Environmental and Human Safety of Major Surfactants

Volume I. Anionic Surfactants

Part 1. Linear Alkylbenzene Sulfonates

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Synopsis

The linear alkylbenzene sulfonates (LAS) represent a substantial portion of today's surfactant market. Approximately 2.15 billion pounds of LAS are consumed annually in North America, Western Europe and Japan.

A number of analytical methods are available for the determination of LAS in water samples. Historically, the assay for methylene blue substances (MBAS) has been the predominant method used. However, physicochemical methods with a high degree of specificity and sensitivity have largely replaced the MBAS method. Although GC techniques have been developed, HPLC with UV detection is the most promising technique for LAS analysis. Recent advances in NMR and MS techniques suggest that use of these techniques will become more popular in the near future.

Results of numerous laboratory studies indicate that LAS is rapidly and extensively biodegraded under aerobic conditions. This is apparent from tests of inherent biodegradability and wastewater treatment simulation tests. Tests using natural freshwater or seawater and concentrations of LAS typically found in the environment also indicate ready biodegradability of LAS, with degradation occurring somewhat more slowly in seawater than fresh water. Biodegradation also occurs readily in soils. Biodegradation of LAS is not observed under anaerobic conditions.

Most field tests of LAS biodegradation involve the study of removal or degradation by wastewater treatment processes. Activated sludge treatment is highly effective in removing LAS, trickling filters somewhat less so. Rapid removal has been observed in river water and ground water field tests.

The rate of biodegradation of LAS is influenced by the position of the phenyl group, branching of the alkyl chain, and the presence of aliphatic, cyclic groups. Biodegradation rates generally increase with

increasing proximity of the phenyl group to the end of the alkyl chain. Biodegradation rates decrease with branching and the presence of cyclic groups. In screening studies, various effects of chain length upon biodegradation rate have been reported. However, tests at realistic environmental concentrations have failed to detect chain length effects on biodegredation rates.

Concentrations of LAS in influent sewage generally range from 2-7.5 mg/L. Concentrations in effluents from activated sludge treatment are generally 0.070 mg/L or less. Concentrations in effluents from trickling filters may range up to 0.76 mg/L.

Concentrations of LAS are further reduced by dilution in surface waters. Results of recent in-stream monitoring for LAS indicate that concentrations of LAS in river waters below sewage effluent outfalls range from 0.01-0.3 mg/L. Concentrations are rapidly attenuated downstream of sewage outfalls due to in-stream biodegradation. Concentrations of LAS in sediments below sewage outfalls range from 1.5 to 174 mg/kg.

LAS toxicity to fish and other aquatic organisms is affected by chain length and phenyl ring position. A decrease in the carbon chain length of LAS surfactants is accompanied by a decrease in toxicity to fish, aquatic invertebrates and algae. LAS toxicity also decreases as the phenyl group is positioned farther from the end of the alkyl chain. By-products generated in the manufacture of LAS (e.g., dialkyl tetralins) follow the same trends. However, the toxicity of these latter materials is considerably less than for LAS isomers.

There appears to be little variation in the acute aquatic toxicity of LAS. Values for LC_{50} generally fall below 10 mg/L for representative test species. Biodegradation of LAS reduces toxicity by 10 to 100 fold, with the more toxic components being most rapidly biodegraded. Fish generally appear to be more sensitive to LAS than invertebrates or algae.

Subacute studies in fish show the gills and the locomotive muscles to be the major sites of LAS toxicity. Effects include reduced operculum movement, cellular changes, hemorrhaging, lethargy and decreased swimming endurance and locomotion. Low levels of LAS induce behavioral changes, such as a disruption in the avoidance response and alterations in odor perception and chemotatic response. Early developmental stages of fish, particularly the feeding sac-fry, are most susceptible to LAS toxicity. Examination of algae exposed to toxic concentrations of LAS revealed cell wall defects and impaired photosynthesis.

Many environmental factors influence the toxicity of LAS. Among the most dramatic is water temperature. Investigators have shown that an increase in temperature increases the toxicity of LAS. Also, LAS toxicity increases as the oxygen tension in the test solution is reduced. LAS was more toxic in hard water than soft water due to an acute change in permeability of gill tissue. A greater than additive toxic effect exists when LAS is combined with zinc, copper and mercury. Addition of solids decreased the toxicity or bioavailability of longer chain (i.e., more sorptive) LAS homologues, particularly with benthic fauna, but had no effect on the toxicity of LAS with shorter alkyl chains. Likewise, exposure of Daphnia to LAS up to seven generations showed no change in LC_{50}'s compared to cultures that did not have previous exposure to LAS.

LAS has a low bioaccumulation potential relative to nonionic, hydrophobic organics. LAS is readily absorbed through the gills and body surface, and is distributed via the blood to various organs and tissues. As time passes, the majority of the LAS accumulates in the gall bladder and the hepatopancreas. Clearance of LAS is usually rapid with a half life of 2-3 days. The uptake of LAS is much grater at low levels than at high levels of exposure, and usually short-chain LAS are accumulated less than long-chain LAS.

Some studies show a possible synergism between LAS and pesticides or petroleum products. However, no substantial experimental evidence has been located to prove that LAS actually enhances the uptake of these agents. No synergistic effects were observed when LAS was mixed with other surfactants.

Phytotoxicity studies have been conducted in a wide range of media and conditions, including hydroponics. At high concentrations, LAS inhibits plant growth but at low LAS levels, limited evidence suggests a possible stimulation of growth.

Recent data on the mammalian toxicity of LAS corroborate the previously observed low order of toxicity of LAS to mammalian species, and add further support to the position that no human safety problem exists from normal use levels of LAS. There are no indications of carcinogenicity, mutagenicity, teratogenicity or other long-term adverse effects resulting from LAS exposure.

LINEAR ALKYLBENZENE SULFONATES

I. INTRODUCTION

The linear alkyl benzene sulfonates (LAS) were introduced as a prime component of detergents in 1965 to replace the tetrapropylene-derived surfactants (ABS), which had achieved widespread use until that time. Compared to the ABS type of surfactants, LAS are almost completely biodegradable and their use has resulted in elimination of environmental problems due to foaming and residual surfactant levels in waterways. Because of their efficiency as cleaning agents, their relatively facile biodegradability and their environmental safety, LAS surfactants are a major component of almost all types of household surfactant products (Matson, 1978).

Production of synthetic surfactants in the USA, Western Europe and worldwide for 1984 was estimated at 4,973.5, 5,809.2 and 17,393.4 metric tons, respectively (Layman, 1984). Anionic surfactants dominate the production market, accounting for approximately two-thirds of total production. About one-third of the anionic market is made up of the linear alkylbenzene sulfonates (Jakobi et al., 1987; Layman, 1984). A recent estimate by Greek and Layman (1989) puts LAS consumption in North America, Western Europe and Japan at 2.15 billion pounds per annum.

Almost 80% of the total U.S. production of LAS is used in household products. The use of LAS had been growing until 1980 due to the increased use in non-phosphate laundry detergents. Sinee that time reformulations of laundry powders have reduced the LAS content or replaced LAS with alcohol ethoxysulfates. However, reeent trends toward inereased use of liquid in laundry detergent formulations have added to the growth in the LAS market. Detergent manufacturers have also found that LAS can be used in multi-functional products, an area formerly believed to be the province of the alcohol based nonionics (Dean, 1985).

This review evaluates the information relevant to LAS that has accrued in recent years in the following areas:

(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.

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II CHEMISTRY

A. Product Chemistry

LAS used in commercial products is a complex mixture of materials with a range of linear alkyl chain lengths, usually from ten to fourteen carbons. The distribution of the phenyl ring position on the alkyl chain varies aecording to the starting materials and processes employed to generate the linear alkylbenzene (LAB) moieties from which LAS are derived by phenyl ring sulfonation. Side reactions produce 5-10 percent of impurities including dialkyl substituted mono- and di-ring structures and some diphenylalkane.

1. Primary Product-LAS

Linear alkylbenzene (LAB) is the precursor for LAS. The production of LAB was traditionally carried out by alkylation of benzene with a mixture of secondary alkyl chlorides in the presence of aluminum chloride, the Friedel-Crafts reaction. More recent methods employ either dehydrogenated paraffins or dehydrochlorination of alkyl halides to obtain a mixture of olefins which are then reacted with benzene using hydrofluoric acid as the catalyst. Both procedures result in a mixture of LAB isomers with various chain lengths, depending on the nature of the alkyl halide or olefin feedstock. Moreover, even if a single isomer or alkyl feedstock is used, a mixture of isomers of different phenyl group position on the alkyl chain results from the alkylation process (Olson, 1960; McGuire and Nicks, 1971).

Since the properties of LAS with regard to wetting, detergency, and biodegradation vary with alkyl chain length and with the position of the phenyl group, the desired constitution of the material finally used in a product can be controlled to some degree by the choice of starting materials and manufacturing process. Table II-1 indicates the

diversity of LAS structures in several typical commercial products. The impact of this diversity on biodegradability and toxicity will be discussed in a later section.

2. Secondary Products

The alkylation of benzene results in a number of side reactions. The known products of these reactions are shown in Figure II-1. The diphenylalkanes and dialkylbenzenes boil at temperatures sufficiently above the linear monoalkylbenzene, facilitating their removal. However, some of the other dialkylbenzene materials cannot be separated from the primary product with ease and, following sulfonation, they remain in the commercial LAS used in detergent products. Dialkyltetralin, and to a lesser extent, dialkylindane and dialkylnaphthalene moieties may represent as much as 5 to 10 percent of the final product.

B. Analytical Methods

LAS has been used commercially for several decades, and a variety of methods are available for LAS analysis. Chemical methods involving complexation of LAS with cationic reagents are used for some environmental analyses; the most widely used reagent is methylene blue. However, physicochemical methods with a high degree of specificity and sensitivity are gaining acceptance as important analytical tools in the surfactant industry. These methods have been demonstrated to work on industrial, product, and environmental samples from a variety of matrices. Although GC techniques involving desulfonation or derivatization of the LAS molecule have been developed, HPLC is the most promising technique for LAS analysis. Detection for routine analysis is

TABLE II-1

CHARACTERISTICS OF REPRESENTATIVE COMMERCIAL LAS PRODUCTS*

(Percent of Total)

	Supplier A		Supplier B		Supplier C		
Carbon Chain length	Product 1	Product 2	Product 1	Product 2	Product 1	Product 2	Product 3
$<C_{10}$	<1	<1	2**		1.0	0.3	
C_{10}	15	11		10**	20.6	8.5	0.5
C_{11}	43	31	85		39.4	17.0	2.0
C_{12}	35	32			31.2	20.0	18.1
C_{13}	7	23	15**		7.3	30.0	52
C_{14}	<1	3		85-90	0.5	15.0	27
$>C_{14}$			2**	5**		0.2	0.4
Phenyl isomer distribution							
2-phenylalkane***	32	30	25	25	28.4	15	12
Tetralins and sulfonated tetralins	6	7	NA	NA	6-10	<1	<1

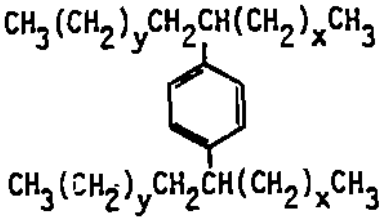
*Manufacturers' specifications

**Maximum percentage

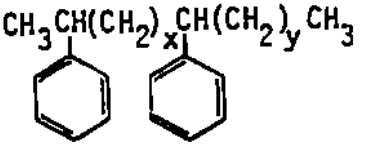
***Remaining phenyl isomer distribution not available

FIGURE II-1

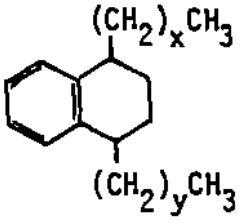
PRODUCTS RESULTING FROM SIDE REACTIONS
IN THE MANUFACTURE OF LINEAR ALKYL BENZENE



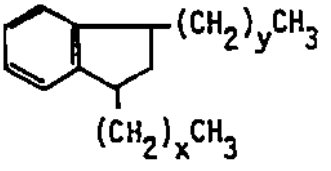
Dialkylbenzene



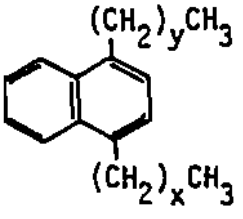
Diphenylalkane



Dialkyltetralin



Dialkylindane



Dialkylnaphthalene

generally by UV. Recent advances in NMR and MS techniques indicate that use of these techniques will become more widespread in the near future.

Swisher (1987), Rosen and Goldsmith (1972), Howard et al. (1975), Higgins and Burns (1976), Llenado and Jamieson (1981), Llenado and Neubecker (1983), Painter and Zabel (1988), and others have described in considerable detail the procedures and potential problems of the analytical methods available for the determination of surfactants, including LAS. The analytical procedures can be categorized into 3 major areas: physical methods, chemical techniques, and physicochemical techniques. The following is a brief examination of some of the more common procedures employed to assess presumptive levels of anionic surfactants, including LAS, in the environment and in laboratory studies.

1. Physical Methods

Determination of foaming potential and measurement of changes in surface tension are two physical procedures used historically to monitor the presence of surfactants. However, these methods are non-specific and the measured effect is not necessarily a linear function of the surfactant concentration.

A transient phenomenon associated with surfactants, foaming is affected by a wide variety of factors such as temperature, humidity, size of the test container, etc. Similarly, although each surfactant has a characteristic ability to lower surface tension, the major drawbacks of the surface tension measurements are: (1) the lack of specificity; (2) insufficient sensitivity to distinguish minute changes in surfactant eoncentration; and (3) the ease with which foreign substances can distort results (OECD, 1964). This latter point makes this procedure unsuitable for analysis of surfactants in waste waters and other environmental samples.

2. Specific Chemical Techniques

Several spectrophotometric and titration methods are currently considered standard procedures in many surfactant laboratories. Although these techniques are reliable and are easy to use for routine product analyses, they are generally considered to be inadequate for trace surfactant measurements requiring identification of specific surfactants and isomers. However, research activities on these methods continue.

a. Spectrophotometric Methods

Spectrophotometric methods for the analysis of anionic surfactants are based on the formation of a solvent-extractable complex of the anionic surfactant and an intensely colored cationic reagent. The most commonly used and accepted method for anionic aurfactants is the Methylene Blue Active Substances (MBAS) method first proposed by Jones (1945) and later modified by Longwell and Maniece (1955) and Abbott (1962). Methylene blue (MB) and anionic surfactants react to form large hydrophobic ion-pair complexes that are readily extracted into $CHCl_3$ forming a blue color that is quantified by absorbance at 652 nm. The MBAS procedure is the standard method for the examination of water and wastewater for anionic surfactants recommended by the American Public Health Association (1985) and is used by the U.S. Environmental Protection Agency. It is also the ASTM standard method for analysis of alkylbenzene sulfonate in water (ASTM, 1986). In waters that are relatively free of interferences, the method is applicable to MBAS determinations in the range of 0.03 to 3.5 mg/L for a 100-mL sample.

Although the MBAS method is quick, sensitive, and easy to perform, significant problems exist (Swisher, 1966; Swisher, 1970):

- MBAS method lacks specificity and cannot distinguish between LAS and other anionic surfactants or any other compounds possessing a strong anionic group and a lipophic base.

- MBAS method cannot detect intermediate degradation products in which the anionic group has been altered or removed.

- Adsorption of surfactants, particularly in waters with high solid contents, results in false colorimetric readings.

- Inorganic and organic components present in effluents may interfere with the MB reaction.

For these reasons, much effort has been directed at improving sample cleanup procedures to remove interfering compounds (particularly for surface water and effluent samples), and examining alternative cationic reagents. However, even these modifications do not generally provide an analysis specific to LAS.

Wickbold (1976), for example, reduced positive interferences by using a sublation step to isolate anionic surfactants and demonstrated improved selectivity in surface water and drinking water samples. Uchiyama (1977) initially extracted LAS into 1,2-dichloroethane as the MB complex, then washed with HCl and reextracted the LAS back into aqueous solution for quantification by absorbance at 222 nm. Good separation from other surfactants was reported with LAS (dodecylbenzene sulfonate) recoveries of 100 $\pm$ 5%. Hycholysis of aqueous samples prior to addition of methylene blue has also been used to destroy organic sulfate interferences such as AS and AES.

Oba *et al.* (1976) extracted anionics in sewage and river water samples into $CHCl_3$ as the MB complex, hydrolyzed the complexes, and released the anionic surfactants from the MB complex by ion exchange. The free anionic surfactants were converted to the sulfonyl derivatives and quantified by infrared (IR) absorbance bands at 640, 618, and 524 cm^{-}, respectively. Similarly, Hellmann (1979) used IR to quantify anionic surfactants in sediments, suspended sediments, and sewage sludge. Anionics were extracted from dried samples with methanolic HCl or methanolic ammonia, isolated by sublation, and reextraeted after formation of MB-surfactant complexes. The MB complexes were cleaned up

by thin layer silica gel chromatography and quantified by IR spectrometry. Chromatography on silica gel was reported superior to acid extraction as a method for releasing the anionic surfactant from the MB complex because it caused no structural change.

Yasuda (1980) determined anionic surfactants by separating and concentrating the surfactant by anionic exchange (Amberlite IRA-GC400), and quantifying the surfactants by the MBAS method. ABS was selectively eluted before LAS, permitting separate determinations of the two surfactant types. In a later paper, Yasuda (1981) reported a process for removing many of the MB-active substances that normally interfere in MBAS analysis. LAS was extracted into ethyl acetate by lowering the pH (~ pH 1) and protonating the molecule. After neutralization and successive washes, a conventional MBAS analysis was performed.

A series of surface water samples were analyzed by Waters (1976), using a modified MBAS method which more specifically determines LAS than the earlier methods; however, the analysis is still not completely specific. The modification consists of preliminary treatments, including extraction after complexing the surfactant with 1-methyl-heptylamine. Methylene blue is used for the final determination, in which levels of LAS as low as 5 μg/L can be detected. Recoveries from surface and distilled water samples range from 89-99+%.

A review and experimental comparison of colorimetric methods for determination of anionic surfactants (including LAS) was published by Wang et al. (1978). They examined comparisons of the standard Methylene Blue Method (SMBM), a Modified Methylene Blue Method (MMBM), and a Modified Azure A Method (MAAM). They ranked them as MMBM > MAAM > SMBM for both precision and accuracy with both distilled water and river water. They also performed an extensive study of interference by large numbers of inorganic and organic substances such as sulfates, sulfonates, phosphates, phenols, etc.

Holtzclaw and Sposito (1978) present a modified methyl green method for the analysis of anionic surfactants in the fulvic acid fraction of sewage sluge. This method was also used for the determination of LAS in soils amended with sewage sludge (Sposito et al., 1982). The method involves hydrolysis in 4N HCl, separation on an anion exchange resin, formation of a methyl-green-surfactant complex, its extraction into benzene, and spectrophotometric determination of the complex at 610 nm. The method is non-reactive to partially degraded LAS.

Crisp et al. (1975) used the bis(ethylenediamine)copper(II) ion to form anionic surfactant complexes that are extractable with $CHCl_3$. Anionic surfactants were initially quantified by determination of copper by colorimetry or atomic absorption (AA); LAS detection limits for fresh, estuarine and seawater samples were lowered (2 μg/L) by using flameless AA (Crisp et al., 1976). Gagnon (1979) used the same reagent for the analysis of anionic surfactants in seawater. The method demonstrated good precision and recovery (92-98%) at the 1-50 μg/L range. Potential interference from copper and natural organic chelators was noted, however. LeBihan and Coustot-Coupez (1977) reported routine determination of anionic surfactants at 10 μg/L using flameless AA and tris(o-phenanthroline)copper(II) as the cationic metal complexing agent.

Several other methods for non-specific determination of LAS have been described in the Japanese literature and reported in Painter and Zabel (1988). Matsueda and Morimoto (1980) described a method in which an ion-pair complex was formed between LAS and thiourea-copper(I) and extracted with methyl iso-butyl ketone. Copper was determined by AA spectrophotometry; the applicable concentration range was 0.001 to 20 mg/L LAS. A second method involves complexation with a sodium reagent, extraction with methyl iso-butyl ketone and determination of sodium by flame photometry. The limit of detection of LAS was reported to be 0.05 mg/L. Abe (1984) reported another method in which the amount of ferrion reacting with the anionic surfactant was quantified.

Other cationic reagents reported (Llenado and Neubecker, 1983) to be used for the spectrophotometric determination of anionic surfactants but not explicitly discussed here include: Rosaniline, Crystal Violet, Methyl Violet, Ethyl Violet, Methyl Yellow, Azure Blue, Azure A, Methyl Green, Billiant Green, Toluidine Blue, and cobalt chelates of 2(2-pyridylazo)-5-aminophenol.

b. Volumetric Methods

The two-phase titration method for the analysis of LAS is also well established and has been extensively reviewed by Vavrouch and Kuban (1984). The method depends on the extraction of the LAS-indicator complex into a non-aqueous solvent (usually $CHCl_3$) in equilibrium with an aqueous solution of the surfactant and the indicator compound. Typical relative standard deviations for this type of analyses is $\leq$ 5% with a detection limit of $\geq$ 25 mg/L. Li and Rosen (1981) reported that titration methods using $CHCl_3$ as the organic phase were not quantitative for anionic surfactants containing fewer than 12 carbons. Quantitative results were achieved, however, with a mixed organic phase of 2:3 (v/v) chloroform:1-nitropropane and multiple extractions.

In the ASTM standard method for determining anionic compounds by cationic titration (ASTM, 1987), the anionic surfactant is titrated in a mixed aqueous chloroform medium with a standard solution of a cationic reagent (Hyamine 1622) and small amount of indicator. The end-point is determined by the transfer of the colored complex from the organic solvent phase to the aqueous phase.

Wang et al. (1973) proposed a two-phase titration method for the analysis of LAS in seawater. Their procedure employs a nonaromatic quaternary ammonium salt and a tetraphenyl boron (TPB) reagent. Although the method is reportedly insensitive to high salinity values, potassium content does produce a negative interference by reacting with the TPB reagent. A later paper by Wang et al. (1975) presents the further development of the two-phase titration method for LAS and ABS in waters and commercial detergents and reports the development of a

simplified kit for field use. The method involves formation of a complex of anionic surfactant and (cationic) cetyldimethylbenzyl-ammonium chloride, and analysis by determination of residual (free) cation by titration in an acidified aqueous/chloroform mixture. Ivanov (1979) determined anionic surfactant concentrations by titrating against a standard acid with a Bromophenol Blue Acid Chrome Dark Blue indicator, hydrolyzing the mixture in concentrated HCl under reflux, and titrating again in the presence of the same indicator.

Taylor et al. (1974) developed a procedure which can distinguish between homologous anionic surfactants by solvent extraction of the surfactants with iron (II) chelates. Selection of the appropriate chelate enables one to readily separate longer-chain surfactants from the shorter-chain compounds.

c. Potentiometric Methods

Ion-selective electrodes are another technique for anionic surfactant analysis. Hoke et al. (1979) prepared an ion-selective electrode for rapid monitoring of pentadecylbenzene sulfonate down to 10^{-7}M. The active material was a nylon membrane (ELVAMID 8064) impregnated with the complex salt of pentadecylbenzene sulfonic acid and Hyamine 1622. Results achieved with this method compare favorably with those from the MBAS method; chloride ion was reported to interfere. Ciocan and Anghel (1976) described the potentiometric titration of sodium dodecylbenzene sulfonate with cetydimethylbenzyl ammonium chloride; end points were determined with Ruzicka-type electrodes filled with dodecylbenzene sulfonate-ferroin complex in o-dichlorobenzene:n- decanol.

Dilley (1980) constructed an electrode with a membrane of 40% PVC and 60% tricresyl phosphate for the potentiometric titration of LAS with Hyamine 1622 as the titrant. Similarly, Dimitriev et al. (1981) designed an electrode with a PVC matrix impregnated with methyl green indicator; analysis was completed in 2-3 minutes with 4-8% relative error. Vytras et al. (1981) constructed a wire-coated electrode using 2-nitrophenyl 2-ethylhexyl ether in a PVC matrix for the analysis of

over 15 anionic and cationic surfactants. Anghel and Popescu (1981) patented the potentiometric determination of ionic surfactants used in petroleum recovery and the various means of constructing the ion-specific sensitive electrodes. Siemroth and Hennig (1981) patented an electrode with a PVC membrane containing the tetraalkyl- or triarylalkyl-phosphonium salt of an anionic surfactant.

3. Physicochemical Techniques

Since LAS generally represent only a portion of the anionic surfactants measured in many of the physical and chemical techniques described above, efforts have been devoted to developing analytical techniques with high specificity for routine determination of LAS in environmental samples.

Isolation and concentration of anionic surfactants in environmental samples is generally the first step in a chemical-specific analytical technique. Swisher (1987) summarizes many of the preconcentration/ purification techniques utilized in analysis of surfactants (e.g., evaporation, sublation, extraction, ion exchange, and others). The most promising concentration techniques used in the analysis of LAS surfactants are: extraction, often promoted by the complexation of anionic surfactant with added cations such as methylene blue; adsorption to activated carbon or synthetic polymer resins such as the Amberlite XAD series, widely favored for aqueous samples; and ion exchange adsorption with subsequent elution designed to isolate the desired compound category from other interfering compounds. Complexation of anionic surfactants with methylene blue and other cationic agents prior to extraction has been discussed previously. Significant advances in other LAS isolation techniques used in conjunction with spectroscopic or chromatographic techniques will be highlighted in the discussions below.

a. Spectroscopic Techniques

Spectroscopic methods are generally used for qualitative determination of LAS or for quantitative characterization in combination with some other technique, such as chromatography. Spectroscopic methods that are commonly used for detection of LAS include infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR), and atomic absorption (AA) spectroscopy. Several of these were discussed previously as detection methods for LAS-cationic or LAS-metal complexes; they will also be mentioned as detection methods in the following section on chromatographic separation. The basic principles of IR, UV, and NMR detection of LAS are discussed below. Mass spectrometry (MS) has exhibited rapid advances as a method of LAS characterization and is gaining acceptance as an important analytical tool. MS is also discussed in this section.

The major use of IR analysis has been to distinguish between ABS and LAS surfactants via minor differences in the absorption spectra. However, the reliability of this procedure is uncertain when one or the other component drops below 10% to 20% of the total (Swisher, 1987). With respect to infrared procedures, the American Public Health Association (1971) proposed an infrared analytical method for detection of low LAS concentrations in water. It is based on the formation of an amine complex with LAS and a carbon adsorption procedure to purify the complex for subsequent analysis. The method is only applicable to raw water samples and not to sewage or industrial wastes.

The benzene rings of LAS show 3 characteristic absorption bands in the UV range: 260 mμ, 223 mμ, and 193 mμ. These absorption bands can be readily detected at LAS coneentrations of 1 ppm or less in aqueous solution (Swisher, 1987). One of the major disadvantages of the UV procedure is that it is susceptible to interference by many naturally occurring inorganic compounds as well as other aromatic species.

NMR is a powerful method for investigation of molecular structure. Although not direetly applicable to quantitative analysis of

environmental samples, NMR has been used for qualitative determinations. Thurman et al. (1987) has developed a method for determination of both branched-chain and linear-chain alkylbenzenesulfonate surfactants in groundwater samples. The method employs XAD-8 resin for concentration, followed by elution with methanol, separation of anionic and nonanionic surfactants by anion exchange, quantification by titration, and identification by ^{13}C- NMR spectroscopy. Laboratory standards and groundwater samples at concentrations as low as 0.3 mg/L were analyzed.

While anionic surfactants are generally too non-volatile and polar to be amenable to GC/MS and other conventional MS techniques, analysis of these compounds by newer MS techniques has been accomplished. Tandem MS (MS/MS), field desorption (FD), direct chemical ionization (DCI), fast atom bombardment (FAB), and collisionally activated dissociation (CAD) are among the recent MS advances (Llenado and Neubecker, 1983).

Lyon (1984) described an MS/MS system for determination of anionic surfactants in which the sample was bombarded with xenon atoms; the resulting ions were separated, quantified, and then subjected to collision with helium atoms for further characterization. In another study, negative ion FD/MS of anionic surfactants was reported to achieve high selectivity for the detection of sulfonate surfactants (Daehling et al., 1982). Cationic surfactants were noted to produce some interferences. Levson et al. (1984) also reported using FD and FAB/MS for the analysis of anionic surfactants.

Ward et al. (1987) applied FAB/MS techniques to environmental samples and reported that analysis was significantly (5 times) faster than HPLC analysis. Using sample preparation procedures such as evaporation and simple extraction, surfactant concentrations as low as 1 μg/L were measured.

Schneider et al. (1984) reported that an approach using the combination of FD and CAD in a tandem MS is well-suited to the characterization of anionic surfactants. Anionic, nonanionic, and cationic surfactants

each desorb at distinctly different emitter temperatures and proper choice of emitter heating current permits partial separation of each class. The method was applied to the analysis of anionic surfactants in surface water and sewage water samples. Full spectra were obtained with 10^{-6} g of the anionic surfactants.

b. Chromatographic Techniques

Separation by chromatography is the basis of a number of analytical techniques that have been developed to improve the specificity and sensitivity of the analysis of anionic surfactants, particularly LAS. Thin layer chromatography (TLC), as well as paper chromatography, have been used extensively in the analysis of anionic surfactants largely in commercial applications. Gas chromatography (GC) or GC/Mass spectrometry (GC/MS) combined with solvent extraction is sensitive and specific for the analysis of individual LAS components but requires desulfonation or conversion of LAS to volatile derivatives. Capillary columns are currently preferred over packed columns for the GC analysis of LAS-derived substances. However, complete resolution of all isomers has not been achieved even with the high efficiency capillary columns (Painter and Zabel, 1988).

High performance liquid chromatography (HPLC) and UV fluorescence detection combined with solvent extraction, XAD adsorption and ion exchange have been used for relatively rapid determination of LAS components in water or sediment. Direct analysis of LAS in water by HPLC and fluorophotometric detection without preconcentration or cleanup has also been developed, but application of this method is limited by the level of sensitivity (Kikuchi et al., 1986). Several of these chromatography methods are described below and summarized in Table II-2.

Gas Chromatography (GC)

Waters and Garrigan (1983) described a modified microdesulfonation/GC procedure that can accurately determine μg amounts of LAS. This

TABLE II-2

SELECTED METHODS FOR LAS-SPECIFIC ANALYSIS

Isolation	Derivatization	Separation	Detection	Matrix	Reference
XAD-8 Adsorption Anionic Exchange	-	-	NMR (quant. by titration)	Groundwater	Thurman et al. (1987)
Extraction	-	FD	CAD/MS Sewage Water Comm. Surfactants	Surface Water	Schneider et al. (1984)
Extraction Evaporation	-	-	FAB/MS	N/A	Ward et al. (1987)
MB-complexation Extraction Ion Exchange	Desulfonation	HRGC	FID	Sewage Sewage Effluent Surface Water	Waters and Garrigan (1983)
XAD-2 Adsorption Extraction Ion Exchange MB-Complexation	Desulfonation	HRGC	FID	Primary Sludge Digester Sludge Stream Sediment	Osburn (1986)
MB-Complexation Ion Exchange	Methylsulfonyl Chloride Derivative	GC	MS	River Water	Hon-Nami and Hanya (1978)
Extraction	Sulfonyl Chloride Derivative	HRGC	FID or EI-MS or CI-MS	Sewage Sludges	McEvoy and Giger (1986)
Extraction	Sulfonyl Chloride Derivative	HRGC	MS	Wastewater Sludges	Giger et al. (1987)
MB-Complexation Ion Exchange	Methyl Sulfonate Derivative	GC	FID or MS	River Sediment Susp. River Part.	Takada and Ishiwatari (1987)
Extraction	-	rp-HPLC	UV Absorption	Comm. Detergent	Nakae et al. (1981)

TABLE II-2 · Continued

SELECTED METHODS FOR LAS-SPECIFIC ANALYSIS

Isolation	Derivatization	Separation	Detection	Matrix	Reference
Ion Exchange	-	HPLC	UV Absorption	Water	Saito et al. (1982)
-	-	HPLC	UV Fluorescence	Sewage Influents Sewage Effluents River Water	Linder and Allen (1982)
MB-Complexation	-	HPLC	UV	River Water	Kobuke (1985)
-	-	rp-HPLC	UV Fluorescence	Sea Water Marine Sediment Fish	Kikuchi et al. (1986)
-	-	rp-HPLC	UV Fluorescence	Detergents Sewage Sludge Soils River Sediments	Marcomini and Giger (1987)
Adsorption	-	rp-HPLC	UV Fluorescence	Wastewater Sludge Soils	Giger et al. (1987)
-	-	HPLC	Sulfur Detector (ICP)	Wastewaters	Irgolic and Hobill (1987)
Solid Phase Extraction (SAX)	-	rp-HPLC	UV	Influent River Water Sludge Sediments	Matthijs and DeHenau (1987)
Solid Phase Extraction (C2 and SAX)	-	rp-HPLC	UV Fluorescence	River Water Effluent	Castles (1987)
MB-Complexation Ion Exchange	-	HPLC	UV Fluorescence	Influent Effluent	Tokada and Ishiwatari (1987)

procedure offers improvements in sensitivity and selectivity over other earlier procedures in aqueous environmental samples including sewage, sewage effluent, and surface waters. The LAS was concentrated as its methylene blue complex by large-scale solvent extraction and freed from potential interferences by a series of clean-up steps (ion-exchange chromatography, hydrolysis, and solvent extraction), prior to its desulfonation with a concentrated phosphoric acid reagent. The resulting alkylbenzene hydrocarbons were then recovered and quantitatively determined by capillary GC. The introduction of clean-up stages resulted in chromatograms free of major interferences, allowing identification of LAS isomers on the basis of relative retention times. The mean recovery of LAS internal standards through the complete procedure was reported to be greater than 90% even in the presence of high levels of suspended solids. The reported method detection limit was less than 10 μg/L LAS in aqueous samples.

Osburn (1986) presented a similar analytical methodology to the Waters and Garrigan method and validated its applicability to primary and digester sludges, as well as to stream sediments. Good precision and recovery values were obtained for spiked river water samples at 50 and 100 μg/L, and >90% recovery of LAS added to sludge samples at total concentrations of 114 to 177 μg/g. Prior to the microdesulfonation-GC procedure, an aliquot of the final cleaned-up extract was taken for MBAS determination and results of the interference-limited (IL)- MBAS and GC determinations were compared. For the influent samples collected at the sewage treatment plant, the two measurements were very similar, with relative percent differences ranging from 3% to 6%. The effluent samples, however, exhibited IL-MBAS values 42% to 94% higher than the GC-LAS values, indicating the presence of non-LAS MBAS material. In river water and stream sediment samples, the difference between the IL-MBAS and GC measurements was reported to increase with increasing distance from the sewage treatment plant outfall; overall concentrations were reported to decrease with increasing distance. These observations indicate significant biodegradation of LAS after discharge. The IL-MBAS measurements on sewage sludge samples were in close agreement with the GC measurements on the same samples.

Sedlak and Booman (1986) also compared LAS concentrations in wastewater samples that were measured using non-specific anionic surfactant methods and other LAS-specific methods. The three methods chosen for comparison were: the MBAS method, interference-limited MBAS (IL-MBAS) involving cation exchange and acid hydrolysis prior to MBAS measurement, and LAS-specific measurement using cation exchange and acid hydrolysis followed by desulfonation-GC. The concentrations generally declined in the order of MBAS > IL-MBAS > LAS-specific methods. Analysis of LAS and AE surfactants by classical, non-specific methods was adequate for liquid stream samples other than effluent samples; the non-specific methods (i.e., MBAS) over-estimated LAS concentrations in wastewater effluents. For LAS, the IL-MBAS method produced results similar to the desulfonation-GC method for all samples, including effluents and sludges.

A GC/MS method was developed by Hon-Nami and Hanya (1978) in which the LAS components were complexed with MB, released from the complex by ion exchange, and converted to the methyl sulfonate derivatives for GC analysis. Individual components were determined by MS. Analysis of river water samples showed good reproducibility at 3 μg/L concentrations.

Levels of LAS in anaerobically and aerobically stabilized sewage sludges were quantitatively determined by McEvoy and Giger (1986). The analyses were accomplished by formation of the sulfonyl chloride derivatives of the LAS compounds, extraction and cleanup, and subsequent high-resolution gas chromatography (HRGC) with flame ionization detection and directly coupled HRGC/MS employing both electron impact and chemieal ionization modes. The advantage of HRGC and HRGC/MS is that complex mixtures can be resolved into individual components. However, these analyses are generally more costly than the HPLC/fluorescence analyses discussed later. Giger et al. (1987) also reported the successful identification of individual LAS compounds in a complex mixture of homologs and isomers using HRGC/MS. Prior to HRGC analyses, the LAS compounds were converted to sulfonylchloride derivatives. This method was used for wastewater and sludge samples.

High Performance Liquid Chromatography (HPLC)

In general, HPLC is a more promising method for the routine determination of the alkyl chain distribution of LAS than GC because derivatization is not required. Nakae et al. (1981) described an HPLC method for determining alkyl chain distributions of LAS in detergents without any pretreatment. Separation of the phenyl isomers of LAS and the determination of the alkyl chain distribution was achieved using a Hitachi Gel 3053 as a stationary phase and gradient elution with aqueous acetonitrile solution containing 0.1M sodium perchlorate as a mobile phase.

A similar method for the analysis of LAS in water using ion-exchange in combination with HPLC yielded favorable results at a detection limit of approximately 40 μg/L (Saito et al., 1982). Water samples containing LAS were concentrated with a weak base anion-exchange resin, then analyzed by HPLC with Shimadzu ZORBAX SIL as the stationary phase and 0.2% ammonia-ethanol as the mobile phase; detection was by UV absorption. The recoveries for LAS were about 92-107%. Positive errors of 1-4% were observed with 6 times excess of non-ionic surfactants or laurylsulfate; 10-50% positive errors were observed with 2-6 times excess of household detergents.

HPLC analyses for both LAS components and sulfophenylcarboxylic acids (SPC), LAS degradation products, were developed by Taylor and Nickless (1979), using cetyltrimethylammonium ion (CTMA) in a paired-ion technique. The use of CTMA in 87.5% methanol as a mobile phase increased resolution of LAS components enormously; reduction of methanol content to 75% gave similar resolution to SPCs.

Linder and Allen (1982) reported a method using HPLC with a UV fluorescence spectrophotometer for the separation and quantitative determination of intact and partially biodegraded LAS. The HPLC method shows good correlations with the MBAS method for intact LAS material; its primary advantage is the ability to quantitatively determine partially degraded intermediates and the disappearance of those

intermediates. The method is selective for aromatic sulfonates with more than one alkyl carbon and the reported limit of detection was approximately 50 μg/L. The data reported were for samples from laboratory biodegradation studies; however, the method was also reported to be appropriate for sewage plants influents, effluents, and river waters.

Kobuke (1985) determined LAS levels in river water by fluorometric analysis and HPLC-UV analysis. LAS was isolated as the MB complex, released from the complex by treatment with HCl, and reextracted with $CHCl_3$ prior to HPLC analysis. Recovery was 101.8% at levels of 10 μg/L 90.4% recovery was achieved at 2 μg/L after an additional concentration step.

Bear (1986) described a reversed-phase ion pair HPLC procedure that allows the separation of alkylbenzenes according to chain length. Optimization of pH, counter ion concentration, and mobile phase polarity resulted in a linear relationship between retention time and alkyl chain carbon number. Within each group, the linear alkyl chain component had the longest retention time and retention time decreased as branching increased. The analysis was also reported to be applicable to synthetic and petroleum sulfonates, as well as ethoxylated alkyl sulfates and carboxylates. Analyses of environmental samples were not reported.

Kikuchi et al. (1986) presented a new analytical procedure for the determination of trace levels of LAS in sea water, marine sediment or fish samples. LAS were extracted from filtered aqueous samples or from methanol extracts of fish and sediment samples with a Bond Elut C_{18} reversed-phase minicolumn and quantified by HPLC with fluorophotometric detection. The procedure was reported to be "simple, rapid, quantitative, sensitive, and specific". The limits of determination reported for each LAS in marine environmental samples were approximately 0.1 μg/L for water, 0.03 μg/g (dry basis) for bottom sediments, and 0.3 μg/g (wet basis) for fish. The reported recoveries were 80% for water, 87% for sediment, and 86% for fish; precisions of

3-4% relative standard deviation were reported for five-replicated analyses of seawater or sea sediment.

Marcomini and Giger (1987) described a reversed-phase HPLC procedure with UV fluorescence detection for routine LAS analysis in large numbers of samples. Simultaneous determinations of LAS, alkylphenolpolyethoxylates (APEO) and nonylphenol (NP) in laundry detergents, sewage sludges, sludge-amended soils, and river sediments were reported with reversed-phase HPLC using a 10 μm octylsilica column. Analysis on this column provided only the homolog distribution of LAS components with no separation of the phenyl positional isomer. On the other hand, use of a 3 μm octadecylsilica column provided both homolog distribution and partial phenyl isomer distribution for LAS. The octadecylsilica column, however, was not satisfactory for simultaneous routine determinations of LAS, APEO, and NP. Therefore, the octylsilica column was recommended for routine analyses of LAS while the octadecylsilica column may be used when phenyl positioned isomer distributions are needed. Recoveries of 85-100% were reported for LAS isolated by Soxhlet extraction from detergent providers, sewage sludges, sludge-amended soils, and river sediments; relative standard deviations for the different matrices did not exceed 6%. Detection limits were 95, 80, and 60 ng injected for NP, LAS, and HPEO, respectively. Giger et al. (1987) used a similar method for quantitative determination of LAS in wastewater and sludge samples.

Irgolic and Hobill (1987) used HPLC separation followed by a sulfur-specific detector for the analysis of sulfur-containing surfactants, including LAS. An inductively coupled argon plasma vacuum emission spectrometer (ICP) monitoring the 180.7 nm sulfur line served as the sulfur-specific detector. The analysis of surfactants demonstrated many homologs and isomeric compounds that were not completely separated. Treated and untreated wastewaters spiked with surfactants were analyzed without difficulty. The detection limits for the HPLC-ICP system (~15 ng sulfur) were approximately 10 times higher than those reported for the HPLC/extraction/fluorescence method.

Matthijs and DeHenau (1987) used a reversed-phase HPLC procedure to characterize LAS homologs in influcnt, river, sludge, and sediment samples. The procedure was basically the same as that described by Nakae et al. (1981) with the addition of a different gradient elution system to provide better separation of the individual LAS homologs; a phase modifying agent was also used to obtain sufficient retention of the LAS on the analytical column. The method reproducibility, expressed as relative standard deviation, was 4% for aqueous samples and 10% for sedimcnt, sludge, and soil samples. The mean recovery of spiked LAS was 94%, 87%, 84%, and 84% for aqueous samples, sediments, sludges, and soils, respectively. The analytical recovery of the individual homologs decreased with increasing alkyl chain length. The level of sensitivity of the method reported for total LAS was 10 μg/L for aqueous samples and 100 μg/Kg for solid samples.

Castles (1987) presented a reverse-phase HPLC method for detection of LAS in aqueous samples that takes advantage of the inherent sensitivity of the fluorescence detector. Solid phase extraction procedures using C2 and SAX cartridges were employed with river water and effluent samples. All isomers of each LAS homolog group (C_{10}-C_{14}) were eluted in a single peak. Recoveries of 88% and 92%, respectively, were obtained for 40 μg/L LAS spike levels in river water and final effluent samples; 100% recovery was obtained for 10 mg/L levels in influent samples.

Takada and Ishiwatari (1987) examined LAS levels in river sediments, suspended river particles, domestic wastes and waste effluents. LAS were isolated from the environmental samples by formation of the MB complex and subsequent removal of the MB by cation exchange column. The LAS in river particulates and sediments were quantified by GC following derivatization as methyl sulfonates; identification of the methyl esters was by flame ionization detector (FID) or GC/MS. LAS in influent and effluent samples were quantified by the HPLC method developed by Nakae et al. (1981) using a fluorescence detector.

Recovery of a 50 μg spike was 98.6 $\pm$ 0.2 % for the GC method and 100 $\pm$ 11% for the HPLC method. The relative standard deviation of triplicate analyses of a sediment sample was ~ 4% for the GC method and ~ 3% for the HPLC method.

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III. BIODEGRADATION

Biodegradation is "the destruction of chemical compounds by the biological action of living organisms" (Swisher, 1970). It can be divided into primary and ultimate biodegradation. Primary degradation is the minimum extent of degradation needed to change the identity of a compound. Ultimate biodegradation (or "mineralization") involves the complete conversion of a compound to carbon dioxide, water and other inorganic compounds. This section will deal with the methods used to study the biodegradation of surfactants both in the laboratory and in the field, and with the various factors capable of affecting the rate of biodegradation. Biodegradation test methods for organic compounds have been reviewed by Painter and King (1985), Lyman et al. (1982), and Howard et al. (1975). Reviews related specifically to surfactants include Swisher (1987, 1976, 1970), Cain (1987, 1974), Gledhill (1974), Higgins and Burns (1975), Woltering et al. (1987), DeHenau et al. (1986), and Gilbert and Kleiser (1986). Much of the material that follows is based on these sources. Laboratory test systems are reviewed first, followed by a discussion of studies specifically related to LAS.

A. Laboratory Tests Used in Surfactant Biodegradation Studies

A variety of tests are used to assess the biodegradability of surfactants. They include screening tests to indicate ready biodegradability, tests of inherent biodegradability, and simulation tests to assess removal by waste treatment processes. In order to easily measure the results, some of these tests are conducted at high concentrations of the test substance (mg/L). Tests can be conducted using radiolabeled surfactants, which allow lower and more environmentally realistic concentrations (µg/L) to be employed and actual environmental matrices to be used. Test results are affected by medium, inoculum, temperature, pH, surfactant concentration and analytical test methods employed.

There are numerous test procedures that can be used to assess the biodegradability of surfactants. Modern methods, including screening tests and tests using radiolabeled carbon (^{14}C) are described first. Older screening tests and tests for simulating wastewater treatment processes are then briefly described. Additional information on test methods can be found in Swisher (1987).

1. OECD Methods

The Organization for Economic Cooperation and Development (OECD) divides biodegradation tests into three types: screening, inherent, and simulation (Painter and King, 1985). Screening tests generally employ a simple aqueous medium containing mineral salts and a small number of unacclimated microorganisms to which the test compound is added. The purpose of screening tests is to provide unequivocal evidence that the test compound will biodegrade in the environment. Compounds passing these tests are considered readily biodegradable because of the stringency of the test conditions--high concentrations (mg/L) of the test compound, use of unacclimated microorganisms at low concentrations, and the absence (usually) of any other carbon source. Generally, no further testing is required of compounds that pass. Failure to pass a screening test does not mean a compound will not biodegrade in the environment, but that further testing is required.

This next level of testing is for inherent biodegradability. These tests employ a higher concentration of microorganisms and may last for several months. Compounds passing the test for inherent biodegradability are also tested using simulation methods, such as continuous activated sludge or trickling filter systems, to assess their behavior during wastewater treatment.

Gerike and Fischer (1979, 1980) studied the biodegradability of 44 compounds in seven biodegradability tests which are part of the OECD Guidelines for Testing of Chemicals (OECD, 1981). The procedures

included the modified OECD Screening, Closed Bottle, Sturm, French AFNOR and Japanese MITI tests, which are considered tests of ready biodegradability, the Zahn-Wellens test for inherent biodegradability, and the coupled units OECD confirmatory test, which is a simulation test. These tests were chosen for comparison because of their legal status, proven record in identifying environmentally compatible chemicals, or for their scientific soundness. Compound loss is monitored in these tests by measuring dissolved organic carbon, CO_2 evolution, chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Most surfactant biodegradation studies, other than those employing radiolabeled substrates, follow one of these test methodologies. Descriptions of these methods can be found in Painter and King (1985), OECD (1981), and the Gerike and Fischer papers (1979, 1981).

The authors concluded that the degree of biodegradation for a specific compound varies with test method, and that the chemical, physical, and biological characteristics of the compound must be considered when choosing the test (Gerike and Fischer, 1979). Nevertheless, they consider the set of tests of ready biodegradability recommended by the OECD to be fail-safe in indicating the environmental acceptability of compounds that pass.

2. Radiolabeled Carbon (^{14}C) Tests

A major drawback of screening tests is the high concentrations (mg/L) of surfactant that must be employed to yield measurable results. To overcome this problem, modern approaches to biodegradation testing have used surfactants radiolabeled with ^{14}carbon (^{14}C), which can be used to conduct tests at $\mu g/L$ concentrations. Mineralization is indicated by the production of $^{14}CO_2$ and complete biodegradation by the radiolabeling of the moiety least susceptible to biodegradation--the benzene ring in the case of LAS (Larson and Meier, 1988). However, the extent of ^{14}C recovery does not equal biodegradation because some ^{14}C will be incorporated in cell biomass or released as soluble biodegradation end

products. The lower concentration of surfactant used in ^{14}C tests more realistically simulates concentrations found in the environment (Larson, 1987) (see Section IV). The technique has been used to investigate biodegradation in surface waters, groundwater, surface and subsurface soils, sediments, and marine environments (Larson and Meier, 1988; Larson, 1983; Shimp and Young, 1987). In addition to using more environmentally realistic concentrations of the test compound, natural media such as groundwater and river water are used in these tests, without inoculation with sewage sludge. Therefore, natural populations of microorganisms are used for testing.

A further advantage of radiolabeling is that it allows the kinetics and mechanism of biodegradation to be more easily studied (Larson, 1984; Painter and King, 1985). However, tests with radiolabeled substrates are expensive and require careful handling of the test substance.

3. Oxygen Uptake -- Biochemical Oxygen Demand (BOD)

The BOD screening test can be employed to estimate the extent of biodegradation in an isolated system of known composition, since the theoretical amount of oxygen required to completely oxidize a surfactant can be calculated. Interpretation of the results is rather tenuous, however, due to the complexity of the metabolic processes involved, the variety of metabolic byproducts formed, and the many other pathways of possible oxygen utilization by the mieroorganisms. BOD values are reported to vary greatly depending on the state of acclimation of the inoculum used (Price et al., 1974). These problems are partially compensated for by subtracting the oxygen demand for a control culture from the result for the test compound to obtain a net value. Therefore, the BOD test is at best semi-quantitative.

There are two standard procedures for determining BOD values - the APHA closed bottle and the Warburg test. Both are described in Swisher (1987).

4. CO Evolution

In some screening tests, the bacterial evolution of unlabeled CO_2 is used to assess ultimate biodegradation. This method is not quantitative because the CO_2 evolved usually falls short of 100% of the theoretical value. Some of the carbon is used for biomass production, which also constitutes biodegradation (Gerike, 1984). Additionally, such factors as molecular structure, the number and type of microorganisms capable of metabolizing the test compound, and toxicity of the test compound may affect the rate and extent of CO_2 production (Gledhill, 1975).

Larson and Perry (1981) noted that CO_2 evolution in eutrophic natural waters generally cannot be measured using titration procedures, due to the high organic carbon background of these waters and the presence of a carbonate buffering system that effectively traps evolved CO_2. They found that an electrolytic respirometer (to measure oxygen consumption) could be used to estimate the extent of biodegradation, with results consistent with screening studies where the surfactant was used as the sole carbon source by a sewage inoculum.

5. Enrichment Cultures

The enrichment culture technique involves selective culturing of microorganisms from river water, soil or sewage which are capable of utilizing a particular surfactant as their sole carbon source. Treccani (1972) proposed an enrichment culture technique as a standard biodegradation test for anionic surfactants. His procedure consists of a series of transfers in which the previous culture is used as the inoculum for the next transfer. The initial concentration of surfactant for each step is 5-7 mg/L of MBAS and each culture is incubated until an 80% drop in MBAS occurs. A surfactant is considered biodegradable if it is degraded 80% within 24 hours, after two transfers, and 100% in 48-72 hours. The standard compound, 3-phenyldodecane sulfonate (a component of LAS) must be 90%-95% degraded in 24 hours, and 100% degraded in 48 hours.

6. Die-Away Tests

Die-away tests are employed to determine the progress of biodegradation in an isolated system. Employing a series of analyses, monitored surfactant concentrations drop or "die away" with the passage of time. The most common of these die-away tests are the river water test, the fortified and inoculated water test, and the shake culture test.

a. River Water and Seawater Tests

The surfactant (1-10 mg/L) is dissolved in a sample (usually one liter of river water), then stored at room temperature either in darkness or semi-darkness to prevent algal growth. The solution is analyzed at intervals to determine the rate of degradation. A disadvantage of this method is that every river will not necessarily yield the same results (Weaver and Coughlin, 1964); and the test may require an extended period of time due to the low number of organisms present.

b. Fortified and Inoculated Waters

A modification of the river water test, the fortified and inoculated water procedures, were developed in order to better control the degradation medium and shorten the duration of the test. The changes in procedure include the addition of a bacterial concentrate (French IRChA test) to river water, and the use of BOD dilution water or other synthetic media which contain inorganic salts and deionized water. These modifications have not produced any dramatic improvement over the original river water test (Swisher, 1970).

c. Shake Culture Test

With free access to the atmosphere, a flask containing a yeast extract medium, the surfactant (usually 30 mg/L) and the bacterial inoculum is aerated on a rotating or reciprocating shaker. The principal advantage

of the shake culture technique is that the investigator has better control of the water source. This is the standard method of the Soap and Detergent Association (1966).

d. British STCSD (Standing Technical Committee on Synthetic Detergents) Test

This procedure involves the use of an air-dried activated sludge inoculum (30 mg/L) which is added to a solution of BOD dilution water containing 10 mg/L of surfactant. The entire mixture is aerated at 20° C and analyzed (by MBAS) over a 21-day period. Variable results have been noted in this procedure due to the source of the specific inoculum used (Swisher, 1970).

e. Swiss EAWAG (Eidgenossische Anstalt fur Wasser und Abwasserforschung and Gewässerschutz) Test

This procedure uses a salt medium containing 89 mg/L phosphorus, 175 mg/L nitrogen and 10 mg/L surfactant added as the sole carbon source. The mixture is inoculated with washed, fresh activated sludge and then aerated for five days (Swisher, 1970). The extent of degradation of LAS is reportedly 5% to 10% lower than with the official German (1976) continuous activated sludge test (Heinz and Fischer, 1967, cited in Swisher, p. 155, 1970).

f. Bunch-Chambers Test

BOD water, fortified with 50 mg/L yeast extract, is inoculated with 10% settled sewage. The mixture containing up to 20 mg/L of the test surfactant is allowed to stand in the open for a period of seven days. The solution is then analyzed (by MBAS) and used as the inoculum for a fresh batch of medium which is subsequently allowed to stand for a seven-day period. This process is repeated twice for a total of four seven-day runs to provide for bacterial acclimation (Swisher, 1970).

7. Simulated Treatment Processes

a. Activated Sludge

The activated sludge process is one of the most commonly used biological waste treatment processes today (Rogers and Kaplan, 1970). The Soap and Detergent Association (SDA, 1964) has adopted a semi-continuous activated sludge method as its official confirming test, and batch, semi-continuous and continuous activated sludge procedures are routinely used in the laboratory to assess surfactant biodegradation. The OECD considers the continuous activated sludge test a simulated treatment process and the semi-continuous procedure a test of inherent biodegradability.

Activated sludge is obtained by aeration of sewage, causing growth of the bacteria through metabolism of sewage nutrients. This gives a flocculent suspension of microorganisms in which cells adhere to each other via the cementing action of metabolic polymers; the flocs are called activated sludge and their suspension is called the mixed liquor (Swisher, 1970). Basically, the activated sludge process involves further addition of sewage to a mixed liquor with further aeration; most of the adsorbable and biologically oxidizable components are removed from solution. After settling, the clear, treated sewage is removed and the settled sludge is recycled. Several processes account for the removal of chemicals by activated sludge wastewater treatment. The most important for surfactant chemicals are adsorption and biodegradation.

There are three major activated sludge procedures used in laboratory studies: continuous (and miniature) flow tests, the batch system, and semi-continuous (fill and draw) method. Laboratory scale, continuous flow units ranging in size from a few hundred milliliters to several liters have been developed to study the activated sludge process.

The official German 21-day method (1976), now accepted by the OECD (1971), is perhaps the most often used continuous activated sludge

procedure. A unique aspect of this test is that the sludge is allowed to develop spontaneously from adventitious bacteria. The influent is introduced at one liter per hour, giving an average retention time of three hours in the three liter aerator. Twenty-four hour composites of the effluent are analyzed with the methylene blue procedure. To fulfill the requirements of biodegradability according to German and OECD regulations, a surfactsnt must steadily degrade a minimum of 80% over a period of 21 days; a prior acclimation period of up to six weeks is permitted.

A variation of the OECD confirmatory test, the coupled units test, has been utilized by Fischer and co-workers (Fischer and Gerike, 1975; Fischer et al., 1975). Two model OECD confirmatory test units run in parallel are started according to the procedure described in OECD (1971) with the desired concentration of test surfactant added to one of the units. Half of the volume of the activated sludge units is interchanged once a day. Once acclimation has occurred, the effluents of the test and parallel units are analyzed via nonspecific analyses: chemical oxygen demand (COD), dissolved organic carbon (DOC). For example, 94% COD removal of 10 mg/L LAS was observed after a six-hour retention period. With a three-hour mean retention time, a 70% COD removal was achieved as well as a greater than 90% MBAS removal (Fischer and Gerike, 1975).

Batch and semi-continuous systems are considerably more economical in terms of space, time and money than a continuous activated sludge procedure. These two processes further depart from full-scale operating procedures. Therefore, the need for establishing correlations between test results and field results is greater.

The batch die-away test is a simple, quick test to determine surfactant biodegradation. Settled activated sludge is mixed with sewage containing the test surfactant. The entire mixture is aerated and analyses are made over a period of hours or days.

The fill and draw or semi-continuous process involves aeration of a mixture of activated sludge, feed and surfactant, usually for 23 hours. The mixture is then allowed to settle and the supernatant is drawn off. Fresh feed is added, aeration is resumed, and the cycle is repeated. Biodegradation is determined by a reduction in surfaetant concentration during each 24-hour cycle (Eden et al., 1968). A similar procedure is used in the standard SDA procedure (1965). The test requires a 24-hour cycle (23-hour aeration; one hour for settling, drawing off supernatant and refilling). The percentage of MBAS removal is caleulated by averaging the daily removal over a period of seven days. To insure that the test is functioning properly, a standard, pure C_{12} LAS is run simultaneously. The test results are taken to be valid if the standard LAS shows a 97.5% removal during the same period.

Several factors can affect activated sludge tests: the adsorption of the surfactant to the sludge (Price et al., 1974); the makeup of the microbial sludge population (Swisher, 1964, 1970); the concentration of surfactant (Banerji, 1971; McClelland and Maney, 1969) and retention time (Hanna et al., 1965).

b. Trickling Filters

Triekling filters (sewage running over an aerated, porous bed of roeks or other materials) are also employed in sewage treatment, and hence in aerobic treatability studies. Biological growth adheres to the substratum in the form of a bacterial film. Although exposure of the influent liquid is brief, solutes are adsorbed from the liquid onto the film, thereby resulting in a longer exposure time. Generally full-scale filter beds are six feet in depth (Swisher, 1970) but laboratory models may be half this depth or less. Gerike et al. (1980) describe the modification of the OECD confirmatory test for use with model trickling filters rather than activated sludge units. Removal of DOC and COD in tests where sludge exchange between the parallel units took place yielded narrow tolerance limits for the results.

Lamb (1970) investigated in pilot plant tests four of the variables which can radically influence the efficiency of LAS removal in trickling filter installations. These variables were (1) frequency of dosing, (2) recycle ratio, (3) filter loading, and (4) filter depth. He concluded that dosing frequency did not significantly influence the removal of MBAS, BOD or suspended solids while recirculation had a beneficial effect on MBAS and BOD removal. The efficiency of BOD and particularly MBAS removal, was responsive to filter loading. Increasing the depth of the bed in the filter appeared to significantly improve the removal of MBAS but exerted little influence on BOD removal. Tang (1974) reported that BOD and LAS removal were significantly correlated for trickling filter processes in both laboratory and field studies.

c. Anaerobic System

Biodegradation of LAS is severely restricted under anaerobic conditions (see Section 4b, page III-21). Since initial oxidation of a hydrocarbon chain is thought to require molecular oxygen, side chain oxidation would not be expected to occur. Because cesspools, septic tanks and anaerobic digesters are extensively utilized as sewage treatment processes, laboratory tests have been conducted to study anaerobic biodegradation.

Several investigators have reported that when the surfactant concentration in raw sludge reaches 1.1 to 1.2% of dry solids, inhibition of anaerobic sludge-digestion processes generally occurs (Swanwick and Shurben, 1969; Osborne, 1969; Klein, 1969; Wood et al., 1970). These results were reported from England and South Africa. However, when Klein (1969) monitored five Northern California sewage treatment plants, four of which had experienced severe and recurring digester problems, he found the MBAS level in raw sludge was similar for all five plants (0.5%, of dry solids) and well below the critical 1% level. It is not clear whether these differences are related to differences in practice in the United States versus other countrias or to other factors.

Anaerobic digestion of sewage sludge was not inhibited by LAS concentrations in sludge of up to about 15 g/kg dry solids (Bruce et al., 1966; Pitter et al., 1971 and Gilbert and Pettigrew, 1984). Between 15 and 20 g LAS/kg, digestion was sometimes impaired. Above 20 g LAS/kg, a more serious inhibition of gas production was observed, especially if other potentially inhibitory compounds were present. However, if the sludge is allowed to be acclimated over a sufficiently long period with gradual, stepwise increases in LAS concentrations, successful digestion could be obtained at concentrations as high as 30 g/kg dry solids (Bruce et al., 1966). Inhibition caused by anionic surfactants has been overcome by the use of stearine amines (Swanwick et al., 1969).

8. Soil

Soil is an important compartment in the biodegradation process, especially in septic tank systems. After settling of the sewage insolubles, the supernatant flows from the septic tank into an underground drainage field where degradation of the soluble and unsettle components occurs, principally through the action of aerobic bacteria present in soil and sewage. Surfactants are also introduced to soils from the spreading of sewage treatment plant sludges.

Test methods for measuring aerobic degradation in soils vary widely. They include tests of soil perfusion, incubation and suspension in aqueous solutions (Lyman et al., 1982). The latter test, however, is really an aqueous system that uses soil microorganisms as an inoculum. The U.S. EPA (1979), under the Toxic Substances Control Act, recommends a $^{14}CO_2$ evolution test. All of these tests are described in Swisher (1987).

9. Influence of Test System Variables

The four principal components of a biodegradation test system are an aqueous medium, a pure or mixed bacterial culture, a test surfactant to

serve as a standard of biodegradability, and an appropriate analytical method or methods.

a. Media

The medium plays an essential role in the activity of a system's bacterial culture which, in turn, initiates the biodegradation of a surfactant.

There are two basic media employed in treatability tests: (1) natural sewage and (2) synthetic sewage, a mixture of organic nutrients. Some investigators prefer a natural sewage medium in the belief that natural sewage most closely simulates conditions present in sewage treatment processes and/or receiving waters and therefore, will provide more ecologically meaningful results.

Synthetic sewage has the advantage of better reproducibility plus the added convenience of being made in the laboratory. The components of media vary, but generally all contain essential elements in forms needed for bacterial growth (Na, K, Ca, Mg, Fe, N, S, P, Cl). Organic nutrients such as nutrient broth or meat extract may be added to media depending on the test method; these may be supplemented with a carbohydrate, and on occasion, a fatty material.

Ordinarily, biodegradation tests are performed in an aqueous medium maintained at a neutral pH (6.5-7.5). Although no adverse effects on the rate of biodegradation have been reported due to variations in pH within this range, Swisher (1970) points out that pH changes outside this range can markedly affect microbial growth, metabolism, etc.

b. Inoculum

Microorganisms are the principal agents of surfactant biodegradation. The genus Pseudomonas occupies a prominent position among the vast number of species present in the environment capable of surfactant

biodegradation. Certain other genera (e.g., Escherichia, Aerobacter, Alcaligenes, Micrococcus, Klebsiella) are also frequently mentioned in reference to surfactant biodegradation. Most biodegradation testing relies on inocula collected from natural microbial communities, which contain assemblages of multiple genera.

c. Temperature

Since the presence of mieroorganisms is an integral component of a biodegradation test, another factor which should be considered is the temperature at which the test is carried out. The predominance of a particular species following a change in the ambient temperature may shift toward a species whose growth is favored at the new temperature which in turn can influence the rate of degradation (Swisher, 1970).

d. Surfactant Concentration

The concentration of surfactant added to the test system can affect the rate of biodegradation. The lower limit of surfactant concentration (a few mg/L or even less) is dictated by the sensitivity of the analytical test method employed while the upper limit (several hundred mg/L) is imposed by such factors as an inhibitory action on bacteria and the production of foam in an aerated system, both of which influence the rate of biodegradation.

Occasionally, a surfactant is unavailable in pure form, requiring the testing of the detergent formulation. A potential problem with this type of testing is that other components of the formulation such as antiseptic or bleaching agents may interfere with the bacterial culture disproportionately to their level in actual sewage systems. Additionally, the concentration of surfactant in a formulation may not be consistent with optimum test conditions (Swisher, 1970).

e. Reference Compounds for Test Validity

In order to ascertain the activity of the inoculum as well as the accuracy of the method of analysis, a surfactant of known biodegra-

dability is often analyzed along with the test compound. The most commonly employed reference compound used in the United States is a commercial type of LAS which is known to be 90%-100% biodegradable (by MBAS) (Arpino, 1969; Swisher, 1970; APHA, 1985). It is also important to have a negative, or less biodegradable, standard. The OECD (1971) procedure uses tetrapropylene-derived benzene sulfonate as its negative standard.

f. Analytical Test Methods

A number of analytical procedures are currently available, each appropriate to certain applications. Each method has its advantages, none is free of limitations, and no one method has the specificity to handle the diverse requirements of biodegradative evaluation; e.g., marine and freshwater systems, industrial effluent, sewage, agricultural wastes, etc. These problems are considered in detail in Section II.

g. Surfactant Sorption

In laboratory test systems, simulated or full-scale treatment processes, or the natural environment care must be taken to distinguish between surfactant removal by biodegradation and by sorption to solids or microorganisms. Results of tests that cannot distinguish between sorption and degradation, either because of the test design or analytical methods employed, should be considered as indicating removal rather than biodegradation. For this reason, treatment plant studies often report removal rather than degradation efficiencies.

B. Results of Laboratory Tests of LAS Biodegradability

The results of a tremendous number of laboratory studies indicates that commercial LAS is easily biodegraded. This is apparent from tests of inherent biodegradability and wastewater treatment simulation tests. Tests using natural fresh water

or seawater and concentrations of LAS typically found in the environment also indicate ready biodegradability of LAS, with degradation occurring somewhat more slowly in seawater than fresh water. Included among these tests are many used for regulatory purposes. Biodegradation also occurs readily in soils. It is not observed under strictly anaerobic conditions.

Numerous studies have been conducted on the biodegradation of LAS in laboratory test systems and in the field. No attempt is made to review all of these studies here. An exhaustive listing of test results can be found in Swisher (1987).

1. Radiolabeled Carbon ^{14}C Tests

Larson and Wentler (1982) found that 75% of the ^{14}C-LAS initially present in Rapid Creek (SD) water was converted to $^{14}CO_2$ degraded in 4 days, a rate considerably faster than that found in acreening tests employing much higher concentrations of surfactant. Larson and Payne (1981) provide a more detailed description of biodegradation studies using water and sediments that were collected from above (ASO) and below (BSO) the discharge of a trickling filter municipal waste water treatment plant on the same river. Water from below the plant showed a higher degree of LAS-degrading activity, an indication of previous acclimation, while water from above the discharge showed a lag period and/or a slower rate of degradation. In both waters, the addition of the corresponding sediment increased the rate of $^{14}CO_2$ production significantly. Half-lives, and asymptotes for percent $^{14}CO_2$ production were:

Water		Alone	With Sediment
ASO	t½	~14 days	2.7 days
	% CO_2	70%	72%
BSO	t½	1.4 days	0.7 days
	% CO_2	73%	80%

These results are comparable to those obtained with Ohio River water, in which sewage plant effluent was much more highly diluted (Larson, 1980).

Stebner (1979) used two surface water models (closed bottle and OECD screening test) and two continuous miniature activated sludge systems, with ^{14}C-ring labeled LAS. The ^{14}C-LAS was comparable in composition to a commercial product. In the surface water tests 55-72% of the ^{14}C was converted to CO_2, depending on time and other conditions. In 19 days, 2.6% of the ^{14}C remained as intact LAS, 19% was found in a fraction containing predominantly sulfophenylcarboxylic acids, and 7% was low molecular weight hydrophilic materials. In four experiments in simulated activated sludge processes, ^{14}C was distributed (after varying periods of time) as: 42-52% CO_2; 2.0-3.3% intact surfactant; 20% as metabolites, largely sulfophenylcarboxylic acids, and 15%, some of which was residual surfactant, in the biomass of the sludge.

In a series of experiments, Huddleston and Nielsen (Huddleston and Nielsen, 1979a,b; Nielsen et al., 1980; Nielsen and Huddleston, 1981) used ^{14}C-ring-labelled LAS (of commercial composition) and unlabelled pure C_{10}, C_{12}, and C_{14} LAS. In a semi-continuous activated sludge system (SCAS), the pure materials showed nearly 100% loss of MBAS and the benzene ring (as measured by HPLC and UVF spectrophotometry). The ^{14}C experiment is reported to have shown 95% cleavage of the ring with products being: CO_2, 62%; cell mass, 18%; water-solubles, 20% (including 15% as "ring fragments" and 5% as "intact rings"). In a series of die-away tests lasting 45 days, MBAS removal was rapid with three types of LAS, but loss of ring structure (measured by HPLC/UVF) was slower and possibly incomplete, especially for the C_{14} compound. However, "100% conversion" to CO_2 was reported. In further work, the same group investigated the fate of alkyl and ring carbons using ^{14}C-labelled substrates (Nielsen et al., 1980). In this series, residual and biomass liquors from the SCAS system were submitted to a die-away test after supplementation with mineral salts, BOD water and yeast extract. In the SCAS units, conversions of both ring and alkyl carbons were similar: 62% to CO_2; 28-30% to biomass; and 8-10% as

soluble residues. In the die-away tests with sludge solids (biomass) and supernatant, further conversion of both alkyl and ring carbons to CO_2 was demonstrated, with a final calculation that 95.9% of the original substrate fed to the SCAS units was converted to CO_2 and 3.8% recovered in biomass. The mineralization of the benzene ring in natural waters has also been well demonstrated by Larson and Payne (1981), using a C_{12} LAS at $\mu g/L$ concentrations uniformly labeled with ^{14}C in the benzene ring.

2. Oxygen Uptake -- Biochemical Oxygen Demand (BOD)

The standard BOD test is rarely performed with LAS. Swisher (1987) gives the result of 59% of theoretical oxygen demand in a 40-day BOD test with a C_{14}-C_{17} LAS mixture. The Warburg technique yields a greater degree of biodegradation. Utilizing this technique, Brink and Meyers (1966, cited in Swisher, p. 145, 1970) found that the theoretical oxygen uptake with LAS was achieved in 24 hours at a microbial cell concentration of 1-2 mg/L (dry weight) with a nitrogen-free medium. Canton and Slooff (1982) reported over 80% removal of DOC in the modified OECD screening test with a C_{9-14} LAS.

a. CO_2 Evolution

A few LAS degradation studies have employed unlabeled CO_2 evolution to assess degradation (Swisher, 1987). In general, the extent of degradation over four weeks ranged from 60-80% depending on test conditions and the surfactant used. Gilbert and Pettigrew (1984) report that 75.0% of ultimate biodegradation was achieved by C_{12}LAS (Marlon A™, a commercial product) in the modified Sturm CO_2 evolution test.

3. Die-Away Tests

a. Fresh Water

Sekiguchi et al. (1975) found that approximately 15 days were needed for 5 mg/L LAS to completely biodegrade (as measured by MBAS) in

samples of Tama River water; it took 40 days for the total organic carbon values to disappear. LAS may account for less than half of the MBAS response material in natural river water (Gilbert and Kleiser, 1986).

b. Seawater

Tests similar to the river water die-away have been conducted with seawater. Vives-Rego et al. (1987) observed the primary biodegradation of LAS in natural seawater; more than 70% of the parent compound (20 mg/L, 22°C) was degraded within 10 days, with an estimated half-life of 6-9 days. In a modification of the river water test that employed natural seawater, 250 g/L of sediment, and 10 mg/L of LAS, Sales et al. (1987) observed LAS biodegradation at various temperatures. Only a few percent remained at 20 and 25°C after 20 days; at 15 and 5°C after 20 days, 60 and almost 100%, respectively, remained.

Degradation of three commercial LAS-based detergent products in saline water (Chesapeake Bay) was evaluated by Cook and Goldman (1974). There were differences in rates of degradation depending in large part on the nature and content of the LAS component. Bock and Mann (1971) studied the degradation of 10 mg/L of sodium dodecylbenzene sulfonate (MARLON A™) in sea water. The surfactant concentration had dropped to 3 mg/L within one week, and within an additional seven days, 97% of the surfactant had degraded (as measured by MBAS). Hon-nami and Hanya (1980) reported over 97% LAS degradation in Tokyo Bay water after 34 days.

The extent of primary biodegradation of LAS in estuarine waters has been examined. In both rivers and estuarine waters, greater than 85% primary biodegradation (as MBAS) of $C_{11.7}$ LAS was achieved in 11 days or less. Degradation was slower in ocean water, requiring 28 days for 85% reduction in MBAS response (Procter and Gamble Company, unpublished data).

c. Shake Culture Test

Biodegradation of LAS in shake culture tests generally occurs within 1-2 weeks if acclimated organisms are employed as the inoculum (Gledhill, 1974; Swisher, 1970). Mann and Reid (1971) reported a range of 71% to 91% degradation for a group of commercial LAS products (Shell DOBANE™ series) with a shake flask procedure.

The biodegradability of varying chain length LAS and the corresponding dialkyl tetralin sulfonates was compared utilizing both shake flask (7-8 days) and river die-away (up to 30 days) procedures. The results (Table III-1) indicate that the lower molecular weight tetralins (C_{10}-C_{13}) are degraded more slowly than their corresponding LAS analogues but eventually do achieve the same degree of primary biodegradation (Vista Chemical Company, unpublished data). In a separate shake flask test, these investigators found that LAS containing 5, 10 or 14% alkyl tetralins had biodegraded (as measured by MBAS) 97, 95 and 96%, respectively, at the end of one week (Vista Chemical Company, unpublished data).

TABLE III-1

PRIMARY BIODEGRADABILITY OF DIALKYL TETRALIN SULFONATES AND THEIR CORRESPONDING LAS ANALOGUES

Surfactant	% MBAS Biodegradability	
	Shake Flask	River Die-Away
C_{10} LAS	>97	99
C_{10} Tetralin	71	96
C_{11} LAS	>96	99
C_{11} Tetralin	74	97
C_{12} LAS	>95	99
C_{12} Tetralin	90	93
C_{13} LAS	>97	98
C_{13} Tetralin	90	99
C_{14} LAS	>95	99
C_{14} Tetralin	96	99
C_{15} LAS	>97	99
C_{15} Tetralin	>96	98

d. British STCSD (Standing Technical Committee on Synthetic Detergents) Test

The range of results obtained from eleven laboratories which used the STCSD test to study the degradation of DOBANE™ JNX-LAS was 83% to 92% in 69 runs, with a mean degradation value of 87% (Eden et al., 1968).

4. Simulated Treatment Processes

a. Activated Sludge

Activated sludge treatment processes achieve a high degree of LAS degradation. Gilbert and Pettigrew (1984), for example, report 87.6% degradation of Marlon A in the OECD semicontinuous activated sludge test. Gerike and Fischer (1981) found 98% DOC removal of LAS in the EPA activated sludge test. Using the German test method, LAS was found to be degraded 91.1% in 21 days (Weaver and Coughlin, 1964).

Mann and Reid (1971) also compared the field test results with the degree of LAS biodegradation noted in standard laboratory procedures. They found 90% to 97%, 88% to 98%, and 71% to 91% degradation with the official German method (continuous), the semi-continuous activated sludge procedure, and the shake flask test, respectively.

The apparent lower biodegradation of 5-phenyldecane sulfonate (as a minor component of Marlon A™, a G_{10-13} LAS) was investigated by Schöberl (1979) in the OECD confirmatory system, a simulation of a continuous flow activated sludge system. By itself, the compound is poorly degraded and is a poor stimulator of enzymes and organisms needed for its degradation. However, in the presence of Marlon A™, or after adaptation to Marlon A™, the compound is readily degraded (>90%) if present in sufficiently high concentration. Other papers, difficult to evaluate without full translation, but apparently presenting little of new import, include the work of Itoh et al. (1979), Hrsak and Johanides (1975), Yakushev et al. (1978, abstract only) and Ohba et al. (1975, abstract only).

b. Anaerobic System

Biodegradation of LAS is limited under anaerobic conditions. Wagener and Schink (1987) reported the absence of sodium decylbenzenesulfonate degradation during the anaerobic digestion of sewage sludge; at concentrations above 10 mg/L methanogenesis was inhibited. However, once the sludge is introduced into an aerobic environment, biodegradation occurs readily (see Ward, 1987 below).

Using an experimental cesspool model, Rismondo and Zilio-Grandi (1968) found that LAS detergents, under anaerobic conditions, exhibited limited (20%) degradation within the 3-6 hour retention period. Slight increases in degradation resulted from an increase in retention time to 12 hours.

Oba et al. (1967) compared the anaerobic degradation of LAS in a shake culture system in which the inoculum used was either activated sludge obtained from a sewage treatment plant or sludge removed from the bottom of a private cesspool. In the sewage plant sludge system, 18% of the surfactant had degraded (as measured by MBAS) by 14 days and 36% had been removed at 28 days. Somewhat slower degradation was seen in the system employing sludge from a private cesspool, i.e., 19% degradation after 28 days.

Oba (1971) carried out a two-year study of the bacterial flora involved in LAS degradation in a cesspool-percolation field test system. Analysis of both soil and cesspool waste flora revealed that Pseudomonas species appear to play the major role in LAS biodegradation. Species of Micrococcus, Aerobacter, Flavobacter, Paracolobactrum and Alcaligenes were also present. In anaerobic sewage treatment systems, such as cesspools and septic tanks, it should be emphasized that the actual biodegradation of LAS occurs not in the anaerobic retention tank, but rather in the soil of the underground drainage field under aerobic conditions.

5. Soil

Larson and deHenau (1988) report C_{13}LAS degradation half-lives ranging from 1 to 20 days in two subsurface soils which had been exposed to LAS. Abe and Seno (1987) investigated the biodegradation of LAS in a soil perfusion system containing clay and sandy loam soils. Degradation was evaluated by measuring the amount of ferroin reagent active substances (FRAS). Biodegradation of LAS began after adsorption onto soils. At high LAS concentrations (50-100 mg/L), biodegradation occurred after a brief lag phase. Subsequent additions of LAS were degraded readily with no lag period.

The ultimate biodegradation of ^{14}C ring labeled C_{10}-C_{14} LAS homologs at realistic environmental concentrations was established by monitoring $^{14}CO_2$ production in sludge-amended agricultural soils (Ward, 1987). The rate and extent of LAS mineralization were comparable to those of typical substrates in sludge-amended soil, such as natural oils, greases, and cellulose. Half-lives of ring mineralization ranged from 3 to 35 days and were independent of the homolog chain length.

Larson (1984a,b) studied the degradation of ^{14}C labeled C_{13} LAS in an aerobic slurry of subsurface soil and groundwater. Degradation of the surfactant (initial concentration of 5 μg/L) was rapid yielding an estimated half-life of 27 hours.

Mansell et al. (1970) atudied the effect of soil aeration upon the biodegradation of LAS solutes as they moved (steady state flow) through vertical columns of stratified aoil. Aerating gas, containing either 0.2%, 5% or 20% oxygen, was applied to the columns of soil. The LAS present in the liquid effluent was detected by ultraviolet techniques, rather than by MBAS measurement. The authors found that the oxidation of LAS was not appreciably affected by the level of oxygen; i.e., the amount of applied LAS recovered in the liquid effluent during the 15-day study was 50.4%, 50.01% and 48.68% for the 0.2%, 5% and 20% O_2 aerations, respectively. Mansell and associates suggested that LAS monolayers at the air-liquid interfaces may have restricted the

transport of oxygen to the soil microbes; thus, the oxygen supply was insufficient to maintain an aerobic environment required for biodegradation.

In studies somewhat related to the Citernesi study (1976) on anaerobic organisms, two groups have investigated the behavior of LAS in soil systems. The work of Archer and Yaron (1977) was done with reference to use of sewage effluent in agricultural irrigation. They explored in detail the kinetics of adsorption and release on two kinds of soils, but the apparent losses to soil were complicated by an unknown, but probably considerable, degree of biodegradation.

Also somewhat related to agriculture, is the work of Inoue et al., (1978) on the mechanisms involved in the reactions of LAS with soil colloids from 19 different soils, and their relationship to degradation. Soils of different types varied greatly in their ability to adsorb LAS. In the presence of two of three soils tested with added sewage supernatant, degradation of LAS was retarded, whereas the third had little or no effect. The authors term this retardation, a "protective" effect. Since their adsorption data show that the non-inhibitory soil had a much lower adsorptive capacity than the other two, this "protective" effect should probably be attributed to firmer binding and less availability of LAS to microorganisms.

Vadoni and Federico (1981) observed the inhibition of nitrifying bacteria in agricultural soils treated with high concentations of LAS (0.01-0.10%). Litz et al. (1987) examined the fate of LAS in a number of different West German soils. Under field conditions, LAS half-lives varied from 5 to 25 days in summer and 68 to 117 days in winter depending upon applied concentrations, the presence of organic matter and soil temperature.

6. Influence of Test System Variables

a. Media

The addition of glucose or other organic components to a synthetic medium may interfere with the acclimation of microorganisms to the surfactant under examination as a result of repression of the enzymes needed to catalyze biodegradation (Cain, 1976). Ciattoni and Scardigno (1968) found that they could delay the onset of biodegradation for C_{11} LAS for a period of 28 days (duration of experiment) by maintaining a glucose concentration greater than 30 mg/L. When the glucose removed by biodegradation was not replenished, biodegradation of the LAS ensued. Normally, biodegradation of C_{11} LAS would have commenced within four days. Another paper reported that the addition of 50 mg/L glucose to the test medium delayed the degradation of DOBANE JNX SULFONATE by 10 days; i.e., 15 days versus the normal 4- to 5-day period (WPRL report, 1965, cited in Swisher, p. 136, 1970).

On the other hand, Mann (1969, cited in Swisher, p. 136, 1970) observed that the addition of a meat extract-peptone-urea mixture enhanced the biodegradation of a (C_{11}-C_{15}) LAS compound, perhaps by adsorption of the LAS onto protein.

b. Inoculum

Gard-Terech and Palla (1986) report the dependence of the rate of primary degradation of C_{10-14} LAS and ABS on the inoculum employed. Both the nature and proportion of the bacterial genera present and the level of cellular adenosine triphosphate (ATP) affected the results, with higher ATP levels favoring biodegradation.

Larson (1983) demonstrated the effect of acclimated and unacclimated microorganisms in natural river water samples on the degradation of ^{14}C-LAS. While test results of O_2 consumption using water collected up stream and downstream of a sewage plant treatment yielded similar

fractions of the theoretical oxygen demand (68.4% and 71.2%, respectively) the rates of degradation were quite different. The estimated half-life for the sample containing acclimated microorganisms (downstream) was 1.3 days, while the corresponding figure for the unacclimated sample was 9.9 days.

Exposure of natural microbial communities to LAS in situ in Acton Lake, Ohio over an LAS concentration range of 0.001 to 10 mg/L had no effect on the microbial biomass. Short-term tests (3-hr) indicated a decreased turnover of glucose in the concentration range 0.1 to 10 mg/L LAS but microbial communities recovered over 21-day exposures and carbon turnover returned to preexposure levels (Procter and Gamble Company, unpublished data).

Goodnow and Harrison (1972) found wide variations in the ability of 45 strains of 34 species in 19 genera of bacteria to degrade $C_{11.8}$ LAS (as measured by MBAS). The rate of biodegradation of LAS or components has been studied in both pure and mixed bacterial cultures. Pure cultures are most useful in obtaining insight into the metabolic pathways of biodegradation and also insuring some degree of reproducibility. Mixed cultures, on the other hand, provide more meaningful results about a surfactant's behavior in the environment in that if one species does not have the necessary adaptive enzymic systems capable of degrading the surfactant or one of its metabolic products, another bacterial species may.

Hrsak et al. (1982) found that enriched, mixed cultures of bacteria were more efficient in primary than in ultimate biodegradation of LAS. From a mixture of bacteria taken from the wastewater of a detergent plant, which consisted of five strains S. Pseudomonas, two of Achromobacter, and two of Acinetobacter, no single strain was found that could degrade LAS completely.

Working with $C_{11.8}$ LAS (DOBANE JNX), Cook (1968) found that a pure culture which she had isolated from a mixed bacterial culture acclimated to JNX, had a considerably lower ability to degrade JNX;

using a bacteriological slope culture technique, 57% of JNX was degraded in 16 weeks with a pure culture while with a mixed culture, 74% was degraded after 15 days.

Rogers and Kaplan (1970) compared the biodegradative activities of a mixed bacterial inoculum obtained from activated sludge versus a pure culture of Pseudomonas crucivae in a shake culture test. They found that the pure culture degraded 53% (as measured by MBAS) of the LAS (dodecene-1-derived LAS) in 17 days while the mixed bacterial inoculum resulted in a more than 90% degradation in less than five days. The authors found that pure cultures of a variety of Pseudomonas species or Achromobacter cycloclastes could degrade LAS as effectively as a mixed bacterial inoculum provided they were acclimatized to 20 mg/L LAS prior to testing.

Although most biodegradation is accomplished by bacteria, a series of papers by Davis and Gloyna (1967, 1969a, 1969b) reported that LAS was degraded in pure culture by several species of blue-green algae (Cyanophyta) and green algae (Chlorophyta). Pure cultures of blue-green algae differed between species of the same genus and between genera regarding degradative capabilities, and wide variations were noted between blue-green and green algae. In a comparison of the degradative capabilities of green and blue-green algae, the authors noted that no distinct pattern was evident.

The biodegradation of ^{14}C-labeled LAS by epilithic microbial communities from the Little Miami River, near the city of Xenia, Ohio, was determined in a flow through system after 0, 10 and 21 days. Exposure concentrations ranged from 0 to 5 mg/L LAS (average chain length 13). Epilithon were found to readily degrade LAS, with adaptation playing a key role in determining biodegradation rates (Procter and Gamble Company, unpublished data).

Willetts (1973b) has reported fungal metabolism of 1-phenylundecane-p-sulfonate and 1-phenyldodecane-p-sulfonate with pure cultures of

Cladosporium resinae and Jigami et al. (1974) found that four strains of Candida grew well on n-alkylbenzenes.

c. Temperature

Kikuchi (1985) found that water temperature affected biodegradation of LAS in a river die-away test using water collected from the Tama River, Tokyo, Japan. LAS were found to biodegrade moderately at high water temperatures (15, 21, 27°C) but poorly at low water temperature (10°C). In a separate study, Yoshimura et al. (1984) found 25°C to be the optimum temperature for LAS biodegradation in river die-away tests; no degradation occured at 10°C or 40°C.

Mann and Reid (1971) found a slightly higher degradation rate during the summer months than in other seasons at a trickling filter sewage treatment plant, while Krone and Schneider (1968, cited in Swisher, p. 25, 1970) found notably less degradation of LAS occurred at 6°C (25% MBAS removal) than at 20°C (96%) with the official German test method. In a field study, however, the authors found 76% MBAS removal across a trickling filter at an ambient temperature of 10°C. Similarly, Stiff and Rootham (1973) reported that LAS, after a period of acclimatization, consistently biodegraded by more than 90% at ambient temperatures of 19.5, 12 and 8°C in a porous-pot activated sludge unit.

Hollis (1976) found that increases in temperature enhanced the biodegradation of LAS provided the thermal limits of the microbial population were not exceeded. Further, he noted that sewage seed acclimatized to temperature alone biodegraded LAS at essentially the same rate as unacclimatized seed between 5 and 35°C. LAS-acclimatized seed, on the other hand, degraded the test surfactant at a more rapid rate at each temperature tested when compared to unacclimatized and thermally acclimatized microbial seed.

d. Surfactant Concentration

Urano and Saito (1985) observed that C_{12}LAS was not degraded at concentrations of 30 mg/L and higher in a 14-day test employing a

synthetic 14-day sewage medium, and acclimated sewage sludge. Larson and Perry (1981) observed an absence of biodegradation of $C_{11.7}$ at concentrations of 20 mg/L and higher in Ohio River water. They attributed this result to toxic effects, which likely explain Urano and Saito's results also.

Pollack and Anderson (1970) reported that after 20 hours incubation, a broth culture containing a very high concentration (5000 mg/L) sodium dodecylbenzene sulfonate and Escherichia coli 11303 became slimy and viscous. The authors suggested this was the result of leakage of intracellular components into the medium. Generally, surfactant concentrations used in biodegradability tests range between 5 mg/L and 20 mg/L (Arpino, 1969), reflecting maximum environmental levels, especially those in sewage. Larson and Maki (1982), however, found that even 5.0 mg/L of LAS had an inhibitory effect on microoganisms found in natural well water. Concentrations in rivers in streams are typical in the μg/L range.

e. LAS Sorption

Removal of LAS in natural systems occurs by other mechanisms in addition to biodegradation. The most important is sorption to solids. Several recent studies have been published on the sorption of LAS to river sediments. Hand and Williams (1987) reported on the sorption of radiolabeled LAS at initial concentrations of 10-1000 μg/L to four river sediments. The sediment/water partition coefficient increased by a factor of 2.8 with each additional methylene group from C_{10}-C_{14} and increased as the phenyl group approached the end of the chain. The partition coefficient varied from 3-26,000 L/kg and varied with sediment type. However, it did not correlate well with the sediment organic carbon faction, unlike most neutral organic compounds.

Mathijs and deHenau (1985) also studied the sorption of LAS to river sediments and found positive correlations between extent of sorption and organic matter, total alkaline material, and clay content of the sediment. The sorption results could be described by Langmuir or

Freundlich isotherms, although irreversible sorption accounted for almost a third of the LAS sorbed. Urano et al. (1984) found that LAS sorption to river sediments could be described by a Freundlich isotherm and was correlated with the sediment organic carbon fraction. A K_{oc} value of 1.9 L/kg (mg/kg organic carbon per mg/L of solution) was reported.

C. Field Studies

Most field tests of LAS biodegradation involve the study of removal or degradation by waste treatment processes. Activated sludge treatment is highly effective in removing LAS, trickling filters somewhat less so. LAS is poorly degraded in anaerobic environments such as septic tanks or anaerobic digestors. Rapid removal has been observed in river water and ground water field tests.

1. Wastewater Treatment

a. Aerobic Treatment

Many, if not most, field tests involving LAS examine its behavior in sewage treatment plants. Rapaport et al. (1987), for example, reported average LAS removals of 98%, 80%, and 27% for activated sludge treatment, trickling filtration, and primary clarification, respectively. Similarly, Woltering et al. (1987) reported corresponding values of 96-99%, 73-87%, and 29%. De Henau et al. (1986) report the same range of removal for LAS in activated sludge plants of Canada, the U.S., and Germany, and note that in one U.S. plant 3% of the removal was associated with sludge.

Concentrations of LAS were measured entering and leaving a municipal activated sludge wastewater treatment plant in Enid, Oklahoma in

October 1983. About 1% of the LAS entering the plant left in the liquid effluent. Another 3% of the LAS left the plant in the sludge removed to a landfill. The LAS concentrations in the digested sludges (4.3-6.7 mg/g) were found to drop to 0.15-0.16 mg/g in dried digested sludge, suggesting the biodegradation of LAS occurred during the drying process (Sedlak and Booman, 1986; Gledhill et al., in press).

Utsunomiya et al. (1986) studied the behavior of free and complexed LAS in a Japanese activated sludge plant. No removal of complexed LAS was observed during primary sedimentation, but a high (58%) removal of free LAS occurred. During aeration and secondary settling, 87% of the complexed and 16% of the free LAS were removed. Longer chain-length LAS was reported not to be enriehed in the sludge.

Dazai et al. (1968) found that within one week, synthetic detergent samples consisting of either 53% dodecene-1-LAS or 46% NALKANE N-500™ (LAS) were degraded 100% and 97.5% (analyzed by Japan Industrial Standard Methods (JIS, 1967)), respectively, in an acclimated activated sludge system; each detergent sample added to the sludge contained approximately 20 mg/L of the active LAS component. These authors also found that within a concentration range of 10 to 100 mg/L of detergent there was no difference between LAS and ABS with respect to the metabolic activity of microorganisms in activated sludge. No inhibition was noted with concentrations of less than 20 mg/L, while at 50 mg/L, 7%-8% inhibition occurred, and at a level of 100 mg/L, 17%-20%.

McEvoy and Giger (1985) determined that the concentration of LAS in sludge samples from eight Swiss Sewage treatment plants ranged from 0.3 to 1.2% of the dry sludge or approximately 2 to 5% of the total organic carbon. Only minor variations were noted in the relative composition of LAS homologs and isomers. These results emphasize the importance of distinguishing between removal and degradation in treatment systems.

Mann and Reid (1971) evaluated a number of LAS products (sodium sulfonates of the Shell DOBANE series of alkylbenzenes: $C_{11.8}$ (DOBANE JNX™), $C_{12.7}$ (DOBANE 055™), $C_{10.9}$ (DOBANE 83™), and $C_{11.6}$ (DOBANE JNB™) in field trials at a trickling filter sewage plant serving a community of 31 homes in the United Kingdom. To assess normal performance, sewage samples were taken for seven weeks prior to initiation of the test. At the beginning of the test period, the specific LAS product was provided to the homeowners. All compounds were quite readily removed (86%-95% removal of MBAS). Repetition with each of the LAS products during a different season of the year indicated a slightly higher degradation rate at higher temperatures.

In a study of removal of zeolite in an operating package activated sludge plant serving 81 homes, Hopping (1978) developed data on removal of MBAS (and other eomponents). MBAS removal was 97 and 98% in baseline and test periods, respectively.

In a series of papers, Renn (Renn, 1965; Renn et al., 1964) examined the biodegradation of LAS in an extended aeration aetivated sludge system which served approximately 90 mobile homes. The entire community volunteered to simultaneously switch from ABS (branched chain alkyl benzene sulfonates) to LAS detergents. A rapid decline in the amount of foam on the surface of the aerator was noted within the first week. MBAS content of effluent samples dropped from approximately 8 mg/L to 3 mg/L. Eventually, LAS removal of slightly above 96% was seen. Residues of ABS were still detectable (via IR) more than two months after the switchover to LAS, probably due to their retention in the system sorbed to sludge.

McGauhey and Klein (1966) and Klein (1969) reported an approximately 30% drop in the synthetic detergent concentration of raw sewage entering a sewage treatment plant following a changeover from ABS to LAS detergents. Values ranging from 15% (Stennett and Eden, 1971) to 34% degradation of LAS have been reported to occur in sewage lines before entrance to the sewage treatment system (Spohn, 1964, 1967;

Knapp and Morgan, 1965, cited in Gledhill, 1974). Direct measurement of LAS radiolabeled with ^{35}S confirmed that 15% of LAS was biodegraded in a 4.17-mile sewer during a 170-minute retention period (Standing Technical Committee on Synthetic Detergents, 1967, cited in Gledhill, 1974).

Oba et al. (1976) analyzed raw municipal sewage and effluent from two Japanese sewage treatment plants for a one year period. MBAS and IR analyses of influent and effluent sewage revealed that the surfactant content of the influent sewage contained 75% LAS. Sewage treatment removed approximately 85% of the LAS during passage through the treatment plant.

b. Anaerobic Treatment

LAS has also been studied in anaerobic treatment environments. Whelan and Titamnis (1982) observed LAS at concentrations of 1.2-6.5 mg/L in the effluent of 6 domestic septic tanks, suggesting the inability of anaerobes to biodegrade LAS. Giger et al. (1987) reported an average LAS concentration of 4 g/kg in the dry matter of 24 anaerobically stabilized sewage sludges, which they attributed to poor anaerobic degradation. While LAS concentrations in anaerobically digested sludge were 20-30% less than in fresh sludge, the authors believed the difference was due to normal variations is LAS concentration in the sludge.

2. Groundwater

Thurman et al. (1986) studied the fate of detergents in a groundwater plume resulting from infiltration beds at Otis Air Force Base, Massachusetts. LAS degradation was found to be rapid, based on concentrations in the plume. None was found more than 600 meters down gradient from the point of infiltration.

The mineralization of LAS was determined as a function of depth in sediment profiles from beneath a laundromat wastewater pond and a

pristine pond. The concentration of LAS was found to decrease markedly with increasing depth in the laundromat profile. Bacterial numbers and activity decreased with depth in both profiles. LAS was mineralized without a lag period at all depths in the laudromat profile; its half-life ranged from 3.2-16.5 days. In the control pond, it was mineralized more slowly (half-life 5.2-1540 days) and only after a lag period of 2-40 days (Procter and Gamble Company, unpublished data).

3. River Water

Sueishi et al. (1988) studied the fate of commercial LAS (chain length not specified) in rivers of Lake Biura Basin in Japan. A decay constant of 0.62/day was employed to account for the biodegradation of dissolved LAS; removal was also accounted for by the settling of suspended matter. Holysh et al. (1986) modelled G_{12}LAS degradation and removal in Rapid Creek, South Dakota. The field data fit a model using a biodegradation rate constant of 0.50/day for river water, and 0.96/day for bottom and suspended sediments.

Games (1982) examined the behavior of C_{10}-C_{14} LAS in a flowing stream as part of a field validation of the EPA's EXAMS exposure model. Total concentrations of LAS in the creek and sediments decreased from 270 to 10 μg/L and 275 to 1.5 mg/kg, respectively, as distance from the sewage treatment plant that was the source of the LAS increased from 0.8 to 87.2 Km down stream. The EXAMS model was found to be successful in predicting stream concentrations but was sensitive to the rate of biodegradation in sediments and the dynamics of the sediment water interface.

4. Seawater

Hon-nami and Hanya (1980) determined LAS concentrations in Tokyo Bay. The proportion of C_{10} and C_{11} LAS was slightly decreased and the proportion of C_{12} and C_{13} LAS was slightly increased when compared to river and estuary waters. Eganhouse et al. (1983) reported the

presence of linear alkylbenzenes in the marine environment off the coast of California. They were believed to be formed from either incompletely formed or desulfonated LAS and to be preserved in sediments for 10-20 years.

D. Effect of Chemical Structure

The rate of biodegradation of LAS is influenced by the position of the phenyl group, branching of the alkyl chain, and the presence of aliphatic, cyclic groups. Degradation rate increases as the phenyl group nears the end of the chain and with less branching and the absence of cyclic groups. In screening studies, degradation rate of individual LAS compounds has been observed to increase with chain lengths from C_6-C_{12}, decrease with lengths of C_{13-15}, and increase again with chain lengths up to C_{18}. Tests at more realistic environmental concentrations (µg/L) indicate an absence of chain length effects. In any case, degradation rates of LAS are rapid.

The rate of biodegradation of a particular surfactant depends upon its specific chemical structure. Highly branched surfactants display increased resistance to biodegradation while degradation is enhanced by increased linearity of the hydrophobic group. Divo (1974) found that biodegradation was almost independent of a surfactant's molecular weight, but it was directly influenced by the surfactant's isomeric distribution. Swisher (1970) cites four variations in chemical structure which can influence the biodegradation of alkylbenzene-sulfonates. They are:

(1) the position of the phenyl group;
(2) the length of the alkyl chain;
(3) the degree of branching, and
(4) the presence of aliphatic, cyclic groups.

It is significant that the LAS homologs and isomers which are degraded more rapidly in mixtures are the more toxic components, particularly as demonstrated with aquatic organisms.

1. Position of Phenyl Group

Several investigators (Huddleston and Allred, 1963; Ruschenberg, 1963a, 1963b; Setzkorn et al., 1964, cited in Swisher, pp. 207-209, 1970) have noted that biodegradation of LAS compounds in which the phenyl group is attached to the terminal portion of the alkyl chain occurs more rapidly than that which results when the phenyl group is attached toward the central portion of the chain. Working with river water cultures, Ripin et al. (1970) found that 1-phenyldodecane sulfonate disappeared more rapidly than the 2-phenyl or 4-phenyl isomers and that this variation was primarily a result of the difference in lag or acclimatization time. Bayona et al. (1986) observed increased rates of biodegradation of C_{11}-C_{14} linear alkyl benzenes by pure strains of Pseudomonas with increasing closeness of the phenyl group to the end of chain.

2. Lenghth of Alkyl Chain

In screening studies using mg/L concentrations of LAS, an apparent relationship between the length of the alkyl chain and the rate of biodegradation has been observed. Degradation occurs at an increasingly more rapid rate for single LAS compounds with chain lengths from C_6 through C_{12}; it slows for C_{12} to C_{15} homologs, then increases again up to a chain length of 18 carbons (Swisher, 1970). However, Kravetz et al. (1982) found that the extent of degradation of the benzene ring in C_{12}LAS was much less than for C_{13}LAS using a modified shake flask proeedure with initial surfactant eoncentrations of 30 mg/L. A single report by de Jong and Testa (1967, cited in Swisher, p. 218, 1970) indicates that this chain length biodegradability relationship may be reversed with a LAS homolog chain lengths greater than 21 carbons. However, the correlation between chain length

and the rate of biodegradation of LAS homologs does not appear to apply to LAS mixtures. Swisher (1970) reports that the degradation of a mixture containing C_{12} plus C_{14} LAS was delayed until acclimation to C_{14} LAS was achieved. Then, both homologs degraded together, although the C_{14} LAS degraded slightly faster. Using his shake flask procedure for determination of CO_2 evolution, Gledhill (1975) found that the rate of ultimate biodegradation for both commercial and pure LAS homologs was affected by the length of the alkyl chain; i.e., the longer the chain length, the slower the rate of ultimate biodegradation (see also Section III-C). The concentration of the surfactant (30 mg/L) appeared to be the cause of this inhibition in that the inhibition could be completely eliminated by incremental feedings of the surfactant.

More recent studies in a variety of media have found no chain length effect at µg/L concentrations of LAS, which are more typical of those found in the environment than are the mg/L concentrations employed in screening tests. Studies reported by Larson and de Henau (1988) and Ward (1987) are examples of such work.

3. Presence of Branching and Groups

In accordance with the above relationship for longer chain LAS compound in screening studies, inclusion of a branched alkyl group into the molecular structure of a surfactant would also tend to retard biodegradation by effectively reducing the length of the linear chain (Swisher, 1970).

Several investigators have noted somewhat slower degradation rates for various LAS-related compounds which contain cyclic groups (Huyser, 1960; Hammerton, 1962, cited in Swisher, pp. 225-226, 1970). Addition of an aliphatic ring structure, although itself biodegradable, results in the formation of a more compact alkyl chain; these cycloalkyl groups were found to be more resistant to biodegradation in an activated sludge aeration unit (Nelson et al., 1961). Nelson postulated that cycloalkyl groups may affect the adsorption of the compound onto the activated sludge which may be a necessary factor in removing resistant

structures from solution; however, adsorption is effective only if the compound is also biodegradable. Swisher (1970) found that condensed cyclic systems - linear dialkylindanes and dialkyltetralins - were readily biodegradable.

When Gledhill (1975) examined the degradation of C_{12} LAS (synthesized from uniformly-labeled ^{14}C-benzene), he found that ring degradation occurred concomitantly with, and at approximately the same rate as, the total CO_2 evolution rate.

E. Metabolic Pathways of Biodegradation

> *The metabolic pathway of LAS degradation has been characterized as ω-oxidation of the terminal methyl group of the alkyl side chain to an alcohol, its subsequent oxidation to an aldehyde, then to a carboxylic acid. Degradation of the side chain proceeds by β-oxidation (possibly in combination with α-oxidation). Degradation of the benzene ring and desulfonation then occur. Other metabolic pathways have also been observed.*

An extensive review of the metabolic pathways of biodegradation is beyond the scope of this report. For a more comprehensive treatment of the subject, the reader is referred to Swisher (1970, 1976, 1987); Cain (1981); Cain et al. (1971); Cain (1976); and Gledhill (1974).

1. Primary

There are three demonstrated points of metabolic attack; the alkyl chain, the sulfonate group on the benzene ring and the ring itself (Cain, 1976). It appears that the first point of attack is often the terminal methyl group of the alkyl side chain (Swisher, 1963; Willetts and Cain, 1962a). Cleavage of the alkyl chain is followed by scission of the aromatic ring and its subsequent degradation (Swisher, 1968; Willetts and Cain, 1972a).

Swisher (1964) characterized primary biodegradation as an initial attack (ω-oxidation) on the terminal methyl group of the alkyl side chain. The methyl group is oxidized to an alcohol, this to an aldehyde, and then to a carboxylic acid. Following ω-oxidation, the side chain is degraded via β-oxidation - a sequence of enzymatically catalyzed reactions in which two carbon units are successively removed. While β-oxidation is the predominant metabolic pathway for side chain degradation, it is not the only available pathway. This was indicated by Cain et al. (1971) who found odd-numbered alkyl chain intermediates arising from the metabolism of even-numbered substrates. These intermediates most likely resulted from a combination of α- and β-oxidation.

Once primary degradation has occurred, LAS ring degradation is initiated. The exact sequence of LAS ring degradation is not fully known but it is presumed to involve oxidation to a catechol derivative and rupture between ("ortho") or adjacent to ("meta") the two hydroxyl groups (Swisher, 1970). The two metabolic sequences result in different intermediate products. The enzymes of each of these pathways have been shown to be inducible in one or another microorganism (Cain and Farr, 1968; Willetts and Cain, 1972a; Thysse and Wanders, 1972; Bird and Cain, 1976).

Cain and Farr (1968) found that 17 species of Pseudomonas isolated from sewage, river water or soil by enrichment culture on either a benzene-sulfonate or toluene-p-sulfonate medium degraded benzenesulfinate, benzenesulfonate and toluene-p-sulfonate by releasing the sulfonate group as inorganic sulfite. Growth on benzenesulfonate and toluene-1-sulfonate elicited a catechol 2,3-oxygenase, which effected a "meta" cleavage of the ring.

Swisher (1967a, 1972), however, did not observe any desulfonation of LAS prior to ring cleavage. In his experiments, the sulfonate group split off just at the point in time of ring degradation and not measurably prior to ring degradation.

In a series of papers, Swisher (1967a; 1967b; 1968) studied LAS ring degradation based on the progressive disappearance of the UV absorption band. He found that both pure 3-phenyldodecane sodium sulfonate and pure 6-phcnyldodecane sodium sulfonate were degraded in river water, with destruction of the benzene ring occurring in the process (Swisher, 1967a). He also observed that the above two compounds showed ring degradation of approximately 90% in continuous and semi-continuous activated sludge systems (Swisher, 1967b) and greater than 80% ring degradation in a standard shake culture method (Swisher, 1968).

In a later paper, Swisher (1972) reported that the 2-, 4- and 5-phenyldodecane sodium sulfonates also underwent ring degradation in activated sludge; however, in a shake flask procedure, ring degradation was nil for the 4-phenyl compound.

Since Heyman and Molof (1968) pointed out that complete ω, β-oxidation of all five isomers of phenyldodecane sulfonate would lead to the same intermediate, sulfophenylsuccinic acid, Swisher (1972) attempted to determine whether acclimation to one of the C_{12} LAS isomers imparted the ability to immediately degrade the rings of the other four isomers. Although the results of the cross-acclimation study showed some consistency, reproducibility was poor. Nevertheless, the results appear to indicate that each of the five isomers has a different key intermediate.

Swisher suggests that multiple degradative pathways are involved in at least three of the five isomers. This may be due to ω-oxidation initiating at one end of the chain or the other to yield two different series of intermediates from each isomer. This results in the formation of two different key intermediates prior to the sulfophenyl-succinic acid stage.

Leidner et al. (1976) have suggested that ring cleavage of C_{12}LAS (Marlon A™) does not occur in the OECD screening system, even after extended incubation periods. Their studies were conducted at high concentrations of LAS (~13 mg/L), using a soil suspension as the

bacterial inoculum. It is quite likely that LAS was toxic to soil microorganisms at this concentration, since toxic effects are observed between 10 and 20 mg/L in biodegradability screening studies. At concentrations greater than those measured in the environment, ultimate degradation of LAS is also less complete than at lower concentrations (Larson, 1979).

Leidner et al. (1976) also report that residual quantities of various sulfophenyl carboxylic acid intermediates were present in the spent OECD supernatant, although quantitative estimates were not made. This result may be related to the fact that soil microorganisms were used instead of sewage microorganisms, and the likelihood that soil systems may not receive the kind of exposure to LAS necessary to develop an acclimated population of LAS degraders. By contrast, the rate and extent of LAS degradation by sewage microorganisms is comparable irrespective of whether sewage influent, effluent or activated sludge is used (Larson, 1980).

In additon to appearing as intermediates in the OECD test, sulfophenyl carboxylic acids were found (qualitatively) in various wastewaters and streams. This suggests that degradation of intermedidates is slower than the degradation of the parent material.

The obvious complexity of the possible metabolic pathways of surfactant degradation is illustrated by Cain (1976) who reported that there may be no less than eight possible degradative routes for alkylbenzene sulfonates alone:

(1) ω- and β-oxidation of the side-chain without desulfonation or ring metabolism.

(2) ω- and β-oxidation of the side-chain with concomitant hydroxylative desulfonation and ring cleavage.

(3) Degradation similar to (2) but with a reduction desulfonation giving rise to phenylalkanoate rather than p-hydroxphenylalkanoate intermediates.

(4) ω-oxidation and desulfonation followed by both α- and β-oxidation giving odd-numbered intermediates from an even-numbered substrate and vice-versa with subsequent ring cleavage.

(5) (4) but without ring cleavage.

(6) "Pantothenate" or "valine"-type branched side-chain degradation with desulfonation and ring cleavage.

(7) Attack initiated by desulfonation of the aromatie nucleus to form the corresponding alkylcatechols followed by 'meta' ring cleavage. This is usually confined to alkylbenzene sulfonates with short ($<C_4$) alkyl side-chain.

(8) Phenol-enhanced cometabolic degradation.

Further work on model substrates (Leidner et al., 1980), showed that very short chain LAS (C_1-C_4) were not degraded by unadapted soil organisms in the OECD screening test (mineral salts medium, containing the substrate as sole carbon source at 8 mg organic carbon per L). On the other hand, the carboxylic acid analogs of these LAS chain lengths were readily degraded. The C_1 to C_4 LAS were still not degraded in the presenee of the carboxylates.

Similar concerns about the degree of degradability of LAS were expressed by Pitter and Fuka (1979) based both on a review of the literature and their own experiments using adapted activated sludge. The experiments were conducted with extremely high levels of LAS (25-65 mg/L) as sole carbon source in an inorganic salts medium. Analyses for residual COD, DOC and UV absorption showed incomplete biodegradation

(50-70%), although MBAS values indicated 95+% primary loss of LAS. They conclude that "the ultimate biodegradability of LAB's appears to be at least debatable." As pointed out in an "extension" of this subject (Swisher, 1980), this conclusion may be more related to the experimental conditions employed by Pitter and Fuka, than to the situation in the real world. The mineralizability of LAS has been demonstrated under close to real-life conditions; e.g., in semi-continuous activated sludge tests. Further, results from environmental sampling show low amounts of the expected intermediates, corroborating that LAS degrades in "real-world" conditions.

F. Summary

There are a number of methods for studying the biodegradability of LAS (and other) surfactants. Tests vary in their pertinence to real situations and ease of use in the laboratory; e.g., determination of "inherent" biodegradability of a compound and the "treatability" of a detergent formulation, including the LAS component. To a greater or lesser degree, these test systems represent models of the processes occurring in bodies of water, rivers and sewage treatment of varying degrees. The predictive value of many of them has been confirmed by field tests.

The extent and chemical pathways of biodegradation are important features of any surfactant, and, of course, LAS compounds were chosen as replacements for ABS (tetrapropylene) because of their greater biodegradability. Commercial LAS's are generally biodegraded to 90% or more in a variety of test systems. Tests employing natural media and LAS concentrations typically found in the environment show LAS to be readily biodegraded. The benzene ring and the alkyl chain are both degraded.

In conclusion, it is clear that linear alkylbenzene sulfonates as a class of surfactants are highly biodegradable, that their levels are low in receiving waters, and, even there, they further degrade.

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IV. ENVIRONMENTAL LEVELS

A. Water Quality Standards

The concentration of anionic surfactants in water is measured as MBAS, however, only a fraction of this would be expected to be LAS. The maximum permissible level of surfactants in drinking water involved in interstate commerce is 0.5 mg MBAS/L. Several states and Canadian provinces also set 0.5 mg MBAS/L as the maximum permitted level. Under the Safe Drinking Water Act the secondary maximum contaminant level for foaming agents in public water systems is 0.5 mg/L. Under the Clean Water Act effluent limitations guidelines have been set for soap and detergent manufacturing. The European Economic Community has a directive which prohibits marketing and use of detergents which have an average level of biodegradation (as measured by MBAS) less than 90%.

1. National Regulations

Under the National Secondary Drinking Water Regulations, the secondary maximum contaminant level (SMCL) for foaming agents in public water systems is 0.5 mg/L. The SMCL is the maximum permissable level of a contaminant in water which is delivered to the free flowing outlet of the ultimate user of a public water system. These levels represent reasonable goals for drinking water quality. States may establish higher or lower levels which are appropriate for local conditions, however, at this time all states are following the federal regulation. (U.S. Code of Federal Regulations, Title 40, Part 143, 1987; American Petroleum Institute, 1985). The regulations recommend that monitoring take place at no less than one year intervals for all community water systems utilizing surface water sources and no less than every 3 years for community systems utilizing ground water sources.

The method of analysis is "foaming agents-methylene blue method" which may be found in Standard Methods for the Examination of Water and Wastewater, 16th edition, p 581 or Methods for Chemical Analysis of Water and Wastes from the U.S. Environmental Protection Agency, p 157, 1974 edition.

Under the terms of the Clean Water Act, effluent limitations guidelines have been set for soap and detergent manufacturing. The limitations establish the quantity or quality of pollutants or pollutant properties which may be discharged by a point source after application of varying levels of pollution control technology. A point source is any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container or vessel from which pollutants are discharged. Within the soap and detergent manufacturing point source category, there are 19 subcategories for various processes and specific types of detergents. The range of effluent limitations as well as standards of performance for new sources may be found in the Code of Federal Regulations, Title 40, Part 417.

The U.S. Public Health Service has set a maximum permissible level of 0.5 mg MBAS/L for drinking waters involved in interstate commerce (1962).

2. State and Local Regulations

A number of states have imposed regulations with respect to MBAS levels in waterways and/or prohibitions of the use of detergents containing specific surfactants. The states establishing guidelines have generally used the U.S. Public Health Service drinking water standard of 0.5 mg/L MBAS applied to all waters, with the exception of New Jersey, whose standard applies to the tidewater sections of the Delaware River.

Indiana bans alkyl benzene sulfonate (ABS) detergents. Wisconsin has a law which prohibits the sale and use of nondegradable detergents containing ABS.

In addition, the state of Oregon prohibits non-biodegradable cleaning agents. Restrictions on the sale of surfactants exist in several localities. Bayville, New York specifically prohibits sale of all surfactants as does the city of New Shoreham, Rhode Island. Both of these areas are islands with special problems of contamination of ground water by sewage effluents. Dade County Florida prohibits the sale of non-biodegradable cleaning agents. New York state has a standard for aquatic species of 40 micrograms/liter for LAS with side chains of 13 carbons or longer. (Soap and Detergent Association, 1988).

3. International Regulations and Guidelines

Canadian regulations provide that each province may determine its own water quality regulations. Among the individual provinces, there is considerable variation in the detail of these regulations, with the province of Saskatchewan, in cooperation with the provinces of Alberta and Manitoba, specifically designating MBAS in criteria standards. Surface waters are allowed to have a maximum of 0.5 mg/L MBAS. For sewage treatment, the following criteria are specified for MBAS:

	Percent Removal	Effluent (MBAS)
Primary Treatment	Nil	-
Secondary Treatment	80-95	0.2 - 0.8 mg/L
Aerobic Lagoons	60-90	0.2 - 1.5 mg/L

Source: Bureau of National Affairs, 1974

The value for aerobic lagoons is a summer value, since most of these water bodies are frozen during the winter (Bureau of National Affairs, 1974).

While there are not direct water quality standards for LAS or MBAS established in European countries, the European Economic Community has published a directive that prohibits the marketing and use of detergents containing surfactants which have an average level of biodegradability (as MBAS) less than 90%, and the use of the permitted surfactants "must not, under normal conditions of use, be harmful to human

or animal health." (Official Journal of the European Communities, No. L 347/51, Directive 73/404/EEC, 1973).

An additional directive (73/405/EEC) prohibits detergents with a biodegradability of less than 80% based on a single analytical method for biodegradation chosen from 3 recommended procedures (Official Journal of the European Communities, No. L 347/53, Directive 73/405/EEC, 1973).

The European Economic Community has published a directive which defines quality requirements that surface fresh water must meet if used or intended for human consumption. For the purposes of this directive, surface waters are divided into three categories which correspond to different treatment methods. These are outlined below.

Definition of the standard methods of treatment for transforming surface water of categories A1, A2 and A3 into drinking water

Category A1. Simple physical treatment and disinfection, e.g., rapid filtration and disinfection.

Category A2. Normal physical treatment, chemical treatment and disinfection, e.g., pre-chlorination, coagulation, flocculation, decantation, filtration, disinfection (final chlorination).

Category A3. Intensive physical and chemical treatment, extended treatment and disinfection, e.g., chlorination to break-point, coagulation, flocculation, decantation, filtration, adsorption (activated carbon), disinfection (ozone, final chlorination).

Water treated by these methods must conform to parameters which are

specific for each category. The parameters include physical, chemical and microbiological characteristics. Values are listed for mandatory standards as well as guidelines. For surfactants reacting with methyl blue in surface water treatment categories A1, A2 and A3, the guidelines are 0.2, 0.2 and 0.5 mg/L as lauryl sulfate, respectively. There are no mandatory values (Official Journal of the European Communities, No. L 194, Directive 75/440/EEC, 1975).

An additional EEC directive sets standards for water intended for human consumption, either in its original state or after treatment, regardless of origin. Both guide levels and maximum admissible concentrations have been set. For surfactants reacting with methylene blue, the maximum admissible concentration is 200 µg/L as lauryl sulfate. There is no guide level (Official Journal of the European Communities, No. L 229, Directive 80/778/EEC, 1980).

The quality of bathing water is addressed in another directive. Bathing water is defined as all running or still fresh waters and sea waters where bathing (swimming) is practiced. These waters are subject to physical, chemical and microbiological testing. For each testing parametcr, there are both guideline and mandatory values. For surface active substances reacting with methylene blue, the guideline value is ≤0.3 mg/L as lauryl sulfate. The mandatory limit states that no lasting foam be present (Official Journal of the European Communities, No. L 31, Directive 76/160/EEC, 1976).

B. LAS Levels in the Environment

Since the change from ABS to LAS, surfactant levels (measured as MBAS) have decreased significantly in most waterways. The levels in most waterways are below the prescribed standard of 0.5 mg/L MBAS. In areas where MBAS levels are elevated, it is indicative of poor sewage treatment, es most LAS is removed during primary and secondary treatment processes.

The substantial increase in the use of anionic surfactants since 1950 has resulted in the occurrence of MBAS in natural environments from the discharge of wastes containing these surfactants. In 1965, the United States manufacturers of detergent products voluntarily switched from the use of the relatively poorly degradable tetrapropylene-derived surfactants to the more biodegradable linear alkylbenzene sulfonates (LAS). Since that time, despite the increased use of LAS, MBAS levels in the environment have actually decreased because of the facile biodegradability of LAS. Because LAS can act as a toxicant in aquatic and terrestrial ecosystems under some conditions, it is important to examine residual concentrations of these surfactants in various environments.

The following is an account of LAS and MBAS levels as related to the use and subsequent discharge of LAS in various natural environments.

1. Waste Water

a. Sewage Treatment Plants

Concentrations of detergent products in washing situations range from 1,000 to 3,000 mg/L (0.1-0.3%). These detergent solutions would be expected to contain 150 to 600 mg/L LAS. The surfactant components of the detergent products, being soluble, eventually reach raw sewage drains at concentrations of 1-7 ppm (Rapaport et al., 1987). These wastes, which are the influent to sewage treatment works, are treated to various degrees (primary, secondary and tertiary), and the surfactants are removed from the waste influent in proportion to the extent of treatment.

Removal rates of LAS during sewage treatment average 98% for activated sludge, 80% for trickling filtration and 27% for primary clarification (Rapaport et al., 1987). Similar results were reported by Matthijs and DeHenau (1987) who found that activated sludge plants removed an average of 98% LAS while trickling filter plants removed 90% LAS.

Rapaport et al. (1987) reported that effluent sewage from activated sludge plants contained 0.06 mg/L LAS while that from trickling filter and primary clarification plants contained 0.6 and 2.1 mg/L, respectively. These results were in agreement with those reported by Matthijs and DeHenau (1987) who found LAS effluent levels of 0.07 and 0.76 mg/L from activated sludge and trickling filter plants, respectively.

In Israel, MBAS concentrations in raw sewage from five sources varied between 9.6 and 11.0 mg MBAS/L (average 10.3 mg/L) and, where secondary treatment was applied, the concentration in the final effluent was between 0.3 and 1.3 mg/L representing 87 to 97% removal (Zoller, 1985).

For Japan, Ogino and Nagao (1979) reported up to 22 mg MBAS/L in raw sewage but, surprisingly, could find no LAS by a "simple GC method." Nara et al. (1983) found that the average sewage production was 120 L/person-day and the anionic surfactant consumption 2.31 g/person-day. From these data the concentration in raw sewage can be estimated at 19.2 mg MBAS/L. The anionic surfactant consumption in Japan of 2.31 g/person-day compares well with the the USA, German and UK figures of 2.6, 2.2 and 2.5 g/person-day, respectively.

Using a liquid-membrane ion-selective electrode method, Hu et al. (1983, 1985) reported concentrations of 5.4 to 15.7 mg anionic surfactant/L in raw sewage in China.

A nationwide water quality survey was conducted by the U.S. Environmental Protection Agency (1987), which included an examination of the concentrations of MBAS in certain water bodies of the United States. They reported that there were 15,438 municipal waste treatment facilities in the United States in 1986. The primary treatment facilities removed ~60% of the incoming BOD and 70% of the solids. Secondary facilities removed ~88% of the incoming BOD and 87% of the solids. Nationwide, 86% of the existing treatment facilities practiced at least secondary treatment in 1986. The levels of treatment and the

population served by municipal treatment facilities are summarized in Table IV-1. These data approximately cover the 73% of the population of the United States served by municipal wastewater treatment facilities.

An analysis of the actual MBAS levels in sewage treatment plants of the activated sludge type was carried out by Klein (1969). MBAS levels of 0.5% on a dry solids basis were found in raw sludge, with levels of 200 to 400 mg/L in the circulating digester sludge (1.0 to 1.5% of dry solids). Bottom sludges contained 1,000 mg/L MBAS (0.5% of dry solids) and were not considered to significantly affect digester performance.

Linear alkyl benzenes (LAB) were determined in 11 samples of Los Angeles settled sewage by two methods, Ag NO_3 TLC/GC and GC/MS; the concentrations were 150 ± 69 and 142 ± 62 µg/l respectively (Eganhouse et al. 1983).

McEvoy and Giger (1986) determined the total concentration of LAS in 12 samples of digested sewage sludge obtained from various sites worldwide. The concentration ranged from 2.9 to 11.9 g of LAS/kg.

In another study, the concentration of LAS in 10 samples of aerobic sewage sludge ranged from 182-432 µg/L, while that in 10 samples of anaerobically digested sludge ranged from 1327-9927 µg/L. This shows that LAS is highly biodegraded in treatment plants and that the levels found in anaerobically digested sludge are strongly influenced by the LAS from the primary settling where conditions for biodegradation are not present (Matthijs and DeHenau, 1987).

Levels of LAS in wastewater and sludge which have been recently reported are outlined in Tables IV-2 and IV-3. These data indicate that typical LAS concentrations in raw sewage, primary effluent, trickling filter plant effluent and activated sludge plant final effluent are 2-4 mg/L, 1.5-2.5 mg/L, 0.5-1.0 mg/L and less than 0.1 mg/L, respectively.

TABLE IV-1

WASTEWATER TREATMENT LEVELS, 1986

Treatment Levels	No. Facilities	% of Total Facilities (15,438)	Pop. Served (millions)
Less than secondary	2,112	13.7	28.8
Secondary	8,403	54.4	72.3
Greater than secondary	3,115	20.2	54.9
No discharge	1,762	11.4	5.7
Other*	46	0.3	10.3
TOTAL:	15,438	100.0	172

*Level of treatment and design capacity data were unavailable for these facilities.

Source: U.S. Environmental Protection Agency, 1987

b. Septic Tank/Tile Field Systems

A secondary pathway into the environment for LAS and other surfactants is through soils from septic tank disposal of domestic and municipal wastes. Much of the information on surfactant concentrations in soils from septic systems relates to ABS. LAS, being much more biodegradable than ABS, would degrade faster in the soil column. The Organization for Economic Cooperation and Development (1964), in their review of septic tank contamination of natural waters, states that ABS surfactants deposited in the soils via septic tank discharge may remain up to 1-3 years without degradation. These surfactants may dissolve later and contaminate water sources. This accumulation in the soil is

TABLE IV-2

ANALYSIS OF WASTEWATER TREATMENT PLANT SAMPLES

Sample Type	Number of Samples	Number of Locations	Country	LAS Concentration (mg/L)	Reference
Primary influent	3	1	USA	2.97	Sedlak and Booman, 1986
Influent sewage	97	17	USA	3.5	Rapaport et al., 1987
	10, 5	10	West Germany	4.0, 7.4	Matthijs and DeHenau, 1987
	--	--	USA	3.8-6.5	Osburn, 1986
	--	3	USA	3.8	DeHenau et al., 1986
	--	3	Canada	2.0	Rapaport et al,1987
	--	8	Germany	4.8	DeHenau et al., 1986
		8	Switzerland	2.4	Giger et al., 1987
Final effluent	3, 3	1	USA	0.02, 0.05	Sedlak and Booman, 1986
Primary effluent	3, 3	1	USA	1.73, 2.51	" "
	16	4	USA	2.1	Rapaport et al., 1987
Trickling filter effluent	25	4	USA	0.6	Rapaport et al., 1987
	--	1	USA	0.14-0.60	Osburn, 1986
	--	1	USA	0.51	Holysh, et al., 1986
Trickling filter final effluent	5	--	West Germany	0.76	Matthijs and DeHenau, 1987
Activated sludge final effluent	--	10	West Germany	0.07	Matthijs and DeHenau, 1987
Activated sludge effluent	66	12	USA	0.06	Rapaport et al., 1987
Effluent sewage	--	3	USA	0.06	DeHenau et al., 1986
	--	3	Canada	0.09	Rapaport et al., 1987
	--	8	West Germany	0.07	Rapaport et al., 1987

TABLE IV-3

ANALYSIS OF SLUDGE SAMPLES

Sample Type	Number of Samples	Number of Locations	Country	LAS Concentration (mg/L)	Reference
Anaerobically digested	59	14	USA	4.68	Rapaport et al., 1987
	1	1	USA	6.66	Sedlak and Booman, 1986
	--	8	Germany	6.2	DeHenau et al., 1986
	2	--	USA	5.2, 6.9	McEvoy and Geiger, 1986
		7	Switzerland	5.9	Giger et al., 1987
		10	West Germany	4.9	Matthijs and Dettenau, 1987
		24	Austria	4.2	Giger et al., 1987
Aerobically digested	1	1	USA	4.25	Sedlak and Booman, 1986
	1	1	Switzerland	2.9	McEvoy and Geiger, 1986
Primary	2	1	USA	5.34, 6.31	Sedlak and Booman, 1986
Secondary (activated sludge)	2	1	USA	0.41, 0.86	Sedlak and Booman, 1986
		1	Japan	0.09	Yoshimura, 1984a
Drying bed digested	2	1	USA	0.15, 0.16	Sedlak and Booman, 1986

probably a result of the dampening effect of the anaerobic environments commonly found in septic tank systems following biodegradation in the soils (Lawton, 1967). The Organization for Economic Cooperation and Development has reported surfactant levels of 0.1 to 1.0 mg/L MBAS from well waters in close proximity to septic tank systems indicating the potential infiltration of the surfactants through soils into water supplies.

A recent study by Thurman et al. (1986) found that MBAS moves conservatively in sand and gravel aquifers and that ABS detergents appear to degrade slowly, if at all, in groundwater. MBAS levels in well waters near septie tank systems ranged from 0.02 to 2.0 mg/L.

Soils of different properties of ionic, mineral, and chemical content have differential adsorption and biodegradation rates. Soils with a low cation exchange capacity and high clay content adsorb the anionic materials well (Fink et al., 1970). Webb and Earle (1972) showed an 80% reduction in the drainage capacities of soil due to the formation of "water lattices" between the sulfonate groups and the water molecules. Furthermore, it has been suggested that the presence of undecomposed surfactants in the soil column indicates the presence of other pollutants (Organization for Economic Cooperation and Development, 1964). In many cases, other chemical pollutants and bacterial concentrations were high in conjunction with high surfactant concentrations in the proximity of septic tank systems. An early study which found that LAS adsorption on soils was directly correlated with high content of organic matter and with phosphate-fixing capacities of specific tested soils (Hurti et al., 1966), have been disputed by a recent study which found that sorption did not correlate well with organic carbon content (Hand and Williams, 1987).

The sorption of LAS to river sediment increased with chain length and phenyl position. Sorption was also increased with increasing LAS hydrophobicity (Hand and Williams, 1987).

LAS, therefore, as discharged from a properly designed septic tank system, will readily degrade if it is in an aerobic soil environment. Degradation and oxidation of organic compounds are affected by variables such as soil pH, permeability, void space, water content, mineral and cation content. If the soils have a tendency to be anaerobic, LAS will not decompose as readily and may give rise to the potential for high concentrations of undegraded LAS in aquifers deriving from these soils.

2. Surface Waters

a. Streams and Rivers

With the changeover from ABS to LAS, concentrations of MBAS generally decreased in surface water environments. This has not been the result of a reduction in use of surfactants, since the use of LAS has increased over the years, but because of the increased biodegradability of LAS. LAS is more completely degraded than ABS both in natural systems and in biological sewage treatment processes. Several reviews show the general decrease year by year of MBAS levels since the change from the ABS to LAS surfactants (Husmann, 1968; Lawton, 1967; Heinz and Fischer, 1968; Brenner, 1968; Waldmeyer, 1968; Sullivan and Evans, 1968; Sullivan and Swisher, 1969; Rapaport et al., 1987).

Some recent data on LAS concentrations in rivers and sediments are listed in Table IV-4.

Mochalov et al. (1984) reported that "anionic surfactants" were present in waters of the Baltic Sea at 0 to 0.093 mg/L. Near to the mouth of the Vistula River concentrations were 0.040 to 0.102 mg/L at 3 m depth and 0.015 to 0.135 mg/L in deep waters. In South America, 0.05 to 4.5 mg MBAS (as Manoxol OT)/L, average 0.7 mg/L, was present in samples of bay water near a large discharge of untreated sewage in the Rio Grande (Brazil), (Kantin et al, 1981). However, the surfactant here is probably tetrapropylene benzene sulphonate, since at that time the surfactants used in Brazil were not biodegradable.

TABLE IV-4

ANALYSIS OF RIVERS AND SEDIMENTS

Sample Type	Number of Samples	Number of Sites	Country	LAS Concentration	Reference
				(mg/kg)	
Sediment below sewage outfall	24	2	USA	174	Rapaport et al., 1987
<5 miles downstream	10	1	USA	11.2	
>5 miles downstream	10	1	USA	5.3	
Sediment below sewage discharges	13	--	Germany	1.5-25	DeHenau et al, 1986
				(mg/L)	
River water below outfall	42	26	USA	0.099	Rapaport et al., 1987
<5 miles downstream	28	8	USA	0.063	
>5 miles downstream	18	1	USA	0.041	
River water	14	11	Germany	0.04	Matthijs and DeHenau, 1987

In Germany, with the introduetion of surfactants which are 80% or more biodegradable, MBAS levels perceptibly decreased in German rivers. Detergent loads as measured by MBAS concentrations decreased in the Rhine River basin by as much as 70% from 1,390 kg/day before 1964 to 435 kg/day by 1967. Furthermore, the loads and concentrations were reduced further downstream indicating additional reduction in the river itself. From 1962-1964, the Emschergenossen Schaft and Lippegenossen Schaft Rivers averaged 5.4 mg/L MBAS which fell to 1.2 mg/L by 1966. Other rivers exhibited similar reductions in loadings and concentrations. In general, concentrations of surfactants in German rivers fell by 67% from 1959 to 1966 and 92% by 1967 (Husmann, 1968). Brenner (1968) also noted that influent and effluent surfactant levels in German water treatment districts had decreased. Heinz and Fischer (1968) reported an 18 to 39% reduction in MBAS levels in the Rhine River basin after the change to LAS. This was followed by further large decreases to very low residual concentrations and to average annual values of 0.02-0.1 mg MBAS/L along the Rhine and its tributaries (Fischer, 1980). Wickbold (1974) also reported a reduction of detergent concentration in the Lippe River from a level of 0.7 mg/L in 1964 to levels between 0.15 and 0.2 mg/L for the interval from 1965 to 1972. Based on data from laboratory scale biodegradations, including gas chromatography analysis for tetralins and indanes, he estimates that 37 to 52% of the MBAS found in the Lippe River may be due to sulfonated dialkyltetralins and dialkylindanes.

Further evidence indicating that the change from ABS to LAS resulted in a decrease in MBAS concentration in surface waters was reported by Huber (1969). In an extensive examination of 8 major rivers and 4 lakes in Bavaria, West Germany, reductions of MBAS concentrations of 21 to 79% were found from 1962 to 1966. Data from 1985 indicates MBAS levels in the Rhine, Neckar, Main and Ruhr Rivers in West Germany have dropped further, with concentrations ranging from 0.01 to 0.03 mg MBAS/L (Gerike, 1987). In England, similar phenomena were observed due largely to the increased biodegradability of LAS in use since 1965 (Waldmeyer, 1968). A mean MBAS level of 0.15 mg/L was found for 35 river sites in the United Kingdom, of which only 26% on average was

attributable to LAS by microdesulfonation/GLC analysis. MBAS levels for the 35 sites ranged from 0.02 to 0.47 mg/L, with only 3 samples from two sites in the vicinity of major sewage effluent discharges containing more than 0.3 mg MBAS/L (Waters and Garrigan, 1983).

In the Guadulquivir river in Spain, the MBAS concentration at two sites was <0.1 mg/L and further downstream it was 0.19 mg/L (Moreno Danvila 1987). In aqueduct waters in Florence, Italy, the average concentration was 0.03 mg MBAS/L, with a maximum concentration in surface waters of 0.1 mg MBAS/L (Mancini et al., 1984). In a second study, (Ruffo et al., 1983) 86% of river samples contained 0 to 0.25 mg MBAS/L, with 2% exceeding 2 mg MBAS/L. In the latter study, it was found that the concentrations were much lower than in 1973.

In a one year sampling program, in 1978-1979, of various sites on the Jadro river in Yugoslavia, Dujmov (1984) found that the concentration of anionic surfactants (method unstated) increased towards the mouth of the river. Three sections of the river were distinguishable, with concentrations along the river of 0.020 to 0.045 mg/L.

In the late 1960's, the 'hard' ABS was replaced with 'soft' ABS which was monitored in Japan by following changes in the ratio of LAS to total ABS in river water using infra-red spectrometry (Miura et al., 1968; Oba et al., 1975). The proporiton of LAS rose in the years 1967 to 1970 from 20 to 70% and reached 90% by 1973.

Untreated domestic wastes are a major source of linear alkyl benzene (LAB) pollution in the rivers of Tokyo. Takada and Ishiwatari (1987) reported that suspended LAS levels in the Sumidagawa and Tamagawa Rivers ranged from 0.5 to 53.8 μg/L. Suspended LAB levels ranged from 37 to 721 ng/L. The amount of LAB coming from untreated wastes ranges from 93-96% of the amount measured in these samples. Tsukioka and Murakami (1983) reported values of trace to 2.5 mg LAS/L. Higher values were associated with sites close to untreated sewage discharges. Higher concentrations were also seen in small streams flowing through "highly developed" areas (Kobuke, 1985). Results show that the lower

the LAS concentration in the river, the lower the proportion of LAS in terms of total MBAS. Uchiyama (1979) followed the fate of LAS along a "small flowing" lake receiving a discharge upstream. Over a distance of 2 km, the LAS concentration fell from 0.6 mg/L to less than the detection limit (unstated) as determined by a UV absorption method. The concentration of MBAS was reduced from 1.46 mg/L to 0.29 mg/L over the same distance. LAS concentrations detected in river water and sediments of the Tama River were trace to 0.38 mg/L (trace to 0.82 mg/L as MBAS) and 2.79 to 10.72 ppm (6.23 to 31.6 ppm as MBAS), respectively. LAS was determined by HPLC with fluorimetric detector (Yoshimura et al., 1984a). In river sediments Yoshimura et al (1984b) found that LAS constituted on average about 30% of total MBAS, while Uchiyama (1979) reported that the proportion of LAS fell from about 25% near the outfall to about 17% two km downstram. Kobuke (1981) found MBAS levels in river water higher in the coastal areas of Osaka Bay and Harima Nada, where population and industry are centralized. Levels greater than 1 mg MBAS/L were observed. Uchiyama (1982) reported MBAS levels in the water of Lake Oze betwean 0.06 and 0.12 mg/L. MBAS levels in samples of the bottom mud of the lake ranged between 14.7 to 30.9 μg/g dry mud.

In the only Korean study (Bae et al., 1982), the concentration of anionic surfactants (method not given) exceeded 2 mg/L in the Han river at Seoul and increased downstream. The concentration of LAS-degrading bacteria was 10^2 to 10^3 cells/ml, and the species identified were Pseudomonas, Aeromonas and Enterobacter.

In the United States, similar changes have been observed. Lawton (1967) reported maximum levels of MBAS of 3.1 mg/L in the Root River of Milwaukee, and average levels of 1.04 mg/L which decreased to 0.06 mg/L. Similar changes were recorded on the Milwaukee River. The results generally show that MBAS levels decreased downstream in rivers and streams due to dilution and degradation, especially after the change from ABS to LAS. The addition of regional sewage treatment facilities with aeration systems to process wastes resulted in additional improvements in MBAS levels.

Brenner (1968) states in a general review that MBAS concentration changes in the United States since 1965 indicated surfactant levels had dropped; furthermore, foam incidents on natural waters were no longer a concern except in those areas where raw or partially treated sewage was introduced.

Sullivan and Evans (1968) have examined MBAS levels in the Illinois River during the period of 1959 to 1966 when the change from ABS to LAS occurred. They found that the mean loading of MBAS at Peoria, Illinois, decreased from a level of 15 to 20 tons MBAS/day in 1959-1965 (pre-LAS), to 9 tons MBAS/day in 1966 (post-LAS). The monthly mean MBAS concentrations at these times were 0.56 mg/L and 0.22 mg/L, respectively. This river system was re-examined in 1968 by Sullivan and Swisher (1969). They found that the mean MBAS levels had fallen to about 0.05 mg/L.

In an analysis of EPA STORET data for 1970 through 1979, MBAS concentrations for both ambient surface waters and effluent levels at publicly owned sewage treatment works, industrial discharges etc., were examined on a national level, by major river basin and by four states and a local area, selected randomly as representative of various regions of the country. There was no attempt to relate the retrieved concentration values to a point source. Instead, the focus was on MBAS values relative to the 0.5 mg/L level established for drinking water. Results of the retrieval are summarized below. It is important to reiterate, however, that MBAS values are not synonymous with LAS concentrations in the environment, but rather, reflect the presence of all anionic materials and/or any of a large number of inorganic and organic substances that interfere with this analytical method. Furthermore, one must temper STORET data with the fact that the reported MBAS values are for surface waters, not drinking water, and secondly, that STORET consists of raw data entries with no mechanisms of quality assurance for such data. A summary of this data can be found in Table IV-5.

TABLE IV-5

EPA STORET DATA FOR MBAS

Area	Level	Year
Minnesota	0.06-0.19 mg/L	1967-74
Mississippi River Delta	0-1.20 mg/L	1964-75
Oregon - Willamette River	0.02-0.07 mg/L	Before 1977
New York State	0.01-0.27 mg/L	1960-75
Northeast, North Atlantic and Southeast Basins	0.5-10 mg/L	1970-1979

Figure IV-1 illustrates that maximum and mean concentrations of MBAS detected in surface waters of the United States from 1970 to 1979. No overall trend is evident, but rather, year to year fluctuations reflecting such factors as rainfall, dilution, stream flow rate, etc.

Four states selected randomly from various regions of the country were examined for a view of MBAS levels from 1970 to 1979. Generally, each state reported mean values below 0.5 mg MBAS/L. An annual mean exceeded 0.5 mg MBAS/L in only one year for three statea during 1970-1979. The results are summarized below:

State	Range of Ambient Mean MBAS (mg/L) 1970-1979	Year Value Exceeded 0.5 mg MBAS/L	Effluent Mean Ranges (mg/L)
California	0.2 - 3.3	1976	0.1 - 2.6
Illinois	0.5 - 0.7	1972	- - - -
Louisiana	0.0 - 0.1	- -	- - - -
New York	0.0 - 1.4	1977	0.0 - 0.1

The New York City area was selected as a highly populated region to examine. A polygon was constructed around the metropolitan area to

retrieve station data aggregated annually over the ten-year period. Of the 800 sampling stations along the New Jersey Coast, Lower Hudson River and Long Island, no more than 1 percent had a year with mean MBAS values higher than 0.5 mg MBAS/L during the time period*. At the 173 effluent stations within the same polygon, 1 or 2 observations had been recorded at or below 0.5 mg/L.

An analysis of 316 randomly selected wells in an area outside the United States (metropolitan Tehran, Iran) during 1974-1975 indicated an average MBAS concentration within 95% confidence limits of 0.12 to 0.16 mg MBAS/L. The range was zero to 1.4 mg MBAS/L, thus exceeding 0.5 mg MBAS/L in some aquifers.

It should be noted, however, that no sewage system exists in Tehran and that detergents used in this area are mostly of the ABS type (Imandel et al., 1978).

An examination of MBAS levels in drinking water in New York from data collected by the U.S. Geological Survey from November, 1970 to April, 1972 reveals that MBAS values reported are far below the standard for drinking water (0.5 mg/L). No county mean exceeded 0.05 mg/L MBAS during the test interval.

New York State monitoring information on MBAS levels from the Department of Environmental Conservation indicates levels of MBAS in the upper Hudson River are significantly lower than those in the lower Hudson River at New York City. MBAS concentrations at Bethlehem, south of Albany, ranged from 0.01 to 0.10 mg/L with a mean value of approximately 0.05 mg/L from 1964 through 1973, with no significant changes from year to year. At the city of Yonkers, however,

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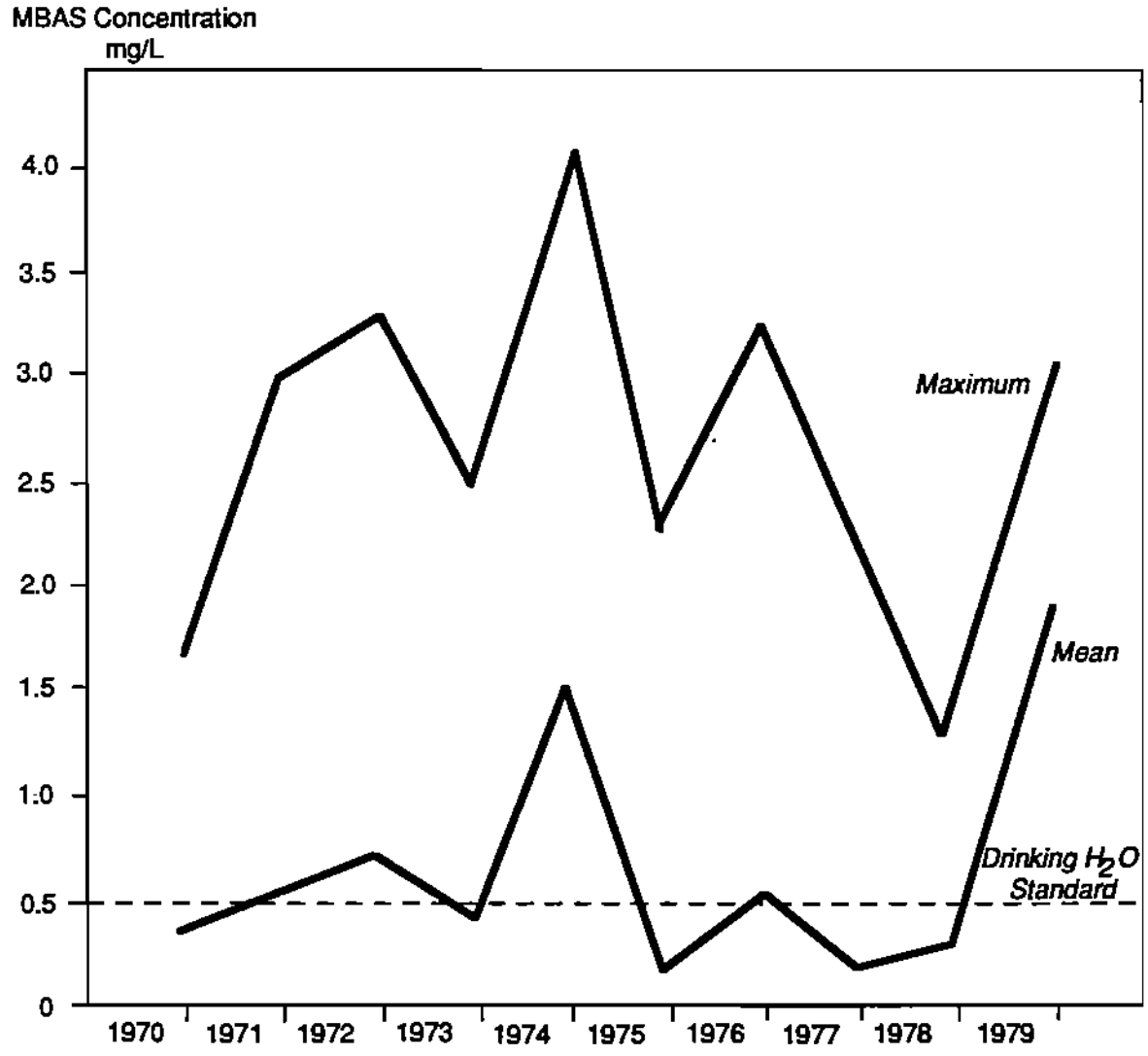
* The dimensions of the polygon encompassed the western half of Suffolk County, which probably biased the results downward.

Figure IV-1

Methylene Blue Active Substances

Maximum and Mean Ambient Concentrations
United States
1970 - 1979



Storet Water Quality Data System.
Public Health Drinking Water Standard, 1962

Number of observations during 1970-1979 period - 71286
Number of Stations - 7671

concentrations ranged from 0.03 to 1.80 mg/L, with a mean value of approximately 0.40 mg/L. The high MBAS levels were most likely associated with inadequate sewage treatment at the Yonkers site.

A 1971 examination of MBAS levels in waterways ranging from creeks to large rivers one mile above the below sewage outfalls revealed that in one set of samples from 447 sites in 48 states, the MBAS levels above and below sewage outfalls ranged from 0 to about 4.5 mg/L. Approximately 93% of the MBAS values from sites above sewage outfalls were <0.5 mg/L and approximately 80% of the sites below outfalls had MBAS levels of <0.5 mg/L. A second set of samples taken at a later time from several east and west coast states revealed a similar pattern with respect to MBAS levels above and below sewage outfalls and indicated a possible general decreasing trend in MBAS levels (Procter and Gamble Company, unpublished data).

Results of a 1978-86 U.S. monitoring survey indicated that the concentrations of LAS in river water below effluent outfall ranged from 0.01-0.3 mg/L. The concentrations less than 5 miles downstream were 0.026-0.15 mg/L while those greater than 5 miles downstream ranged from less than 0.005-0.12 mg/L (Rapaport et al., 1987).

b. Estuaries

In the mouths of the Elbe, Eider, and Ems Rivers in Germany, anionic surfactants were found by Bock and Mann (1971) to be as high as 0.3 mg MBAS/L. In the Elbe, surface samples yielded MBAS concentrations of 0.1 to 0.3 mg/L. Biodegradation measured by MBAS of over 97% within 14 days after discharge of surfactants into sea water and a corresponding increase in surface tension to normal levels were observed.

Sediments containing MBAS-reactive materials have been found at depths of up to 30 m in Tokyo Bay in Japan. Degradable detergents are reported to be stable in anaerobic environments, and apparently remain undecomposed for long periods of time (Ambe, 1973).

Hon-nami and Hanya (1980) determined the LAS/MBAS ratios in Tama River water and water from Tokyo Bay to be 0.4 to 0.85 and less than 0.2, respectively. A combination of gas-liquid chromatography and mass spectrometry were used.

Kikuchi and coworkers (1986) measured between 0.8 and 29.9 μg/L LAS in Tokyo Bay water, with the highest concentration detected in coastal areas. LAS concentrations of less than 0.2 to 69 μg/g (dry basics) for sediment and below 0.3 μg/g whole body (wet basis) for fish samples (Konosirus punctatus) were also reported. The method of detection was high performance liquid chromatography with fluorophotometric detection. In two Japanese bays, Katsuura and Moriura, the concentration ranged from 0.003 to 0.016 mg LAS/L (Arimoto et al 1980), and 0.008 mg LAS/L was measured in coastal waters near Hiroshima (Okamoto and Shirane, 1982).

The concentration and biodegradation of anionic surfactants in the Hudson estuary of the New York City area have been studied (Lever Brothers Company, unpublished data), to estimate MBAS, and specifically LAS, concentrations in saline waters as well as biodegradation rates at various salinities. In surface samples from the Hudson River estuary with salinities ranging from 1.3 to 23 parts per thousand, MBAS levels ranged from 0.02 to 0.19 ppm and were directly correlated at the sampling points (sewage outfalls) with the efficiency of sewage treatment of the effluents discharged into the estuary. The greatest municipal discharges were from the Westchester Sewage Primary Treatment Plant in Yonkers (63 mgd) and from the untreated Canal Street sewage outfall (23 mgd) which had MBAS levels of 0.150 and 0.110 mg/L, respectively, in surface water samples. Coliform levels at Canal Street outfall were as high as 11,400/100 ml. Outfalls from most other sewage treatment facilities had lower MBAS levels. In subsurface samples, MBAS levels tended to increase as did coliform counts. Subsurface concentrations of MBAS were as high as 0.190 mg/L near the Yonkers Plant and coliform counts were the highest in the estuary (170,000/100 ml).

In a subsequent investigation of the Hudson River estuary in 1975, the average MBAS level for surface waters from all 20 sites, including plants with no treatment, primary or secondary treatment, was 0.085 mg/L (Lever Brothers Company, unpublished data). The highest MBAS levels were recorded at the Ft. Lee, New Jersey outfall, 2.360 mg/L, and at the 114th street outfall (raw sewage), 0.706 mg/L. The lowest MBAS level was 0.007 mg/L at Haverstraw, New York (primary treatment). Total coliform counts ranged from 9,500/100 ml (primary treatment), to 20,000,000/100 ml. Fecal coliform counts ranged from 100/100 ml to 1,100,000/100 ml. The two stations with the highest fecal coliform count also exhibited the highest MBAS concentrations. Interstitial waters from bottom sediments had MBAS levels ranging from 1.145 mg/L to 0.017 mg/L with an average of 0.251 mg/L for all 20 stations, which is 3 times the average for MBAS concentrations in surface waters. Concentrations of MBAS in bottom sediments ranged from 11.4 ppm (dry wt. basis) to 57.5 ppm, with an average of 22.3 ppm, 90 to 260 times the interstitial and surface water concentrations, respectively. At the Lever Brothers loading pier, the MBAS concentration in the bottom sediment was 35 ppm with LAS (as determined by the Ambe IR method) being 42% of the measured MBAS of 14.7 ppm. Discharge from detergent processing and poor tidal circulation and silting at the Lever Brothers pier would lead to conditions resulting in deposition of detergent in bottom sediment. LAS could not be confirmed in any other bottom sediment. With multiple site sampling, it was observed that surface MBAS levels were influenced by stream current, tide flow and outfall configuration. Regression analysis for data on surface waters showed a high positive correlation between MBAS and total coliform counts, with no significant relation between MBAS and fecal coliforms. The reasons for this lack of correlation are not understood. MBAS also showed a significant negative correlation with dissolved oxygen levels. The presence of MBAS with high coliform counts in waters seems to indicate contamination from untreated sewage.

c. Groundwaters

A number of studies have investigated contamination of ground waters with ABS, presence of ABS in domestic water supplies, correlation of ABS persistence with other pollutant contamination, and coliform movement through soils containing ABS. Studies have not been as extensive for the fate and influence of LAS in ground waters.

Lawton (1967) reports that after the changeover to LAS there was a signifieant decrease in the MBAS content of well water in Wisconsin.

An extensive study was undertaken between 1961 and 1968 by the U.S. Geological Survey in conjunction with the Suffolk County Water Authority on the Suffolk County ground water resource because of the presence of surfactants in that resource. MBAS was present in the entire saturated thickness of the shallow aquifer in that county because of sewage discharge into the surface soils. Concentrations of MBAS in waters from shallow publie supply wells ranged from 0.1 to 1.3 mg/L, showing an upward trend from 1961 to 1966 and a general decrease by 1968. The major influences on the surfactant concentrations were aquifer flow lines, recharge rates from recharge areas and seasonal pumping. The lower aquifers did not appear to be affected, and MBAS levels in the water withdrawn were not greater than drinking water standards (0.5 mg/L) because of the mixing of waters from the deep and shallow aquifers (Perlmutter and Guerrera, 1970).

Cohn (1968) reported on the behavior of various surfactants at 6 sites in Nassau and Suffolk County, Long Island, Naw York. The sites included 3 cesspools, 2 septic tanks and 1 septic tank followed by a cesspool. Considering LAS, the reductions of MBAS from tha effluent sites to ground water plumas ranged from 20 to 38%. Thus, under the special ground water conditions existing in Long Island, neither cesspools nor septic tanks were sufficiently effective in the biodegradation of LAS.

Preliminary results from a study to determine the effects of septic tank effluents on ground water quality in Dade County, Florida, have shown that no MBAS values exceed 0.2 mg/L at 5 sites. Samples were analyzed downgradient and upgradient from each of the sites.

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V. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

Since the change in 1965 from tetrapropylene-derived alkylbenzene sulfonates (ABS) to linear alkylbenzenesulfonate (LAS) surfactants, there have been a number of research efforts investigating the environmental toxicity of LAS. Much of the research on surfactants prior to this time was concentrated on the relatively undegradable ABS surfactants. The existing laboratory derived toxicity information for LAS deals largely with acute studies, although investigations of chronic toxicity, pathological effects, and aquatic interactions have been reported. Even though many of the biological effects of branched-chain ABS surfactants have been investigated, their relevance to LAS is limited since the different chemical structure and biodegradation rates of LAS result in different biotic effects.

Determinations of the acute and chronic toxicity of intact LAS for aquatic organisms in the laboratory and the direct use of such values to establish water quality criteria do not reflect the actual situation in the environment. While LAS exhibits toxicity to a variety of marine and fresh water organisms in the laboratory, the likelihood of substantial concentrations of toxic LAS moieties occurring in natural waters is low because of facile biodegradation of LAS in waste treatment plants and in waterways. The homologs of LAS which exhibit the highest toxicity for aquatie species (longer chain lengths with more terminal phenyl group sites) are those which undergo the most rapid biodegradation. [See Section III of this report.]

For this reason, any assessment of the environmental safety of LAS should include information on the form and toxicity of LAS residues after biodegradation. Such information provides an appropriate perspective to consider any potential problems which may arise from the entry of LAS and its biodegradation products into the environment. Where available, information on the alkyl chain length and phenyl position is provided in this review.

1. Methodology

The most commonly accepted acute toxicity tests include the LC_{50} test, the TLm test and the LT_{50} test, all of which report the concentration of an agent required to kill 50% of the test population in a given period of time, usually 24-96 hours. The EC_{50} is the concentration required to induce a particular sublethal phenomenon in 50% of the test population over a specified length of time. This is generally the value reported in chronic studies.

Acute toxicity tests are conducted to estimate the concentration of material that has a detrimental effect on a certain fraction of organisms in a particular environment. Because the death of an organism is an easily observable effect and is related to the survival of representatives of the total population of a particular species, the lethal threshold or acute mortality test is most often used to determine whether toxic effects of a chemical or a mixture of chemicals could be elicited in natural ecosystems. The LC_{50} is defined as the concentration of an agent required to kill 50% of tested individuals in 24 to 96 hours and is the most common and acceptable method of determining acute toxicity (U.S. Environmental Protection Agency, 1975). The median tolerance limit (TLm) is the concentration that results in 50% survival over a certain time interval and is another way investigators have used to measure acute toxicity. It is usually equivalent to the LC_{50}. Toxicity can also be expressed in terms of the lethal time for 50 (LT_{50}) or 100% of the population at a particular concentration.

In measuring effects other than death of an organism, an EC_{50}, the concentration required to induce the measured phenomenon in 50% of the tested individuals in a specified length of time, is often used. In general, the EC_{50} and its sublethal effects are monitored in chronic studies. NOEC (No Observable Effect Concentration) and LOEC (Lowest Observed Effect Concentration) are other measures of toxicity routinely determined in chronic effects testing.

The difficulty of controlling environmental factors, the lack of concurrence between the results from different laboratories and the lack of studies at extreme concentrations make the evaluation of these acute toxicity data problematic in terms of extrapolation to set levels for aquatic safety. Concomitant histological examinations of organisms studied in acute toxicity experiments have not been widely performed, but would help to determine possible modes of action of toxic chemicals.

In an effort to standardize methods used for the assessment of acute toxicity of chemical agents to aquatic organisms, the Environmental Protection Agency supported an analysis of the problem by an expert committee. This group published its findings in the form of detailed protocols for the performance of acute aquatic toxicity studies (U.S. Environmental Protection Agency, 1975).

2. Intact LAS Structure-Activity Relationships

LAS toxicity is affected by chain length and phenyl ring position. A decrease in the carbon chain length of LAS surfactants is accompanied by a decrease in toxicity to fish, aquatic invertebrates and algae. LAS toxicity also decreases as the phenyl group is positioned farther from the end of the alkyl chain. Side products generated in the manufacture of LAS follow the same trends. However, the toxicity of these materials is considerably less than for LAS isomers.

The acute toxicities of the different types of LAS compounds vary according to the length of the alkyl chain and the position of the benzene ring on this alkyl chain. An increase in toxicity of LAS to various aquatic species with an increase in the length of the carbon chain has been documented by a number of investigators. The results

are summarized in Table V-1. Swisher et al. (1964) and Marchetti (1968) have also provided data to support this finding. Decreased toxicity at chain lengths longer than 16 carbon units has been observed in several studies, but the reason for this reduction is not known. LAS surfactants with chain lengths of 16 or more are not usually found in commercial products.

An abstract by Kikuchi and Wakabayashi (1984) revealed that "the lipophilic chain length affected LAS toxicity in Oryzias latipes," but no other details were provided. Oba et al. (1977) made a similar qualitative statement.

Initially, Hirsch (1963) showed that LAS toxicity increases as the phenyl group occurs closer to the end of the alkyl chain. Borstlap (1967) found that LC_{50} values for the guppy (Lebistes reticulatus) with dodecylbenzene sulfonate isomers with phenyl groups at the 2, 4 or 6 carbon of the alkyl chain were 3, 7 and 10 mg/L, respectively.

Divo (1974) examined the acute toxicity of individual LAS homologs and isomers to a fish species (unidentified) and confirmed that the toxicity of LAS increases with increased chain length and as the phenyl group is located nearer to the end of the chain.

Takita (1985) also confirmed this trend in algae. An inhibition of the sporlings of Porphyra yezoensis was noted following exposure to LAS (concentration not stated). The longer alkyl chain homologs and the other phenyl isomers showed higher degrees of toxicity than shorter chain homologs and inner phenyl isomers. Gas chromatography revealed that the more toxic components decreased more rapidly than others during biodegradation in sea water.

A study by Maki (1979c) reported 96 hr LC_{50} values for $C_{11.8}$ LAS and C_{13} LAS of 3.94 and 2.19 mg/L for Daphnia magna. No observed effect concentrations (NOEC) for 21 day exposure to these two materials were

1.18 and 0.57 mg/L, respectively. Furthermore, the 48 hr LC_{50} to Daphnia magna ranged from 29.5 mg/L for C_{10} LAS to 0.11 mg/L for C_{16} LAS. There was a slight, statistically insignificant decrease in toxicity to 0.12 mg/L with C_{18} LAS (Maki and Bishop, 1979).

The Procter and Gamble Company (unpublished data) also reported results for Daphnia exposed to LAS with chainlengths varying from C_{10} to C_{14} for 14 days. NOEC values were established. Review of a summary of this study confirmed previous findings of an increase in toxicity with an increase in chainlength (see Table V-2).

Holman and Macek (1980) reported 96 hr LC_{50} values for fathead minnows of 12.3, 4.1 and 0.86 mg/L for $C_{11.2}$, $C_{11.7}$ and $C_{13.3}$ LAS, respectively. No observed effect concentrations for embryos and larvae of this species were 5.1-8.4, 0.48 and 0.11-0.25 mg/L, respectively, in life-cycle tests.

Eggs and fry of the fathead minnow (Pimephales promelas) continuously exposed to LAS of varying chain lengths from 2 days after fertilization to 30 days after hatching showed toxicity was directly related to carbon length chain. The C_{10} LAS was the least toxic [minimum threshold concentration was between 14.0 and 28.0 mg/L] and the C_{14} LAS was the most toxic [minimum threshold concentration was between 0.05 and 0.10 mg/L] of the compounds tested. A commercial blend of surfactants with carbon chain lengths of 11 to 14 had a minimum threshold concentration between 1.02 and 2.05 mg/L indicating that the toxicity of surfactant blends containing relatively long carbon chains can be reduced by including in the blends surfactants of shorter chain lengths (Monsanto Company, unpublished data).

In the process of LAS manufacture, a number of side products are generated which remain in the final commercial detergent products. These compounds, dialkyltetralins, dialkylindanes and alkylnaphtalenes have not been extensively studied for their aquatic toxicity. Divo (1974) found increased toxicity with an increase in chain lengths among

TABLE V-1

ACUTE TOXICITY OF INTACT LAS. EFFECT OF CHAIN LENGTH

LAS Homolog	48-Hour LC_{50} (mg/L)[1] Fathead Minnow (Pimephales promelas)	96-Hr LC_{50} (mg/L)[6] Fathead Minnow (Pimephales promelas)	LC_{50} (mg/L)[2] Gold Fish (Carassius auratus)	LC_{50} (mg/L)[3] Guppy (Lebistes reticulatus)	LC_{50} (mg/L)[4] Goldorfen (Idus melanotus)	96-Hr LC_{50} (mg/L)[5] Bluegill Sunfish (Lepomis macrochirus)
C_{10}	43.0	100.0	61.0	50	16.6	21.2-47.5
C_{11}	16.0	28.0	22.5	-	6.5	11.6
C_{12}	4.7	6.0	8.5	5	2.6	1.18-6.5
C_{13}	0.4	2.4	3.3	-	0.57	1.11
C_{14}	0.4	0.6	-	1	0.26	0.25-0.42
C_{16}			0.087	1	0.68	
C_{18}			0.38		15	

[1] Kimerle and Swisher, 1977.
[2] Gafa, 1974.
[3] Borstlap, 1967.
[4] Hirsch, 1963.
[5] Procter and Gamble Company, unpublished data.
[6] Taylor, unpublished data

* LC_{50} values of individual LAS homolog dependent on phenyl group position. Lower LC_{50} values correspond to LAS with higher proportions of 2-phenyl isomers.

TABLE V-2

EFFECT OF CHAINLENGTH OF LAS TOXICITY TO DAPHNIA

LAS Chainlength	NOEC (mg/L)
C_{10}	9.8
C_{12}	4.9
$C_{12.6}$	0.9
$C_{13.1}$	0.8
C_{14}	0.1

Source: Procter and Gamble (unpublished data)

a series of tetralins with chains from 10 to 13 carbon units. Kimerle and Swisher (1977) confirm this trend with 48-hour LC_{50} values in fathead minnow (Pimephales promelas) of 86.1, 21.5 and 5.3 mg/L for C_{10}, C_{12} and C_{14} dialkyltetralinindane sulfonate mixtures, respectively. However, these side products are considerably less toxic than LAS isomers of comparable chain length.

3. Acute Toxicity

There appears to be little variation in the acute toxicity of LAS. LC_{50} values generally fall below 10 mg/L. The egg and fry stage of development are usually more sensitive to LAS than the adult. Histological examination of gills from fish exposed to lethal concentrations of LAS show a matted condition, with occasional blood masses and loss of gill mucosa cells. Sublethal concentrations of LAS effect locomotor activity and ventilation. Biodegradation of LAS reduces its toxicity by 10 to 100 fold with the most toxic components of LAS being most rapidly biodegraded. However, toxicity of LAS and its biodegradation products (as measured

by MBAS) are not so easily determined in actual environmental situations. LAS toxicity testing in invertebrate species produces results similar to results from fish studies. Sensitivity is greater in the early stages of development while adults are less sensitive. In Daphnia, the presence of food during testing reduces the toxicity of LAS. Sublethal concentrations of LAS to shrimp affect locomotor activity and respiration. LAS is also strongly irritating to worms. In algae, LAS toxicity varies between species with diatoms being more sensitive to LAS than bluegreen algae. Green algae are the least sensitive.

a. Fish

i. Intact LAS

In considering the reported data on the acute toxicity to fish of LAS and LAS-containing detergents, a number of factors should be weighed in the examination of experimental results, especially in relation to the use of these data to set aquatic safety standards. Abel (1974) has reviewed in detail the problems surrounding the assessment of toxicity of synthetic detergents to fish and aquatic invertebrates. In addition to wide variations in experimental protocols with respect to water temperature and chemistry and exposure patterns (static vs. continuous flow; water volume to organism mass ratio; nominal vs. measured test levels), the lack of adequate chemical characterization of the LAS samples tested and the wide range of susceptibilities among different aquatic species raise genuine difficulties for the comparative evaluation of acute toxicity studies.

In a series of continuous flow-through bioassays, Thatcher and Santer (1967) determined acute toxicities of an LAS preparation (SDA Interim Reference Sample: LAS Lot No. 1-1, 60.8% surfactant and 36.1% sodium

sulfate), to several species of freshwater fish (Table V-3). The authors contended that "sufficient difference in sensitivity to LAS exists among species of fish to warrant attention to this factor when assessing the potential hazard of LAS to aquatic populations."

TABLE V-3

ACUTE TOXICITY OF LAS TO FRESH WATER FISH

Species	Common Name	TLm (mg/L)	95% Confidence Limits (mg/L)
Notropis atherinoides	Emerald shiner	3.0	2.96-3.56
Lepomis macrochirus	Bluegill	4.0	3.70-4.30
Pimephales promelas	Fathead minnow	4.2	Not given
Notropis cornutus	Common shiner	4.9	4.58-5.18
Icatalurus melas	Black bullhead	6.4	6.08-6.68

Source: Thatcher and Santer, 1967.

Pickering (1966) investigated the effects of the same type of LAS preparation used by Thatcher and Santer (1967) on eggs of the fathead minnow (Pimephales promelas) in a continuous-flow bioassay. The results expressed as 9-day TLm (the concentration that results in 50% survival over a specified time interval) values for survival of hatched fry ranged from 2.3 to 2.6 mg/L in 4 replicate tests. The 1-day TLm value was 3.6 mg/L, with the threshold of mortality at 0.9 mg/L. The results with this species indicate that the egg and fry stages are more sensitive to LAS than are adults.

Dooley (1968) also examined the acute toxicity of this same LAS sample on mosquito minnows (Gambusia affinis). At an LAS concentration of 0.1% (1000 mg/L), the survival time for males was 9 minutes and for females, 17 minutes. As the concentrations of LAS were reduced, survival times increased until the populations exhibited 72-hour survival at a level of 0.00078% (7.8 mg/L). The gills of fish killed by LAS were damaged showing a matted condition, occasional blood masses and loss of gill mucosa cells.

TABLE V-4

ACUTE TOXICITY OF LAS

Species	Common Name	LAS	LC_{50} (mg/L) 6 hr	48 hr	96 hr	Authors
Lepomis macrochirus	Bluegill	$C_{11.8}$	-	-	1.67	Lewis & Perry (1979)
			-	-	6.5	Lubinski et al. (1974)
Salmo gairdneri	Juvenile Rainbow Trout	C_{10-15}	-	-	0.36	Brown et al. (1978)
Carassius auratus	Goldfish	-	-	-	6.2	Tsai & McKee (1978)
		C_{12}	8.4	-	-	Marchetti (1968)
		C_{14}	7.0	-	-	Marchetti (1968)
Phoxinus phoxinus	Minnow	C_{12}	-	6.0	-	Lundahl & Cabridenc (1978)
		C_{12}	-	6.4	-	Lundahl & Cabridenc (1978)
Salvelinus alpinus	Arctic Char	-	-	-	5 ppm	Olsen & Hoglund (1985)
Poecilia reticulatus	Guppy	-	-	-	5.6-10	Canton & Slooff (1982)
Oryzias latipes	-	-	-	-	10-18	Canton & Slooff (1982)
Misgurnus anguillicaudatus	Loach	-	-	12.6 ppm	4 ppm	Lee & Chin (1984)

The acute toxicity of LAS has been reported by numerous investigators (see Table V-4). Lewis and Perry (1979) reported a 96 hr LC_{50} for bluegill (Lepomis macrochirus) of 1.67 (95% CL: 1.58-1.77) mg/L using $C_{11.8}$ LAS. Brown et al. (1978) reported a 96 hr LC_{50} of 0.36 (95% CL: 0.25-0.5) mg/L for juvenile rainbow trout (Salmo gairdneri) exposed to C_{10-15} LAS in sewage effluent. Tsai and McKee (1978) found a 96 hr LC_{50} of 6.2 mg/L for goldfish (Carassius auratus) exposed to LAS. The 48 hr LC_{50} values for minnow (Phoxinus phoxinus) were 6.0 and 6.4 mg/L for two samples of C_{12} LAS (Lundahl and Cabridenc, 1978). Olsén and Höglund (1985) reported a 96-hr LC_{50} value of 5 ppm in 1-year old Arctic Char exposed to LAS. The 96 hour LC_{50} values of LAS in the fish, Poecilia reticulata and Oryzias latipes, were 5.6-10 mg/L and 10-18 mg/L, respectively (Canton and Slooff, 1982).

The LC_{50} values of LAS on the larvae of the loach, Misgurnus anguillicaudatus were 12.6 ppm for 48-hour exposure, 4 ppm for 96-hour exposure and 1 ppm for 120-hour exposure (Lee and Chin, 1984).

The fact that there appears to be little variation in the acute toxicity of LAS to fish was confirmed by Reiff et al. (1979). These authors tested several species of fish under varying conditions and found that the LC_{50} values for LAS ranged from 0.1-7.6 mg/L.

In addition to determining 6-hour LC_{50} values of 8.4 and 7 mg/L in goldfish (Carassius auratus L.) with C_{12}- and C_{14}-LAS products, respectively, Marchetti (1968) examined swimming activity. The concentrations of C_{12} and C_{14} LAS necessary to reduce swimming activity to zero in 6 hours in the test system used by Marchetti were 4.7 and 3.2 mg/L, respectively. In this study, the lethal effects and subacute effects on locomotor activity appeared to be related.

The effect of LAS ($C_{11.8}$ and C_{13}) on ventilation rate in juvanile bluegills was investigated by Maki (1979a). This author found that the lowest significant effect levels for 24 hr mean ventilation rates were 2.19 mg/L for $C_{11.8}$ LAS and 0.39 mg/L for C_{13} LAS (as measured concentrations). A two day exposure to 2.0 mg/L $C_{11.8}$ LAS resulted in

an increase in ventilation rate the first day which then decreased to the vicinity of control levels. Maki suggested that bluegill were exhibiting an acclimation response to LAS which is consistent with the work of Kimerle et al. (1979) demonstrating the facile metabolism of LAS by fathead minnows.

Lubinski et al. (1974) also examined the toxicity of LAS to the bluegill (Lepomis macrochirus) in a continuous flow bioassay and found a 96-hour LC_{50} value of 6.5 mg/L. They also developed an additive toxicity model based on fractions of the 96-hour LC_{50} values of each of the identified toxicants in the Illinois River. They speculated that the Illinois River water is not normally toxic to bluegills and that the major source of potential toxicity for fish would probably come from ammonia and cyanide, with LAS, copper, fluoride and zinc also contributing fractional toxicity.

ii. Biodegraded LAS

In the first systematic study of the toxicity of degraded LAS to aquatic organisms, Swisher et al. (1964) examined the effect of biodegradation on toxicity to bluegill (Lepomis macrochirus) fingerlings. They found that addition to continuous flow activated sludge units of as much as 100 mg/L C_{12}- or C_{14}-LAS resulted in effluents that did not exhibit any lethal toxicity. LAS concentrations analyzed in the test tanks ranged from 0.1 to 0.9 mg/L. Toxicity tests of effluents from acclimated, as well as unacclimated sludge yielded toxic levels of LAS at 1 to 2 mg/L. A minimally altered LAS, mixed isomers of sulfophenylundecanoic acid disodium salt, gave a 96-hour TLm of 75 mg/L indicating that even a single oxidative alteration of the alkyl chain of LAS is sufficient to markedly reduce toxicity.

Swisher et al. (1978) conducted acute toxicity tests on two anticipated biodegradation products of LAS in the fathead minnow (Pimephales promelas). Results revealed a 24 to 96 hour LC_{50} range of >1000 <1500 mg/L for sulfophenylundicanoic acid (C_{11}) and >24,000 <32,000 mg/L for sulfophenyl butyrate (C_4).

Borstlap (1967) found that the acute toxicity (minimum lethal concentration) for guppies (Lebistes reticulatus) decreased markedly from 5 mg/L to >1000 mg/L with the biodegradation of the commercial LAS product DOBS-C-300 (sulfonate of Dobane C®-300). Similar sharp reductions in toxicity of other commercial LAS products following their biodegradation have been reported for guppies (Poecilia reticulatus) and harlequins (Rosboral supp.) (Shell Research Ltd., London, unpublished data) as well as for rainbow trout (Salmo gairdnerii) (Unilever Ltd., unpublished data).

Cairns and Dickson (1973) have reported on a series of toxicity tests with intact and biodegraded LAS on bluegills and snails using high (HLAS) and low (LLAS) molecular weight products. The 96-hour LC_{50} value for intact HLAS was 0.72 mg/L. After biodegradation to 25% of the initial surfactant concentration (MBAS), the 96-hour LC_{50} for the bluegill was increased to 1.64 mg/L and to 2.3-7.2 mg/L with 92% degradation. Undiluted biodegraded HLAS resulted in LC_{50} values of 4.6 mg/L or less for 24 and 48 hours. At 50% and 90% biodegradation levels, 24-hour LC_{50} values were 5.0 mg/L or greater. A test using intact LLAS gave a 3.89 mg/L LC_{50} in 96 hours for bluegills. This LAS product also showed decreased toxicities for biodegradation products, with LC^{50} of 10.3 mg/L MBAS.

Divo and Cardini (1980) isolated the alkanoic acid derivatives of 2-sulphophenyl C_{13} LAS and 4-sulphophenyl C_{13} LAS in nearly pure form and tested them against Carassius auratus (goldfish). The 6 hour LC_{50} values for the 2 LAS isomers were 2.0 and 4.6 mg/L, respectively, whereas the corresponding alkanoic acids caused no deaths at 800 mg/L, the highest concentration tested, even after 48 hours. These results show the toxicity of the two intermediates were at least 500 and 200 times less than those of the two original LAS homologs.

Kimerle and Swisher (1977) obtained evidence that toxicity of a commercial LAS preparation (C_{12}-C_{14}) to Daphnia magna decreased from an LC_{50} of 3 mg/L for the parent product to a level of 6 mg/L for a

partially (50%) degraded product. Further biodegradation resulting in 80 to 90% removal of the initial concentration of MBAS produced LC_{50} values of 20 to 35 mg/L. Moreover, they showed that certain presumptive LAS biodegradation intermediates have little or almost no toxicity to either Daphnia magna or fathead minnow (Pimephales promelas) (Table V-5).

TABLE V-5

ACUTE TOXICITY OF LAS AND PRESUMPTIVE BIODEGRADATION INTERMEDIATES

	48-Hour LC_{50} (mg/L)	
	Daphnia magna	Pimephales promelas
Intact LAS-C_{11}	5.7 ± 0.6	16.0
Sulfophenylundecanoic acid, disodium salt (mixed isomers, 6-through 10-phenyl)	208 ± 85	76.6 ± 12.4
3-(Sulfophenyl)butyric acid, disodium salt	~6,000	~10,000
4-(Sulfophenyl)valeric acid, disodium salt	~5,000	~10,000

Source: Kimerle and Swisher, 1977

The preparation of a set of computations designed to predict the acute fish toxicity of complex mixtures of LAS from a knowledge of their molecular composition led Divo (1974) to several conclusions with respect to the relationship of biodegradation of LAS to toxicity:

- The most fish-toxic components of a LAS are also the ones most rapidly biodegradable.

- The biodegradation, even a partial one, reduces greatly the toxicity of the surface-active agent.

- Different LAS isomers with different initial fish toxicity tend to be reduced and to become equal upon biodegradation.

- The value of the LC_{50} of any LAS tends to increase considerably with the progress of biodegradation.

The work by Brown et al. (1978) is consistent with these conclusions. These authors found that the 96 hr LC_{50} for C_{10-15} LAS added to detergent-free control effluent and diluted with hard water was 0.36 (95% CL: 0.25-0.51) mg/L for juvenile rainbow trout. After achieving greater than 95% removal (as MBAS) in an activated sludge unit, residue and degradation products resulted in a nominal 4-day, LC_{50} of 29.5 (95% CL: 24.0-36.3) mg/L. This concentration is expressed in terms of the concentration of the surfactant in the influent to the activated-sludge treatment units.

Dolan and Hendricks (1976) reported similar reduction and eventual elimination of toxicity after treatment of LAS in acclimated sludge. These authors found that the TLm for intact LAS to bluegills was 0.85 mg/L for a 24-hr exposure. After the surfactant had degraded 76% (as MBAS), the 24-hr TLm was estimated at 1.4 mg/L. No deaths occurred in 24 hours after 92% biodegradation (as MBAS), but one death (of 10) occurred after 96 hours. A similar pattern was observed for snails (Gonobasis sp.), but the increment in 24-hr TLm values from 4.6 to 5.0 mg/L was not as distinctive. The authors suggested that the reduction in toxicity was associated with a change in homolog-isomer distribution.

Schoberl and Kunkel (1977) investigated the toxicity of various fractions of biodegraded LAS. Using 94-98% degraded C_{10-13} LAS (as measured by MBAS), the authors separated the residual surfactant from the non-surfactant metabolites (mainly sulfophenyl carboxylic acid). The surfactant fraction was tested on goldorfen (Leuciscus idus melanotus) at 10 mg/L and 20 mg/L; no mortality or other observable effects were seen by 48 hr. The non-surfactant metabolites were tested

at 100 mg/L and 200 mg/L; no effects were observed at 48 hours. A combination of 20 mg/L and 200 mg/L of the respeetive fractions was also found to have no effect in this time period. The intact C_{10-13} LAS had an LC_{100} of 4-5 mg/L during the 48 hr test.

Danvila (1977) reported biodegraded LAS to be less toxic to aquatic organisms than intact LAS. Furthermore, biodegradation of LAS in seawater, as measured by a reduction in toxicity to Artemia salina, was slower than in fresh water systems.

There has also been some assessment of the toxicity of secondary products of LAS manufacture. Table V-6 lists LC_{50} values for Daphnia

TABLE V-6

ACUTE TOXICITY OF DIALKYLTETRALIN SULFONATES TO DAPHNIA

Compound	Molecular Weight	LC_{50} (mg/L)
C_{10} tetralin sodium sulfonate	216	420
C_{11}	230	195
C_{12}	244	110
C_{13}	258	50
C_{14}	272	27

Source: Vista Chemical Company (unpublished data)

magna exposed to alkyltetralin sulfonates for 24 hours (Conoco, Inc., unpublished data). The secondary products are considerably less toxic than the corresponding intact LAS. The shorter carbon chain compounds are less toxic than the longer chain compounds, paralleling findings for LAS.

Swisher et al. (1978) also examined the toxicity of intermediate biodegradation products of LAS and found them considerably less toxic than LAS. In addition, these investigators found the products to be

less toxic than noted above and attributed this result to an increased purity of the samples tested. Table V-7 summarizes their results.

iii. MBAS in Sewage Effluents

The data summarized above clearly show that LAS is rapidly degraded in laboratory simulations of the activated sludge sewage treatment process. The biodegradation of LAS results in a 10- to 100-fold reduction in acute toxicity to fish. In the actual environment, the situation is considerably more complex because of wide variations in treatment of waste waters and in the extreme diversity of effluents reaching natural water bodies with respect to amount and characteristics of materials other than LAS. Thus, the toxicity to aquatic organisms of sewage effluents and waters containing these effluents cannot be readily attributed to LAS even though these waters contain MBAS. The problems surrounding the use of MBAS as an analytical tool for LAS in natural waterways, especially those which receive sewage effluents, have been considered above (Section II.B).

Esvelt et al. (1971), in a study of San Francisco Bay, determined toxicity to estuarine fish (golden shiner, Notemigonous chrysoleucas) related to sewage plant effluents and found significant reductions in MBAS levels after biological sewage treatment and concomitant reductions in toxicity. Toxicity attributable to MBAS in this study was difficult to separate from the overall toxicity of sewage effluents. There was a significant difference in concentrations of MBAS in effluents from primary and other more extensive treatment processes. Mean MBAS values in effluents from four primary treatment plants in the San Francisco Bay area were 10.9, 5.0, 7.2 and 7.3 mg/L. For activated sludge plants, the average was 1.1 mg/L, occasionally reaching levels of 6.7-7.3 mg/L. It was determined from a mathematical model that MBAS and ammonia nitrogen were significantly correlated with toxicity of primary effluents. However, the direct addition of LAS to primary effluents had little effect on its toxicity lending further support to the view that MBAS levels are not conclusive measurements related to acute toxicities of primary effluents.

TABLE V-7

CONCENTRATIONS (mg/L) AT WHICH TOXIC EFFECTS OF SULFOPHENYL CARBOXYLIC ACIDS WERE FOUND IN AQUATIC ORGANISMS

Effect	LAS C_{10-13}	3-sulfophenylbutyric acid, disodium salt	4-sulfophenylvaleric acid, disodium salt	3-sulfophenylheptanoic acid, disodium salt	sulfophenylundecanoic acid, disodium salt
Acute LC_{50}					
Daphnia (24-hr)	6.9	>22,000	~22,000	~12,000	2,000
Fathead minnow (96-hr)	4.6	~28,000			1,200
Chronic*					
Daphnia (4-wk)					
Survival*	>0.63	>2,000			>200
Reproductive*	>0.63	>2,000			>200
Fathead egg-fry (30-day)					
Effect**	2	>1,400			>52
No-effect**	1	>1,400			>52

*Minimum concentration at which effect was observed compared to controls.

**Effect on fry survival compared to control; egg hatchability and fry growth were less sensitive.

Source: Swisher et al., 1978

In a study at the Elm Farm Sewage Treatment Plant, which employs an activated sludge treatment process, it was found that MBAS levels contributed by LAS were generally reduced by greater than 95%. Testing of this effluent for toxicity in fathead minnows (Pimephales promelas) resulted in complete survival (Renn, 1974). In an effluent solution of only 36% MBAS removal, all of the tested individuals died. In this instance, MBAS concentrations were 11.5 ppm (Colgate-Palmolive Company, unpublished data).

Large concentrations of MBAS alone do not appear to exert any adverse effects on various fresh water fish. Rainbow trout, goldorfen, goldfish, bream, tench, loach, perch, carp, raffe, dace, chub, pike, rudd, gudgeon, stoneloach, spined loach and bullhead fish have been reported to survive, grow and breed in two small, artificially created lakes (Colworth Lakes). The main source of water in the lakes is sewage effluent which, due to the nature of the site, contains high MBAS levels (3 mg/L), higher than normal BOD and a large amount of total dissolved solids (Unilever Ltd., unpublished data).

An additional study indicating that toxicity to aquatic organisms of sewage effluents cannot readily be attributed to LAS was carried out by Calabrese and Davis (1967) with oysters (Crassostrea virginica).

Although not a fish study, the results are appropriately considered in this discussion. Effluents from treatment of sewage containing LAS degradation products equivalent to 20 mg/L of LAS had approximately the same toxicities to oysters with respect to survival of larvae, development of eggs and increase in length of larvae as an equal volume of effluent containing no degradation products. Thus, biodegraded LAS did not contribute products to the toxicity of the sewage effluents.

b. Invertebrates

Daphnia, a commonly tested invertebrate, showed no effect from exposure to LAS at concentrations less than 1 mg/L. The 24 hr LC_{50} value for

this species was 3.46 (95% C.L.: 2.31-5.22) mg/L (Shell Chemical Company, unpublished data). Petresa (1987) reported a 24 hours LC_{50} value for $C_{11.74}$ LAS as 10 mg/L.

Lewis and Perry (1979) studied the effects of water hardness on the toxicity of $C_{11.8}$ LAS to Daphnia magna. The 48 hr LC_{50} values decreased from 5.6 mg/L to 2.7 mg/L as water hardness increased from 35 to 340 mg/L as $CaCO_3$.

In order to determine the toxicity of LAS in Daphnia in relation to age, Barera and Adams (1983) conducted acute toxicity tests in Daphnia ranging from 6 hours of age to 216 hours of age. Results indicated that age did not greatly alter the EC_{50} values (measured effect not stated) and the maximum difference observed between the 6-hour and 216 hour age group was a factor of 3.9. The EC_{50} values ranged from 2.2-10.1 mg/L. It was concluded that daphnids up to 48 hours of age can be used to conduct static acute toxicity tests with no loss in the sensitivity.

Diet was shown to have a statistically significant effect on the sensitivity of Daphnia magna to LAS with the presence of food reducing acute toxicity (Taylor, 1985). The 48-hour fasted LC_{50} of $C_{11.8}$ LAS range between 3.6 and 4.7 mg/L while LC_{50} values for daphnids fed during the acute test ranged from 4.4 to 8.1 mg/L. Daphnia cultures receiving lower concentrations of food were less sensitive to LAS than Daphnia cultures receiving higher food concentrations.

Lal et al. (1983, 1984a) reported an exceptionally low 48-hour LC_{50}value of 0.013 mg/L in D. magna which could not be accounted for. A 40% mortality was noted in the control population and it also appeared that LAS in the free acid form may have contributed to the low LC^{50} value.

The 96-hour medium tolerance value for LAS in the clam, Tapes philippinarum, was 10.5 ppm (Seko and Ishii, 1981).

The effects of LAS (60.8%) on the oyster (Crassostrea virginica) have been studied by Calabrese and Davis (1967). At concentrations of LAS greater than 0.025 mg/L, the development of fertile eggs was reduced significantly and the percentage survival and growth of larvae decreased significantly at 1.0 and 0.5 mg/L, respectively.

Maki (1979b) exposed oyster embryos (Crassostrea virginica), juvenile pink shrimp (Penaeus duorarum), and blue crabs (Callinectes sapidus) to $C_{11.8}$ LAS in static bioassays. The 48-hr EC_{50} value (causing abnormal development) for oyster embryos was 7.4 mg/L. For juvenile shrimp and crabs, the respective 96 hr LC_{50} concentrations were 11 and 29.9 mg/L. Sublethal effects were noted for the shrimp and included increased locomotor and respiratory activity.

In an acute bioassay, Renzoni (1974) exposed gametes and larvae of the sea squirt (Ciona intestinalis) to C_{12} LAS. The resultant six hour LC_{50} value was 1 mg/L.

Swedmark et al. (1971), in a broad-ranging study of marine organisms, studied the effects of LAS (uncharacterized) on a number of marine bivalves and crustaceans. The LC_{50} values at 6 to 8°C are shown in Table V-8. Other than the cockle and scallop, adults of the species examined were markedly insensitive to LAS. These data parallel the findings in fish with respect to increased sensitivity of early developmental stages. At an LAS concentration of 5 mg/L for 6 hours, siphon retraction was completely abolished in the cockle, while the same exposure resulted in only a slight reduction in this response in the clam. Among the crustaceans, the swimming ability of larval stages of the spider crab and barnacle were reduced severely (100-fold) by LAS at a concentration of 10 mg/L.

As part of the chronic study on the effects of an LAS-containing detergent (LAS-14.0%, alcohol ethoxylate-2.3%, sodium soap-2.5%) on 3 invertebrate species, Arthur (1970) reported 96-hour TLm values for the amphipod Gammarus pseudolimnaeus and for the snails Physa integra and

TABLE V-8

ACUTE TOXICITY OF LAS TO MARINE BIVALVES AND CRUSTACEANS

Species	96-Hour LC_{50} (mg/L)
Mussel (Mytilus edulis)	>100
Clams (Mya arenaria)	70
Cockle (Cardium edule)	15
Scallop (Pecten maximus)	<5
Decapod (Leander adspersus)	50
Decapod (Leander squilla)	>100
Hermit crab (Eupagurus bernhardus)	>100
Spider crab (Hyas areneus), adult	>100
stage I zoea larvae	9
Shore crab (Carcinus maenus)	>100
Barnacle (Balanus balanoides), adult	50
stage II naupluis larvae	3

Source: Swedmark et al. (1971)

Campeloma decisum of 7, 9 and 27 mg/L, respectively, based on the LAS content of the detergent. The possible toxicity of other components of the detergent was not considered. Moffett and Grosch (1968) reported that brine shrimp (Artemia sp.) exhibited a 50% lethality in 22 hours following an 8-hour exposure to 5 mg/L LAS. Dolan et al. (1974) found the 96-hour LC_{50} to larvae of the mayfly (Isonychia sp.) to be 5.33 mg/L (Litchfield-Wilcoxon confidence limits, 4.23-6.72) for a well defined sample of LAS (C_{10}-13.2%, C_{11}-32.7%, C_{12}-37.9%, C_{13}-13.2%, C_{14}-3.0%)

The acute toxicity of LAS ($C_{11.8}$) in aquatic invertebrates showed the oligochaete, Dero, and the flatworm, Dugesia to be fairly sensitive with reported 48-hour LC_{50} values of 1.7 and 1.8 mg/L respectively. The isopod, Asellus, was insensitive to LAS, as shown by a 48-hour LC_{50} of 270 mg/L (Lewis and Suprenant, 1983).

The toxic effects of a 20% LAS solution (Parnol J liquid) in the plankton, Diaptomus forbesi, and the worm, Brachiura sowerbyi, were reported by Chattopadhyay and Konar (1985). The plankton was most susceptible to LAS as shown by a lethal concentration range of 0.005-0.574 mg/L (LC_5-LC_{95}). The lethal concentration of LAS in worms ranged from 0.06-2.305 mg/L. LAS was highly irritating and, upon exposure, the worms suddenly coiled and rubbed their bodies. Degeneration of the tail was observed, and in some worms only the head was found living.

Hendricks et al. (1974) found that a high molecular weight LAS (C_{13}, MW-362) was more toxic to the snail (Goniobasis sp.) than a low molecular weight LAS ($C_{11.6}$, MW-342). The respective 24 hr LC_{50} values were 19.4 (95% confidence limits of 14.6-38.9) and 92 mg/L.

Following a 72-hour LAS exposure to the snail, Lymnaea vulgaris, Misra et al. (1984) found a 34.4% decrease ($p < 0.001$) and a 37.7% decrease ($p < 0.001$) in ^{45}Ca uptake in living shells and in tissue, respectively. No change in calcium content was found in dead shells or in control snails. The authors suggest that detergent can affect the active exchange of calcium by inhibiting the activity of carbonic anhydrase, thus reducing the rate of shell formation and interfering with cellular metabolism.

c. Algae

Although algae are not aquatic fauna, their critical role as the lowest trophic level of the aquatic food chain makes their discussion appropriate at this point. Hall (1973) has examined the effects of

surfactants on phytoplankton and found that results from toxicity assays provide useful data for prediction of aquatic environmental safety. For the 3 species examined, Selenastrum capricornutum, Microcystis aeruginosa and Navicula seminulum, the 5-day minimum algistatic concentrations of LAS were 1000 mg/L, 500 mg/L and 50 mg/L, respectively.

The 4-day EC_{50} values of C_{12} LAS and C_{13} LAS in algae ranged from 0.9-29.0 mg/L and 1.4-116 mg/L, respectively (Procter & Gamble Company, unpublished data). Toxicity varied with species; diatoms were more sensitive to LAS than bluegreen algae. Green algae was least sensitive to LAS.

The LC_{50} range of LAS in the green algae, Selenastrum capricornutum, the blue-green algae, Microcystis aeruginosa, and the diatom, Nitzschia fonticola, were reported as 50-100, 10-20, and 20-50 mg/L, respectively (Yamane et al., 1984).

Dhaliwal et al. (1977) exposed the blue-green alga, Plectonema boryanum, and the green alga, Chlamydomonas reinhardi, to $C_{11.2}$ LAS concentrations from 1 to 30 mg/L in short-term bioassays. The growth rate was reduced at 30 mg/L for blue-green algae, and at 20 mg/L for green algae. No morphological changes were observed.

Takimoto et al. (1982) investigated the effects of LAS and its proposed metabolites on the growth of two marine algae, Skeletonema costatum and Heterosigma sp. Growth of Heterosigma sp. was inhibited at all concentrations tested (concentrations not stated) while S. costatum was stimulated at low concentrations and inhibited at higher concentrations (again, the duration and levels of LAS were not provided).

The short-term effects of surfactants on phytoplankton communities were reported by Lewis (1986). The effect of $C_{11.8}$ LAS on a phytoplankton community was compared to results achieved in conventional single species laboratory toxicity testing. LAS was tested at 0, 0.01, 0.02,

0.24, 0.8, 27 or 108 mg/L. An induction of growth stimulation was observed at the 0.24 and 0.8 mg/L LAS levels, but not at the higher LAS levels. Overall, based on abundance, the green algae were the least affected by LAS, followed by the blue-green algae, with the diatom being most sensitive.

Kondo et al. (1984) found that upon exposure to 10 mg C_{12} LAS/L, Thallassiosira pseudonana and Chorella pyrenoidosa have a lower photosynthetic activity and ATP content, and chlorella pyrenoidosa have a lower photosynthetic activity, decreased ATP and chlorophyll content. Kikuchi (1979) also reported a lowering of cell division and chlorophyll content with some cell death in colonies of Pleodorina californica exposed to low, unstated concentrations of LAS for an unstated period. Organelle disintegration and colony death was reported at higher LAS concentrations (levels unstated).

Pybus (1973) used a detergent containing a mixture of C_{12} LAS and C_{12} AES in a study with the alga, Laminaria saccharina. A concentration of 50 mg/L of the surfactant mixture prevented the swimming of algal zoospores after 7 minutes; after 30 minutes exposure to 5 mg/L, the zoospores were swimming normally. A concentration of 10 mg/L resulted in reduced zoospore settlement and a reduction in growth rate of mature algae. The effects of each individual component of the detergent were not studied on L. Saccharina.

Surfactants have also been studied for their effects on Gymnodinium breve, a red tide dinoflagellate, as a means to control its population. Hitchcock and Martin (1977) found that 0.025 mg/L of C_{13} LAS resulted in nearly 100% mortality of G. breve in 24 hours. Eng-Wilmot et al. (1979) reported C_{13} LAS to have no effect on G. breve at up to 0.1 mg/L, but was toxic at 0.15 mg/L. C_{12} LAS was 25% toxic at 0.08 mg/L in 20 hours at 25°C and 100% toxic at 0.1 mg/L.

d. Amphibians

LAS had an LC_{50} value for exposure to the South African clawed frog, (Xenopus laevis) of 5.6-10 mg/L (Canton and Sloof, 1982).

4. Subacute Toxicity

Subacute studies in fish show the gills and the locomotive muscles to be the major sites of LAS toxicity. Effects include reduced operculum movement, cellular changes, hemorrhaging, lethargy and decreased swimming endurance and locomotion. Low levels of LAS induce behavioral changes, such as a disruption in the avoidance response and alterations in odor perception and chemo-attraction. Early developmental stages, particularly the feeding sac-fry, are most susceptible to LAS toxicity. Egg fertilization is generally insensitive to LAS, except in the mussell, Mytilus edulis, where fertilization and early development were inhibited at concentrations of 0.05 and 0.1 mg/L, respectively. One early study reports gross developmental abnormalities in five invertebrates. However, the effects were not described and the results were never duplicated.

a. Systemic Effects

Chattopadhyay and Konar (1985a) studied the toxic effects of a 20% LAS-containing solution (Parnol J liquid) in the fish Tilapia mossambica. The lethal concentration of the detergent ranged from 1.276-1.750 mg/L. Observation of exposed fish revealed lethargy and a tendency to stay at the bottom of the tank. Fish frequently surfaced to gulp atmospheric air for 4-6 seconds, then ejected the air as bubbles before returning to the bottom of the tank. Operculum movement

nearly stopped before resuming suddenly in a rapid and labored fashion. Blood was observed at the base of the pectoral and pelvic fins and also on the head of fish exposed to high LAS concentrations. The maturity index of both male and female fish exposed to 0.25-1.10 mg/L LAS was significantly decreased, while fecundity decreased significantly only in fish exposed to 0.57 mg/L. Concentrations of LAS as low as 0.25 mg/L (19.6% less than the LC_{50} value) significantly reduced the growth rate of fish.

Examination of goldfish (Carassius auratus) gills exposed to LAS revealed marked edema on the respiratory epithelium of the secondary gill lamellae and hyperplasia of the global cell masses in the secondary gill lamellae after 10 days. Fish exposed to LAS for 30 days retained large quantities of mucus-like material in proliferated cells (Fukuda, 1983).

Several other sublethal effects have also been reported. Swedmark et al. (1976) found that the lowest median concentration of LAS to affect locomotion in cod (Gadus morrhua) was 0.5 mg/L as compared to the 96 hr LC_{50} of 1.6 mg/L. Tatsukawa and Hikada (1978) estimated a threshold of avoidance in ayu (Plecoslossus altivelis) of 0.11 mg/L for formulation LAS and 1.5 mg/L for pure reagent LAS.

Saboureau and Lesel (1977) tested the effect of sublethal concentrations of C_{10-15} LAS on the swimming endurance of rainbow trout. The 24 hr LC_{50} for this species was reported to be 1.9 mg/L. Swimming endurance was tested at concentrations of 0.2-1.9 mg/L LAS. At low concentrations (0.2-0.4 mg/L), the decline in endurance time was rapid. This decrease was slower for moderate concentrations (0.4-1.6 mg/L) and increased again at concentrations of 1.6-1.9 mg/L. The authors suggested that toxicity manifested itself differentially on the various muscle groups used in locomotion, depending on concentration.

Maciorowski et al. (1977) found that C_{18} LAS had no effects on the sphaeroid clam (Pisidium casertatum) at concentrations of 0.01 and 0.1

mg/L. Intestinal damage occurred at 1 and 10 mg/L with a loss of cilia, cell vacuolization, sloughing of column epithelia, and a marked reduction in cell size.

b. Behavioral Effects

Hidaka and Tatsukawa (1986) found that the avoidance response to LAS exposure did not vary between male and female medaka fish (Oryzias latipes). However, the avoidance of small fish to LAS was significantly lower than large fish indicating a size-dependent behavioral effect.

Behavioral toxicity tests in Arctic char were carried out in sublethal concentrations ranging from 0.02 to 2 ppm LAS (Olsén and Höglund, 1985). The investigators found that sublethal concentrations of LAS had a significant effect on odor perception at the lowest concentrations tested and disturbed the chemo-attraction in Arctic charr. This disturbance of olfactory senses was shown to be partly reversible.

c. Developmental Effects

Hokanson and Smith (1971) studied the toxicity of a well defined LAS sample (90% active; C_{10}-16.0%, C_{11}-33.5%, C_{12}-29.5%, C_{13}-18%, $\geq C_{14}$-2.5%) in Mississippi River water to various developmental stages of the bluegill (Lepomis macrochirus) ranging from sperm and unfertilized egg to fingerlings. They found that the feeding sac-fry were most sensitive to LAS; eggs and fingerlings exhibited intermediate sensitivity; egg fertilization was the least sensitive developmental step (Table V-9).

Arima et al. (1981) showed eggs of Cyprinus carpio to be highly susceptible to LAS at the initial stage of development (no additional information was provided).

TABLE V-9

EFFECTS OF LAS ON BLUEGILL (LEPOMIS MACROCHIRUS)

Development Stage	TLm (mg/L)	Median Response (mg/L)
Fingerling	3.80 (24 hr)	
Sac-Fry		
5-day	3.4 (24 hr)	
1-day	2.3 (6 day)	
Newly hatched fry	>5.6 (24 hr)	
Fertilized eggs*		3.7-4.0 (hatching)
Fertilization		10
Sperm		5.4-5.7 (active swimming - gyration)

*Eggs burst at LAS concentrations >4.0 mg/L.

Source: Hokanson and Smith, 1971.

Vailati et al. (1975) found that toxicity varied with exposure time and developmental stage. However, these investigators found that eggs of rainbow trout were the most resistant, followed by adults, and then by fry with partially-absorbed yolk sacs. The mean 24 hr lethal concentrations for these respective stages were 10.8, 2.2 and 1.0 mg C_{12} LAS/L. Over a longer exposure time, the eggs were the least resistant, with a 4-day mean lethal concentration of 0.12 mg/L.

In an extensive study of marine fishes and their responses to LAS, Swedmark et al. (1971) investigated 3 species; i.e., cod (Gadus morrhua L.), flounder (Pleuronectes flesus L.) and plaice (P. platessa L.). In continuous flow assays at 6-8°C, 96-hour LC_{50} values were 1.0, 1.5 and between 1.0 and 5.0 mg/L LAS, respectively. Tests conducted at 15-17°C gave 96-hour LC_{50} values of less than 1.0 mg/L for cod and plaice. As

for fresh water fish, early developmental stages were more sensitive than adults. Concentrations of LAS of 0.1 and 0.3 mg/L significantly reduced survival time of cod and plaice, respectively, in the stages from hatching to yolk absorption. Sublethal responses such as impaired swimming activity and breathing rate as well as reduced opercular movement were observed in cod after exposure to 0.5 mg/L LAS for 24 hours. In contrast, flounder were more resistant, exhibiting normal swimming behavior after 21 days in 0.5 mg/L LAS.

In a subsequent examination of the mussel (Mytilus edulis) by Granmo (1972), fertilization and early developmental stages were inhibited at concentrations as low as 0.05 mg/L and larval growth was depressed at an LAS concentration of 0.1 mg/L.

Moffett and Grosch (1967) have reported that LAS induces "gross developmental abnormalities" in larvae of 5 genera of marine invertebrates at 1 to 3 mg/L LAS. The genera studied were Arbacia (sea urchin), Asterias (starfish), Spicula (sponge), Chaetopteris (annelid) and Molgula (tunicate). The exact nature of the gross abnormalities was not described.

5. Chronic Toxicity

Reports indicate fish are relatively insensitive to long term LAS exposure. No effect was seen on five-week growth, egg production, hatchability, hatched egg survival, mean fry length or mean fry wet weight following exposure to LAS or LAS biodegradation intermediates. Developmental defects of the spine were reported following exposure to a 10% LAS-containing detergent, however, other components of the detergent were not evaluated. Review of limited data on invertebrates and algae indicate adverse effects to LAS. LAS was corrosively lethal to soft bodied zooplanktons and

bottom dwelling organisms while exposure of a 14% LAS-containing detergent to an amphipod produced an adverse effect and reduced the survival of the F_1 and F_2 progeny at concentrations as low as 0.2 mg/L. Little or no effect was reported in snails following long-term exposure to this detergent. Again, no other components of the detergent were evaluated for toxicity. Examination of algae exposed to LAS revealed cell wall defects and impaired photosynthesis.

a. Fish

i. Intact LAS

One of the first reports of LAS chronic toxicity to fish was by Bardach et al. (1965) who found that LAS at concentrations of 0.5 mg/L for 24 days resulted in damage to the chemoreceptors of the taste buds of yellow bullheads (Ictalurus natalis). The study of Pickering and Thatcher (1970) on the effects of LAS (LAS-60.8%) to fish remains among the first to examine the chronic toxicity of LAS. They examined a number of responses with the fathead minnow (Pimephales promelas) in continuous flow systems including 5-week growth, egg production, hatchability and fry survival. Five-week growth, egg production and hatchability were not affected by mean LAS concentrations up to 2.7 mg/L. In agreement with other studies, the fry were more sensitive than other stages with deaths occurring at levels of LAS of 0.63 mg/L or above, and the greatest sensitivity at 7 to 14 days. The authors noted that even during the two 96-hour static TLm tests that 80 to 90% of the LAS as measured by MBAS was lost. For chronic studies, difficulty was encountered because of the increasing efficiency of biodegradation even though a dilution device was used to feed LAS. Standard deviations of MBAS values in 7-day composite samples amounted to 25% of the MBAS values.

Effects on young carp kept for up to 125 days in 5 mg/L LAS included small reductions in red blood cell counts (13%) and hemoglobin (8%) by the end of the study. Mean corpuscular volume decreased 8-12% as early as day 7 while up to a 50% decrease in white blood cells was noted after 14 to 125 days. Fish lost their appetite, swam lower and became disinterested in their environment. There was little recovery when fish were returned to an LAS-free enviroment (Walczak et al., 1983).

A study by EG&G (1978) used fathead minnow eggs and fry in 15 day exposure to LAS. The results showed that hatchability of eggs and percentage survival, mean total length, and mean wet weight of fry were not affected at concentrations up to 0.63 mg/L LAS.

A NOEC value of 0.9 mg/L $C_{11.8}$ LAS was calculated for fathead minnows by Maki (1979c) in a one-year toxicity test. For larval minnows, however, survival was impaired at 0.74 mg/L. The no-observed effect concentration (NOEC) of LAS in the fish, Poecilia reticulata, was 3.2 mg/L (Canton and Slooff, 1982).

Woltering (1984) also reported the chronic effects of LAS on the fathead minnow. Fry was the most sensitive stage of development with the LOEC-NOEC (Lowest Observable Effect Concentration-No Observable Effect Concentration) range listed in Table V-10.

TABLE V-10

LOEC-NOEC RANGES FOR LAS IN THE FATHEAD MINNOW

	LOEC-NOEC
LAS Homologue	1.2 - 0.63 mg/L
$C_{11.2}$LAS Homologue	8.4 - 5.1 mg/L
$C_{11.7}$LAS Homologue	0.48 - 0.31 mg/L
$C_{13.3}$LAS Homologue	0.25 - 0.11 mg/L

Source: Woltering (1984)

Holman and Macek (1980) conducted life-cycle and embryo-larval toxicity tests with fathead minnows. The respective 96 hr LC_{50} values derived for $C_{11.2}$, $C_{11.7}$ and $C_{13.3}$ LAS were 12.3, 4.1 and 0.86 mg/L. NOEC (from life-cycle or embryo-larval tests) decreased similarly with chain length to 5.1-8.4 mg/L, 0.48 mg/L and 0.11-0.25 mg/L, respectively. Again, the embryonic and larval stages were the most sensitive to LAS. In the life cycle test, however, no effects were observed at 1.09 mg/L with the $C_{11.7}$ LAS.

The effect of 30 days' exposure of 4 species of fresh water fish to a detergent containing LAS (14%), alcohol ethoxylate (2.3%), sodium soap (2.5%) and inorganic salts was examined by McKim et al. (1975). Statistically significant reductions in the 30-day standing crop were noted (Table V-11).

The long term effect of a common household detergent containing 10% LAS (Teepol®) on the morphogenesis of the dulong Mirogobius lacustris Herre was studied by Vallejo (1986). Embryos were allowed to develop in 15 or 20 mg/L Teepol® until the yolk-sac larva stage. Bent body axes were observed in 25 and 37% of the embryos treated with the low and high dose of Teepol®, respectively. Spinal defects were associated with hemorrhage around the site of spinal curvature, vertebral fracture and tetany of spinal muscles. Almost total cessation of swimming activity occurred in deformed embryos. Other major effects of Teepol® included reduction or complete loss of lower jaw bones, desquamation of the body surface, and erosion of the fins. Vallejo theorized that the effect of Teepol® on the bone was due to an LAS-induced Vitamin C competition between collagen metabolism and microsomal-mixed function oxidase. This effect would result in an increased use of Vitamin C in the microsome mixed function oxidase system in order to detoxify the LAS contamination. Vitamin C in bone would decrease, thereby reducing the collagen content and making bones more fragile.

TABLE V-11

EFFECTS OF AN LAS-CONTAINING DETERGENT ON 30-DAY STANDING CROP*

Fish Species	LAS Concentration (mg/L) Showing Decrease in Crop ($P < 0.05$)
White sucker (*Catostomus commersoni*)	<0.5
Fathead minnow (*Pimephales promelas*)	0.5
Northern pike (*Esox lucius*)	0.5
Smallmouth bass (*Micropterus dolomieu*)	2.3

*Total weight of live fish at the end of 30 days divided by the original number of exposed larvae.

Source: McKim *et al.*, 1975

These studies, which approach the problem of the possible chronic toxicity of LAS to aquatic organisms, indicate that long-term exposure may result in toxic effects because of the increased sensitivity of early developmental stages; e.g., sac-fry and larvae, as compared to adult organisms. Concentrations of 0.63 to 1.67 mg/L were lethal to minnows in long-term experiments, while invertebrate survival was reduced at 0.4 to 4.4 mg/L. The NOEC value for fathead minnows was 0.9 mg/L; in embryo-larval life cycle tests, the NOEC ranged from 0.11 to 8.4 mg/L, depending on chain length.

ii. LAS Biodegradation Intermediates

Eggs and fry of the fathead minnow (*Pimephales promelas*) were continuously exposed to the LAS biodegradation intermediates, sulfophenylundecanoic acid (C_{11}) and sulfophenylbutyrate (C_4).

Exposures were initiated within 48 hours of egg fertilization and continued through thirty days post-hatching. Concentrations as high as 52 mg/L sulfophenylundecanoic acid or 1400 mg/L sulfophenylbutyrate had no effect on hatchability, survival or growth rate. Based on these results, the minimum threshold concentration (MTC) for fathead minnows and sulfophenylundecanoic acid and sulfophenylbutyrate were estimated to be >52.0 and <1400 mg/L, respectively (Monsanto Company, unpublished data).

b. Invertebrates

In Daphnia magna exposed to either $C_{11.8}$ LAS or C_{13} LAS for 21 days, the 96 hr and 21-day LC_{50} values were 3.94 and 1.67 mg/L, respectively, for $C_{11.8}$ LAS; and 2.19 and 1.17 mg/L, respectively, for C_{13} LAS (Maki, 1979c). Taylor (1985) reported the 21 day LC_{50} value to be between 2.2 and 4.1 mg/L LAS, while Woltering and Ritchie (1984) reported the 14 day LC_{50} value to be between 1.5 and 2.6 mg/L LAS. Woltering and Ritchie determined actual concentrations of LAS in the test solution which may explain the lower LC_{50} values reported. Canton and Sloof (1982) reported the 21 day LC_{50} value to be as high as 18 mg/L and a no-observable effect concentration (NOEC) for LAS in Daphnia as 10 mg/L.

Hatori et al. (1984) found that 30 mg/L C_{12} LAS had a significant effect on reproduction of D. magna. Braginshii and Shcherban (1985) reported that low concentrations of LAS (levels not stated) lowered the resistance of D. magna to attack by filamentous green algae, which overgrew daphnids in detergent-containing water, but not control water.

Maki and Bishop (1979) showed that acute toxicity of LAS to D. Magna was not affected by previous exposure to LAS at a level of 0.4 mg/L for a period of up to 7 generations.

In chronic testing (14 days) using river water Woltering and Ritchie (1984) confirmed that higher LAS homologs were more toxic. It was also found that toxicity of LAS to D. Magna was essentially the same in laboratory media and in surface water except for the effect of water hardness.

The 28-day no-observable-effect level of $C_{11.8}$ LAS to mysid shrimp was 0.38 mg/L (Procter & Gamble Company, unpublished data).

The chronic effects of LAS were studied in the aquatic ecosystem by Chattopadhyay and Konar (1985b). Water quality was not affected by LAS. However, the corrosively lethal action of LAS was shown in soft bodied zooplanktons and bottom dwelling organisms. Ostracods exhibited an avoidance reaction when detergent was present at high concentrations but appeared not to perceive the presence of the detergent at low concentrations, thus allowing absorption of the toxicants which resulted in severe body damage and death. Similarly, severe damage to the chirognomid population was seen at LAS concentrations of 0.38 mg/L or greater.

Arthur (1970) studied the effects of a detergent containing LAS (14%), alcohol ethoxylate (2.3%), sodium soap (2.5%) and inorganic salts. The results were reported in terms of LAS concentrations alone for the invertebrate test organisms which were the amphipod (Gammarus pseudolimnaeus) and 2 species of snails (Campeloma decisum and Physa integra). The organisms were exposed acutely followed by a 6-week exposure of survivors. Survival of Physa was unaffected at LAS concentrations up to 4.4 mg/L, whereas Gammarus was affected at 0.4 mg/L and Campeloma at between 1.9 and 4.4 mg/L. Survival of F_1 and F_2 progeny of Gammarus was reduced at the lowest LAS concentration employed (0.2 mg/L). The possible toxicity of detergent components other than LAS was not considered.

c. Algae and Aquatic Plants

Electron-microscopic examination of the freshwater algae, Scenedesmus quaurdicauda, exposed to levels of LAS above 0.05% revealed various cell wall defects. Fine warts were found along the cell wall surface and empty cells with gaps between adjoining cells were also evident. At higher LAS levels (up to 0.2%) cell damage was more extensive (Chawla et al., 1986).

Further investigation in Scenedesmus quaurdicauda by Chawla and Viswanathan (1987) revealed LAS to be a growth promoter at doses as low as 0.02%. However, toxicity did manifest at the 0.1% treatment level with altered functional organization in the chloroplasts and impaired photosynthesis being most prevalent. Additional testing suggested chlorophyll synthesis to be a major target of toxicity.

Lewis and Hamm (1986) evaluated the effects of $C_{11.8}$- and C_{13}-LAS on plankton photosynthesis and the effects of changing environmental conditions in a lake ecosystem during a 2-year study. Algae studied included the green algae Selenastrum capricornutum, the blue-green algae Microcystic aeruginosa, and the diatom Navicula pelliculosa. The photosynthetic response to each surfactant varied as much as 40.5x depending on monthly changes in water temperature (range = 17-28°C) and seasonal differences in phytoplankton composition. The lake photosynthesis 3-hour EC_{50} values for $C_{11.8}$ and C_{13}LAS averaged 3.4 and 1.9 mg/L, respectively. In a laboratory growth study used for comparison, the 96-hr EC_{50} value for $C_{11.8}$LAS was 29.0 mg/L and the value for C_{13}LAS was 116.0 mg/L. Overall, LAS was less toxic during periods of lower water temperature, diatom dominance and low algae density.

Seven-day EC_{50} values in duckweed (Lemna minor) were determined by Bishop and Perry (1981). The median effect concentration of $C_{11.8}$ LAS to reduce frond count, dry weight, and root count was 2.7 mg/L. Labus and Kohler (1981) also found aquatic macrophytes more sensitive to LAS than terrestrial plants.

6. Mode of Action

The exact mechanism of action of LAS in fish is not known but gill damage and loss of gill function appear to be the major cause of death which may be due all or in part to LAS exposure. Numerous theories exist on the mode of action of LAS. Earlier work showed LAS to form LAS-protein complexes when in contact with gill protein which impaired gill function and caused death from oxygen deficiency. More recent work shows LAS to interfere with energy requiring respiratory mechanisms in the gill epithelium and inhibit adrenergic dilatation in the gill vasculative. Changes in skin structure have also been reported following LAS exposure and include increased active mucous cells and enhanced mucous secretion which result in impaired activity of respiratory enzymes in the epidermis and alterations in skin metabolism. This increased mucous production is also responsible for the reduction in swimming speed of fish following LAS exposure. Other studies show LAS to alter the permeability of cell membrane. Whatever the mechanism, the effect appears to be a decreased selectivity of the cell membrane. In fact, in algae, disruption of the cellular membrane is considered the critical factor for detergent toxicity.

The means by which LAS exerts a toxic effect on fish and other aquatic organisms is not known in detail. The effects of LAS on gill tissue in fish have been recognized from the earliest studies (Swisher et al.,

1964), and alterations in gill structure have been found in surviving fish at doses of 0.18 mg/L (Brown et al., 1968). Thus, while gill damage is a consistent finding associated with LAS toxicity to fish, it is not clear that this gill damage is responsible for the death of organisms. Abel (1974) has considered the problem of the mode of action for the toxicity of surfactants to fish and he states that "it remains to be established that death is primarily caused by loss of any gill function and not by some form of internal poisoning to which gill damage may be only a contributing or complicating factor."

Several studies hava proposed theories for the mode of action of LAS in aquatic species. Tomiyama (1974) observed that addition of extraneous protein to the test medium delayed LAS-induced death, auggesting that toxicity under normal test conditions may be due to the formation of an LAS-protein complex upon LAS contact with gill protein.

In subsequent work, Tomiyama (1978) postulated an initial formation of complexes of surfactanta (RSO_3 er RSO_4 groups) with protein components in the gills, resulting in impairment of gill function and death due to oxygen deficiency. This author tested his theory using several different approaches. He expoaed fish to LAS and then took an erythrocyte count, which is inversely correlated with oxygen availability. He found an increased erythrocyte count in fish that died upon exposure to LAS. In addition, by using various proteins, he found that previous formation of surfactant complexes can prevent formation of a complex with the gills, thus reducing toxicity. Complexation appears to be promoted in hard, acidic water.

The effect of LAS on respiratory enzyme activity in the gills of Heteropneustes fossilis (Bloch) was studied by Zaccone et al. (1985). Exposure to 1.0 or 2.5 mg/L LAS resulted in localized swelling and progressive separation of the lamellae. Other deleterious effects on the gills included vascular stasis and hemorrhaging. A marked decrease in succinate dehydrogenase, malate and isocitrate dehydrogenase activity was noted within 48 hr of exposure, while lactate dehydrogen-

ase activity was elevated. Results of this study indicate that LAS interferes with energy-requiring respiratory mechanisms in the gill epithelium. Elevated lactate dehydrogenase levels also reveal the possible presence of anaerobic pathways which represent an adaptive response to lower oxygen consumption. The specific mechanism of action of the mitochondrial enzymes and the anaerobic pathway are not currently known.

LAS has been shown to inhibit adrenergic dilatation in the gill vasculature of fish at concentrations of 1 mg/L (Bolis and Rankin, 1980) and 100 μmole/L (Pârt et al., 1985). Bolis et al. (1984) expanded these findings by examining the effect of 1 mg/L LAS on two peptide-mediated actions in gill blood flow. Both vasoactive intestinal polypeptide (VIP), which promotes vasodilation of perfuse trout gills, and β-endorphin, which potentiates the vasodilatory effects of noradrenaline, were studied. Little effect was noted on the vasodilatory response of the arterio-arterial pathway to VIP but a large reduction in the vasodilatory response to noradrenaline occurred. Noradrenaline response was almost completely inhibited following 48 hr of LAS exposure. Addition of β-endorphin greatly reduced the effect of LAS on nonadrenaline. These results show LAS inhibits vasodilation at the adrenergic level and had no effect on vasodilation at the arterio-arterial pathway. Stagg and Shuttleworth (1986) found only β-adrenergic receptors inhibited following LAS perfusion of the gills of the marine teleost, Platichthys flesus L. No effect was seen on the α-receptors.

Operculum movement and schooling patterns were studied by Lal et al. (1984) as an index for behavioral toxicity in control and detergent exposed 90-day old fish fingerlings (Cirrhina mrigala). Opercular movement and lactic acid content were significantly increased ($p < 0.02$ ppm and $p < 0.05$ ppm, respectively) in detergent exposed fingerlings. No marked change in schooling pattern was noted. Muscular expansion and contraction of the buccal and opercular cavities maintain a flow of water over gill surfaces in fishes. Respiratory

movements are under physiological control of the respiratory centre and are modified by both internal (O_2 consumption, CO_2 concentration, pH) and external (temperature, O_2 depletion) factors. The increased rate of opercular movemant and the decrease in dissolved oxygen shown in detergent-exposed fingerlings indicates physiological stress. This stress leads to the accumulation of lactic acid in the gills along with an increase in lactic acid dehydrogenase activity. Detergent stress also apparently leads to a higher metabolic rate and additional energy expenditure.

Other work in this area has pointed to an effect of LAS on the permeability of the cell membrane. Manner and Muehleman (1976) found that the diffusion and uptake of tritiated uridine through the chorion in fathead minnow was reduced upon exposure to 15 mg/L $C_{11.2}$ LAS. These authors hypothesized that surfactants adsorb to the cell membrane, and thus depolarize it and affect transport.

The physiology of rainbow trout gills exposed to lethal concentrations of LAS (100 μmoles/L) was described by Pärt et al. (1985). Exposed gills were severely damaged with swollen epithelial tissue lifting away from the underlying basal membrane. Secondary lamellae were fused and edematous, which were attributed to impaired oxygen diffusion capacity. At concentrations of 0.2 μmoles/L, LAS inhibited adrenergic dilatation in the gill vasculature. This increased vascular resistance was thought to be the result of the surfactant inhibiting the action of adrenalin in the gill. A markedly negative Na+ net flux was also shown in the presence of LAS which indicated an increased Na+ efflux from the gill and corresponding increase in ion permeability.

Jackson and Fromm (1977) also found that treatment with LAS affected permeability. They exposed isolated gill arches of rainbow trout to 5-100 mg/LAS, and observed an exponential increase in uptake of tritiated water. They concluded that LAS either interacted with the mucous coating of the gill, directly affected the epithelium, or both. Since the mucous serves as a diffusion barrier, they suggested that

increased uptake of water would occur. This increased uptake of water might not be lethal in itself, but these authors proposed that it could burden the kidneys.

Epidermis mucous of fish regulates swimming speed by controlling the hydrodynamic resistance of the skin surface. Mucous can also act as a defense against pathogenic organisms and is associated with osmoregulation. Misra et al. (1987) found an increased number of active mucous cells and enhanced mucous secretion in Cirrhina mrigala fingerlings exposed to 0.005 ppm LAS (25% of the LC^{50}).

Examination of the epidermis of catfish, Heteropneustes fossilis (Bloch), revealed both histological and biochemical changes attributable to sublethal concentrations of LAS (Zaccone et al., 1985). The number of mucous goblet cells increased causing an increase in mucous secretion and a thickening of the mucous covering the skin surface. Club cell rupture in the mid-epidermis was also evident. LAS was shown to impair the activity of respiratory enzymes in the upper epidermis which may have injured the overall metabolism of the skin. Biochemical studies revealed that the pentose phosphate pathway increased at the expense of the citric acid cycle in order to cope with the metabolic requirements of the epidermis for the increased mucous synthesis.

The fine structure of the head epidermia of juvenile rainbow trout (Salmo gairdneri Richardson) expoaed to LAS was studied by Pohla-Gubo and Adams (1982). Fish were exposed to 1.0 or 2.5 mg/L LAS and were examined electron-microscopically after 24, 48, 96 or 192 hours. Cell death and altered structure of the mitochondria were both prominent in treated fish. Only fish treated with the low dose of LAS and allowed a regeneration time of 8 days showed a decrease in epidermal damage.

With respect to the possible importance of internal levels of LAS in fish and their relationship to the toxicity of LAS, there is a large gap in information. Bellassai and Sciacca (1973) have measured MBAS

levels in the tissues of a variety of marine organisms using a procedure designed to minimize interference. Among the fish examined, mullet (Mugil cephalus) and boba (Boops boops) had a clearly demonstrable MBAS content (1 to 2 mg/kg). These fish are usually found near shore, especially mullet which are often found in muddy coastal waters near sewage outfalls. Several other species had MBAS contents ranging from 0.01 to 1.0 mg/kg, with the food fish mackerel (Scomber scombrus) and sole (Solea vulgaris vulg.) in this group.

Specimens of mollusk commonly used as a food in Italy, mussel (Mytilus edulis), taken from a polluted ocean site after 12 hours immersion, had MBAS in their flesh (1.4 to 1.8 mg/kg). However, if the exposed mussels were removed to clean sea water for 1 or 2 days, tests for MBAS were either negative or only weakly positive. A companion assay performed on mussels bought in a market gave a positive test for MBAS (0.5 to 1.2 mg/kg). While the tissue levels of MBAS were considered to be quantitatively insignificant, their presence was taken as an indicator of a wider problem of inadequate waste water treatment and possible microbial pollution. The question of the possible importance of the MBAS levels found in the various species with respect to toxicity to the organisms themselves was not addressed.

Kimerle et al. (1975) have considered the problem of acute toxicity and bioaccumulation of LAS and their relation to partition coefficients. In Daphnia magna, the acute toxicity of pure LAS homologs as well as commercial blends of LAS was correlated with the octanol:water partition coefficient as a direct logarithmic function. Further, bioaccumulation measured using a ^{14}C-benzene-labeled LAS varied with partition coefficient in a similar fashion in this organism as well as in fathead minnows (Pimephales promelas) when accumulation in whole fish was considered. The analysis of specific fish tissues indicated wide variations from levels of 0.4 to 4.0 $\mu g/g$ in muscle to 1000 to 3000 $\mu g/g$ in gall bladder. Direct analysis of the ^{14}C-residues in the gall bladder showed that less than 5% was in the form of LAS, with the remaining material presumably occurring as shorter alkyl chain

carboxylates. The introduction of Daphnia and minnows to clean water resulted in a clearing of the ^{14}C activity within 3 days.

Biedlingmaier et al. (1987) found that the disruption of the cellular membrane is the critical factor for detergent toxicity. Some green algae, such as Chlorella fusca 211-8b and 211-11n, Scenedesmus obliquus 276-3a and Scenedesmus communis 276-4b, all have a thick cell wall and a continuous sporopollenin layer which appears to increase their resistance to C_{10} LAS by 10 to 100 times. The sporopollenin layer is thought to act as a protective coat which prevents the chemical attack of detergents on algal cells.

While a consensus has not yet been reached as to the mode of action of LAS in aquatic species, it seems clear that the gills are affected in some way. Whatever the mechanism, the effect appears to be decreased selectivity of the cell membrane.

7. Effects of Environmental Conditions on Toxicity

Many environmental factors influence the toxicity of LAS. Among the most dramatic is water temperature. Investigators have shown an increase in temperature greatly increases the toxicity of LAS. Also, LAS toxicity increases as the oxygen tension in the test solution is reduced. LAS was more toxic in hard water than soft water due to an acute change in permeability of gill tissue. Therefore, hardness of culture water may effect LAS toxicity. A more-than-additive toxic effect exists when LAS is combined with zinc, copper and mercury. Addition of solids decreased the toxicity of C_{14}-C_{18} LAS but had no effect on the toxicity of LAS with shorter alkyl chains. Likewise, exposure of Daphnia to LAS for 7 generations had no effect on toxicity response.

The toxicity of a chemical in an aquatic environment, natural or experimental, is dependent on physical, chemical and biological conditions. Different species of fish exhibit various degrees of tolerance to toxic pollutants depending on temperature, water hardness, dissolved oxygen and heavy metals.

A number of investigators have noted the dramatic effects temperature changes exert on the acute toxicity of LAS to a variety of organisms. Marchetti (1968) reported an increase with temperature in lethal threshold of C_{12} and C_{14} LAS preparations to goldfish (Carassius auratus). Upon raising the temperature from 15 to 28°C, the lethal threshold at 250 minutes was altered from 9.3 to 1.8 mg/L for the C_{12} LAS and from 12.9 to 0.1 mg/L for the C_{14} LAS. Swedmark et al. (1971) observed a similar effect in several species of marine fish and invertebrates when the temperatures for acute toxicity testing were raised from around 6 to 8°C, up to 15 to 17°C.

In a study which examined a number of factors influencing the toxicity of LAS to the bluegill (Lepomis macrochirus), Hokanson and Smith (1971) did not observe any statistically significant increase in toxicity of LAS in raising the test temperature from 15 to 25°C. However, there was an increased number of lethalities at short exposure times at the high temperature. These authors also examined the effect of dissolved oxygen and water hardness on toxicity. For both fingerlings and sac-fry, the sensitivity to LAS increased as the oxygen tension in the test solutions was reduced. For fingerlings, the 48-hour TLm value at 7.5 mg/L of oxygen was 2.2 mg/L LAS, while at 2.0 mg/L of oxygen the TLm dropped to 0.4 mg/L LAS. With respect to water hardness, the toxicity of LAS to bluegills was greater in hard water than in soft.

Eyanoer et al. (1985) found that LAS was more toxic to Puntius gonionotus Bleeker fingerlings than detergents which degrade slowly. The 96-hr LC_{50} values of LAS were 13.6, 11.8 and 11.4 mg/L in soft, moderate and hard water, respectively, while the 96-hr LC_{50} values of a slower degrading ABS detergent were 24.7, 13.6 and 10.5 mg/L in soft, moderate and hard water, respectively. These results show that in soft

water, LAS was significantly more toxic than ABS while in hard water, both detergents had a similar acute toxicity value. Fish in hard water absorbed more anionic detergent due to an acute change in permeability of gill tissue. The immediate cause of death from acute detergent poisoning where extensive gill damage occurred was due to either asphyxiation or loss of osmotic or ionic stability.

Gafa (1974) reported a decrease in the LC_{50} of a C_{12} LAS to goldfish (Carassius auratus) from 15.0 mg/L at 0 degrees of hardness (mg $CaCO^3$equivalency unknown) to 5.7 mg/L at 50 degrees of hardness.

Lewis and Perry (1979) showed that the 48 hr LC_{50} to Daphnia decreased from 5.6 to 2.7 mg/L as hardness increased from 35 to 340 mg/L $CaCO_3$. Holman and Macek (1980), however, found water hardness was not a particularly significant factor in determining chronic toxicity to fathead minnows. In looking more closely at this effect, Maki and Bishop (1979) found that toxicity in Daphnia magna was related to the hardness of the culture water as well as the test water. In hard test water (350 mg/L as $CaCO_3$), toxicity was independent of culture conditions; 48 hr LC_{50} values ranged from 3.2 to 4.0 mg/L. In soft test water (25 mg/L as $CaCO_3$), toxicity was related to the hardness of culture water (see Table V-12). This finding may explain previous reports which sometimes showed contrary results.

TABLE V-12

THE EFFECT OF CULTURE WATER HARDNESS ON LAS TOXICITY IN DAPHNIA

Culture Water	Hardness (mg/L $CaCO_3$)	48 hr LC_{50} (mg/L)
soft	50	7.1
moderately hard	125	4.2
hard	225	2.0
very hard	350	1.8

Source: Maki and Bishop, 1979

Brown et al. (1968) have examined the effects of zinc on the toxicity of LAS to rainbow trout (Salmo gairdnerii). The 72-hour LC_{50} for LAS was 0.5 mg/L when tested in the absence of zinc. However, the administration of LAS with 0.8 mg/L zinc resulted in an LC_{50} of 0.33 mg/L. For copper and mercury, Calamari and Marchetti (1973) report a "more-than-additive" effect with LAS in rainbow trout in 24-hour and 14-day LC_{50} studies.

Maki and Bishop (1979) also studied the effects of suspended solids on the toxicity of LAS to Daphnia magna. They found that the addition of 50 mg/L suspended solids (as kaolin) significantly reduced toxicity for C_{14} and C_{18} LAS, but had no effect on C_{11} LAS. For example, the 48 hr LC_{50} for C_{14} LAS in Daphnia magna was increased from 1.0 to 1.4 mg/L with kaolin, and for C_{18} from 0.09 to 0.18 mg/L with kaolin. There was no decline in toxicity for C_{11}.

The effects of previous exposure to LAS on toxicity was studied by Maki and Bishop (1979). Daphnia magna were exposed to 0.4 mg/L $C_{11.8}$ LAS for up to seven generations. The 48 hr LC_{50} value for D. magna was 2.6 (95% CL: 1.8 - 3.3) mg/L for cultures exposed for seven generations, and 3.0 (95% CL: 2.0 - 3.8) mg/L for cultures not previously exposed to LAS.

8. Bioaccumulation

LAS is readily absorbed through the gills and body surface, and is distributed via the blood to various organs and tissues. As time passes, the majority of the LAS accumulates in the gall bladder and the hepatopancreas. Clearance of LAS is usually rapid with a half life of 2-3 days. The uptake of LAS is much greater at low levels than at high levels of exposure, and usually short chain-LAS are accumulated less than long-chain-LAS.

Kikuchi et al. (1978) examined the uptake of radiolabelled C_{12} LAS in carp exposed to 1.1 mg/L. They found that LAS was rapidly taken up through the gills and body surface, distributed via blood to various tissues and organs, transported to the hepatopancreas, and subsequently carried to the alimentary canal with the bile. Bioconcentration factors (BCF) of 40, 1.7 and 0.5 were observed in the gills, hepatopancreas and gall bladder, respectively, at 2 hours. After 24 hours, the BCF decreased to 13 in the gills and increased to 9.7 in the hepatopancreas and 1000 in the gall bladder.

Kimerle et al. (1979) reported an LAS bioconcentration factor (BCF) of 104 for the bluegill fish. LAS accumulation was greatest in the gall bladder which had a BCF of 5100.

In a study assessing the relationship between the exposure concentration of LAS and its concentration in the carp, Cyprinus carpio, Wakagbayashi et al. (1981) found a concentration factor of 16 and 400 in fish exposed to 9.9 and 300 $\mu g/L$ LAS, respectively.

Bishop and Maki (1978) looked at bioconcentration using two different methods. They found a range of BCF (whole body) of 120-260 for bluegill exposed to 0.063-0.64 mg/L LAS. The uptake rate constant at the lower expoaure was twice as great as that at the higher LAS concentration. The authors suggested that a sublethal effect may influence the uptake rate at higher concentrations. Over 99% of accumulated material was eliminated by 336 hours, and a clearance half-time of 30 hours was estimated.

In a related study, whole body BCF (based on total ^{14}C-residue) for bluegill exposed for 168 hours to ^{14}C-labelled-C_{12}LAS (0.062 mg/L) were 8603, 610, 124 and 60 for gall bladder, viscera, carcass and muscle, respectively. BCF values were not significantly different for bluegill ranging in size from 0.5 to 5.0 g wet weight (Procter and Gamble Company, unpublished data).

Kimerle et al. (1979) reported similar results in bluegill to those reported by Bishop and Maki (1978) and Kikuchi et al. (1978).

Comotto et al. (1979) studied the uptake and depuration of LAS by Daphnia magna and fathead minnow using ^{14}C-labelled LAS surfactants (C_{12} LAS, C_{18} LAS and light and heavy blend ^{14}C-LAS). In Daphnia, steady-state was reached in 1 day; in fatheads, three days were needed. In both cases, bioconcentration was dependent on chain length and exposure concentration; shorter chain lengths were accumulated less. The bioconcentrations factors in Daphnia ranged from 500-4000. In fathead, the dry weight bioconcentration factors were as follows: muscle, 79-372; whole fish, 269-1223; gall bladder, 21,000-70,000. Clearance was rapid in both species upon removal to clean water. These authors also determined the chemical species composition of the accumulated radioactivity. Much of that in Daphnia was in the form of intact LAS. However, fish tissue analysis indicated that the ^{14}C content detected in the tissues was not entirely LAS. The following percentages of intact LAS were found: gills, 25-75%; carcass, 50-70%; viscera, 15-35%; and gall bladder 2-3%. Presumably the non-LAS fraction of the ^{14}C was in the form of LAS metabolites. Thus, it is apparent that fathead minnows metabolized LAS more readily than daphnids.

These results suggest that LAS is rapidly taken up by fish and concentrated in tissues and organs. Clearance is also rapid, with a half-life of 2-3 days. It should be pointed out that all of these experiments were conducted using radiolabelled LAS. Thus, the reported accumulation factors assume that the detected radioactivity was associated with intact-LAS. However, one study showed that LAS is at least partially metabolized by fish and the eliminated products may well include some secondary products.

9. Interactions with Other Chemicals

LAS significantly increased the toxicity of parathion and certain parathion-related pesticides

but had no effect on endrin or dieldrin toxicity. Studies with DDT were inconsistent. Toxicity of No. 2 and No. 4 fuel oils were also significantly increased in the presence of LAS. These studies show a possible synergism between LAS and pesticides or petroleum products. However, no substantial experimental evidence has been located to prove that LAS actually enhances the uptake of these agents. Equal ratios of LAS and chloramines were slightly synergistic at low concentrations but additive at higher concentrations. For unequal ratios, the two chemicals were strongly synergistic. Likewise, for copper and LAS mixtures, an additive effect was reported at equal ratios or at high concentrations of unequal ratios while a synergistic effect was reported at low concentrations of unequal ratios. One study reported an antagonistic effect between LAS and copper and LAS and zinc in the developing cod embryo. No synergistic effects were observed when LAS was mixed with other surfactants.

Several studies have been conducted to investigate the possible interactions of LAS with pesticides and other potential aquatic contaminants to determine whether some synergistic or antagonistic effects occur.

Dugan (1967) studied a number of surfactants for the effects of chronic exposure on pesticide toxicity in goldfish. The single reported study with LAS (4 mg/L, 37 days) indicated that the chronic toxicity of 50 ng/ml of p,p'-DDT was substantially enhanced by prior exposure to LAS.

The effects of LAS on the acute toxicity of several insecticides to the fathead minnow (Pimephales promelas) have been examined by Solon et al. (1969). They found that LAS (1 mg/L) increased the toxicity of

parathion by 100% and that a concentration of LAS of 0.5 mg/L gave a significant increase. In contrast, no synergism was observed with endrin. The results with DDT were too inconsistent to discern any synergism with LAS.

Subsequently, Solon and Nair (1970) reported on the interaction of LAS with several organophosphate pesticides related to parathion. Of the 8 pesticides tested, 5 (parathion, methyl parathion, ronnel, trithion, trichloronat) exhibited synergism of acute toxicity with LAS (1.0 mg/L), while dicapthon, guthion and EPN (Ethyl para-Nitrophenyl Phenylphosphonothioate) did not. Hille (1970) found no synergism between LAS and dieldrin toxicity in the bluegill (Lepomis macrochirus) and no correlation between uptake of dieldrin into fish tissue and LAS concentration.

LAS applied topically to houseflies (Musca domestica) with parathion, diazinon or dieldrin in a 1:1 or 10:1 ratio did not affect the toxicity of these insecticides. Synergistic effects were observed, however, when LAS was mixed into soil treated with parathion and diazinon. Maximum synergistic effects were observed when both constituents were at concentrations of 2 ppm. At this point, toxicity to Drosophila melanogaster was increased by factors of 2.39 and 1.64, respectively (Lichenstein, 1966).

Katz and Cohen (1976) attempted to determine the effect of chlorination on the toxicity of LAS to mosquito fish. Static bioassays were conducted with a 1 mg/L solution of LAS which had been allowed to react with an excess of chlorine. Chlorination did not affect the toxicity of LAS to mosquito fish.

Since surfactants may be used to aid in the cleaning of oil spills in aquatic environments, the question has arisen as to whether the presence of surfactants may enhance the toxicity of the petroleum products to fish. Hokanson and Smith (1971) found that the addition of 1 mg/L LAS to No. 4 grade fuel oil increased the toxicity from 91 mg/L

for the oil alone to 51 mg/L with oil and LAS. A similar finding was reported by Rehwoldt et al. (1974) who determined that the acute toxicity of No. 2 and No. 4 fuel oils were significantly increased by performing the tests in the presence of 1-5 mg/L LAS. Six species of fresh water fish indigenous to the Hudson River of New York were used.

Panigrahi and Konar (1986) also found that oil refinery effluent and the detergent, LAS, when present in combination have a much greater toxicity than either compound alone. Zooplankton population was significantly reduced at all concentrations and was totally absent after treatments containing a mixture as low as 0.40% oil refinery effluent and 1.01 ppm LAS. As the oil refinery effluent concentration increased (LAS content remained at 1.01 ppm) toxicity increased. Chironomid population was also reduced at high concentrations.

The results of these studies with pesticides and petroleum products show the possibility for synergism between LAS and other potential aquatic toxicants at doses of LAS not of themselves toxic to aquatic species. The hypothesis has been offered that LAS may enhance the uptake of these agents, but there is no substantial experimental evidence as yet for this view.

Nakanishi et al. (1985) found that C_{12} LAS increased the BCF of PCB and two nitrophenyl ethers in the willow shiner (Gnathopogon caerulescens) by approximately 1.5 fold. However fish in this study preexposed to LAS showed a reduced uptake of other chemicals.

Due to problems with evaluating dsta, it is unclear whether any of the above studies has shown synergistic effects, or merely additive effects. Studies by Tsai and McKee (1978) and Lewis and Perry (1979) have looked at such interactions somewhat more rigorously. Tsai and McKee (1978) investigated the effects on goldfish (Carassius auratus) of various chemical interactions that might occur in a stream receiving chlorinated sewage effluents. They utilized mixtures of chloramines, LAS and copper as representative of possible interactions and found

that equal ratios of LAS and chloramines were slightly synergistic at lower concentrations, but additive at higher concentrations; LAS concentrations ranged from 1.8 to 6.5 mg/L. For unequal ratios, the two chemicals were strongly synergistic. When LAS was combined with copper, the toxicity was additive at equal concentrations and at a ratio of 2:1 (LAS:copper). However, then the ratio was 1:2, the effects were additive at high concentrations and synergistic at low. The ternary mixtures of chloramine, copper, and LAS in various ratios were all additive at high concentrations and synergistic at low concentrations.

Swedmark and Granmo (1981) tested the effects of LAS alone and in combination with the heavy metals, zinc and copper, on the development of cod (Gadus morhua L.). Results indicate an interaction between the metals and LAS giving a weak antagonistic effect for zinc and a strong antagonistic effect for copper. Topcuoglu and Birol (1982) found that LAS significantly increased the accumulation of zinc in young goby, Proterorhinus marmoratus.

Surfactants are known to alter the permeability of biological membranes to water and ions and may modify the uptake of heavy metals in fish. Since cadmium (Cd) is taken up primarily through the gills of freshwater fish, Pärt et al. (1985) studied Cd uptake in freshwater trout (Salmo gairdneri) exposed to LAS. Results showed that 0.14 μmoles/L LAS more than doubled the Cd transfer into the gill while 100 μmole/L LAS marked reduced Cd transfer. Results indicate that fish exposed to low levels of LAS and Cd (well below LC_{50} values) take up lethal concentrations of Cd. This increased uptake of Cd is due to the destructive effects of LAS on the gill increasing the permeability to Cd. Modification of Cd transfer in both "low dose" and "high dose" LAS experiments suggest LAS affects Cd transfer by a specific mechanism, most likely the result of LAS interacting with proteins involved in Cd transport in the gills.

Lewis and Perry (1979) examined the effects of surfactant mixtures on Daphnia magna and bluegill. Using $C_{11.8}$ LAS, Neodol®45-7 (n-pri-

$C_{14-15}AE_7$) and C_{12-14} monomethyl-dihydroxyethyl ammonium chloride, these authors found that a ternary mixture and three binary mixtures were less than additive or additive, depending on the statistical method of analysis used.

B. Effects of LAS on Terrestrial Plants

Phytotoxicity studies have been conducted in a wide range of media and conditions, including hydroponics. The relevance of some of these tests to "real world" conditions is difficult to ascertain. At high concentrations, LAS inhibits plant growth but at low LAS levels, limited evidence suggests a possible stimulation of growth. The growth of pea plants, in terms of weight and length, was inhibited by 50% following treatment with 50 mg/L LAS. The same dose of LAS also significantly reduced rice plant production. Examination of the inner pericarp wall of pepper fruit treated with 25 μM LAS showed a decreased elasticity of the cell wall and reduced hydraulic conductivity. Orchid seedling growth was reduced 60% after treatment with 100 mg/L LAS. Histological damage following treatment with 1000 mg/L LAS for 48 hours included alterations in chloroplast membranes and internal structure which became more extensive with time. Growth of Norfolk Island pines was also reduced and brown shoots occurred following LAS treatment. However, in five species of hardwood trees, 500 mg/L LAS had no effect on translocation or absorption. The majority of data show LAS to adversely effect the growth of plants; however, one study did report a stimulation of growth in bean and tomato plants watered with 25 and 40 mg/L LAS, respectively.

Several investigators have examined the effects of LAS on plants. Lichtenstein et al. (1967) examined the effects of LAS on translocation of insecticides in pea plants. LAS alone at a concentration of 0.005% (50 mg/L) inhibited growth weight and length by 50%. LAS did not affect the uptake by the roots of the pesticides lindane or aldrin. Ethyl parathion uptake was significantly reduced.

An examination of the effect of LAS on orchid seedlings (Ernst et al., 1971) revealed that LAS at 100 mg/L reduced growth by 60% as compared to untreated controls. At 10 mg/L a 30% reduction in fresh weight was found. In a companion study, Healey et al. (1971) exposed orchid seedlings to 1000 mg/L LAS for 48 hours. In 4 hours, a number of changes were observed in chloroplast membranes and internal structure. In 48 hours, extensive aberrant cellular changes were found.

Taniyama and Nomura (1978) reported a signifieant reduction in paddy rice production for plants watered with 50 mg/L LAS solutions. The reduced grain yield was due to a reduced number of grains per panicle (7 g vs 30 g for controls) and a significant decrease in the percentage of ripened grains (9.5% compared to 77% in controls) in LAS-treated plants. In separate experiments with potted rice plants, exposure to 50 mg/L LAS solutions produced no effects 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.

In a recent study by Gilbert and Kleiser (in press), solutions containing 0, 0.01, 10, 100 or 1000 mg/L LAS were applied to the soil and leaves of several crop plants. No effects were seen at concentrations of 100 mg/L or less with the exception of the cucumber which showed effects (not specified) at 100 mg/L. Visible and retardation effects were observed at the 1000 mg/L level on barley, radishes, peas, tomatoes and lettuce.

A similar study by Unilever (1987, unpublished data) studied the effects of adding LAS to soil equivalent to 1, 10, 100 or 1000 mg/kg dry soil on the growth of Sorghum bicolor (sorghum), Helianthus annuus (sunflower) and Phaseolus aureus (mung bean). The LAS used contained C_{11}, C_{12}, and C_{13} homologs and had a molecular weight of 343. There was no growth reduction over 21 days by 100 mg/kg LAS for any of the species and the 21 day EC_{50} (growth) values were 167, 289 and 316 mg/kg LAS for sorghum, sunflower and mung bean, respectively. At 1000 mg/kg LAS 69, 91 and 95% of shoots emerged, respectively, but in each case several emerged shoots were dead.

Sivak et al. (1982) and DeHenau et al. (1986) both found that LAS is generally not inhibitory to most plants at concentrations below 10 mg/L. This concentration is nominally equivalent to 2.2 mg LAS/kg which is typical for values reported in soils. In practice, however, this value was thought to represent a higher soil concentration.

The effects of LAS in the giant subepidermal cells of the inner pericarp wall of pepper fruit (Capsicum annuum) were studied by Rygol and Luettge (1984). LAS at 25 μM significantly reduced the hydraulic conductivity during the first 2 to 6 hours of treatment. A decrease in the elasticity of the cell wall was also attributed to LAS treatment.

An experiment with 18-month-old Norfolk Island pines (Araucaria heterophylla) examined the effects of varying LAS concentrations and salinity on growth (Dowden et al., 1978). Aqueous solutions of LAS at concentrations of 100, 1,000 or 10,000 mg/L reduced growth and produced browning in the shoots more than treatment with seawater alone; when LAS was combined with seawater, the effects were even more pronounced.

In another study to determine whether surfactants could increase absorption or translocation of two pesticides, an LAS preparation at 0.5% (5000 mg/L) did not increase either parameter in 5 species of hardwood trees common to central Louisiana (Hall, 1973).

Lopez-Zavala et al. (1975) observed no effect on growth in barley plants watered with solutions of 10, 25 or 40 mg/L C_{10-13} LAS but did note stimulation of growth in bean and tomato plants watered with 25 and 40 mg/L solutions of LAS, respectively.

Popa et al. (1967) showed no effect on quality or yield when 100 mg/L LAS was added during the blooming and fructification stages of hydroponically grown tomatoes. Addition of 500 mg/L resulted in a 50% reduction in yield. When added throughout the growth period, 100 mg/L LAS caused a slight reduction in yield (Popa and Gruia, 1968). Concentrations of up to 500 mg/L did not affect the quality of the fruit.

Vandoni and Goldberg-Federico (1973b) found germination and growth by four species of plants unaffected by up to 100 mg/L LAS.

The preponderance of data suggest that on average LAS may affect plant growth at concentrations of about 50 µg/L, conditions not likely to be attained in the environment. As with other biota, the toxicity of LAS to plants may depend on alkyl chain length, as well as other factors such as growth medium and frequency of toxicant renewal. Some data also suggest that LAS may stimulate plant growth in some cases.

C. Effects on Birds and Wildlife

No specific information was found with respect to the effects of LAS on birds and mammalian wildlife.

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VI. HUMAN SAFETY

In 1966, Swisher concluded for LAS that "the margin of safety is very great and there is no indication that hazard exists" from the trace amounts of surfactants which are found in the environment or from the normal use of surfactant products. The substantial volume of experimental information that has been generated in the intervening years supports this view of the safety of LAS. Berth et al. (1972), Gloxhuber (1974) and Tomiyama and Oba (1972) have summarized portions of this information, and they have also concluded that present day detergent use is not a threat to human health.

Evaluation of the extensive safety data available dealing with various measures of mammalian toxicity as indicators of human safety, as well as actual human exposure, agrees with the view that the use of LAS in consumer products does not represent a hazard to human health.

A. Animal Studies

LAS is moderately toxic to rodents. The oral LD_{50} values range from approximately 400-2000 mg/kg. A subacute study has shown that exposure of rats to LAS at 5000 ppm (0.5%) in the food for up to 12 weeks results in no abnormal changes. In another study, a six-month oral exposure of rats to LAS at 0.2% induced slight kidney damage which increased at a dose of 0.6%. However, chronic exposure of rats to LAS at doses up to 0.5% of the diet for two years, resulted in no pathological responses. The chronic studies indicate that a very large factor of safety exists in long-term low-level exposure.

The rapid clearance of LAS following oral exposure and the absence of any substantial absorption (<1%) through the skin after percutaneous administration indicate little likelihood of tissue accumulation.

A 10% LAS solution is an acute irritant for rabbit skin, while at 1 percent no skin irritation is observed. At concentrations above 0.1%, LAS causes irritation in rabbit eyes. Below this concentration there is little or no reaction.

Two-year feeding studies in rats and chronic skin painting experiments in mice show no evidence of any systemic or cutaneous carcinogenic effect. The mutagenicity studies that have been carried out would suggest no problems of genetic effects from LAS exposure. The reports of teratogenic effects of exposure to detergents containing LAS emanating from the laboratories of Mikami at Mie University in Japan are not supported by a number of other investigations which have examined the phenomenon (Charlesworth, 1975). A variety of studies with LAS alone or with the same detergents that Mikami used have not shown any teratogenic risk attributable to LAS exposure. Except for doses of LAS which exhibit toxicity in pregnant dams, no reduction in reproductive performance is found following LAS exposure.

1. Acute Toxicity

a. Oral

Early studies of the oral toxicity of LAS had reported 650 and 1260 mg/kg as the acute LD_{50} in rats (Swisher, 1968). Buehler et al. (1971) reported an oral LD_{50} in rats of 900 mg/kg for a product 98.1% pure consisting of C_{10} to C_{14} chain lengths. Similar LD_{50} values in rats of 700 to 2480 mg/kg were obtained for a number of commercial samples of LAS (Continental Oil Company, Monsanto Company, Procter and Gamble Company, unpublished data). Tiba (1972) has reported an acute oral LD_{50} of 2300 mg/kg in mice. Kobayashi and co-workers (1973) examined

the acute oral toxicities of LAS and ABS in two varieties of Wistar strain rats and ddY/s strain mice. The data for LAS, shown in Table VI-1, are comparable to earlier findings with respect to LD_{50} values in rats and mice. In the study with specific pathogen free SLC Wistar rats, the LD_{50} values decreased with increasing body weight, and some differences were noted among the two varieties of Wistar rats employed in the study. Survivors of LAS treatment gained weight as rapidly as control animals during the 32-day interval following treatment. Considering the oral toxicity of commercial detergent products, which contained approximately 15% LAS, estimates of 3000 to 10,000 mg/kg have been made for LD_{50} values of detergents in rats (Calandra and Fancher, 1976).

TABLE VI-1

ACUTE ORAL TOXICITY OF LAS IN RATS AND MICE

Strain	Age (weeks)	Sex	Mean Body Weight (grams)	LD_{50}±0.05 (mg/kg)	Confidence Limits p=0.05 (mg/kg)
SLC Wistar Rats*	6	M	125.6	873	(773-906)
	7	M	151.9	659	(560-774)
	10	M	235.7	404	(359-454)
	6	F	99.3	760	(675-855)
	7	F	111.1	670	(584-769)
	10	F	145.5	409	(362-462)
Wistar Rats	8	M	166.4	1525**	(1317-1766)
	8	F	123.6	1820**	(1099-1491)
ddY/Mice	7	M	27.2	1665	(1508-1838)
	8	F	22.0	1950	(1540-2480)
	7	M	27.4	1575	(1433-1731)
	7	F	23.0	1850	(1674-2044)

Kobayashi et al., 1973

*Specific pathogen free.

**This value is outside the reported confidence limits.

Some acute toxicity tests on commercial detergent products possibly containing LAS have been carried out in mice, rats, dogs and pigs (Muggenberg et al., 1974). The results of these tests are not reported here, since it is not possible to ascribe the effects observed to any particular component of the products employed. In a study which examined a household hand dishwashing detergent containing 15% dodecylbenzene sulfonate and 13% ammonium fatty alcohol polyglycolether sulfate, the LD_{50} of the detergent solution for mice was 12.6 ml/kg and for rats was 7.5 ml/kg. For dogs, a lethal dose was 400 ml/kg while a level of 100 ml/kg was without effect (Leuschner et al., 1969).

b. Inhalation

A single, 30-minute aerosol exposure of guinea pigs to 1% (w/v) C_{12} LAS produced no lethality or significant pulmonary lesions. A combination of LAS with an aerosolized proteolytic enzyme solution (1 mg/ml Bacillus subtilis protease), however, resulted in markedly increased mortality (29% vs. 3% for protease alone) and altered pulmonary pathology compared to responses seen with either protease or LAS alone. In vitro studies indicated that LAS inhibits serum protease inhibitors and this effect may be responsible for the observed effects in vivo (Markham and Wilkie, 1979).

c. Percutaneous

i. Acute Dermal Lethality

The minimum acute lethal dose in rabbits of commercial LAS samples tested as 20% aqueous solutions on unabraded skin was in the range of 200 to 1260 mg/kg (Monsanto Company, unpublished data).

No evidence of systemic toxicity or deaths were observed when 2 mg/kg of 5, 10, and 25% w/v aqueous LAS solutions were applied occluded to rabbit skin for 24 hours. Moderate skin irritation was observed at the

two highest doses. The results indicate that the acute dermal LD_{50} of LAS in rabbits is greater than 500 mg/kg (Procter and Gamble Company, unpublished data).

ii. Skin Irritation

Tests for irritation using the Draize procedure have shown that undiluted commercial samples of LAS applied as a moistened powder are primary skin irritants (Continental Oil Company, Monsanto Company, unpublished data). In contrast, at a concentration of 1%, which is above normal domestic use levels, LAS is non-irritating for rabbit skin or when applied to the vaginal tissue of dogs (Ethyl Corporation, unpublished data), and an aqueous emulsion containing 0.4% LAS was non-irritating to guinea pig skin (Sujbert and Fodor, 1970). Application of an occluded patch containing 0.1 ml of a 2% aqueous solution of 97.9% pure C_{12} LAS to the shaved backs of guinea pigs for 24 hr resulted in moderate to severe skin irritation; i.e. a skin irritation score of 3 out of a possible 4. A slightly less severe reaction (skin irritation score = 2.75) was seen with a mixed LAS sample (99.8% pure; C_{10}-7%, C_{11}-36%, C_{12}-33.7%, C_{13}-23.4%) under the same test conditions (Imokawa, 1979).

With a 30% solution of LAS applied to the skin of guinea pigs for 10 minutes or two hours, epidermal hyperkeratosis with epidermal hypertrophy and edema were noted. Recovery from these effects was not complete after 168 hours. At a concentration of 10% LAS, similar changes in treated skin were observed; however, recovery was complete at 168 hours (Iimori et al., 1971).

iii. Skin Sensitization

Guinea pigs injected intradermally with a 1% w/v aqueous solution of LAS (DOBANE JN™) and topically challenged showed no skin sensitization (Shell Research Limited, unpublished data).

Similar studies (Magnusson-Kligman Maximization) were conducted in guinea pigs using LAS injection induction doses from 0.6% to 5% (w/v aqueous). At a 1% patch challenge concentration, LAS produced positive sensitization results in up to 76% of the animals tested. When the Buehler test for guinea pig sensitization was used, LAS produced negative to moderately positive sensitization reactions in animals at induction concentrations of 2% w/v or greater. No evidence of sensitization was observed in animals induced and challenged with LAS concentrations of 1.4% or less (Robinson et al., in press). These results suggest that LAS is a weak sensitizer in guinea pigs under exaggerated exposure conditions. Clear no-effect levels for induction of sensitization have been identified which are above anticipated LAS dermal exposure levels from detergent usage. No evidence of positive sensitization reactions have been observed in humans patched with LAS solutions or exposed to LAS-containing products (see Section B.2).

d. Ocular Irritation

At concentrations above 5% instilled into the rabbit eye according to the Draize procedure, LAS produces an irritation. Some congestion and edema have been noted at levels of 0.5 to 1.0%, while at or below 0.1%, LAS resulted in mild to no reaction (Oba et al., 1968; Iimori et al., 1972; Procter and Gamble Company, Ethyl Corporation, Continental Oil Company, unpublished data).

e. Other Routes of Exposure

The intravenous injection of rabbits or the intraperitoneal injection of rats with a 2 mg dose of LAS (PERLAN ALB™) induced an increase in body temperature in both species within one hour. The body temperature of the rabbits returned to normal in three hours. The body temperature of the treated rats was still elevated five hours following treatment, but returned to normal in 24 hours. In addition, structural changes were noted in the polynuclear granulocytes of treated animals (Bordas and Bretter, 1973).

Ito et al. (1978) compared the acute oral, subcutaneous and intravenous toxicities of the magnesium and sodium salts of C_{10}-$_{13}$ LAS in ICR mice and Sprague-Dawley rats (see Table VI-2). Oral LD_{50} values in rats were comparable to values previously reported. No sex differences were observed for either species, regardless of route of administration, but both formulations were more toxic to rats than mice. Mg-LAS was considerably more toxic than Na-LAS by the intravenous route, particularly in rats. In terms of toxicity, the following rank was observed: intravenous > subcutaneous > oral route.

TABLE VI-2

ACUTE TOXICITIES OF Mg-C_{10-13} LAS IN MICE AND RATS

Species	Compound	Route	Sex	LD_{50}(mg/kg) ± 95% C.L.	
ICR mice	Mg-LAS	Intravenous	M	98	(91-106)
			F	151	(113-200)
	Na-LAS		M	207	(129-330)
			F	298	(276-320)
	Mg-LAS	Subcutaneous	M	1520	(1338-1727)
			F	1550	(1336-1798)
	Na-LAS		M	1250	(1078-1450)
			F	1400	(1239-1582)
Sprague-Dawley rats	Mg-LAS	Intravenous	M	27.2	(22.4-33.4)
			F	35.0	(33.3-38.0)
	Na-LAS		M	119	(106-134)
			F	126	(97-166)
	Mg-LAS	Subcutaneous	M	710	(602-838)
			F	730	(620-847)
	Na-LAS		M	840	(712-991)
			F	810	(664-988)
	Mg-LAS	Oral	M	1900	(1597-2261)
			F	1840	(1559-22171)
	Na-LAS		M	1460	(1207-1767)
			F	1470	(1256-1720)

Ito et al., 1978

2. Subacute Toxicity

a. Oral

The feeding of LAS to rats at levels to 5000 ppm in the diet for periods of 33 days to 12 weeks did not influence body weight, food consumption, hematological parameters or urine clinical chemistry. Histopathological examinations did not reveal abnormal changes (Kay et al., 1965; Oser and Morgareidge, 1965). At maturity, the 5000 ppm level provides a dosage of 250 mg/kg/day which is approximately 25% of the mean acute oral LD_{50} for LAS in rats.

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

In a six-month feeding study in Wistar rats, Yoneyama et al. (1973) reported that 0.07% LAS in the diet, equivalent to approximately 40 mg/kg/day, resulted in no adverse effects. At 0.2% LAS, minor histological changes in the kidneys were found. The severity of these lesions increased as the dose of LAS was increased to 0.6% and 1.8%. At the highest dose, decrease in weight gain, as well as tissue damage in the caecum and liver, was noted in addition to an increased severity of kidney pathology; specifically, glomerular atrophy and destruction of urinary tubules.

The effect of LAS on the liver and kidneys of male and female albino rats was studied by Gupta et al. (1986) and Mathur et al. (1986), respectively. In both studies, the animals were given oral doses of 50, 100 or 250 mg/kg/day LAS for 10 weeks. Histopathology was evaluated in the females only. At the highest dose level, the kidneys showed mild degeneration and desquamation of the tubular epithelium and

there was a moderate degree of fatty change in the liver as well as proteinaceous degeneration. The activity of adenosine triphosphatase was inhibited with increasing dose in both sexes while the activities of alkaline phosphatase and acid phosphatase were increased with increasing dose. The activity of lactate dehydrogenase (LDH) was significantly inhibited at all dosage levels in females but was not measured in males. Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were significantly decreased in females at the 100 and 250 mg/kg dosage levels. SGPT inhibition in males was significant at the 250 mg/kg level only. The authors surmised that these alterations in enzyme activity may be caused by surfactant interaction with proteins which disturb the metabolism or binding of the enzymes.

Leuschner et al. (1969) examined the subacute oral toxicity of a product containing 15% sodium dodecylbenzene sulfonate and 13% ammonium fatty alcohol polyglycolether sulfate in rats and dogs. The inclusion of the detergent solution in the drinking water to give a dose of 0.5 ml/kg/day in rats had no effect on growth or on any of the hematological or urinary clinical chemistry values examined. At a dose of 2.5 ml/kg/day for nine weeks followed by 3.75 ml/kg/day for an additional nine weeks, only a slight depression in growth rate in males was noticed. Elevation to a dose of 5.0 ml/kg/day in both males and females at 18 weeks resulted in a rapid weight loss. Following a return to the control diet at 22 weeks, the test animals gained weight and achieved control values by the twenty-sixth week. Histopathological analysis of this group revealed some mild necrosis of intestinal mucosa with hemosiderosis of spleen, liver and kidney. These lesions were absent in a group of rats receiving 0.5 ml/kg/day. In dogs, doses of 10, 100 and 1000 mg/kg/day of the detergent were included in the diet for six months. Body weight gains were similar to controls for all treatment groups other than females at a dose of 1000 mg/kg/day which exhibited a slight decrease in weight gain. None of the treated groups had alterations in blood or urine chemistry values.

Histologically, hemorrhagic necrosis of the intestine with infiltration of chronic inflammatory cells was noted at the 10 mg/kg dose and hemosiderosis of the liver and spleen at 100 mg/kg and 1000 mg/kg.

In another study, Watari et al. (1977) gave DDY-strain mice 100 ppm LAS in their drinking water for 6 months. Mice were killed at 1, 2, 3, 6 or 8 months after initiation of the study. Even after 1 month, ultrastructural studies indicated hypofunctional hepatic cells and treatment-related hepatic injury consisting of atrophy of the Golgi apparatus, degranulation of the rough-surfaced endoplasmic reticulum, disappearance of the nucleolonema, and degeneration of mitochondria. Ultrastructural recovery of hepatic cells was noted, however, two-months after cessation of LAS treatment.

Ito and co-workers (1978) orally administered either 0, 155, 310 or 620 mg/kg/day Mg-C_{10-13} LAS or 0, 125, 250 or 500 mg/kg/day Na-C_{10-13} LAS to groups of twelve male and twelve female Sprague-Dawley rats for one month. Body weight gain and feed efficiency were decreased in all treatment groups, but blood and clinical chemistry findings were comparable to those of control animals. Liver weights were increased in the 620 mg Mg-LAS/kg group as well as the 500 mg Na-LAS/kg group compared to controls. Two females and one male in the 620 mg Mg-LAS/kg group died during treatment. In a second experiment (Ito et al., 1978), groups of 20 male and 20 female Sprague-Dawley rats were given 0, 75, 150 or 300 mg Mg-C_{10-13} LAS/kg/day orally for 6 months. Body weight gain was suppressed and slight decreases in serum protein, albumin, and calcium were observed, but were within normal physiological ranges. Other biochemical and hematological parameters and organ weight values were all comparable to controls.

b. Inhalation

Data on the inhalation toxicity of LAS were reported by Coate et al. (1978). Five male and four female cynomolgus monkeys (Macaca

fascicularis) were exposed 6 hr daily 5 days per week for 6 months to an atmosphere containing 100 mg/m^3 of a synthetic detergent dust containing 13% (w/w) C_{12} LAS. The mass median diameter of the dust particles (3 μm) was in the respirable range. Gross signs of respiratory distress, pulmonary histopathological effects and pulmonary function impairment were noted. Histological alterations consisted of chronic bronchiolitis characterized by infiltration of mononuclear macrophages and lymphocytes. The walls of the respiratory bronchioles were moderately to markedly fibrosed and there was a diffuse non-suppurative alveolitis in the proximal alveoli adjacent to respiratory bronchioles. Basal cell hyperplasia and focal squamous metaplasia of the trachea were also noted. The proliferative changes were considered to be a result of irritation. Cumulative tidal volume for nitrogen washout was significantly increased (1.10 to 1.27 L) but returned to normal when animals were held without further exposure. Dust exposure had no apparent effect on flow resistance, or diffusion capacity. Two mortalities were recorded during the study; one animal died during Week 22 of exposure; another was killed in moribund condition during Week 24. No treatment-related effects were noted with respect to hematology, clinical chemistry, urinalysis, skin sensitization tests or chest radiographs. The presence of builders and additives in the dust formulation, however, compromised the direct application of the data to LAS safety assessments.

An analysis of sera and the lungs of monkeys from the above study suggested no clear-cut evidence of antigen-specific IgE or precipitating antibodies in sera nor deposits of immunoglobulins, complement or fibrinogen (as detected by immunofluorescence) in the lungs of these LAS-exposed monkeys (Cashner et al., 1980).

c. Percutaneous

No severe skin damage was noted after a 15-day application of a 20% aqueous solution of LAS to clipped adult rat skin. In contrast, 30%

solutions resulted in fairly pronounced skin damage and decrease in body weight (Sadai and Mizuno, 1972). Although a reduction of oxygen consumption was found in the skin of hairless mice treated with a 2% LAS solution for one or four weeks as compared to controls, this reduction was not statistically significant. Oxygen consumption is indicative of changes in skin metabolism (Brown, 1969).

Ito et al. (1978) reported no adverse effects other than slight erythema of the skin and suppreased body weight gain in Sprague-Dawley rats treated percutaneously with 5% Mg-C_{10-13} LAS in polyethylene glycol (0.1 ml/rat/day) for six months. Hematological and biochemical parameters, as well as pathological findings were within normal ranges.

LAS solutions up to 10% were applied daily to rabbit skin (2 mg/kg). LAS was applied to abraded skin for 28 days (6 hr/day, 5 days/week) or to intact skin for 91 days (6 hr/day, 5 days/week). No systemic toxicity was observed upon histopathological evaluation of 14 organs and blood values. Severe skin irritation at the site of application was the only remarkable finding. With a 10% solution (2 mg/kg) of a hand dishwashing liquid containing 19% LAS and 19% tallow alkyl ethoxylate sulfate only moderate skin irritation was found following a 91-day exposure of intact rabbit skin or a 28-day exposure of abraded rabbit skin under the same conditions (Procter and Gamble Company, unpublished data).

When NaLAS was applied to mouse dorsal skin for 7 consecutive days, pustules, crust scabs, scale formation and erosion were observed. Microscopically, desquamation of the epidermal cells, inflammatory cell infiltration and ulcer formation were found. No abnormalities were seen in the liver, kidney, heart, lung, testes, thymus or adrenal glands. One mouse out of the five tested died on the third day of the experiment. No details are available (Uchida and Sasaki, 1980).

Immersion of guinea pigs in solutions of detergents containing LAS caused erythema, edema, cracking and scaling of the skin. Two different types of LAS containing detergents were tested. The amount of LAS contained in each detergent was not reported. The animals were immersed up to their necks in solutions of 1%, 2.5% or 5% for 1.5 hours daily for 5 consecutive days. The reaction to both types of detergent was similar. The 5% solution caused definite erythema, edema, cracking and scaling in both cases. These signs were very slight in both 2.5% groups. Very slight erythema was seen in the 1% groups and also in the controls. Skin enzymes were also measured. In the group exposed to one detergent at 5%, there was a statistically significant increase in G6PDH and GPT. In the other group, a statistically significant increase in acid phosphatase, GPT and GOT was observed at all levels indicating liver dysfunction. Increases in G6PDH were significant at the 2.5 and 5% levels only (Mathur and Gupta, 1986).

Slight skin irritation occurred in a cumulative open patch test in guinea pigs with 97.9% pure C_{12} LAS and a mixed LAS sample (99.8% pure; C_{10}-7%, C_{11}-36%, C_{12}-33.7%, C_{13}-23.4%). Application of 0.1 ml of a 2% aqueous solution of either surfactant to the shaved backs of the test animals twice daily for a total of nine treatments resulted in skin irritation scores of 0.58 and 1.42 of a possible 4 points for the mixed and C_{12} LAS samples, respectively. Details are unavailable (Imokawa, 1979).

In another study, three 6-hour applications of a 1% (w/v) aqueous solution of LAS (DOBANE JN™) produced primary skin irritation in rabbits. No effect was noted after the first application but by the third treatment, moderate to severe erythema and moderate edema were evident and persisted at 7 days. A moderate degree of hyperkeratosis and epidermal acanthosis with crusting focally was observed histologically at 7 days (Shell Research Limited, unpublished data).

d. Other Routes of Exposure

Kikuchi (1978) subjected male and female C57BL/TW strain mice to repeated subcutaneous injections of LAS from the day of birth. The injection schedule was as follows:

0.02 ml of 1% LAS, days 1-10;

0.04 ml of 1% LAS, days 11-20;

0.02 ml of 10% LAS for 5 injections over the next 10 days (days 21-30); and

0.04 ml of 10% LAS every other day for next 30 to 60 days.

Treated mice exhibitad epilation around the site of injection with dermatitis frequently notad after several injections. Survival, growth and reproductive parameters were not affected. Necropsy at 60 days revealed an increase in adhesions (60.6% vs. 0% in controls) between some organs, particulary between spleen and kidney. Interestingly, Kikuchi noted no adhesions in related experiments if injections were initiated on day 11 of life or later. The relative weights of kidney and liver were increased, and the spleen in females, but not males, was markedly enlarged, especially in mice with dermatitis at the site of injection (See Table VI-3). Histopathological examination of liver, kidney, spleen, adrenal and thyroid revealed no treatment-related changes.

TABLE VI-3

RELATIVE ORGAN WEIGHTS OF C57BL/TW MICE
INJECTED SUBCUTANEOUSLY WITH LAS FROM DAY 1 TO DAY 60 OF LIFE

Treatment	No.	Sex	Mean Body Weight (g)	Relative Organ Weight (mg/20 g bw)		
				Kidney	Liver	Spleen
LAS	32	M	22.97	315.3	1240	105.9
	12	F	17.48	302.4	1240	144.7
Control	18	M	22.1	278.0	1130	101.8
	3	F	17.3	--	1130	129.3

Adapted from Kikuchi, 1978

Simultaneous oral and subcutaneous administration of C_{10}-$_{13}$ LAS to male and female rhesus monkeys (Macaca mulatta) for 28 days resulted in no adverse effects. Groups of three male and three female monkeys were simultaneously administered aqueous solutions of 0, 30, 150 or 300 mg/kg C_{10-13} LAS orally and 0, 0.1, 0.5 or 1.0 mg/kg C_{10-13} LAS, respectively, by subcutaneous injection. At the highest treatment level, the monkeys frequently vomited, usually within 3 hours of administration. An increased frequency of loose or liquid feces was noted in animals in the top two treatment levels. No significant decrease in mean body weight gain was seen, but individual animals in the top treatment group did show a slight depression in body weight gain. Fibrosis of the injection site was seen in all groups, the incidence and severity being dose-related. No other treatment-related responses with respect to histopathology, hematology, urinalyses or

opthalmology were reported (Heywood et al., 1978). This study adds the monkey to the list of species in which subchronic oral administration of LAS results in no permanent adverse effects.

3. Chronic Toxicity

a. Oral

The exposure of rats to 100 ppm (0.01%) of sodium dodecylbenzene-sulfonate in their drinking water for 100 weeks did not result in any detrimental effects on body weight or cause any increase in organ pathology, including the occurrence of tumors (Bornmann et al., 1961). A study of similar length was carried out by Buehler et al. (1971) who examined the effects of LAS included in the diet of rats for two years at levels of 0.02, 0.1 and 0.5%. They found that such feeding of LAS did not affect body weight, hematological values or induce any unusual gross or microscopic tissue lesions. Tiba (1972) also reported a two-year feeding study in rats with LAS levels in the drinking water of 0.01, 0.05 and 0.1%. Body weight gains were normal in all treated groups and histopathologic changes were not remarkable.

4. Carcinogenicity and Co-Carcinogenicity

The chronic oral toxicity studies described above give no indication of any carcinogenicity which could be ascribed to ingestion of LAS (Bornmann et al., 1961; Buehler et al., 1971). Since the levels used in these studies (1000 to 5000 ppm) did not induce tumors and were several orders of magnitude in excess of the amounts allowable in water supplies, there appears to be no increased risk for the direct induction of neoplastic disease from consumption of any minute amounts of LAS that may occur in some water supplies.

The percutaneous application of aqueous solutions of a detergent containing 15.6% LAS and 18.6% tallow alkyl ethoxylate sulfate at concentrations of 0.1, 1.0 or 10.0% to Swiss ICR mice three times

weekly for 18 months did not result in any carcinogenic response either on the skin or systemically. At the 10% level, acanthosis and/or hyperkeratosis of the treated skin was noted along with the occurrence of only a single benign papilloma in a group of 50 test animals (Procter and Gamble Company, unpublished data).

Takahashi et al. (1968, 1970, 1973) have examined the co-carcinogenic effect of oral administration of LAS with either 4-nitroquinoline-N-oxide (NQO) or with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in rats. The esophageal intubation of 80 mg LAS with 1 mg of NQO three times weekly for 18 weeks resulted in a substantial incidence of tumors of the glandular stomach and squamous cell carcinomas of the forestomach in comparison to the absence of these tumors in animals receiving the carcinogen alone. The incidence of forestomach papillomas induced by NQO was not influenced by LAS. The authors suggest that the surfactant may cause a carcinogen to penetrate the protective mucous barrier of the stomach and attack the surface epithelium of the stomach directly. In contrast, co-administration of 1000 mg/l LAS with 100 mg/l MNNG in the drinking water of rats for 63 weeks did not increase the incidence of adenocarcinomas of the glandular stomach as compared to animals receiving MNNG alone (Takahashi et al., 1975).

5. Teratogenesis and Reproductive Effects

A large number of studies were conducted between 1971 and 1980 to evaluate the teratogenic and reproductive effects of LAS. The majority of these have reported negative results. These studies are outlined in Table VI-4. Those studies giving some positive results, as well as all recent studies are discussed below.

a. Oral

The effect of oral administration of LAS and a commercial light-duty liquid detergent containing 17% LAS and 7% sodium dodecylethoxy-sulfate

to pregnant mice, rats and rabbits were examined in detail by Palmer et al. (1975a). Effects of LAS and the commercial detergent on litter parameters were found only at doses that resulted in toxicity to the pregnant dams. These doses for LAS were 300 and 600 mg/kg/day for mice (days 6-15 of gestation) and rabbits (days 6-18 of gestation), and 600 mg/kg/day for rats (days 6-15 of gestation). Toxic doses for the commercial detergents ranged from 1200 to 3200 mg/kg/day. While these levels were toxic to the dams and resulted in abortion or resorption of fetuses, lower dosages which caused slight or no maternal toxicity did not influence litter size or fetal weight. Other than a higher incidence of skeletal anomalies in mice at a maternally toxic LAS dose of 300 mg/kg, no increase in major malformations, minor visceral anomalies or skeletal anomalies was found. A dose level of 2 mg/kg in all three test species had no toxic effect on litter parameters and did not induce any teratogenic response.

Kiozumi et al. (1985) conducted a series of studies to evaluate the effects of LAS on pregnant ICR mice. In the first experiment, LAS caused no significant difference in the incidence of implantation disturbance when compared to controls. The animals received oral doses of approximately 14, 70 or 350 mg/kg once daily on days 1 through 3 or on day 3 only. In the second experiment, a single, oral dose of 350 mg/kg was administered on the third day of gestation and the distribution was investigated 4 hours and 8 hours after administration. LAS was not detected in reproductive organs at any time.

In contrast, Mikami and his associates have reported on the teratogenicity to mice and rats following exposure to a number of commercial detergents, some of which contain LAS. The work dealing with the LAS-containing detergents was first published by Iseki (1972) and later explained by Mikami et al. (1973). In one study, three detergents containing LAS were administered by gavage in ICR/JCL mice from day 6 to 11 of gestation. The uterine contents were analyzed on the 17th day of gestation. The detergents contained, in addition to other surfactants, varying amounts of LAS resulting in doses of LAS

ranging from 12.6 mg/kg to 189 mg/kg. In all caaes of detergent treatment, increased fetal deaths were noted with an increase in palatoschisis and other skeletal defects. The results presented by Iseki (1972) are summarized in Table VI-5. It is clear from these data that there is no dose response with respect to LAS on any of the parameters measured. These findings would indicate that the teratogenic responses were not the result of exposure to LAS, but rather may be due to some other component of the commercial products used in the test.

b. Dermal

Masuda et al. (1974) observed no evidence of teratogenicity even after dermal application of high concentrations of LAS. LAS at concentrations of 0.85, 1.7, 2.55 or 3.4% was applied to pregnant ICR-JCL mice once daily (0.5 ml/mouse) from days 1 to 13 of gestation. In a separate experiment, ddY strain mice were similarly treated with LAS concentrations of 0.017, 0.17 or 1.7% LAS from days 2 to 14 of gestation. Controls received distilled water. No suppression of body weight gain or visceral defects of dams was observed in any group tested. A reduced rate of pregnancy was observed in the 3.4% group, in which considerable skin irritation at the site of application was seen; the pregnancy rate for this group was 33% compared to 69% in controls. Slight growth suppression of live fetuses was noted in ICR mice at the 0.85, 2.55 and 3.4% levels (but not 1.7%). No significant difference in fetal anomalies was observed, although an increased frequency of retarded ossification of sternebrae was noted in ICR fetuses at the 2.55 and 3.4% levels (25% and 27%, respectively, compared to 11% in controls). No adverse effects were reported for ddY-strain fetuses.

When a 20% LAS solution or a 27% solution of a detergent containing LAS and an alcohol sulfate was applied twice daily to the dorsal side of pregnant JCL:ICR mice during preimplantation period (days 0-2), a significant number of embryos collected on day 3 showed severe

TABLE VI-4

TERATOGENESIS AND REPRODUCTIVE STUDIES WITH NEGATIVE RESULTS

Species	Dose/Route/Duration	Author
Rats	0.1% LAS in drinking water for 26 weeks - 2 generations	Bornmann et al., 1961
Rats	0.5% LAS in the diet for 84 days - 3 generations	Buehler et al., 1971
Mice	2.2% LAS applied dermally on days 0-13 of gestation	Sato et al., 1973
Mice	15-20% aqueous solution of a detergent containing 17% LAS applied dermally on days 1-13 of gestation	Iimori et al., 1973
Rabbits Rats	A mixture of 55% tallow alkylethoxylate and 45% LAS orally administered to: - rabbits at doses up to 300 mg/kg on days 2-16 of gestation or continuously for 2 generations - rats at doses up to 800 mg/kg on days 6-15 of gestation or continuously for 2 generations	Nolen et al., 1975

TABLE VI-4 - Continued

TERATOGENESIS AND REPRODUCTIVE STUDIES WITH NEGATIVE RESULTS

Species	Dose/Route/Duration	Author
Rats Rabbits Mice	Doses of LAS up to 60 mg/kg in rats, 500 mg/kg in mice and 90 mg/kg in rabbits applied dermally during the first 2/3 of gestation	Palmer et al., 1975b
Mice	Oral doses of 10, 100 or 300 mg/kg NaLAS on days 6-15 of gestation	Shiobara and Imahori, 1976
Rats	0.1 or 1.0% LAS in the diet	Tiba et al., 1976
Rats	0.1 or 1.0% LAS in the diet on days 0-20 of gestation	Chiba et al., 1976
	Oral administration of 500 ppm on days 6-18 of gestation	Kuwano et al., 1977
Rats	1, 2 or 10 mg/kg/day applied dermally throughout gestation or 20, 100 or 400 mg/kg/day applied for 30 minutes daily throughout gestation	Daly et al., 1980

deformity or remained at the morula stage (an early stage of embryonic development). Most of the abnormal embryos were fragmented or remained at the 1-8 cell stages and were either dead or dying. It should be noted that these are very irritating doses (Nomura et al., 1980; Nomura et al., 1987).

TABLE VI-5

EFFECTS OF LAS-CONTAINING DETERGENTS ON REPRODUCTION IN ICR/JCL MICE

LAS Dose (mg/kg)	Average Implants	Average Live Fetuses	Palatoschisis (% of Live Implants)	Other Malformations (% of Live Implants)
0	13.1	12.0	0	0
12.6	11.6	4.66	52.9	1.4
107	12.3	5.41	33.8	10.8
189	13.0	6.92	35.5	6.6

Iseki, 1972

A study to examine the effects of percutaneous administration of a detergent containing 17% LAS as well as other surfactants in ICR/JCL strain mice and Wistar rats was reported by Mikami et al. (1973) without a complete description of the methodology or detailed exposition of results. It was claimed that the teratogenic effects described were the result of percutaneous detergent application. However, the absence of data on the incidence of these lesions in control groups in comparison to treated groups and the absence of any relationship of dose to effect prevent an adequate evaluation of this particular report. The absence of teratogenic effects in the experiments described by Palmer et al. (1975a,b) which were performed with the same detergent products used by Mikami et al. (1973), and similar independent evaluation of the literature on detergents containing LAS do not support the view that LAS and detergents containing LAS are teratogenic hazards (Charlesworth, 1975).

Several investigators have evaluated the effects of dermal application of LAS to pregnant animals in an attempt to replicate the results of Mikami group indicating that skin application of LAS to pregnant mice and rats was teratogenic. These studies are described in the following paragraphs. Again, the alkyl chain lengths of the tested materials were not given.

Nishimura (1976) reported that results of a joint study on the effects of dermal application of LAS to pregnant Wistar rats failed to support earlier reports by Mikami of LAS-induced abnormalities in mice and rats dermally treated with LAS during gestation. Four study groups (including Mikami) conducted parallel studies utilizing identical LAS samples, animal strain and the Mikami protocol. Pregnant rats were treated with 0.5 ml of 0, 1, 5 or 20% LAS, applied daily on days 0-20 of gestation to the backs of unrestrained rats. All rats were killed on day 21. All four study groups, including Mikami, obtained highly analogous results indicating no teratogenic effects. Dams treated with 20% LAS (~600x normal use levels) exhibited reduced feed consumption during the first half of gestation and a primary-type contact dermatitis at the site of application; fetuses in this group had reduced body weights which may have been secondary to decreased feed consumption in the dams, and one of four study groups noted delayed bone ossification at this exposure level which may be related to irritancy produced at such high doses leading to maternal stress. No other noteworthy effects were seen in the other treatment groups. Findings by Mikami's group of bleeding under the skin in fetuses were demonstrated to have been artificially induced during extraction of the fetuses from the uteri.

A consensus report, written by participants in the joint study, regarding earlier published findings of Mikami summarized their conclusions as follows:

(1) The design of the Mikami experiments and the criteria used to assess results were not sufficiently detailed;

(2) Reported data were insufficient;

(3) Data were interpreted non-systematically or illogically;

(4) No statistical evaluation was done;

(5) Reported abnormalities were most likely artificially produced during extraction of the fetuses from the uteri; and

(6) A joint study group using identical test protocols and LAS wes unable to reproduce the findings reported by Mikami.

c. Other Routes of Exposure

In addition to the oral and percutaneous routes, Masuda and Inoue (1974) administered LAS to pregnant mice by subcutaneous injection. Mice were injected with 0.4, 2, 10 or 50 mg/kg LAS subcutaneously once daily on days 7 to 13 of gestation. No suppression of body weight gain was seen and pregnancies were well maintained. Fetal mortality, neonatal growth, pathology and incidence of skeletal and soft tissue anomalies in the LAS group were comparable to those in control mice. Mice in the 0.4, 2 and 10 mg/kg groups did show a higher incidence of fetuses with a 14th rib, but mice in the top treatment level were comparable to control (39.4, 35.8, 34.7%, and 25.7% for the 0.4, 2, 10 and 50 mg/kg groups, respectively, compared to 23.3% in controls). A significant incidence of retarded ossification of calcaneous or talus in the 10 mg/kg group and the talus in the 2 mg/kg group was seen, but once again, these changes are not dose dependent and are not likely due to LAS exposure.

Thus, the results of the studies described above indicate that LAS, and detergents containing LAS, do not pose a teratogenic hazard to laboratory test animals at doses which are several orders of magnitude above those occurring in the environment. Reproductive effects are observed only at doses at which significant irritation was produced in

acute studies. The effect of this dermal irritation on maternal stress cannot be discounted. The percutaneous absorption of LAS is negligible. Thus, it is unlikely that effects observed with high dermal doses are mediated systemically.

6. Mutagenicity

Bacterial assays with Salmonella typhimurium strains TA 100 and TA 1535 (base-pair mutants) and TA 98, TA 1537 and TA 1538 (frameshift mutants) exposed to n-pri-C_{12-13} LAS at concentrations up to 2000 µg/plate were negative with and without added microsomal activation (Shell Toxicology Laboratory, unpublished data). Similar findings were reported by Swisher (1980) for C_{10-13} LAS and the disodium salts of two carboxylated LAS degradation intermediates, 3-sulfophenylbutyric acid and sulfophenylundecanoic acid, in tests with the five Salmonella tester strains at 50 µg/plate with or without added microsomal fraction. Negative results were also observed for C_{10-14} LAS (200 µg/plate) in S. typhimurium TA 98 and TA 100 (Inoue et al., 1980). Sunakawa et al. (1981) and Yam et al. (1984) also reported negative findings in the Ames test.

Mutagenicity tests with 2000 µg/plate C_{12-13} LAS in Escherichia coli WP2 and WP2 uvrA, 5 mg/ml C_{12-13} LAS for mitotic gene conversion in the yeast Saccharomyces cerevisiae JD1 and 100 µg/ml of a commercial LAS formulation in a "rec" assay with Bacillus subtilis H17 and M45 were all negative (Shell Toxicology Laboratory, unpublished data; Oda et al., 1977).

In vitro studies with mammalian cells exposed to LAS have also proved negative. No chromosomal aberrations were noted in rat liver cells exposed to n-pri-C_{12-13} LAS at concentrations up to 100 µg/ml (Shell Toxicology Laboratory, unpublished data). In another study, Inoue et al. (1980) reported no induction of morphological transformation of hamster embryo cells exposed in culture to 0.5, 1, 5, 10, 20 or 50 µg C_{10-14} LAS/ml, but did observe cytotoxic effects at the 50 µg/ml level.

In a micronucleus test, C_{12}-LAS did not effect the number of polychromatic erythrocytes with micronuclei (MNPCE). Oral pretreatment of the animals for 4 days did not influence the number of MNPCE induced by Mitomycin C. Neither the species of animals used nor the dose administered was reported (Kishi et al., 1984).

Kiozumi et al. (1985) evaluated the mutagenicity of LAS during pregnancy in mice. A single oral dose of 2 mg/kg on day 3 of gestation caused no increase in micronuclei in maternal bone marrow or in fetal liver or blood. A subcutaneous dose of 1,2 or 10 mg on day 17 also had no mutagenic effect.

In another study, Hope (1977) reported that the incorporation of C_{10-15} LAS 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. Chromosome studies of bone marow cells after 9 months dietary administration of 0.9% LAS showed no induction of chromosome abnormalities in either ICR mice, Wistar or Sprague-Dawley rats (Masubuchi et al. 1976).

The potential mutagenicity of a commercial detergent containing 16% LAS was studied in several systems including (1) dominant lethal assay in CDF_1 mice, (2) in vivo cytogenetic changes in marrow of Sprague-Dawley rats, (3) in vitro exposure of human lymphocytes in culture, and (4) in a host-mediated assay of human lymphocytes in rats for determination of cytogenetic lesions.

The dominant lethal assay was performed in mice at single oral dosages up to 1000 mg/kg of LAS-containing detergent and at 200 mg/kg for 5 consecutive days in a subacute protocol. The detergent contained 16% LAS. No increase in mutagenic index was found in the detergent-treated groups, while treatment with trimethylphosphate as a positive control resulted in a significant increase in the mutagenic index. Similarly, no induction of chromosome abnormalities was noted in rat marrow cells following a single oral administration of 1000 mg/kg of the detergent.

Karyotype analysis revealed a typical normal rat chromosome constitution (Procter and Gamble Company, unpublished data). Human peripheral leukocytes treated in vitro as well as in vivo in a host mediated assay in the rat did not exhibit increases in cytogenetic lesions following treatment at 1.5-1500 mg/kg with the same detergent containing LAS (Procter and Gamble Company, unpublished data).

A dominant lethal study conducted with ICR mice fed 0.9% LAS for 9 months also showed no increase in the mutagenic index (Masubuchi et al., 1976).

7. Pharmacology

a. Absorption and Metabolism

The metabolism of LAS was studied in rats following oral administration of an ^{35}S-labeled LAS commercial preparation. Three days after treatment less than 0.1% of the original ^{35}S-dose was still in the carcass. The urine appeared to be the primary route of excretion with no evidence for lymphatic system transport or large scale excretion in the feces via the bile. The primary urinary LAS metabolite recovered was identified as 4-(4'-methylsulfophenyl) pentanoete methyl ester. Unchanged LAS in the feces amounted to 19% of the original dose, and no inorganic ^{35}S-sulfate was found in the urine (Michael, 1968). Similar studies with ^{14}C-phenyl-labeled LAS showed that, in rats, over 90% of the orally administered label was excreted in 72 hours (Procter and Gamble Company, unpublished data). Pretreatment of test animals by feeding an LAS-containing detergent in the diet for one year did not influence the rapid and complete excretion of ^{14}C-labeled LAS. No significant tissue binding of ^{14}C-label or occurrence of radioactivity in expired air was found (Procter and Gamble Company, unpublished data). The intraperitoneal injection of ^{14}C-LAS in rats gave similar results with 78% of the dose excreted in 24 hours in the urine and no radioactivity in expired air. The remaining ^{14}C-label was associated with the carcass (Howes, 1975).

Cresswell et al. (1978) examined the disposition of [^{14}C]-LAS in four adult rhesus monkeys following single and repeated oral or subcutaneous administration. A single 30 mg/kg oral dose of LAS (mean mol. wt. 349) was rapidly excreted principally in urine during the first 24 hours (66.5% in males; 72.1% in females). Mean excretion values at 5 days were 71% in urine, 23% in feces. Similarly, a single subcutaneous injection of [^{14}C]-LAS resulted in the excretion of 64% of the radiolabel in urine and an additional 11% in feces within 5 days, with most excretion occurring within the first 24 hours. No unchanged LAS was detected in urine samples after oral or subcutaneous doses. Five major radioactive components were detected in urine; all were apparently more polar than LAS but were not sulfate or glucuronide conjugates. Similar peak plasma concentrations of 34, 41 and 36 μg/ml of [^{14}C]-LAS were noted, all at 4 hours, after single oral doses of 30, 150 or 300 mg/kg, respectively. These results could indicate nonlinear absorption processes, or alternatively, the absorption may be saturated at the 30 mg/kg level. With single subcutaneous doses, peak plasma concentrations increased almost proportionately, representing 0.16, 0.72 and 1.13 μg/ml for the 30, 150 and 300 mg/kg doses, respectively. No accumulation of plasma radioactivity was seen during seven consecutive daily oral (30 mg/kg/day) or subcutaneous (1 mg/kg/day) doses. Mean peak plasma concentrations and biological half-lives were similar after the first and seventh doses.

The effect of other surfactants on the absorption, distribution and excretion of LAS in rats was studied by Lin and DeSalva (1981). A single oral dose of ^{14}C-LAS and AES resulted in an increase in LAS absorption in comparison to the administration of ^{14}C-LAS alone. The addition of lauric/myristic monoethanolamide/sodium xylene sulfate to the ^{14}C-LAS/AES combination decreased the absorption of LAS. The results of a separate experiment suggested that in the dose range of 20-100 mg/kg, LAS is absorbed and eliminated by first-order kinetics.

Studies with isolated rat skin preparation as well as in vivo investigations of percutaneous administration of LAS have demonstrated

that penetration through skin and subsequent systemic absorption of this surfactant does not occur to any significant extent in 24 to 48 hours (Howes, 1975; Procter and Gamble Company, unpublished data). In rats, rabbits or guinea pigs using LAS with either ^{14}C or ^{35}S label, more than 90% of the recoverable label was located in or on the skin of the test animals, with less than 1% appearing in urine and feces.

In another study, Drotman (1977) applied a single cutaneous dose of radio-labelled $C_{12}LA^{35}S$ to the skin of rats, rabbits and guinea pigs. The test material was left in place for 72 hours; the animals were restrained to prevent ingestion. Only traces of radioactivity appeared in urine and feces with recovery from the site of application being 78% in the guinea pig, 82% in the rabbit and 93% in the rat.

Javed et al. (1984) evaluated the effect of LAS on the uptake and distribution of ^{65}Zn in guinea pigs. The animals were immersed in 1% solutions of neutralized LAS for 15 days at 90 minutes per day. A patch impregnated with 0.2 ml of ^{65}Zn was then applied to the ventral surface of each animal for an additional 15 days. Skin reactions such as erythema, edema, scaling and cracking were observed in some of the animals. The distribution of ^{65}Zn in skin, liver, kidney, brain, lung, spleen and blood was increased in LAS-treated animals as compared to controls, but it was not statistically significant. Skin on the ventral side (the application site) showed maximum amounts of ^{65}Zn, indicating mild percutaneous absorption.

The amount of LAS and polyoxyethylene alkyl ether (PAE) adsorbed on the skin (species not identified) was examined with ^{14}C-labeled compounds. The amount of adsorption of LAS was decreased by a factor of 4 or 5 when PAE was present (Kawai and Okamoto, 1982).

b. Hematological Effects

The concentration of LAS necessary to hemolyze rabbit erythrocytes *in vitro* to a 50% level in comparison to water was found to be 2.65×10^{-5} moles/liter (~10 ppm) (Oba *et al.*, 1968). *In vivo*, a single oral dose of 600 mg/kg LAS to rats induced hemolysis and haptoglobin outflow from the blood, although daily administration of 200 or 400 mg/kg LAS for two or four months did not induce these hematological changes (Dakay *et al.*, 1973).

The possible synergistic effects of 0.8% butylhydroxytoluene (BHT; 2,6-ditertiarybutyl-p-cresol) and 0.2% LAS given in the diet to rats were studied with regard to effects on blood clotting and prothrombin time three days after the start of feeding. Spontaneous bleeding in testis, nose and abdominal cavity was observed in 50% of the treated males after approximately 70 days of treatment. Direct addition of LAS to blood prolonged the prothrombin time with a Ki of 0.60 mM, while BHT had no effect at 10 mM. LAS also inhibited thrombin esterase activity *in vitro* in a non-competitive manner on a synthetic substrate with a Ki of 9 mM. Thus, the clotting inhibitor in rats fed LAS and BHT may have been LAS itself, although no data were presented to indicate that LAS alone influenced blood clotting times *in vivo* (Takahashi *et al.*, 1974).

c. Enzyme Activities

Total lipid and free fatty acid content of sera, and glucose-6-phosphatase and glucose-6-phosphate dehydrogenase activities of liver were unaffected in male albino rats fasted for 3 days, then fed a diet supplemented with 0, 250 or 2500 mg/kg C_{12} LAS for 4 days (Selmeci-Antal and Balskovits, 1977).

d. Glucose Tolerance

In a series of studies, Antal (1970, 1971, 1973) observed that the inclusion of LAS in the diet of male and female rats at 250 mg/kg/day

for up to three months resulted in increased liver weight and fasting blood sugar levels. A single high dose of 940 mg/kg in fasted control and treated rats resulted in increased blood glucose levels similar to those obtained by glucose loading (610 mg/kg). Females were generally more sensitive than males in exhibiting reduced glucose tolerance. Pretreatment with LAS had no remarkable influence on the blood glucose pattern following LAS or glucose loading.

e. Dye Uptake

The effects of LAS on the absorption of various dyes in ligated sections of rat large intestine was examined by Bornmann and Stanisic (1962). A 2% solution of LAS injected with the test dye resulted after one hour in increased uptake of phenol red and Ponceau 6R while the uptake of methyl violet was inhibited and Congo red was unaffected. At an LAS concentration of 0.01%, no effect was observed with phenol red.

B. Human Studies

At concentrations of LAS well above normal use levels (1% or less), little or no skin irritation is observed in patch tests in humans, and there is no evidence that LAS exerts any sensitization reaction on human skin. The rapid and complete excretion of LAS following a single oral dose and the absence of any substantial percutaneous absorption (<0.01%) in human subjects closely parallels the findings in experimental animals and indicates little likelihood of tissue accumulation in humans.

1. Skin Irritation

Nixon et al. (1975) conducted a comprehensive examination of the skin responses of humans, rabbits and guinea pigs to a number of materials, including several detergents containing from 4 to 17% LAS. For many

materials, the rabbit showed substantially stronger reactions than the human and seemed to be unduly sensitive. The guinea pig appeared to be less sensitive than the rabbit, but in some cases it failed to predict significant human response. Among the three LAS-containing detergents examined, no correlation was found between LAS content and skin irritation in humans. Two of these detergents exhibited negligible irritancy, even on abraded human skin.

In a similar study, Brown (1971) found a lack of agreement in response among the different animal tests, and between human and animal tests, which suggested that caution be exercised in the use of animal tests as predictors for skin irritancy in humans.

LAS induces the release of arachidonic acid from 2 different types of mammalian cells in culture. In a study by DeLeo et al. (1987), C3-10T$_{\frac{1}{2}}$ cells and human keratinocytes were prelabeled with ^{3}H-arachidonic acid. Cells were incubated for 2 hours in media with or without LAS. In C3H-10T$_{\frac{1}{2}}$ cells, LAS in 5-50 μM concentrations stimulated 2-10 times the release of ^{3}H-arachidonic acid as compared to controls. Similar results were obtained with human keratinocytes. Arachidonic acid metabolites mediate the inflammatory reaction which causes irritancy in human and animal skin.

Imokawa et al. (1975a) evaluated the relative intensity of skin roughness produced on the surface of the forearm of human volunteers by contact with LAS of varying alkyl chain length (C_8, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}). Skin response was characterized mainly by gross visible changes. C_{12} LAS produced more skin roughening than LAS with alkyl chains longer or shorter than 12. Imokawa and co-workers (1975b) also found that the relative degree of skin roughening *in vivo* correlated with the extent of protein denaturation measured *in vitro*. Also the degree of adsorption of anionic detergents parallels skin roughness caused by the surfactant (Imokawa, 1974).

A study by Tavss et al. (1985) demonstrated a correlation between surfactant-induced in vitro epidermis curling and in vivo skin irritation. A 2.4% solution of LAS caused strips of epidermis to twist and curl while a 10% solution applied to the forearm of subjects caused severe irritation within 1 day. A later in vitro study by the same investigators (1986) suggested that the addition of positively charged proteins might increase the mildness of solutions containing anionic detergents without reducing their foaming and detergency properties.

Using dilute solutions of surfactants (0.1-1.0%) to simulate actual use conditions, Smeenk (1969) found that there was some agreement between the increase of thiol group availability (a measure of keratin dissolution) from callus powder, release of potassium ion (a measure of cell permeability increase) from isolated skin and in vitro patch test response. However, based on tests with a number of surfactants, the relationship between the in vitro tests indicated that only the immersion test was suitable for prediction of skin irritancy in humans. Patch tests on humans with a 1% LAS solution showed a low level irritation in eight of 50 subjects with no irritation in the others. Wood and Bettley (1971) found a rapid increase in free thiol group following treatment with 0.04% solution of LAS in vitro; however, there was little correlation with epidermal penetration of the surfactant.

A correlation between desquamative skin changes in vivo and inhibition of invertase activity was noted among several anionic surfactants, including LAS. A concentration of 0.003% LAS completely inhibited invertase activity (Okamota, 1974).

2. Skin Sensitization

Although LAS is a contact sensitizer in guinea pigs under exaggerated exposure conditions (including intradermal injection of the compound in adjuvant), LAS produces no evidence of sensitization in humans under normal usage conditions. The skin sensitization potential of LAS in humans was evaluated using the Human Repeat Insult Patch Test (Stotts,

1980). LAS active concentrations from 0.01% to 0.113%, selected on the basis of the highest minimally irritating concentration, were used. Out of 2294 panelists tested, there was no evidence of sensitization in these individuals. In addition, 17,887 subjects have undergone human sensitization testing with LAS-containing detergent products (LAS patch concentration 0.001-0.09%). No evidence of sensitization was observed in these subjects (Robinson et al., in press). These studies demonstrate that LAS does not produce sensitization reactions at minimally irritating doses in humans under the exaggerated conditions of multiple, occluded patch test exposures.

An examination of the sensitization potential of LAS for human skin revealed that, at 0.05% and 0.2% aqueous concentrations of active LAS, no sensitization was found in 71 and 81 human subjects, respectively. Repeated patch tests at these LAS concentrations produced mild to moderate primary irritation (The Procter and Gamble Company, unpublished data). In another study (The Procter and Gamble Company, unpublished data), a 0.1% aqueous LAS preparation caused no sensitization in 86 subjects, whereas a 0.1% solution in 50% ethanol/water induced a sensitization response in 6 of 86 subjects. Further studies with the 50% ethanol solution alone demonstrated that the positive response was due to the ethanol alone.

3. Pharmacology

There is only very limited information on the metabolic fate of LAS in humans. With respect to the primary route of exposure, it was found that, following a single cutaneous dose of ^{35}S-labeled LAS, 99% was removed from the application site and less than 0.01% of the radio-activity was found in the urine and feces after 144 hours (The Procter and Gamble Company, unpublished data). Howes (1975) found that LAS did not penetrate skin as measured by an in vitro test using ^{14}C-labeled LAS. Iimori (1971) observed that, although penetration of LAS into human skin did not readily occur, adsorption was pH dependent. In the range of pH 7.0 to 11.0, adsorption of LAS decreased with

increasing alkalinity of a post-treatment rinse. In a similar study, Tomiyama (1975) observed that following an exposure of fingers to ^{35}S-labeled LAS, washes of increasing alkalinity removed increasing amounts of LAS from the skin.

After a single oral administration, excretion of ^{35}S-labeled LAS in the urine and feces was found to be over 90% complete in 144 hours. Approximately 50% of the dose was absorbed and excreted in the urine, mostly in the first 24 hours after administration (The Procter and Gamble Company, unpublished data).

4. Epidemiology

a. Accidental Exposure

The general problems associated with accidental ingestion of detergents have been reviewed using data of the National Clearing House for Poison Control Centers through 1970 (Calandra and Fancher, 1976). While a large number of detergents contain LAS, the toxic effects observed following detergent exposure cannot with certainty be attributed to the surfactant. Of the 3446 incidents of accidental ingestion of soaps, detergents and cleaners, less than 10% (278 cases) reported any symptoms, and of these, only 1.6% (54 cases) were due to products which may have contained LAS in addition to other materials. For 1973, a total of 6509 inquiries were reported by Poison Control Centers with 1094 cases requiring treatment or consultation. Of these cases, 852 presented no symptoms and no fatalities were recorded (National Clearing House for Poison Control Center, 1973). A similar experience was noted by Krienke (1974) for Berlin, West Germany in 1971. Of approximately 600 inquiries classified as detergent ingestions, no fatalities were indicated and only 2% of the incidents resulted in "moderately severe" symptoms. The nature of these symptoms was not reported. In 1986, 5808 cases of accidental ingestion of detergents containing anionic/nonionic surfactants were reported. Over 80% of these cases were in children under 6 years of age. There were no fatalities and 47% reported no symptoms (Litovitz et al., 1987).

The data compiled by the National Electronic Injury Surveillance System (NEISS) of the U.S. Consumer Product Safety Commission for the fiscal year ending June 30, 1975, for cases seen in emergency rooms of the 119 participating hospitals were examined. In the overall category of "Home and Family Maintenance Products," there were 4683 incidents. However, of these, only 73 were attributed to laundry soaps and detergents, products likely to contain LAS. These statistics must be evaluated with considerable care since the individual case reports are unverified.

b. Occupational Exposure

One account of the effects of occupational exposure to LAS has been published (Rosner et al., 1973). A group of 60 workers exposed to a work atmosphere of 8.64 mg surfactant per cubic meter were tested for serum lipid and sugar content as well as for activities of selected serum enzymes. Among the parameters studied, total plasma lipids and plasma cholesterol were slightly lower in the exposed group compared to controls. No differences were noted for blood sugar, plasma phospholipid, plasma lipoprotein, α-amylase, leucineaminopeptidase or pseudocholinesterase. The duration of the exposure prior to testing was not indicated. The authors concluded that exposure to the work environment was not injurious to health.

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