7</td>
</tr>
<tr>
<td>10</td>
<td>166.1</td>
<td>84.2</td>
</tr>
<tr>
<td>11</td>
<td>119.5</td>
<td>100.4</td>
</tr>
<tr>
<td>12</td>
<td>238.9</td>
<td>194.8</td>
</tr>
<tr>
<td>13</td>
<td>198.5</td>
<td>141.7</td>
</tr>
<tr>
<td>14</td>
<td>163.9</td>
<td>110.3</td>
</tr>
<tr>
<td>15</td>
<td>77.9</td>
<td>41.3</td>
</tr>
</tbody>
</table>

Prottey and Ferguson, 1975

Brown (1971) investigated a number of animal tests for assessment of irritation due to surfactants. A 7 day occluded exposure to a 1 percent solution of a sodium salt of a broad cut coconut alcohol sulfate (C₁₂ and C₁₄) was highly irritating to rabbits, although a 0.1% solution was not. In rats, a 5 percent solution of the AS did not elicit a response after occlusion for 16 hours. In uncovered exposures on rabbits and guinea pigs, a 1 percent solution of surfactant was not irritating. Similarly, repeated percutaneous exposure to hairless mice for 28 days showed no histochemical skin changes.
The data from several studies indicate that AS are not skin sensitizing agents. Brown and Muir (1970) found that topical application of various ethanolamine and ammonium salts of alcohol sulfates did not sensitize guinea pigs. They were administered as 0.17% w/v active material in water. Intradermal treatment with mono- or triethanolamine salts of the surfactant did sensitize, whereas the ammonium salt did not. Thus, the authors suggest that sensitization may be due to the presence of free amines rather than the surfactant.

In two other studies using several different procedures, confirmation was obtained that AS are not sensitizing materials (Magnusson and Kligman, 1969; Procter and Gamble Company, unpublished data).

d. Intraperitoneal

The acute intraperitoneal LD_{50} in mice of a series of AS of carbon chain length from C_{8} to C_{18} (2 carbon increments) ranged from 284 to 477 mg/kg. There appeared to be no trend relating toxicity to chemical structure of the surfactant (Gale and Scott, 1953).

Ciuchta and Dodd (1978) examined the irritation induced by sodium, ammonium or triethanolamine salts of C_{12} AS in a mouse writhing test. Mice were injected intraperitoneally with 0.2 mL of various concentrations of aqueous solutions of these surfactants and observed until a positive response was elicited or for a period of five minutes. The calculated concentrations to produce writhing in 50% of the animals were 0.086%, 0.086% and 0.1% for the sodium, ammonium and triethanolamine salts of C_{12} AS, respectively.

e. Ocular

Alkyl sulfates cause irritation to rabbit eyes ranging from mild at low concentrations to severe at higher concentrations.
The administration of slightly branched chain AS at 4 or 8 percent concentrations gave rise to corneal necrosis in rabbits as determined by fluorescein staining (Smyth et al., 1941).

Solutions of 20 to 35 percent of a variety of AS (C<sub>10</sub> to C<sub>18</sub> carbon length) tested according to the Draize procedure resulted in scores indicating primary irritation. At 1 percent concentrations, there was little or no irritation from instillation of AS in rabbit eyes (Continental Oil Company, Ethyl Corporation, Procter and Gamble Company, unpublished data; Brown and Muir, 1970).

Davies et al. (1976) found that the critical exposure time before corneal damage was produced in the rabbit eye after instillation of 0.1 mL of a 10% aqueous solution of C<sub>12</sub>AS was approximately 4 to 10 seconds. Considerable conjunctival erythema and edema were produced, however, even when the eye was irrigated four seconds after instillation of the surfactant. Conjunctival irritation did subside more quickly, however, in rabbits exposed to the surfactant for shorter periods of time; complete conjunctival recovery was noted within four days in eyes irrigated after four seconds, compared to nine days for eyes irrigated either after 30 seconds or not at all.

Instillation of 0.1 mL of 2, 10 or 20% aqueous solutions of the sodium, ammonium or triethanolamine salts of C<sub>12</sub>AS into rabbits' eyes produced mild irritation at the 2% level and moderate to severe irritation which persisted at 7 days at the higher surfactant concentrations (Ciuchta and Dodd, 1978). The relative irritancy of the three samples could not be determined since they were fairly well grouped together.

Recent studies of sodium C<sub>12</sub>AS found it to be irritating to mouse eyes when applied in concentrations of 0.2, 1.0 or 5.0% (Etter and Wildhaber, 1985). It also produced opacity when in contact with the epithelium side of isolated bovine cornea (Muir, 1987).
In a study of surfactant penetration into rabbit eyes, sodium C_{12\text{ave}} AS labeled with sulfur-35 was found to penetrate the cornea. Within 0.5 hours of a single application, the surfactant was found in all ocular tissues. The highest levels were seen in the cornea followed by the choroid, retina and iris. The rate of loss was slow with label still remaining after 48 hours. Repeated applications led to increased binding. Permeability, ocular and systemic uptake were all greater in juvenile rabbits than in adult rabbits (Clayton et al., 1985).

2. Subacute Toxicity

a. Oral

The inclusion of AS in the diet of rats at 1 percent for 91 days yielded no adverse effects (Procter & Gamble Company, unpublished data).

Walker et al. (1967) administered a C_{12\text{ave}} and a C_{12-C_{16}} mixture of AS to rats in the diet for 13 weeks. At a dose of 5000 ppm (0.5 percent) some increases in organ weights were noted along with changes in serum urea levels. No histopathological changes were found. At 1000 ppm (0.1 percent), no changes were observed.

Sodium C_{12\text{ave}} AS fed to rats at 2 and 4 percent of the diet for 16 weeks gave rise to reduced weight gain in treatment groups as compared to controls. No other toxic effects were noted. Administration of the surfactant at 8 percent in the diet resulted in death of the test animals within 2 weeks (Fitzhugh and Nelson, 1948).

No adverse effects on histopathology were noted in Sprague-Dawley rats administered dietary levels of 0, 0.025, 0.25 or 2.5% w/w of a liquid dishwashing detergent for 13 weeks. The detergent contained 15% NH₄ LAS, 13% mg (AS)₂ and 13% NH₄ C_{12-16} alcohol ethoxy sulfate. The only effect was an increase in the relative liver weight at the 2.5% level (Scailteur et al., 1986).
A commercial AS (IRIUM™) was fed to rats in their diet for 5 weeks at levels of 30 or 60 mg/rat/day. At the end of five weeks, the weight gain was reduced in both treated groups and histological examination of the liver revealed swollen liver cells and prominent hepatocyte nuclei in the treated groups. Kidneys were normal in all groups (Hatton et al., 1940).

Several branched chain AS were included in the drinking water of rats for a thirty day interval. The daily doses in several test groups were estimated to range from 230 to 1510 mg/kg/day. At the end of the study, the major pathology observed was seen in the kidneys with light cloudy swelling and secretion in the tubules. Blood counts were normal in all treatment groups. The doses at which no toxic effects were seen ranged from 230 to 440 mg/kg/day (Smyth et al., 1941).

Fogelson and Shock (1944) found no loss of body weight nor alteration in red or white blood cell counts, hemoglobin, blood proteins or urine of dogs given 200 mg/day of a mixture of C_{12}, C_{16}, C_{17} and C_{18}AS for 10 months.

3. Chronic Toxicity

a. Oral

The inclusion of sodium C_{12} AS in the diet of rats at 0.25, 0.5 or
1.0 percent for one year did not give rise to any pathological changes that could be ascribed to treatment with the surfactant (Fitzhugh and Nelson, 1948).

The kidneys were found to be the target organs of toxic effects when a detergent based on sodium C$_{12}$-AS was fed to dogs at a dose of 0.5 mg/kg/day for 110 days. Histomorphological changes relating to glomerular reactions were observed (Potakar, 1980).

4. Carcinogenicity

There is no conclusive evidence that alkyl sulfates are carcinogenic. In one study which reported positive results, the small number of animals used prevents an accurate conclusion from being drawn.

Histopathologic examination of rats fed diets containing 0.25, 0.5 or 1.0 percent sodium C$_{12}$-AS for one year did not reveal any excess of tumors in the treated groups (Fitzhugh and Nelson, 1948).

Fukushima et al. (1974) exposed male Wistar rats to N-methyl-N-nitro-N'-nitroso-guanidine (MNNG) (50 mg/L) with and without sodium dodecyl sulfate (0.25 percent) in their drinking water for 26 to 30 weeks. With MNNG alone, 3 of 6 animals had stomach adenocarcinomas, while the inclusion of the surfactant with MNNG gave rise to adenocarcinomas in 8 of 10 rats. In addition the group treated with both MNNG and NaC$_{12}$-AS had 3 stomach sarcomas, while none was present in rats given MNNG alone. The authors suggest that the surfactant may have increased the absorption of the carcinogen in the stomach tissue and this could be the cause of the increased tumor yield. However, the small number of animals involved in this study does not allow definitive conclusions to be drawn.

Boutwell and Bosch (1957) in an abstract reported the tumor promoting effect of repeated applications on mouse skin of a 25 percent solution of sodium dodecyl sulfate. It was later found that the mice had been reared in creosote-treated boxes, so that the tumors occurring without
carcinogen initiation were most likely due to exposure of the test animals to creosote. Although the direct skin carcinogenicity of the surfactant is unlikely, its tumor promoting effect for mouse skin remains a possibility (R.K. Boutwell, personal communication, 1976).

In a two year skin painting study in mice the total tumor yield or individual tumor types occurring were not different in control and treated groups (Procter and Gamble Company, unpublished data).

5. Teratogenesis

Percutaneous treatment of pregnant mice with high concentrations (10-20%) of AS during early gestation appears to result in either death or normal survival. Treatment with high AS concentrations during later stages of pregnancy is neither embryotoxic nor teratogenic. At lower AS levels, a decreased number of implantations is seen but the significance of this result cannot be presently defined.

Daily application of 0.1 mL of a 20% aqueous solution of AS (alkyl chain length not identified) to the dorsothoracic area of pregnant ICR/Jcl mice on days 1 to 10 of gestation was observed to interfere with embryonic development at the cleavage stage (Nomura et al., 1980). Implanted embryos were found in only 1/26 (3.9%) of AS-treated dams compared to 18/20 (90%) of water-treated controls. Application of a lower concentration of AS (2%) to the skin of mice on days 1 through 17 of gestation also reduced the number of pregnancies (14/22; 63.6%), but this reduction was not statistically significant, because the number of animals compared was too small. The percentage of early and late deaths, the percentage of living fetuses and the incidence of malformation in AS-treated groups were comparable to those noted for control mice. Body weights of living fetuses in the 2% AS group were comparable to controls, but were significantly reduced in the 20% AS group (male: 1.21 g; female: 1.21 g vs. control male: 1.37 g; control female: 1.30 g).
Application of 10% AS twice a day to the backs of pregnant mice prior to implantation (days 0 to 3) interrupted cleavage of eggs and retarded fetal development. A significant number of embryos were in the oviducts of AS-treated dams (18.6%) compared to control mice (2.1%) with the majority in the morula stage in contrast to the late blastocyst stage of control embryos. AS treatment also resulted in an elevated incidence of deformed embryos compared to controls, mostly in the one to eight-cell-stage (29.1% vs. 4.9% in controls). Application of 2% or 20% AS to mice during late pregnancy (days 12 to 17), however, did not interrupt gestation. The 20% AS treatment reportedly retarded growth of suckling mice, but this effect disappeared after weaning (Nomura et al., 1980). A recent study by the same investigators reported similar results (Nomura et al., 1987).

The potential of orally administered AS to cause teratogenic effects was examined by Palmer et al. (1975). The treatment schedule was daily oral administration of doses of 0.2, 2.0, 300 and 600 mg/kg from day 6 to day 15 of pregnancy in rats and mice and to day 18 in rabbits. At a daily dose of 600 mg/kg, marked maternal toxicity was observed with principal effects on the gastrointestinal tract. Reduced litter size and fetal loss occurred only at this dose. At 300 mg/kg, the maternal toxicity was less severe and in all three species there were no significant differences in litter parameters as compared to controls. With respect to abnormalities in the delivered pups, an increased incidence of minor skeletal abnormalities was found in mice from litters of dams given 600 mg/kg. Even at maternally toxic doses (600 mg/kg) of AS in rats and rabbits, no increase in teratogenic abnormalities was observed in pups from these litters.

In one study to measure the anti-fertility action of sodium dodecyl sulfate, male mice fed diets containing 0.1% for 6 weeks or 1.0% for 2 weeks experienced no impairment of epididymal spermatozoa (Hemsworth, 1981).
6. Mutagenicity

Alkyl sulfates have given negative results in various mutagenicity tests. Sodium lauryl sulfate, sodium heptadecyl sulfate, sodium tetradecyl sulfate and TEA lauryl sulfate have given negative results in Salmonella/microsome assays. Sodium lauryl sulfate was also negative in a DNA repair test with A. subtilis and in an in vitro cytogenic study with hamster lung fibroblasts (Yam et al., 1984). Hope (1977) reported that the incorporation C\textsubscript{12} AS into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosomes of rat bone marrow cells.

7. Pharmacology

Sodium dodecyl sulfate has been widely used for many decades as a tool in biological laboratories. Thus, the literature dealing with the effects of this particular alkyl sulfate in biological systems is voluminous, and its exhaustive review is outside the scope of this report. The areas selected for review below are those which may have some relevance to the evaluation of AS for its safety to humans.

a. Metabolism

Prottey and Ferguson (1975) examined the absorption and excretion of 16.3 \( \mu \text{Ci} \) of \(^{14}\text{C}\)-labelled sodium dodecyl sulfate following skin application to guinea pigs and rinsing of the treated area ten minutes later. Under these conditions less than 1 percent of the label was found in each of urine, feces, and exhaled carbon dioxide. The label was approximately equally distributed between the rinsings and the skin at the site of treatment. For a 24 hour exposure, 85 percent of the label was found excreted in urine, faces and carbon dioxide in agreement with earlier studies.

Using \(^{14}\text{C}\)-labelled sodium dodecyl sulfate, Howes (1975) examined the percutaneous absorption in isolated rat skin and in live rats. At a concentration of 7.3 mg/mL (0.73 percent), no detectable penetration
through rat skin was observed in 24 hours using a penetration cell and monitoring passage of $^{14}$C-label from the epidermal side through to the dermal side. The absorption of the $^{14}$C-label through rat skin in vivo shows a binding to skin of $202 \pm 37 \mu g/cm^2$ of skin area and a penetration of $0.26 \pm 0.09 \mu g/cm^2$ based on $^{14}$C levels excreted in 24 hours in the urine, feces and as carbon dioxide. Following intraperitoneal administration of $^{14}$C-sodium dodecyl sulfate, 77 percent of the label was recovered in the urine in 24 hours with 15 percent remaining in the carcass, 2.6 percent in the feces and 1.5 percent expired as carbon dioxide.

Using the same method as Howes (1975), Black and Howes (1979) measured the percutaneous absorption of $^{14}$C-labelled NaC$_{12}$AS and NaC$_{16}$AS in rats. They applied 150 µl of 1% w/v AS and measured the $^{14}$C levels in urine collected over 48 hours. Penetration of NaC$_{12}$AS was $0.26 \pm 0.14 \mu g/cm^2$ while that of NaC$_{16}$AS was $0.08 \pm 0.04 \mu g/cm^2$. In experiments in which application was continued for up to 20 minutes, skin penetration was proportional to the duration of contact. It was also proportional to the number of applications.

The administration of potassium dodecyl $^{38}$S-sulfate orally or intraperitoneally to rats at a level of 1 mg per rat (no weight of rats was given) was followed by a rapid excretion of $^{38}$S-label in the urine. More than 80 percent of the label is excreted in 24 hours, with over 90 percent eliminated in 48 hours. Less than 1 percent of the label was found in the feces and 0.4 percent in the carcass at 48 hours. A major metabolite was the sulfate of 4-hydroxy-butyric acid which was excreted unchanged when given orally to rats indicating that the liver may be the primary site of oxidative degradation of the C$_{12}$AS (Denner et al., 1969).

Similar findings were reported by Burke et al. (1972, 1975) who worked with potassium salts of C$_{10}$ and C$_{14}$AS. Over 80 percent of the $^{38}$S-label of these compounds was excreted in the urine in 48 hours with both compounds regardless of route of administration (oral, intravenous or intraperitoneal; 1 mg/200 g rat). With oral administration, these
authors found 4 to 6 percent of the label in the feces. The carcass contained 2 to 16 percent of the \(^{35}S\)-label. Whole body autoradiography studies showed that the liver and kidney were early primary sites of labeling. By 6 hours after administration only traces of the \(C_{10}\) compound were found in the kidney whereas it took 12 hours for the \(C_{18}\) compound to diminish to trace levels in the kidney.

Burke et al. (1976) also examined the metabolic pathway of \(^{35}S\)-labelled \(C_{11}\)AS. Similar to the even-numbered carbon chain AS, over 80% of the \(^{35}S\)-label was excreted in the urine within 48 hours after oral, intraperitoneal or intravenous administration to rats (1 mg/200 g rat). Following oral administration, 10% of the label was found in the feces and less than 3% in the carcass. Whole body autoradiography studies indicated concentration of \(C_{11}\)AS in the liver. Unlike the even-numbered alkyl chain AS, however, some \(C_{11}\)AS was eliminated via the bile although notable biliary excretion occurred in the female only: 2.5 (95% confidence limit: 2.1-2.8) percent of intravenously injected \(^{35}S\)-label in males, 9.9 (95% confidence limit: 6.9-14.9) percent in females. The rate of elimination of metabolites was slower with \(C_{11}\)AS. The reason for this slower rate is unclear but the authors speculate that possible secretion of \(C_{11}\)AS or its metabolites into the gastrointestinal tract may be a contributory factor. The major radioactive component of urine was identified as propionic acid 3-\([^{35}S]\) sulfate. A second urinary metabolite has been tentatively identified as retinoic acid 5-\([^{35}S]\) sulfate.

Merits (1975) administered \([1-^{14}C]\) \(C_{18}\)AS (4.4 mg/kg) and \([^{86}S]\) \(C_{18}\)AS (2.9 mg/kg) intravenously as the sodium and trimethyl ammonium salts to dogs and orally as the erythromycin salt (14.4 mg/kg) to dogs and rats. In rats, both labeled materials were well absorbed and rapidly excreted in the urine: 87-94% in urine, 4-5% in feces. A similar picture was seen in dogs following intravenous administration. Oral administration of either \(^{14}C\) or \(^{86}S\)-labelled \(C_{18}\)AS in dogs, however, resulted in the excretion of considerable amounts of unmetabolized \(C_{18}\)AS in the feces by 72 hours. With the \(^{86}S\)-\(C_{18}\)AS, 50-54% of the label was recovered in urine, 33-41% in the feces; with the \(^{14}C\)-labelled material, 50-79% of
the label was found in the urine, 12 to 40% in the feces. The main metabolite in both dogs and rats was the sulfate ester of 4-hydroxybutyric acid; δ-[¹⁴C]-butyrolactone was also isolated as a minor metabolite present in dog and rat urine in animals given ¹⁴C-labelled \( \text{C}_1\text{gAS} \). An additional metabolite, the sulfate ester of glycollic acid, made up about 20% of the urinary radioactivity in dogs but not in rats.

Burke et al. (1978) examined the effect of substitution at the \( \omega \)-carbon on the distribution, metabolic fate and mode of excretion of \( \text{AS} \). MRC hooded rats received 1 mg/200 g body weight of either 10-undecenyl \( [^{35}\text{S}] \) sulfate or 10-phenyldecyl \( [^{35}\text{S}] \) sulfate by intravenous injection. With 10-undecenyl sulfate, approximately 78% of the injected radioactivity was recovered in urine, largely as the sulfate ester conjugate (68%); an additional 10% was present as inorganic sulfate. Significant amounts of radioactivity were also detected in bile (9.9-11.5%), mainly as the sulfate ester conjugate. Thus, introduction of a terminal unsaturated linkage did not prevent metabolism.

The presence of a terminal phenyl group on the alkyl chain in 10-phenyldecyl sulfate, however, blocked \( \omega \), \( \beta \)-oxidation and resulted in biliary elimination of conjugated hydroxylated products. Biliary excretion, principally as the sulfate ester conjugate, accounted for 43.8% and 73.9% of the administered radioactivity in male and female rats, respectively. Urinary recovery varied from 10.4 to 23.5% of the dose. Rats were killed 6 hours after injection.

b. Gastrointestinal

Lish and Weikel (1959) examined the influence of \( \text{NaC}_{12\text{ave}} \text{AS} \) on intestinal absorption of the anionic dye phenol red and the cationic dye methyl violet through the colon of rats. These solutions were injected directly into colonic sacs isolated by ligation. The absorption of phenol red was markedly enhanced from little or none absorbed in the absence of surfactant to 77 percent absorption with \( \text{NaC}_{12\text{ave}} \text{AS} \). Absorption of methyl violet was unaffected.
Using a surgical procedure in cats to allow direct irrigation of the esophagus and stomach, Berenson and Temple (1975) found that a 10 percent solution of NaC\textsubscript{12}~AS gave rise to edema, congestion and inflammation within 15 minutes. A 20 percent solution of the surfactant caused extensive mucosal disruption, ulceration and necrosis. A detergent preparation containing 24 percent NaC\textsubscript{12}~AS, 60 percent sodium tripolyphosphate and 16 percent soap produced only slight changes in the esophagus but produced lesions in the stomach comparable to those elicited by 20 percent NaC\textsubscript{12}~AS alone.

Necheles and Sporn (1966) investigated a variety of alkyl sulfates (DUPANOL\textsuperscript{TM}) for their effect on gastric motility in dogs. They found that single doses of 50 to 100 mg depressed gastric motility for periods of a few minutes to as long as several hours depending on the material used. Several C\textsubscript{12}~AS induced inhibition for 2 to 3 hours.

c. Neuromuscular

In a series of \textit{in vitro} studies examining the effects of AS on neural transmission and on smooth and striated muscle, Gale and Scott (1953) reported no effect on nerve impulse conduction in sciatic nerve of the frog at dilutions of 1:100 of AS with carbon chain lengths of C\textsubscript{8} to C\textsubscript{18}. On frog gastrocnemius muscle, a potassium-like effect was elicited by the surfactants at 1:100 dilutions. Experiments with isolated rat intestinal segments showed that the AS induced an increase in contractile activity at dilutions ranging from 1:100 for C\textsubscript{18}AS to 1:20,000 for C\textsubscript{12}AS. No effects on isolated turtle heart were found with 1:100 dilutions of the C\textsuperscript{8} to C\textsuperscript{18}AS tested.

The effects in cats on pulmonary arterial pressure after intravenous injection of a series of sodium salts of alkyl sulfates (C\textsubscript{8}, C\textsubscript{10}, C\textsubscript{12}, C\textsubscript{14}) was examined by Schumacher \textit{et al.} (1972). The C\textsubscript{8} and C\textsubscript{14} surfactants were not effective, while the C\textsubscript{10} and C\textsubscript{12} compounds caused a dramatic increase in pulmonary arterial pressure with infusions of 1 mg/kg/min. The inhibition of this increase by papaverine indicated
that the response was due to a direct effect on smooth muscles of blood vessels and bronchi.

d. Cardiovascular Effects

Administration of 250 mg/kg C₁₂AS by gavage to male New Zealand white rabbits daily for two months was found to slightly inhibit the progression of cholesterol-induced atherosclerosis. Serum cholesterol levels (3000 mg/100 mL) were elevated, however, in rabbits fed the high cholesterol diet plus C₁₂AS by gavage compared to rabbits on the high cholesterol diet alone (2100 mg/100 mL). All rabbits were fed 100 g of a standard commercial rabbit pellet daily. Group 1 received the pelleted diet alone; Group 2 received the diet supplemented with 1% cholesterol and 6% peanut oil by weight; and Group 3 received the supplemented diet plus C₁₂AS by gavage. At two months, the rabbits were killed, aortas removed and serum and tissue cholesterol measured. The percent of aortic surface area affected by atherosclerosis was calculated by planimetry. The extent of cholesterol-induced atherosclerotic lesions in the aorta was reduced by C₁₂AS (32% atherosclerotic lesions compared to 72% in controls fed the high cholesterol diet). Cholesterol levels in aortic tissue for these two groups were 12.5 mg/g and 18 mg/g, respectively. Data for the group given the pellets alone were not given (Kwak et al., 1975).

The mode of action of AS in the inhibition of the progression of cholesterol-induced atherosclerotic lesions is not clear. Morin et al. (1974) have reported that 10⁻⁵-10⁻³ M concentrations of NaC₁₀AS, a homolog of C₁₂AS, inhibits cholesterol esterification by aortic microsomes in swine arteries in vitro. These surfactants may, therefore, reduce the accumulation of cholesterol esters in aortic tissue in vivo.
e. **Hormone Effects**

The intramuscular injection into rats of sodium alkyl sulfates (C₈, C₁₀, or C₁₂) enhanced the reduction of blood sugar caused by treatment with insulin, although the surfactant alone had no effect on blood sugar levels. The doses administered (1 mL of 3.44 mM C₈, 0.76 mM C₁₀, 0.17 mM C₁₂) indicated increasing potency with longer chain length. The surfactants did not influence the binding of insulin to cells, but may have enhanced cellular penetration of the hormone (Strausser et al., 1968).

f. **Cellular Effects**

Hemolysis of erythrocytes from dogs, sheep and rabbits by alkyl sulfates was studied by Tomizawa and Kondo (1971). The hemolytic capabilities of the surfactants increased with increasing carbon chain length, differing by an order of magnitude as the carbon chain length increased by two unit increments (Table VI-3).

**TABLE VI-3**

**HEMOlytic CONCenTrATIONS OF SODiUM ALCOHOL SULFATES**

<table>
<thead>
<tr>
<th>Carbon Chain Length</th>
<th>Dog</th>
<th>Rabbit</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3.91 x 10⁻²M</td>
<td>3.58 x 10⁻²M</td>
<td>3.41 x 10⁻²M</td>
</tr>
<tr>
<td>10</td>
<td>2.31 x 10⁻³M</td>
<td>2.70 x 10⁻³M</td>
<td>2.29 x 10⁻³M</td>
</tr>
<tr>
<td>12</td>
<td>2.15 x 10⁻⁴M</td>
<td>4.70 x 10⁻⁴M</td>
<td>2.13 x 10⁻⁵M</td>
</tr>
</tbody>
</table>

Tomizawa and Kondo (1971)

These results were confirmed by Ossipov et al. (1978) who found that the hemolytic activity of a homologous series of AS (C₈ to C₁₅) on dog and human erythrocytes increased with alkyl chain length, reaching maximum activity at twelve carbons. Further elongation of the hydrophobic chain did not produce any marked changes.
Prottey and Ferguson (1975) found that sodium C$_{12}$-AS released histamine from rat mast cells at a concentration of 0.3 mM.

Alkyl sulfates have also been tested for their ability to cause granule lysis in rabbit polymorphonuclear leukocytes. In a homologous series, lytic ability increased with chain length. The maximum chain length tested was C$_{16}$. With pure surfactants of the same chain length (C$_{12}$) but different functional groups, the lytic ability of the surfactant increased with the polarity of the head group (Gibson, 1980).

B. Human Studies

At concentrations of 10% or greater, alkyl sulfates are moderate to strong skin irritants. At 1% they are slightly irritating. The C$_{12}$ homologue produces the most irritation. Of the different salt forms, sodium and ammonium are the most irritating. An anecdotal 1982 report linking the use of rug shampoo with Kawasaki syndrome has been discounted by recent controlled studies. However, other studies have shown that rug shampoos containing sodium dodecyl sulfate caused symptoms of respiratory irritation. In these cases, the shampoos were used in excessive quantities.

I. Percutaneous Absorption

Measurements of percutaneous absorption of alkyl sulfates indicates a low absorption.

Blank and Gould (1959) investigated the penetration of broad cut sodium dodecyl sulfate into human abdominal skin using an in vitro procedure and found that from unbuffered mildly alkaline solutions, sodium dodecyl sulfate penetrated skin only slightly and was retained by the stratum corneum. At pH 10.5, the surfactant penetrated to the dermis.
In another in vitro study with human epidermis and sodium $^{14}$C-dodecyl sulfate (7.3 mg/mL) little penetration occurred during the first 24 hours. During the second 24 hours of exposure, penetration increased rapidly and was accompanied by tissue swelling (Howes, 1975).

The penetration of sodium $C_{12\text{ave}}$ AS into human skin surfaces was investigated by Moran et al. (1967) using uptake of the dye safranin 0 as an indicator. The data showed that superficial and lower layers of hand skin bound more dye following soaking in 2 percent sodium $C_{12\text{ave}}$ AS for 15 minutes than did skin soaked in water. Repetitive soaking did not increase the uptake of dye over that obtained by a single exposure to surfactant.

With an in vitro procedure, Bettley (1965) found that exposure of human epidermis to 0.04 M Na$C_{12\text{ave}}$ AS induced a 50 percent penetration of potassium ion. In a later study, Wood and Bettley (1971) reported that exposure of isolated epidermis to sodium $C_{12\text{ave}}$ AS (0.04 M) produced an increase in titratable thiol groups after incubation for several hours. Prottey and Ferguson (1975) reported that 10 mM sodium $C_{12\text{ave}}$ AS caused a denaturation of human callus keratin as measured by an increase in titratable thiol groups. Under the same conditions, a 1 mM solution had no effect.

Malaszkiewicz and Gloxhuber (1970) found that the ability of the surfactants to elute amino acids from intact human skin was dependent on carbon chain length and showed a decreasing trend with increasing chain length. Defatting the skin with ethyl ether greatly increased the total nitrogen eluted. Extrapolation of dose response data with sodium $C_{12\text{ave}}$ AS indicated a no effect level of 0.06 percent.

2. Skin Irritation

The damaging effects of surfactants on the skin include dryness, roughness and scaling. Symptoms of inflammation can also develop. In severe cases, tissue necrosis occurs. These damaging effects can be traced to the biochemical properties of surfactants. The skin is
defatted by the ability of surfactants to emulsify lipids which results in the partial or complete removal of the lipid surface film. This causes a disturbance in the barrier function of the skin leading to increased chemical permeability and water loss (Bartnik and Kunstler, 1987).

It has been demonstrated that surfactants in solution induce the swelling of isolated human stratum corneum. The highest levels of swelling were induced by anionic surfactants. For a homologous alkyl sulfate series (C₉-C₁₄), maximal swelling was produced by the C₁₂ homologue. This is similar to in vivo irritancy tests where the C₁₂ alkyl sulfate is also the most irritating. This suggests that swelling of the stratum corneum may be an important part of the mechanism of surfactant-induced irritation. A recent study found that swelling increased with time, was concentration-dependent, saturable and reversible. Saturation for sodium C₁₂₄₅₆AS occurs near the critical micelle concentration. In C₁₂-C₁₄ alkyl sulfates, increased ethoxylation causes a reduction in swelling. On an equimolar lauryl sulfate basis, the sodium salt produced the greatest swelling response. The TEA and Mg⁺² salts produced significantly less swelling (Rhein et al., 1986; Blake-Haskins et al., 1986).

For some surfactants, swelling is accompanied by curling of the edges of the epidermis. A study by Tavss et al. (1985) demonstrated that a 2.4% solution of sodium lauryl sulfate caused strips of epidermis to twist and curl.

Stratum corneum swelling caused by anionic surfactants can be reduced by the addition of amphoteric surfactants such as cocoamidopropyl betaine and lauryl dimethylamine oxide. Swelling by NaC₁₂₄₅₆AS has been reduced by the presence of either of these amphoteric (Rhein et al., 1986; Garcia-Dominguez et al., 1981; Blake-Haskins et al., 1986)
With some surfactants, the degree of swelling corresponds to their irritancy. The anionic surfactants sodium and ammonium lauryl sulfate cause the most swelling and are the most irritating (Blake-Haskins et al., 1986).

Smeenk (1969) and Brown (1971) have reported several \textit{in vitro} and \textit{in vivo} studies with human skin to evaluate the effects of surfactants including sodium dodecyl sulfate. In each of the tests, 1 percent solutions of a broad cut dodecyl alkyl sulfate gave rise to increases in response as compared to controls. In patch tests with 0.25 mL of 1 percent solution of the surfactant, 37 of 50 volunteers exhibited little or no response. The arm immersion test was carried out with 2 groups of 15 subjects, each at 0.1 percent concentrations. With 30 minute exposures on each of 5 consecutive days, scaling and/or erythema were observed.

In a patch test using a 1 percent solution of \(\text{NaC}_{12}\text{aveAS}\), Oba et al. (1968) report erythema and fissure of the treated skin after 24 hours.

Valer (1968a, 1968b, 1969) examined the effects of commercial surfactants used in detergents in Hungary, among them sodium \(\text{C}_{12}\text{aveAS}\), using a patch test and a stripping procedure. Concentrations of 1 percent of the surfactants gave rise to little or no response in the patch test. On stripped skin, at the same concentration, slight to moderate reactions occurred including localized hyperemia and exudative infiltration.

With a \(\text{C}_{14}-\text{C}_{18}\text{AS}\), moderate irritation was observed with application of 0.5 mL of an 0.05 percent solution. Under the same conditions, a coconut oil derived material exhibited mild irritation (Procter and Gamble Company, unpublished data).

A 48 hr patch test in 100 twin pairs (54 monozygotic; 46 dizygotic) with a more dilute 0.5% \(\text{C}_{12}\text{AS}\) resulted in no reaction in 50% of the subjects and slight, non-inflammatory changes to mild erythema on the
upper arm of remaining test subjects. No differences in response could be distinguished between twin groups (Holst and Moller, 1975).

Fisher and Maibach (1975) found that application of aqueous 0.5, 1 or 2% solutions of C_{12}AS to the backs of healthy male volunteers produced epidermal hyperplasia. Treatment with a 1% concentration produced an approximately 30-fold increase in mitotic activity which peaked 48 hr post-treatment. Treatment with either 0.5% or 2% C_{12}AS produced similar changes but to a smaller degree.

Dahl and Trancik (1977) induced moderate to intense erythema on the forearms of 10 human volunteers in a 24 hr occluded patch test with a 10% aqueous solution of C_{12}AS. The mean irritation scores were significantly higher at 26 hr (2.85 of possible 8 points) and 28 hr (2.88) than at 24 hr (2.00) when the patches were removed. Irritation decreased by 48 hr, with a significant drop in the intensity of inflammation apparent by 96 hr.

The reaction of surfactants with proteins leads to their denaturation and is a cause of skin roughness.

Imokawa et al. (1974, 1975a) evaluated the relative intensity of skin roughness produced in the surface of the forearm of human volunteers from contact with 1% aqueous solutions of AS with varying alkyl chainlength (C_6, C_{10}, C_{12}, C_{14}). Skin roughening potential increased with increasing alkyl chain length, reaching maximum intensity at twelve carbons. In later studies (Imokawa et al., 1975b; Imokawa and Mishima, 1979), these investigators noted that the relative degree of skin roughening in vivo correlated with the extent of protein denaturation, but did not coincide with the irritancy potential as evaluated by the closed patch test.

In another study, Dominguez et al. (1977) found that maximum adsorption on human callus occurred with AS having a hydrophobic chain length of twelve carbons. The extraction of proteins from human callus was also
a function of chain length; $C_{12}AS$ and $C_{14}AS$ were very active protein extractors compared to $C_8$, $C_{10}$ and $C_{18}AS$.

Ito et al. (1969) studied the skin roughness induced by various surfactants, including sodium $C_{12}$AS, using three separate exposure procedures, i.e., immersion, drip and a cup technique. The cup procedure was found to be not appropriate for skin testing of surfactants. At concentrations of 0.05 to 0.35 percent, sodium $C_{12}$AS induced skin roughness which was roughly correlated to defatting rate in lanolin. Tronnier et al. (1970) showed an increase in skin roughness with exposure to 2 percent sodium $C_{12}$AS. This concentration of surfactant also resulted in water removal from the skin.

In a recent study, daily application of a 4% solution of sodium dodecyl sulfate to the lower leg for 2 weeks resulted in decreased stratum corneum hydration and increased surface scale/roughness. These were accompanied by significant changes in stratum corneum lipid composition (Fulmer and Kramer, 1986).

3. Mucosal Irritation

A patch test was conducted to determine the sensory irritation of AS to oral mucosa. At 7.5 percent concentration, irritation was noted in 4 minutes, while at 1 percent, only a slight sensory perception of irritation was noted after 20 minutes (Hatton et al., 1940).

4. Pharmacology

a. Absorption and Metabolism

Absorption of orally administered erythromycin salt of [1-$^{14}$C]-labelled $C_{16}$AS proceeded differently in two male human volunteers. The administered dose (360 mg $C_{16}$AS with 48.4 $\mu$Ci $^{14}$C-labelled $C_{16}$AS) was well absorbed in one individual (80% excreted in urine, 7% in feces by 72 hours), but poorly absorbed in the other test subject (20% in urine,
73% in feces by 118 hours). Fecal radioactivity was identified as unmetabolized C₁₆AS. The main urinary metabolite was the sulfate ester of 4-hydroxybutric acid with minor amounts of δ-[¹⁴C]-butyrolactone and glycollic acid sulfate also present (Merits, 1975).

b. Effect on Peptic Activity

Fogelson and Shock (1944) reported that administration of 200 mg of an AS mixture (C₁₂, C₁₆, C₁₇ and C₁₈AS) every 2 hours effectively inhibited pepsin secretion in vivo. Kirsner and Wolff (1944) also found an appreciable, but temporary decrease in peptic activity following administration of 780 mg/hour of this mixture to ulcer patients on a low fat diet (lipids interfere with the inhibitory effects of AS on peptic activity). Pepsin levels, however, returned to pre-test levels within 30 minutes after the administration of AS was halted. Contrary to Fogelson and Shock, Kirsner and Wolff (1944) found that treatment with alkyl sulfates provided no apparent beneficial effect on the healing of gastric ulcers. They did note, however, the absence of toxic effects in humans despite ingestion of exaggerated levels of AS; e.g., administration of AS levels as high as 8.5 g/day for a total consumption of 258 g in 38 days produced only slight nausea, decreased appetite and mild to moderate diarrhea.

5. Epidemiology

a. Accidental Exposure

Berenson and Temple (1974) report an oral exposure to a detergent preparation that contained broad cut C₁₇₄₈AS - 24 percent, soap - 16 percent and sodium tripolyphosphate - 60 percent. Members of a family that had ingested this detergent on two occasions within a 24 hour interval had oropharyngeal burns, esophageal lesions and hematemesis. One month later, barium contrast diagnostic procedures revealed no gastrointestinal strictures.
A 1982 study reported that the use of rug shampoo may be a risk factor for Kawasaki syndrome. Kawasaki syndrome is an acute, febrile illness of unknown etiology which occurs predominantly in children under the age of five. Symptoms include fever, changes in the mouth consisting of erythema and fissuring of the lips and strawberry tongue, erythema and induration of hands and feet, erythematous rash and enlarged lymph nodes. Patriarca et al. (1982) reported an outbreak in Colorado in 1982 in which 48% of 23 reported cases had been exposed to rug shampoo at home in the 30 days prior to disease onset. In all but one case, the children had walked or crawled on the rugs within 2 hours of the time they were shampooed. In all cases, the shampoos contained anionic detergents.

Patriarca's findings have been disputed by recent controlled studies by other investigators. In a case-control study of 15 cases and 30 matched controls in Wisconsin, only one patient had been exposed to shampooed carpet within 30 days before the onset of illness (Klein et al., 1986). In another study, none of 11 children with Kawasaki syndrome had been exposed to shampooed carpets (Rogers et al., 1985) and in a Maryland study, rug shampoo exposure within one month or 6 months of illness was not significantly greater in the 30 epidemic and seven sporadic cases than in controls (Lin et al., 1985).

Rug shampoo has also been associated with respiratory illness. Kreiss et al. (1982) reported two outbreaks of respiratory irritation due to carpet shampoo in which the main ingredient was sodium dodecyl sulfate. In each case, the shampoo used was insufficiently diluted. Symptoms included coughing, irritated or watering eyes, sore throat, headache, sneezing and difficult breathing. In both outbreaks, symptoms persisted for weeks to months and resolved when the shampoo residue was wet extracted. In another case, Robinson et al. (1983) reported symptoms of coughing, sneezing, eye irritation, sore throat and headache in motel guests. Investigation revealed that a chemical shampoo was used to clean rugs approximately one week earlier. The problem was eliminated when excess shampoo was removed from the carpets. In both cases, excessive application of the shampoos led to respiratory symptoms.


CODE OF FEDERAL REGULATIONS, Title 21, Food and Drugs.

CONTINENTAL OIL COMPANY, unpublished data.


ETHYL CORPORATION, unpublished data.


VI-30


PROCTER & GAMBLE COMPANY, unpublished data.


APPENDIX A

AS Nomenclature and Abbreviations
Throughout Part 3, the designation AS has been used to indicate alkyl sulfates. The number of carbon atoms in the alkyl chain is numerically designated via a subscript. Mixtures of various alkyl chain lengths are indicated by a numerical range and, if available, the ratio of each carbon chain length is given in parentheses immediately thereafter. Primary (pri-) and secondary (sec-) AS are also indicated. For example:

**Na n-pri-C_{12-14} (80:20)AS** - The sodium salt of a linear, primary alkyl sulfate consisting of 80% C_{12} and 20% C_{14}.

To distinguish between broad-cut sodium lauryl sulfate and the well-defined sodium dodecyl sulfate, the abbreviation "ave" has been used to designate broad-cut-derived material (i.e., C_{12ave}AS). Sodium dodecyl sulfate is designated C_{12}AS.