PB-301 193

HUMAN SAFETY AND ENVIRONMENTAL ASPECTS OF MAJOR SURFACTANTS

A Report To The
SOAP AND DETERGENT ASSOCIATION

May 31, 1977

Arthur D Little, Inc.

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Human Safety and Environmental Aspects of Major Surfactants.

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This review was assembled to assess the depth and scope of available environmental and human safety data for a group of surfactants which have either present commercial importance or possible future wide use. With respect to present commercial importance, the four surfactants in major use can be ranked according to the amounts synthesized and compounded into commercial products. This ranking inclues (1) linear alkylbenzene sulfonates, (2) alkyl sulfates, (3) alcohol ethoxylates, and (4) alcohol ethoxy sulfates. Among the surfactants of lesser commercial importance at the present time, three were considered in this review: (5) alkylphenol ethoxylates, (6) alpha olefin sulfonates, and (7) secondary alkane sulfonates. For each surfactant considered, the authors have reviewed four major areas of interest: (1) environmental distribution and fate, (2) biodegradation, (3) environmental effects of surfactants and biodegradation products, and (4) human safety as judged from studies of animal toxicity and pharmacology and from human exposure.

KEYWORDS: *Surfactants, *Water pollution, *Sulfates, *Sulfonates, *Toxicology, *Water quality, *Toxic substances.

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PREFACE

This review was assembled to assess the depth and scope of available environmental and human safety data for a group of surfactants which have either present commercial importance or possible future wide use. With respect to present commercial importance, the four surfactants in major use can be ranked according to the amounts synthesized and compounded into commercial products. This ranking includes (1) linear alkylbenzene sulfonates (LAS), (2) alkyl sulfates (AS), (3) alcohol ethoxylates (AE), and (4) alcohol ethoxy sulfates (AES). Among the surfactants of lesser commercial importance at the present time, three were considered in this review: (5) alkylphenol ethoxylates (APE), (6) alpha olefin sulfonates (AOS), and (7) secondary alkane sulfonates (SAS).

For each surfactant considered, we have reviewed four major areas of interest:

(1) environmental distribution and fate,

(2) biodegradation,

(3) environmental effects of surfactants and biodegradation products, and

(4) human safety as judged from studies of animal toxicity and pharmacology and from human exposure.

We have collected and reviewed the open literature published up to the end of 1976 as well as unpublished data supplied to us by the Soap and Detergent Association and its member companies. As new technical information

becomes available, we will re-evaluate the areas affected by the new data.

In addition to a specific review of LAS, Chapter 1 also contains a broadly applicable discussion of methodology for chemical analysis, biodegradation, aquatic toxicity, and a consideration of the human safety of surfactants as components of detergent products.

For the major surfactants, LAS, AS, AE and AES, there were sufficient data in each of the areas reviewed to support the view that these substances as a class pose no threat to either human health or to the environment at large. The primary reasons for this are the low order of mammalian acute toxicity, the general absence of chronic effects in mammalian test systems, and the facile biodegradation of the surfactants in the environment.

With respect to APE, AOS and SAS, there is less complete experimental support for the human safety and environmental acceptability of these materials. However, the similarity of general structural features between these surfactants and the others considered above would suggest a similar degree of safety and acceptability.

CONTENTS

LIST OF ABBREVIATIONS

The following abbreviations have been used throughout the text:

ABS	Alkylbenzene sulfonate, usually with alkyl group in the detergent range; i.e., above C_6.
AE	Alcohol ethoxylate
AES	Alcohol ethoxy sulfate
AI	Active ingredient
AM	Active material
AOS	Alpha olefin sulfonate, α-olefin sulfonate
AS	Alkyl sulfate
AVE	Average; used to designate broad-cut-derived alkyl sulfates
BIAS	Bismuth iodide active substance
BOD	Biochemical oxygen demand
br-	Branched
CL	Confidence limits
cm	Centimeter
CMC	Critical micelle concentration
CO_2	Carbon dioxide
COD	Chemical oxygen demand
conc	Concentration
CTAS	Cobalt thiocyanate active substance
DOC	Dissolved Organic Carbon
Enz	Enzyme
EO	Ethylene oxide
EPN	A phenylphosphonothioate insecticide

LIST OF ABBREVIATIONS (continued)

E_x, E_n	Polyethylene glycol or ethoxylate nonionic averaging x (or n) EO units per molecule
FIR	Far infrared spectroscopy
GC	Gas chromatography
g	Grams
hr	Hours
IR	Infrared spectroscopy
kg	Kilogram
l	liter
LAB	Linear alkylbenzene
LAS	Linear alkylbenzene sulfonate
LC_{50}	The concentration required to kill 50% of test individuals
LD_{50}	The dose required to kill 50% of test individuals
m^3	Cubic meter
MAC	Maximum allowable concentration
MBAS, MB	Methylene blue active substance
mg	Milligram
mgd	Million gallons per day
min	Minute
ml	Milliliter
mM	Millimole
mol. wt. M.W.	Molecular weight
n-	Normal, linear
Na	Sodium
NH_3	Ammonia, ammonia liberation
NMR	Nuclear magnetic resonance

LIST OF ABBREVIATIONS (continued)

O_2	Oxygen, oxygen uptake
PC	Paper chromatography
PEG	Polyethylene glycol
pH	A measure of hydrogen ion concentration
ppm	Parts per million; used interchangeably with milligrams per liter
pri-	Primary
Rf	In paper chromatography, the ratio of the movement of a given spot to that of solvent boundary.
SAS	Secondary alkane sulfonate
SDA	Soap & Detergent Association
sec-	Secondary
STCSD	Standing Technical Committee on Synthetic Detergents
tert-	Tertiary
TES	Tallow alcohol ethoxy sulfate
TLC	Thin layer chromatography
TLm	The concentration that results in 50% survival over a specified time interval
TOC	Total organic carbon
tp-	Tetrapropylene
UV	Ultraviolet spectroscopy
µ	Micron
µCi	Microcurie
µg	Microgram
µmol	Micromole
w/v	Weight in volume
w/w	Weight in weight

LIST OF ABBREVIATIONS (continued)

>	Greater than
<	Less than
∿	Approximately
♂	Male
♀	Female

Characterization of the various animal species and subspecies cited in the Human Safety sections of this report can be found in: Handbook on Genetically Standardized JAX Mice, Earl T. Green, Ed. Bar Harbor Times Publishing Co., Second edition, February, 1971.

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF FIGURES

LINEAR ALKYLBENZENE SULFONATES

Synopsis

The linear alkylbenzene sulfonates (LAS) represent a substantial portion of the total surfactant market. In 1973, approximately seven hundred million pounds of LAS were produced in the United States. These surfactants are a complex mixture of isomers and homologues whose proportion is dependent on starting materials and the conditions of reaction for preparing the linear alkylbenzenes which are the precursors of LAS. The alkyl chains of commercially available LAS mixtures generally range from 10 to 14 carbons in length and the phenyl groups are placed at various internal carbon positions in the alkyl chains. The biodegradable LAS surfactants have been in major use since 1965, when they were introduced by the detergent industry as a constructive environmental action to replace the highly branched, less biodegradable tetrapropylene-derived alkylbenzene sulfonates in wide use at that time.

Although a number of analytical methods are available for the determinations of LAS in water samples and in experimental studies, the primary method in use is the assay for methylene blue active substances (MBAS). This method is dependent upon the formation of a complex between the anionic LAS and cationic methylene blue and the solubility of this complex in chloroform. Since the reaction of LAS with methylene blue is not specific and anionic materials other than LAS can readily bind to methylene blue, the reliability

of MBAS values, especially in the assay of environmental samples, may be open to question. Alternative methods such as infrared and ultraviolet spectroscopy have also been used. However, in complex environmental samples, interference from other absorbing materials prevents spectroscopic assays from achieving maximum effectiveness. In order to characterize LAS samples adequately, a desulfonation-gas chromatography technique has been devised. This research procedure allows the identification of LAS components of different carbon chain lengths as well as components with different phenyl ring positions; however, it is not suited for routine environmental analyses. For the determination of environmental levels of LAS, there has been an almost complete reliance on the MBAS analytical procedure, although this assay technique does not discriminate between LAS and other anionic surfactants or other anionic substances which may be found in waterways.

With respect to environmental standards of water quality, the United States Public Health Service drinking water standard for water involved in interstate commerce is 0.5 mg/l as MBAS. Several states and Canadian provinces have also set 0.5 mg/l MBAS as maximum permitted levels. Although the European Economic Community has set no direct water quality standards for LAS, this organization prohibits the marketing and use of detergents containing surfactants which have an average level of biodegradability less than 90% as MBAS.

The U.S. Environmental Protection Agency, State Agencies, and other institutions, have collected a considerable data base of MBAS levels for different bodies of water. These show that during the change from ABS to LAS, there was a gradual decrease in MBAS levels in most waterways that were examined with any degree of thoroughness. In many cases the MBAS levels are presently below 0.05 mg/l. Where levels higher than 0.5 mg/l MBAS are found, these waterways usually received inadequately treated or untreated sewage.

The original selection of LAS as a replacement for the tetrapropylene-derived ABS was based on the more rapid and complete biodegradation of LAS. A number of test systems are employed to study the biodegradation of LAS. These systems are models for the processes which actually occur in natural waterways and in various levels of sewage treatment. The field studies that have been carried out on LAS biodegradation confirm the value of the laboratory tests as capable of predicting the response of LAS in biological degradation systems. It has been found that LAS mixtures are readily biodegradable (over 90%) in both laboratory and field situations. Although certain chemical characteristics of LAS (alkyl chain length, phenyl group position) influence the rate of biodegradation to some degree, there do not appear to be any forms of LAS now commercially used that are highly resistant to biodegradation.

The study of the acute toxicity of single LAS components to various species of fish has revealed certain general structure-activity relationships. The LAS components exhibiting the highest degree of toxicity to aquatic organisms are those with longer alkyl chains and those with the more terminal phenyl group substitutions. However, the risk to aquatic species from these more toxic LAS components is not great, since these isomers are those which are most rapidly biodegraded to relatively innocuous substances. The LC_{50} values for adult fresh water fish range from 20 to 40 mg/l for C_{10} LAS to 0.4 to 1.0 mg/l for C_{14} LAS. For commercial LAS mixtures, LC_{50} values fall in the range of 1 to 10 mg/l for a number of fresh and salt water fish. A similar range of values is obtained for some aquatic invertebrates, although adult marine crustaceans and bivalves are quite resistant to LAS with LC_{50} values of >100 mg/l. The few studies on partially biodegraded LAS show a marked and rapid reduction in toxicity to adult fish as compared to intact LAS. Model degradation compounds exhibit up to a thousand-fold lower toxicity than LAS. The major concern of aquatic toxicity of LAS rests in the sensitivity of juvenile life stages of some aquatic organisms to levels of LAS below 0.05 mg/l. In several laboratory studies, enhancement of toxicity by concomitant exposure of fish to subtoxic doses of LAS and toxic doses of some pesticides and petroleum products has been reported. The limited information on the effects of LAS on plants indicates that no problems should be anticipated with exposure to LAS at environmental levels.

The studies examining the toxicity of LAS to mammalian species have shown that the acute LD_{50} values for rodents range from approximately 650 to 2000mg/kg. Long-term feeding studies at levels of LAS up to 0.5% of the diet

of rats have shown that continuous exposure does not result in any deleterious effects in these animals, at levels that exceed estimated human consumption by over a thousand-fold. Although undiluted LAS samples have been demonstrated to be primary irritants in rabbit skin tests, irritation and sensitivity tests in humans do not indicate any substantial problems from cutaneous exposure to LAS in humans. The rabbit eye tests conducted according to the Draize protocol have shown that LAS at levels of 0.5 and 1.0 percent can induce immediate congestion and edema which are reversible. However, the severity of this test procedure in relation to the expected extent of accidental exposure in humans raises problems for the interpretation of these rabbit eye studies for human safety evaluations.

The examinations of LAS for possible carcinogenicity by either oral or cutaneous exposure have been completely negative as have been tests for mutagenicity, although only a limited number of mutagenicity tests have been done. The enhancement by LAS of gastric tumor induction by nitroquinoline-N-oxide in rats has been reported; however, it is unclear whether this effect is due to enhanced carcinogen absorption or some other physiological mechanism. A number of teratology investigations with LAS and detergents containing LAS have been performed. Other than the report from a single laboratory (Y. Mikami, Mie University, Japan), which could not be reproduced by other investigators, there is no evidence that LAS induces birth malformations or affects reproduction in experimental animals.

Because of its low order of toxicity to mammalian species and its facile biodegradation in waste treatment plants and natural waterways, resulting in environmental levels well below the established standard for drinking water, LAS remains an acceptable surfactant for consumer and industrial use with respect to both human health and environmental safety.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

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 significant environmental improvements in terms of foaming and residual surfactant levels in waterways. Their efficiency as cleaning agents, their relatively facile biodegradability and their environmental safety have kept these materials as a major factor in the detergent market up to the present time. It has been a number of years since the environmental fate, distribution and safety of LAS have been examined in some detail, and this review was prepared to evaluate the information that has accrued since these earlier reports 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.

With respect to the amount of material produced on an annual basis, the U.S. International Trade Commission (1975) figures indicate a total U.S. LAS production of 6.78×10^8 pounds for 1973. If one assumes a reasonable balance between production and use, it would appear that approximately 7×10^8 pounds of LAS may be introduced into waste water treatment facilities of varying effectiveness in any single recent year.

A review of the substantial body of data on LAS has revealed that the levels of methylene blue active substances (MBAS) in waterways remain acceptably low. Except for areas that have poor or no waste-water treatment, MBAS levels are generally well below 0.5 mg/l, the U.S. Public Health Service standard for drinking water. These low levels of MBAS are primarily a consequence of the ease of biodegradation of LAS, even in natural waterways.

A. Chemical Characterization of LAS

An assessment of the fate and effects directly due to LAS in the environment is difficult because of several factors. First, the LAS that is used in commercial products is a complex mixture of materials with a range of linear alkyl chain length, usually from ten to fourteen carbons. Further, the distribution of the phenyl ring position on the alkyl chain varies according to the starting materials and processes employed to generate the linear alkylbenzene (LAB) moieties from which LAS are derived by phenyl ring sulfonation. Second, a number of side reactions occur resulting in a mixture of dialkyl substituted mono- and di-ring structures and some diphenylalkane. Some of these impurities can be removed, but those remaining may constitute 5 to 10 percent of the final surfactant. Finally, LAS represents a complex mixture of chemical entities which exhibit considerable variations in biodegradation and aquatic toxicity, thereby complicating the evaluation of the effects of LAS in the environment.

1. Primary Product-LAS

Linear alkylbenzene (LAB) is the precursor for LAS. The production of LAB has been carried out by alkylation of benzene with a mixture of secondary alkyl chlorides in the presence of aluminum chloride, the classical Friedel-Crafts reaction. More recently, alternative procedures have also been used. These 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 isomers of LAB with various chain lengths depending on the nature of the alkyl halide or olefin feedstock. Moreover, even if a single isomer of 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 that is finally used in a product can be controlled to some degree by the choice of starting materials and manufacturing process. Table 1-A indicates the diversity of LAS structures in several typical commercial products. This diversity of structure has an impact on biodegradability and toxicity as will be discussed below. A substantial portion of the LAS manufactured for detergent use has a rather narrow range of alkyl chain lengths from ten to fourteen carbon atoms. The distribution of phenyl group positions is determined by the reaction conditions in the production of linear alkylbenzene. However, the character of the commerical linear alkylate is a consequence of the alkylation step and of the subsequent isomerization of the phenyl alkanes in the reaction mixture.

TABLE 1-A

CHARACTERISTICS OF REPRESENTATIVE COMMERCIAL LAS PRODUCTS*

(Percent of Total)

Carbon Chain length	Supplier A		Supplier B		Supplier C	
	Product 1	Product 2	Product 1	Product 2	Product 1	Product 2
$<C_{10}$	<1	<1	2**			
C_{10}	15	11	85	10**	15	<1
C_{11}	43	31			43	2
C_{12}	35	32			35	12
C_{13}	7	23	15**	70-90	7	46
C_{14}	<1	3	2**			38
$>C_{14}$				5**		1
Phenyl isomer distribution						
2-phenylalkane	32	30	25	25	33	29
3-phenylalkane	NA***	NA	NA	NA	20	17
4-phenylalkane	NA	NA	NA	NA	18	16
5-phenylalkane	NA	NA	NA	NA	18	17
6-phenylalkane	NA	NA	NA	NA	11	15
7-phenylalkane	NA	NA	NA	NA		6
Tetralins and by-products	6	7	NA	NA	6-10	6-10

*Manufacturers' specifications

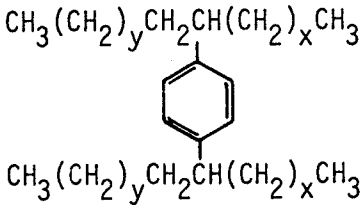
**Maximum percentage

***Not available

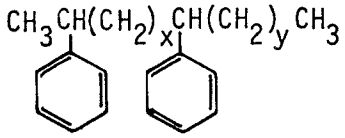
FIGURE 1-1

PRODUCTS RESULTING FROM SIDE REACTIONS

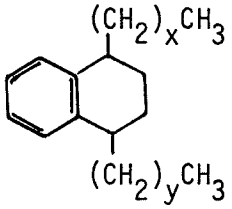
IN THE MANUFACTURE OF LINEAR ALKYL BENZENE



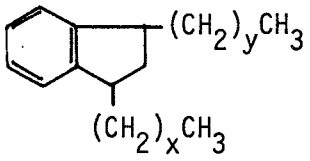
Dialkylbenzene



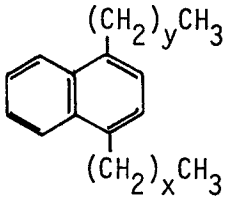
Diphenylalkane



Dialkyltetralin



Dialkylindane



Dialkylnaphthalene

2. Secondary Products

The alkylation of benzene results in a number of side reactions. The known products of these reactions are shown in Figure 1-1. The diphenylalkanes and dialkylbenzenes boil at temperatures sufficiently above the linear monoalkylbenzene so that they are readily removed. 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 that is used in detergent products. Dialkyltetralin, dialkylindane and dialkylnaphthalene moieties may represent as much as 5 to 10 percent of the final product.

3. Inorganic Sulfate

While not directly due to LAS manufacture, the presence of inorganic sulfate in commercial detergents along with the sulfate from biodegradation of LAS results in an addition of this anionic species to the environment. The contemporary interest in sulfate from other sources suggests that this chemical entity not be disregarded in the consideration of the aquatic safety of LAS. If one assumes that all the LAS manufactured in one year in the United States is completely degraded to inorganic sulfate yielding 2.1×10^8 pounds of sulfate and that an additional 20×10^8 pounds of sulfate derives from detergent formulations, the maximum possible annual contribution of detergent products to sulfate in waterways would be 2.2×10^9 pounds or about 10^6 metric tons.

While this may appear to be a substantial amount, it is negligible compared to other sources, primarily precipitation of sulfur dioxide in rain. Taking a single waterway for example, the annual precipitation loading of sulfate in the Niagara River to Lake Ontario alone amounts to 4.5×10^6 metric tons (Shiomi and Kuntz, 1973), or nearly five times the maximal expected amount of sulfate from detergents in the entire United States.

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II. ENVIRONMENTAL LEVELS

The concentrations of anionic surfactants in most surface waterways as measured by methylene blue active substances (MBAS) have shown a general decreasing trend since 1965 when LAS was introduced by the detergent industry to replace the less degradable tetrapropylene-derived ABS. Although MBAS assays lack specificity and are prone to multiple positive and negative interferences, this procedure remains the mainstay for the determination of LAS concentrations in environmental samples. Except for areas receiving either poorly treated or untreated sewage effluents, MBAS levels are usually well below the drinking water standard of 0.5 mg MBAS/l.

A. Analytical Methods

Swisher (1970) and Rosen and Goldsmith (1972) have described in considerable detail the procedures and problems of the analytical methods available for the determination of surfactants, including LAS. Recent studies by Howard et al. (1975) and Higgins and Burns (1976) also summarize this information. The analytical procedures can be categorized into 3 major areas: physical methods, specific chemical techniques and physicochemical analyses. 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 biodegradation studies.

1. Physical Methods

Determination of foaming potential and measurement of changes in surface tension are the two principal physical procedures utilized to monitor biodegradation.

Although the residual foaming potential of a partially degraded surfactant can be used as a measure of biodegradation, the usefulness of this method is limited. A transient phenomenon, foaming can be affected by a wide variety of factors such as temperature, humidity, size of the test container, etc.; furthermore, foaming is not a linear function of surfactant concentration (Swisher, 1970).

Changes in surface tension can also be used as a measure of biodegradation. This procedure is based upon the fact that the presence of a few parts per million of a surfactant significantly lowers the surface tension of water. The magnitude of this change increases (although not in a linear fashion) with an increase in the concentration of surfactant until a critical micelle concentration is reached; above this concentration, further increments of surfactant produce little or no change in surface tension.

Measurement of surface tension is quick and qualitative in that each surfactant has a characteristic ability to lower surface tension. The major drawbacks of this method are: 1) the lack of specificity; 2) insufficient sensitivity to distinguish minute changes in surfactant concentration as

biodegradation proceeds; and 3) the ease with which foreign substances can distort results (Organization for Economic Cooperation and Development, 1964). This latter point makes this procedure unsuitable for analysis of biodegradation in waste waters.

2. Specific Chemical Techniques

a. Methylene Blue Active Substances (MBAS)

The MBAS procedure for determination of anionic surfactants was first proposed by Jones (1945) and later modified by Longwell and Maniece (1955). Because of its simplicity and high degree of sensitivity (0.1 ppm), it has become the primary method for measurement in environmental analysis and for following biodegradation studies. The MBAS procedure is the Official Method for the Examination of Water and Wastewater for anionic surfactants by the American Public Health Association (1971) and this method is used by the U.S. Environmental Protection Agency. It is also the standard method of testing of the American Society for Testing Materials (1974) for alkylbenzene sulfonate in water.

However, the methylene blue reaction lacks specificity. It cannot distinguish between ABS and LAS surfactants (Swisher, 1966). Additionally, the reaction is so responsive that any compound possessing a single strong anionic center and a sufficiently lipophilic group can bring the salt produced into the organic extraction solvent (Swisher, 1970), thus resulting in a positive interference. For the same reason, the procedure requires

appropriate washing steps for seawater analyses, since high concentrations of chlorides could result in positive interference (Swisher, 1970). Wickbold (1976) recently noted that the presence of humic substances in surface waters which pass through the purification steps in the Longwell-Maniece procedure also produces a positive interference which is inversely proportional to the reduction in surfactant concentration. Wickbold overcame this error by isolating the surfactant via a sublation procedure in which a fine stream of nitrogen loaded with ethyl acetate vapor is bubbled through the water sample. Negative interferences also occur in the MBAS analysis in the presence of amines or other cationic substances, such as proteins, which may compete with the cationic methylene blue for the anionic surfactant (Swisher, 1970).

Borstlap (1967) points out that one of the drawbacks of the methylene blue procedure is its inability to detect some intermediate degradation products; i.e., it can only detect the amount of anionic surfactant in which the strongly anionic group has not been altered. Removal of the sulfonate group from the benzene ring or oxidation of the terminal methyl group of the alkyl side chain to a carboxyl group can also eliminate the methylene blue reaction (Gledhill, 1974). With randomly labeled ^{14}C-dodecylbenzene sulfonate, it was found that when 90% MBAS was no longer present, 75% of the radioactivity still remained (Allred et al., 1964; cited in Gledhill, 1974).

Because the methylene blue reaction lacks specificity, the entities detected with this procedure are referred to as methylene blue active substances (MBAS). For example, in a comparison of the methylene blue method and the total organic carbon analysis, Sekiguchi et al. (1972) found that

LAS lost 97.5% of its MBAS in 5 days, while 34.1% of its organic carbon disappeared in the same time period and 69.8% had disappeared at 31 days. In another study, comparable LAS degradation values were noted using the river die-away test (>97 % MBAS removal by 11 days) and the shake flask test (97.2% MBAS removal; duration unspecified). In contrast, when measured as the percentage of theoretical CO_2 evolved, LAS was degraded 56% after 15 days (Ethyl Corporation, unpublished data).

Another potential source of error arises from the fact that detergents adsorb at interfaces, particularly in water with a high solids content (e.g., sewage, activated sludge). This adsorption of surfactant to particles in suspension results in false colorimetric readings of the actual surfactant concentration (Organization for Economic Cooperation and Development, 1964).

b. Other Methods

Anionic surfactants present in surface waters have also been determined via the azure A colorimetric method (Den Tonkelaar and Bergshoeff, 1969; Wang and Ross, 1974). Based on the principal that acidic solutions of anionic surfactants form a blue-colored complex with azure A, this procedure is subject to positive and negative interferences similar to those noted for the MBAS method.

Another procedure commonly used in the determination of anionic surfactants is the two-phase titration method. The anionic surfactant is titrated in a mixed aqueous chloroform medium with a standard solution of a cationic

reagent such as Hyamine 1622 (ASTM:D3049 -'75) 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) have proposed a two-phase titration method as a substitute for the MBAS method for the analysis of LAS in seawater. Their procedure employs a nonaromatic quaternary ammonium salt and a tetraphenyl boron (TPB) reagent and is reportedly insensitive to high salinity values. High potassium content, however, does produce a negative interference by reacting with the TPB reagent.

Taylor et al. (1974) have 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.

Another chemical procedure utilized to evaluate LAS biodegradation is the measurement, either colorimetrically or turbidimetrically, of the sulfate ion (Cordon et al., 1968 1970). Cordon's turbidimetric procedure determines the amount of sulfate ion formed during biodegradation of surface active organic sulfates or sulfonates.

3. Physicochemical Analyses

Thin layer, paper and gas chromatography have all been utilized in the study of surfactant biodegradation. Although anionic surfactants are not sufficiently volatile for useful results with gas chromatography, they are

susceptible to desulfonation by boiling with concentrated phosphoric acid at 200°C (Swisher, 1966). The volatile products are trapped in hexane, the solvent evaporated in a stream of air, and the residue examined by gas chromatography. This desulfonation-gas chromatographic technique can detect 1 μg or less of LAS, but to minimize handling problems, sample sizes of 0.1 to 1 mg are generally used (Swisher, 1966, 1970). This procedure has provided vital information on intermediate products as well as the metabolic routes of LAS biodegradation.

Infrared (IR) and ultraviolet (UV) spectroscopy have also been applied to the study of biodegradation. The major use of IR anlaysis has been to distinguish between ABS and LAS surfactant 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, 1970). With respect to infrared procedures, the American Public Health Association (1971) has proposed a tentative 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. However, the method is applicable only to raw water samples and not to sewage or industrial wastes.

The benzene rings of LAS show 3 characteristic absorption bands in the ultraviolet range: 260 mμ, 223mμ and 193 mμ (Swisher, 1967). The absorption bands can be readily detected at LAS concentrations of 1 ppm or less in aqueous solution (Swisher, 1967). Biodegradation of the benzene portion of a molecule is accompanied by a proportional disappearance of these bands (Swisher, 1972).

This procedure is more applicable to later stages of biodegradation, however, in that the molar absorbance remains relatively constant as long as the benzene ring is present in the system (Swisher, 1970). One of the major disadvantages of this procedure is that it cannot be utilized when working in an undefined system due to the presence of many interfering substances (Gledhill, 1974).

A method for the determination of 2-15,000 µg/l of anionic surfactants in fresh, estuarine and seawater samples has recently been reported by Crisp et al. (1976). The surfactant anions are extracted into chloroform as an ion-association compound with bis(ethylenediamine) copper (II) cation. The copper in the chloroform layer is then determined directly by atomic absorption spectrometry with graphite furnace atomization. Insensitive to ionic interference, this method is applicable to salt water samples without modification.

Another analytical technique which has been applied specifically to the study of LAS biodegradation is the use of stable or radioactively-labeled surfactants. The most commonly used label has been ^{35}S in the sulfonate group, but ^{14}C and ^{3}H have also been used (Huddleston and Allred, 1963, cited in Swisher, 1970; Swisher, 1970). This method is extremely sensitive and free from interferences. However, using the disappearance of ^{35}S-sulfonate as an indication of complete biodegradation may be uncertain in that, reportedly, the sulfonate group has been removed from the benzene ring both prior to and at the same time as ring cleavage (Cain et al., 1971; Oba, 1971).

Tsuji et al. (1975) have recently reported on a potentiometric method for the microdetermination of surfactants. The procedure is based on the

inhibition of the cholinesterase-butyrylthiocholine iodide system by anionic surfactants; nonionics seemingly weaken this inhibition. The degree of suppression of the inhibitory effect of a fixed amount of C_{15}-LAS is taken as a direct measure of the concentration of nonionic surfactant. The procedure is quick and requires no complicated apparatus, but suppression is difficult to evaluate at low concentrations and, due to potential interferences, the method could not be utilized in the field.

B. Water Quality Standards

1. National Regulations

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).

In 1973, the U.S. Environmental Protection Agency considered establishing waterway levels for LAS and for foaming agents (U.S. Environmental Protection Agency, 1973). The criteria were based on aquatic toxicity data from a few selected studies (Pickering, 1966; Arthur, 1970; Pickering and Thatcher, 1970; Hokansen and Smith, 1971). One of the studies, cited by the U.S. Environmental Protection Agency as support for chronic effects of LAS (Arthur, 1970), did not employ a pure LAS or even a commercial LAS mixture, but rather used a detergent preparation containing another surfactant and considerable quantities of inorganics (>60%) in addition to LAS (14%). Moreover, the proposed standard did not account for the widely varying toxicities of LAS homologues of different carbon chain length which can range from an LC_{50} of 61.0 mg/l for

Carassius auratus with a C_{10} LAS mixture to 0.26 mg/l for Idus melanotus with a C_{14} LAS mixture (See Table 1-C and Section IV for a more extensive consideration of LAS structure-aquatic toxicity relationships). Finally, no standard method for determining LAS concentrations was described. With the development of the Criteria for Water Quality in the intervening years, the standard originally proposed for LAS is no longer a part of the Quality Criteria for Water (U.S. Environmental Protection Agency, 1976).

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 "

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 no 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).

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 (Illinois, Michigan, Minnesota, New Jersey and Ohio) 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.

In addition, the state of Oregon prohibits non-biodegradable cleaning agents.Restrictions on the sale of surfactants exist in several localities. Suffolk County on Long Island in New York State 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 sewage effluents. Dade County, Florida prohibits the sale of nonbiodegradable cleaning agents (Soap and Detergent Association, 1975).

C. LAS Levels in Natural Water Bodies

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 alkylbenzenesulfonates (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 and their biodegradation products in various environments.

Surfactants are discharged into natural waterways from a large variety of sources: industrial sources, e.g., surfactants used in processing textiles and leather, and in dyeing and finishing, manufacturing of surfactants and compounding of detergent products; use by laundries and households; agricultural uses in relation to pesticides and insecticides; and municipal treatment plants treating wastes from industry, households and public cleaning units (Organization for Economic Cooperation and Development, 1964).

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

1. Pathways for LAS into the Environment

a. Sewage Treatment Plant

Concentrations of detergent products in washing situations range from 1,000 to 3,000 ppm (0.1-0.3%). These detergent solutions would be expected to contain 150 to 600 ppm LAS. The surfactant components of the detergent products, being soluble, eventually reach raw sewage drains at concentrations of 10-15 ppm. 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. In Germany, of the domestic sewage accounted for in 1964, 23.8% was not subject to any treatment, 44.9% was mechanically treated, and 31.3% was biologically treated (Husmann, 1968). Esvelt et al. (1971) found for sewage treatment facilities in the San Francisco Bay area that chemical precipitation of raw sewage removed approximately 54% of the influent MBAS, biological treatment removed 84% MBAS, and primary treatment was relatively ineffective. He reported MBAS levels for effluents from the various degrees of sewage treatment as follows:

Primary.	8.0-9.5 mg/l
Chemical precipitation.	4.5 mg/l
Activated sludge.	0.16 mg/l

A nationwide water quality survey was conducted by the U.S. Environmental Protection Agency (1974), which included an examination of the concentrations of MBAS in certain water bodies of the United States. They reported that there

were 21,000 municipal waste treatment facilities in the United States through February, 1974. The primary treatment facilities removed 20-35% BOD and secondary facilities removed 65 to 95% BOD. The Water Quality Act Amendments of 1972 require that by 1977 all publicly-owned treatment facilities will achieve at least 85% BOD removal efficiency. Nationwide, over 75% of the existing treatment facilities practiced at least secondary treatment in 1973. However, considerable variation was observed among individual states. In 12 states, 90% of facilities practice secondary or better treatment, while in 6 states, less than half the facilities treat sewage at the secondary level. The 1973 statistics indicate the following levels of treatment and the population served (Table 1-B). These data cover approximately 80% of the population of the United States.

Thus, a substantial degree of LAS biodegradation can be expected on a national scale since LAS biodegradability in secondary sewage treatment systems as measured by MBAS generally ranges from 70% to over 90% (Husmann, 1968; Lawton, 1967; Heinz and Fischer, 1968; Brenner, 1968; Waldmeyer, 1968).

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.

TABLE 1-B

WASTEWATER TREATMENT LEVELS, 1973

Treatment Levels	No. Facilities	% of Total Facilities (21,065)	Pop. Served (millions)
Primary	2,723	12.9	54.6
Adequate* secondary	2,575	12.2	
Inadequate* secondary	3,287	15.6	105.0
Unclassified* secondary	10,153	48.2	
Tertiary	795	3.8	2.7
No treatment	1,532	7.3	3.2
Total	21,065	100.0	165.5

*Treatment characterization not defined.

U.S. Environmental Protection Agency, 1974

b. Septic Tank Systems

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

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. LAS adsorption on soils is directly correlated with high content of organic matter and with phosphate-fixing capacities of specific tested soils (Murti et al., 1966).

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, and will be aided by soils with high organic matter and phosphate-fixing capacities. If the soils have a tendency to be anaerobic (anoxic), 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. MBAS Concentrations in the Environment

a. Surface Waters - Streams and Rivers

With the changeover from ABS to LAS, concentrations of MBAS have 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 recent 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).

In Germany, with the introduction of surfactants which are 80% or more biodegradable, MBAS levels perceptibly decreased in German rivers. Detergent loads as measured by MBAS concentrations have 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 reduction 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. 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. In England, similar phenomena were observed due largely to the increased biodegradability of LAS in use since 1965 (Waldmeyer, 1968).

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 with dilution and degradation, especially after the change from ABS to LAS. The addition of regional aeration systems to process wastes resulted in additional improvements in MBAS levels.

Barth and Ettinger (1967) examined MBAS levels in effluents from 5 municipal sewage treatment plants during the interval from February, 1965, to July, 1966, and found a reduction in biologically resistant MBAS.

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. They also addressed one of the continuing problems in the assessment of environmental levels of LAS using MBAS as the analytical standard. In a comparative analysis using a gas chromatographic analytical procedure, they report that "no more than 10 to 20% of the MBAS is LAS; and whatever LAS is present is still undergoing biodegradation."

EPA STORET data from the state of Minnesota have been examined. Although pre-1965 MBAS levels were not available, data from 1967 to 1974 were analyzed. MBAS levels for "water quality limited" waterways in the state were significantly greater than effluent limited water bodies, since water quality limited waterways were already degraded by pollution sources. Overall concentrations for all stations monitoring MBAS levels for the state of Minnesota ranged from 0.06 to 0.19 mg/l from 1967 to 1974.

EPA STORET monitoring data on MBAS for the Mississipi River Delta between 1964 and 1975 show concentrations of MBAS ranging from 0.00 to 1.20 mg/l. No particular downward trend from year to year was noted in this interval, but average MBAS concentrations were generally quite low, in the range 0.05 to 0.07 mg/l.

EPA STORET data on the Willamette River in Oregon show concentrations of MBAS ranging from 0.02 to 0.07 mg/l, with no significant changes from year to year but with somewhat lower MBAS levels than either the Mississippi River Basin or the New York State area.

Similar data for New York State for the interval 1960 to 1975 show overall average MBAS values ranging from 0.01 mg/l to 0.27 mg/l, with no clear overall trends. Although there appears to be no evidence of significant or sustained reduction in MBAS levels during this interval, the levels are below 0.1 mg/l for all years except 1966 and 1968.

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, 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.

An examination of MBAS levels in waterways ranging from creeks to large rivers one mile above and below sewage outfalls was undertaken starting in 1971 as part of an environmental monitoring program (Procter and Gamble Company, unpublished data). 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.

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 significant 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 public 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) has reported on the behavior of various surfactants at 6 sites in Nassau and Suffolk County, Long Island, New York. The sites included 3 cesspools, 2 septic tanks and 1 septic tank followed by a cesspool. Considering LAS, the reductions of MBAS from the effluent sites to ground water plumes 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.

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/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).

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), both of which contain high levels of coliform bacteria in their effluent. These two outfalls 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). At the Canal Street outfall, subsurface water MBAS concentrations were 0.150 mg/l. Interstitial water from sediment samples reached MBAS levels of 1.250 and 1.20 mg/l at West Point and Yonkers outfalls, respectively, and were lowest at 0.00, 0.03, and 0.113 mg/l at the Haverstraw, Croton Bay and Peekskill outfalls, respectively. Methylene blue active substances other than LAS were thought to have contributed to the high MBAS levels in interstitial water samples.

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 at the Westchester Sewage Plant outfall at Yonkers (primary treatment), to 20,000,000/100 ml at the Ft. Lee outfall. Fecal coliform counts ranged from 100/100 ml at Westchester and Bayonne, New Jersey outfalls to 1,100,000/100 ml at the 114th Street Manhattan outfall. 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 at the Battery to 0.017 mg/l at the West Point outfall, 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 MBAS or 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 in waters seems to indicate contamination from untreated sewage with a high coliform count.

D. Summary

The growing body of data on the environmental levels of surfactants depends almost exclusively on the use of an analysis for methylene blue active substances (MBAS). In natural waters, any other substances containing strongly anionic substances containing hydrophobic groups will interfere with this analysis

and result in values for MBAS considerably in excess of the actual levels of LAS. The limited data available suggest that in natural waters the contribution of LAS to MBAS is probably less than 50% and may, in some instances, be as low as 10% (Sullivan and Swisher, 1969).

Thus, any evaluation of environmental levels of "LAS" obtained by the MBAS analytical procedure must be tempered by the possibility that the true LAS level is lower by as much as an order of magnitude. Nevertheless, an examination of the diverse collection of data on MBAS levels in various water bodies reveals a number of general conclusions:

- Since the change from ABS to LAS, surfactant levels, measured as MBAS have decreased significantly in most waterways.
- A substantial majority of waterways for which there are data are well below the prescribed standard of 0.5 mg/l MBAS.
- Areas where levels of MBAS are higher than 0.5 mg/l are most often those that exhibit other measures of severe pollution (coliform, BOD, turbidity).
- The use of MBAS as an analytical method to detect water levels of LAS is inadequate and provides data that are not accurately descriptive of the low levels of LAS in the environment.

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III. BIODEGRADATION

Biodegradation [as defined by Swisher (1970)] is "the destruction of chemical compounds by the biological action of living organisms." Biodegradation can be subdivided into primary and ultimate biodegradation. Primary degradation relates to 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 utilized to study the biodegradation of surfactants both in the laboratory and in the field, and with a variety of factors capable of affecting the rate of biodegradation. Much of this work has been reviewed by Swisher (1970, 1976), Cain (1974), Gledhill (1974), Howard et al. (1975), and Higgins and Burns (1976).

A. Laboratory Test Systems Used in LAS Studies

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

Since the theoretical amount of oxygen required to completely oxidize a surfactant remains constant, the BOD value can be employed to estimate the extent of biodegradation in an isolated system of known composition. Interpretation of the results is rather tenuous, however, due to the complexity of the metabolic processes involved, the variety of metabolic byproducts formed, as well as the many other pathways of possible oxygen utilization by the microorganisms. BOD values are also 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 value for a control culture from the results to obtain a net value for the surfactant. 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. In the closed bottle test, using an aqueous solution containing a bacterial inoculum, the initial amount of oxygen and the test surfactant are known. The bottle, at a specified temperature, is allowed to stand for a specified interval of time (usually five days, but occasionally up to 20 days). At the end of the test period, the amount of oxygen remaining is determined and the net O_2 uptake is calculated.

The major problem with this assay is that the initial concentration of oxygen available for oxidation is limited by its solubility (9 mg/l if saturated with air at 20°C) (Swisher, 1970). Therefore, the amount of added surfactant is limited to that amount (usually 2-3 ppm) which reacts with a considerable portion of the oxygen yet leaves a sufficient amount unreacted so as to make possible detection of the net oxygen uptake (Swisher, 1970).

The Warburg technique has an advantage over the closed bottle test in that the amount of added surfactant is not limited by the solubility of oxygen. A liquid phase contains the test compound and the inoculum, while an excess of atmospheric oxygen is available in this closed system. Oxygen uptake is measured as a drop in pressure of the gas phase. Endogenous oxygen uptake is determined from control cultures.

Since the carbon dioxide produced by the bacteria would interfere with this procedure, it is generally trapped in KOH. However, 1%-2% CO_2 should be allowed to remain in view of Gaffney's finding (1965, cited in Swisher, p. 144, 1970) with mixed microbial cultures, that complete absence of CO_2 inhibits bacterial food assimilation.

Utilizing the Warburg 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 cell concentration of 1000-2000 ppm (dry weight) with a nitrogen-free medium.

2. CO_2 Evolution

The bacterial evolution of CO_2 can be utilized to assess ultimate biodegradation. This method is not quantitative because the CO_2 evolved usually falls short of the 100% theoretical value because some of the carbon is used for normal cellular processes. Additionally, such factors as molecular structure, the number and type of microorganisms capable of metabolizing the test compound, toxicity, (Gledhill, 1975) may affect rates and extent of CO_2 production.

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.

When Gledhill 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. (See, however, Section III-C.)

3. 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 ppm 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% in 48 hours.

Cain (1974) has some reservations about the enrichment technique in general. One disadvantage of the enrichment culture procedure is that usually

growth is indicated by the development of a visible turbidity in the liquid culture; the concentration of surfactant required to produce a microbial population capable of producing this turbidity may well be inhibitory in nature. Therefore, a smaller population density which can occur without visible turbidity must be measured via direct, viable or Coulter counting, any of which are more or less time-consuming.

4. Die-Away Tests

Die-away tests are commonly employed to determine the progress of biodegradation in an isolated system. Employing a series of analyses, the decreasing surfactant concentration is monitored as the surfactant content drops or "dies 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 Test

The surfactant (1-10 ppm) 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. Using Ohio River water, Weaver and Coughlin (1964) reported that at 20 days, a degradation of greater than 90% (MBAS) was seen with a commercial LAS product. 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. Sekiguchi et al. (1975) found that approximately 15 days were needed for 5 mg/l LAS to completely biodegrade (MBAS) in samples of Tama River water; it took 40 days for the total organic carbon values to disappear. Fuhrmann et al. (1964, cited in Swisher, p. 148, 1970) reported several variables in the river water test which could slow LAS degradation: (1) increased initial concentration of LAS, (2) decreased bacterial count, (3) increased nutrient, (4) increased storage time (>5 days) of river water prior to use, (5) aeration with oxygen (but not with air), (6) decreased temperature, and (7) addition of phenol.

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

The principal advantage of the shake culture technique is that the investigator has better control of the water source. With free access to the atmosphere, a flask containing a yeast extract medium, the surfactant (usually 30 ppm) and the bacterial inoculum is aerated on a rotating or reciprocating shaker. This is the standard method of the Soap and Detergent

Association (1966). Biodegradation of LAS 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 LAS products (Shell DOBANE^{TM} series) with a shake flask procedure.

The biodegradability of varying chain length LAS and the corresponding alkyl tetralin sulfonates was compared utilizing both shake flask (7-8 days) and river die-away (up to 30 days) procedures. The results (Table 1-C) 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 (Continental Oil Company, unpublished data). In a separate shake flask test, these investigators found that LAS containing 5, 10 or 14% alkyl tetralins had biodegraded (MBAS) 97, 95 and 96%, respectively, at the end of one week (Continental Oil Company, unpublished data).

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

This procedure involves the use of an air-dried activated sludge inoculum (30 ppm) which is added to a solution of BOD dilution water containing 10 ppm of surfactant. The entire mixture is aerated at 20° and analyzed (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). The range of results obtained from eleven laboratories which used this test

TABLE 1-C

BIODEGRADABILITY OF ALKYL TETRALINS

AND THEIR CORRESPONDING LAS ANALOGUES

	% MBAS Biodegradability	
Surfactant	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

to study the degradation of DOBANE^{TM} JNX-LAS was 83% to 92% in 69 runs, with a mean degradation value of 87% (Eden et al., 1968).

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

This procedure uses a salt medium containing 89 ppm phosphorus, 175 ppm nitrogen and 10 ppm added surfactant 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 with LAS is reportedly 5% to 10% lower than 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 ppm yeast extract, is inoculated with 10% settled sewage. The mixture containing up to 20 ppm of the test surfactant is allowed to stand in the open for a period of seven days. The solution is then analyzed (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).

5. Simulated Treatment Processes

a. Activated Sludge

The activated sludge system is one of the most commonly used biological waste treatment processes today (Rogers and Kaplan, 1970). SDA (JAOCS, 1965) 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 LAS biodegradation. 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.

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 utilized 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 surfactant must steadily degrade a minimum of 80% over a period of 21 days; a prior acclimation period of up to six weeks is permitted.

Although erratic results may be encountered with the official German test, reproducibility is generally good within a laboratory (Swisher, 1970); however, due to the fact that airborne inoculation leads to variations in the composition of the bacterial species present in the sludge, results might be expected to vary from one laboratory to another. Other disadvantages of this procedure include the costly and time-consuming need to handle 24 liters of synthetic sewage and 24 liters of treated effluent per day, the bulky nature of the equipment, plus the necessity of a 10- to 20-day acclimation period (Weaver and Coughlin, 1964).

Using the German test method, LAS degraded 91.1% in 21 days, while ABS degraded less than 20% (Weaver and Coughlin, 1964).

Stennett and Eden's (1971) modification of the German test procedure overcame another problem associated with this test; i.e., the difficulty of maintaining a satisfactory retention of sludge. This was accomplished by replacing the aeration vessel in which the sludge is retained with a combination of an inner porous unit surrounded by an outer impervious vessel into which the effluent flows.

Miniature continuous flow units save space as well as considerable time and labor in the preparation, storage and handling of feeds and effluents. Minor objections to miniaturization are the limitation on the size of samples which may be withdrawn for analysis and the posssibility of a further departure from the characteristics of a full-scale treatment plant (Swisher, 1970).

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 procedure (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 unspecific analyses (COD, DOC). For example, 94% COD removal of 10 ppm 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 surfactant 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 calculated 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 Mancy, 1969) and retention time (Hanna et al., 1965).

A factor which must be taken account of in activated sludge analyses is the adsorption of the surfactant to the sludge. For example, working with an SDA semi-continuous unit, Swisher (1970, unpublished data) found that 17.7 mg of a 20-mg sample of LAS was adsorbed from solution onto the sludge within two minutes after its addition. However, witnin 24 hours, the adsorbed surfactant had been degraded. The percent adsorption is a function of the concentrations of both surfactant and sludge.

The microbial population of activated sludge is inevitably diverse as to the individual species and strains present. Rogers and Kaplan (1970) isolated and identified 20 bacteria in a shake culture inoculum originating from activated sludge; Pseudomonas species predominated, but Achromobacter, Paracolobactrum, Flavobacterium and Moraxella species were also found. An exhaustive study would multiply this number several fold.

The relative number of different species and state of acclimation of the microbial population can also vary with changes in the environment such as the amount and nature of the nutrients available, the concentration of oxygen, temperature, flow rate, and degree of agitation.

The removal of organic pollutants from waste water can be affected by influent concentrations greater than 10 mg/l of LAS in a laboratory-scale activated sludge system (McClelland and Mancy, 1969). The extent of the effect appears related to the structure and concentration of the surfactant. Banerji (1971) suggests three possible mechanisms by which the rate of substrate utilization might be reduced: toxicity of the surfactant to the microorganisms, enzyme inhibition, or reduced mass transport. McClelland and Mancy (1969) suggest a fourth possible mechanism; i.e., the surfactant adsorbed at the floc-solution interface interferes with the production and availability of extracellular hydrolytic enzymes and acts as a barrier between the substrate molecule and its respective enzyme, at least at influent levels of 10 mg/l LAS.

During biological stabilization of waste, proteolysis and deamination produce ammonia which in turn is either utilized in bacterial cell synthesis, or oxidized to nitrite which usually is further oxidized to nitrate. Baillod and Boyle (1968) found that LAS (42.1% sodium dodecyl benzene sulfonate), at concentrations up to 10 mg/liter, tended to promote complete oxidation of ammonia to nitrate in an activated sludge system. Control units produced approximately equal amounts of nitrite and nitrate. The authors suggest that one possible mechanism might be that LAS stimulates the transfer of ammonia into the cells of nitrite-forming bacteria. This would result in the establishment of this group in the sludge. However, according to this hypothesis, LAS concentrations higher than 10 mg/liter would produce a decrease in nitrite and nitrate formation by impairing the transfer rate, resulting in a diminished number of ammonia-oxidizing organisms.

b. Trickling Filters

Trickling filters (sewage running over an aerated, porous bed of rocks 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.

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. Generally full-scale filter beds are six feet in depth (Swisher, 1970) but laboratory models may be half this depth or less.

Lamb also found a relationship between MBAS and BOD removals in the pilot plant studies. MBAS removals averaged 50% at BOD removals of approximately 75%; with BOD removals greater than 75%, MBAS removals increased

sharply. Lamb's data suggest that relatively high BOD removal (>90%) is required to insure moderate removal (80%) of MBAS. Lamb postulates that the differences reported in MBAS removal between trickling filter plants and activated sludge plants may be due to the higher BOD removals associated with activated sludge plants.

Tang (1974) reported that BOD and LAS removal were significantly correlated for both activated sludge and trickling filter processes in both laboratory and field studies.

Brenner (1965) cites the State of California Detergent Report which concluded that the standard rate trickling filter should be found to remove 85% to 90% of the added LAS.

c. Anaerobic Systems

Biodegradation of LAS is severely hampered under anaerobic conditions. Since initial oxidation of a hydrocarbon chain is thought to require molecular oxygen, side chain oxidation would not be expected to occur. Therefore, since cesspools, septic tanks and anerobic digesters are extensively utilized as sewage treatment processes, laboratory procedures have been developed to study anaerobic biodegradation.

Using an experimental cesspool model, Rismondo and Zilio-Grandi (1968) found that LAS detergents, under anerobic 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 (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. With respect to anaerobic sewage treatment systems, 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.

Several investigators have reported that when the surfactant concentration in raw sludge reaches 1.1-1.2% 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 countries or to other factors.

To date, the only proven means of overcoming detergent inhibition of the digestion process is by the daily addition of a cationic amine, stearine amine, which partially neutralizes the anionic detergent (Swanwick and Shurben, 1969). This process can be rather expensive; Osborne (1969) reported that approximately 200-340 lbs of stearine amine had to be added daily to a digester in order to correct a sludge digestion problem.

6. Soil

Soil is an important factor in the biodegradation process, especially in the septic tank treatment system. After settling of the sewage insolubles, the supernatant flows from the septic tank into an underground drainage field where degradation of the soluble and unsettled components occurs, principally through the action of aerobic bacteria present in soil and sewage.

Mansell et al. (1970) studied the effect of soil aeration upon the biodegradation of LAS solutes as they moved (steady state flow) through vertical columns of stratified soil. 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, without performing correlation with MBAS values. 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.

7. 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. One negative aspect of a natural sewage medium is its instability upon storage; however, deterioration can be minimized through refrigeration or via sterilization either by heat or irradiation (Swisher, 1970).

Synthetic sewage has the advantage of greater 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 are frequently added to media; these may be supplemented with a carbohydrate, and on occasion, a fatty material is added. Sometimes, the surfactant being tested serves as the sole organic source.

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.

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, 1974). Swisher (1970) reports that the range of compounds which produce this phenomenon is not known. Swisher further states that perhaps the entire problem is pragmatically unimportant since these organic compounds exist in nature and are capable of affecting biodegradation in the environment; and yet degradation of surfactants does occur in the field. It should also be noted that the SDA semi-continuous activated sludge test uses a culture medium relatively rich in nutrients, including glucose.

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 ppm. 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 ppm 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 (1968, 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

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.

Microbial metabolism of alkylbenzene sulfonates, lower molecular weight sulfonates, and a variety of hydrocarbons has been widely reported in the scientific literature (Willetts, 1973a; Willetts and Cain, 1972a, 1972b; Horvath and Koft, 1972; Bird and Cain, 1972, 1974; Sariaslani et al., 1974; Cain and Farr, 1968; Pollack and Anderson, 1970; Thysse and Wanders, 1972; Ripin et al., 1971).

Goodnow and Harrison (1972) found wide variations in the ability of 45 strains of 34 species in 19 genera of bacteria to degrade (as MBAS) $C_{11.8}$ LAS.

Bacterial degradation of benzenesulfonate by Pseudomonas species was reported to involve the release of the sulfonate group as a sulfate with subsequent aromatic ring scission (Ripin et al., 1971; Cain and Farr, 1968; Bird and Cain, 1972). Ripin et al. (1971), however, detected no accumulation of sulfate in the medium contrary to the findings of Cain and Farr (1968).

Analogous studies have also been carried out with toluene-p-sulfonate (Cain and Farr, 1968; Bird and Cain, 1974).

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.

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 as to 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.

Working with DOBANE JNX LAS ($C_{11.8}$ sodium sulfonate), 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% (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 ppm 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.

c. Temperature

Since the presence of microorganisms is an integral component of a biodegradative 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).

Mann and Reid (1971) found a slightly higher degradation rate during the summer months in 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

The concentration of surfactant added to the test system can affect the rate of biodegradation. The lower limit of surfactant concentration (a few ppm or even less) is dictated by the sensitivity of the analytical test method employed while the upper limit (several hundred ppm) 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. Pollack and

Anderson (1970) reported that after 20 hours incubation, a broth culture containing 5000 ppm 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, the surfactant concentrations used range between 5 ppm and 20 ppm (Arpino, 1969), reflecting maximum environmental levels, especially those in sewage. Higher or lower levels may be used in special studies.

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 biodegradability 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). It is also important to have a negative 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., polluted lakes, industrial effluent, sewage, agricultural wastes, etc. These problems are considered in detail in Section II.

B. Field Tests

Field studies are equally or more subject to error compared to laboratory tests due to the inherent variability of operating parameters of the various treatment plants. Another problem is the analytical interference due to the variety of surfactants present in the incoming sewage.

The two major techniques used to overcome the problem of interference are substitution and spiking. Substitution requires replacing all detergents used within the area served by the sewage treatment plant. This is an expensive undertaking and requires cooperation of a great number of individuals. The spiking technique involves adding known amounts of a test surfactant just prior to entry into the treatment facility. Analyses are performed prior to the addition, prior to treatment, and following treatment. Despite all these difficulties, field tests of biodegradation are an integral part of validating laboratory studies for their predictive values.

With respect to the extent of LAS degradation, field study findings are generally in agreement with the results obtained in the laboratory. Dazai et al. (1968) found that within one week, synthetic detergent samples consisting of either 53% dodecene-1-LAS or 46% NALKANE N-500^{TM} (LAS) were degraded 100% and 97.5% (analyzed by Japan Industrial Standard Methods , respectively, in an acclimated activated sludge system; each detergent sample added to the sludge contained approximately 20 ppm of the active LAS component. These authors also found that within a concentration range of 10 to 100 ppm 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 ppm, while at 50 ppm, 7%-8% inhibition occurred, and at a level of 100 ppm, 17%-20%.

Mann and Reid (1971) evaluated a number of LAS products (sodium sulfonates of the Shell DOBANE series of alkylbenzenes: DOBANE JNX, $C_{11.8}$; DOBANE 055^{TM}, $C_{12.7}$; DOBANE 83^{TM}, $C_{10.9}$; DOBANE JNB^{TM}, $C_{11.6}$) 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 degraded (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.

Mann and Reid 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.

Using three separate methods of analysis, Janicke (1971) measured the degradation of (C_{10}-C_{13}) LAS in a continuous activated sludge system. He found that effluent MBAS levels did not coincide with either total organic carbon or dichromate values. MBAS analysis indicated a higher degree of degradation than that observed with the other two procedures. The difference may be due to the presence of intermediate products which MBAS analysis cannot detect.

In a series of papers, Renn (Renn, 1965; Renn et al., 1964) examined the biodegradation of LAS in an extended aeration activated 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 ppm to 3 ppm. Eventually, LAS removal of slightly above 96% was seen. It is noteworthy that residues of ABS were still detectable (via IR) more than two months after the switchover to LAS.

Klein (1969) reported an approximately 30% drop in the synthetic detergent concentration of raw sewage entering a sewage treatment plant following a change-over 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 with LA^{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.

Very few biodegradation studies have been carried out in either brackish or sea water (Cook and Goldman, 1974; Bock and Mann, 1971). 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. The authors' analyses showed that the LAS contents were equivalent to 19.5%, 22.5% and 21.5%, respectively, of sodium dodecylbenzene sulfonate. LAS in the first product was the most rapidly attacked.

Bock and Mann (1971) studied the degradation of 10 ppm of MARLON A™ (sodium dodecylbenzene sulfonate, adopted by the OECD (1971) as its "soft" standard) in sea water. The surfactant concentration had dropped to 3 ppm within one week, and within an additional seven days, 97% of the surfactant had degraded (MBAS).

C. Effect of Chemical Structure

The rate of primary and ultimate 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 alkylbenzenesulfonates. 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 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.

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.

There also appears to be a definite relationship between the length of the alkyl chain and the rate of biodegradation. 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). A single report by deJong and Testa (1967, cited in Swisher, p. 218, 1970) indicates that this chain length biodegradability relationship may be reversed with a LAS homolog chain length 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.

In accordance with the above relationship, 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 and unpublished results) found that condensed cyclic systems - linear dialkylindanes and dialkyltetralins - were readily biodegradable.

D. Metabolic Pathways of Biodegradation

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); Cain et al. (1971); Cain (1974); and Gledhill (1974).

There are three demonstrated points of metabolic attack; the alkyl chain, the sulfonate group on the benzene ring and the ring itself (Cain, 1974). It appears that the first point of attack is often the terminal methyl group of the alkyl side chain (Swisher, 1963; Willetts and Cain, 1972a). 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, 1974).

Cain and Farr (1968) found that 17 species of Pseudomonas isolated from sewage, river water or soil by enrichment culture on either a benzenesulfonate 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-phenyl-dodecane 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, but 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 sulfophenylsuccinic acid stage.

The obvious complexity of the possible metabolic pathways of surfactant degradation is illustrated by Cain (1974) who reported that studies to date indicate there may be no less than seven possible degradative routes for

alkylbenzene sulphates 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-hydroxyphenylalkanoate 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 aromatic 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.

E. Summary

There are a number of methods for studying the biodegradability of LAS (and other) surfactants. Different tests vary for the sake of pertinence to real situations; e.g., determination of "intrinsic" 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. Continuing studies of these correlations and predictions are essential.

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. Most LAS's are biodegraded to 90% or more in a variety of test systems and field environments. Chemical structure has considerable effect on the rates of biodegradation, but to date no highly recalcitrant LAS isomer or homolog has been found. The benzene ring is degraded, as well as is the alkyl chain.

In conclusion, it is clear that the LAS surfactants as a class are highly biodegradable, that their levels will thus be low in receiving waters, and, even there, they will be further degraded.

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IV. 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 toxicity information for LAS deals largely with acute studies, although some 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 toxicity of 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, 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 homologues of LAS which exhibit the highest toxicity

for aquatic species (longer chain lengths with more terminal phenyl group sites) are those which undergo the most rapid biodegradation. [See Section III of this report and Gledhill (1974) for a discussion of structure-activity relationships with respect to biodegradation. Gledhill (1974) has also reviewed the aquatic toxicity of LAS in some detail.]

For this reason, any assessment of the environmental safety of LAS must include information on the acute or chronic toxicity of LAS residues after biodegradation. Such information, limited though it is at this point, provides an appropriate perspective to consider any potential problems which may arise from the entry of LAS and its biodegradation products into the environment.

1. Acute Toxicity

a. Methodology

Acute toxicity tests are conducted to determine 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 observed 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 toxic effects of a chemical or a mixture of chemicals such as are found in natural waterways. 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 phenomenom in 50% of the tested individuals in a specified length of time, if often used. 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 the setting levels for aquatic safety. Concomitant histological examination 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 has supported an analysis of the problem by an expert committee. This group has published its findings in the form of detailed protocols for the performance of acute aquatic toxicity studies (U.S. Environmental Protection Agency, 1975).

b. Intact LAS Structure-Activity Relationships

The acute toxicities of the various 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 1-D. Swisher et al. (1964) and Marchetti (1968) have provided additional data to support this finding. Decreased toxicity at chain lengths longer than 16 carbon units has been observed in several studies, but the reasons for this change in trend of toxicity are not known. LAS surfactants with chain lengths of 16 or more are not usually found in commercial products.

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) has examined the acute toxicity of individual LAS homologues and isomers to a fish species (unidentified) and has confirmed that toxicity of LAS increases with increased chain length and as the phenyl group is located nearer to the end of the chain. Further, he has made some calculations based on the toxicity data of these individual chemical species of LAS and the chemical constitution of complex commercial mixtures of LAS and, for 8 separate products, there is close agreement between the predicted and experimental toxicity values.

In the process of LAS manufacture, there are generated a number of side products which remain in the final commercial detergent products. These compounds, dialkyltetralins, dialkylindanes and alkylnaphthalenes have not

TABLE 1-D

ACUTE TOXICITY OF INTACT LAS. EFFECT OF CHAIN LENGTH

LAS Homologues	48-Hour LC_{50} (mg/l)[1] Pimephales promelas	LC_{50} (mg/l)[2] Carassius auratus	LC_{50} (mg/l)[3] Lebistes reticulatus	LC_{50} (mg/l)[4] Idus melanotus	96-Hour LC_{50} (mg/l)[5]* Lepomis macrochirus
C_{10}	43.0	61.0	50	16.6	21.2-47.5
C_{11}	16.0	22.5	-	6.5	11.6
C_{12}	4.7	8.5	5	2.6	1.18-6.5
C_{13}	0.4	3.3	-	0.57	1.11
C_{14}	0.4	-	1	0.26	0.25-0.42
C_{16}			1	0.68	0.087
C_{18}			15		0.38

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

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

been extensively studied for their aquatic toxicity. Divo (1974) found increased toxicity with an increase in chain lengths among 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} dialkyltetralin-indane sulfonate mixtures, respectively. However, these side products are considerably less toxic than LAS isomers of comparable chain length.

c. Acute Toxicity to Fish - 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), 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 fresh water fish (Table 1-E). The results indicated 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."

Pickering (1966) has 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 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 LAS sample obtained from the SDA 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 was 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.

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

TABLE 1-E

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

Thatcher and Santer, 1967.

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.

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 1-F).

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 a concept of aquatic toxicity based on fractions of the 96-hour LC_{50} values of each of the identified toxicants in the Illinois River. They determined 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.

In the only 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

TABLE 1-F

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.

Hokanson and Smith, 1971

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. Sub-lethal 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.

Considering the available data on the acute toxicity of intact LAS to fish, the LC_{50} values for fingerlings and adults of a number of fresh water and marine species range from 1.0 to 10.0 mg/l. For those fresh water and marine species that have been examined, early developmental stages (e.g., sac-fry) are more sensitive to the acute toxic effects of LAS. Acute effects of LAS on sub-lethal manifestations of toxicity (swimming, breathing rate, opercular movement) occur at concentrations at or slightly below the LC_{50} values for the two species that have been examined for these responses.

d. Acute Toxicity to Fish-Biodegraded LAS

Although intact molecules of LAS are readily biodegraded in waste waters and waste water treatment plants as well as in natural waterways, there is a paucity of reliable information dealing with toxicity of degraded LAS to aquatic organisms.

In the first systematic study of this problem, 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. MBAS concentrations 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 sulfophenyl-undecanoic 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.

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^{TM}). Similar sharp reductions in toxicity of other commercial LAS products following their biodegradation have been reported for guppies (Poecilia reticulatus) and harlequins (Rosboral spp.) (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), 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 ranging from 4.6 to less than 4.6 mg/l for 24 and 48 hours. At 50% and 90% biodegradation levels, 24-hour LC_{50} values were 5.0 and greater than 5.0 mg/l. 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.

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 1-G).

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 has led Divo (1974) to several conclusions with respect to the relationship of biodegradation of LAS to toxicity. These conclusions agree with the data from the few studies performed to date:

TABLE 1-G

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

Kimerle and Swisher, 1977

- 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.

e. MBAS-Related Acute Toxicity to Fish 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 4 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.

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 amounts of MBAS alone do not appear to exert any adverse effects on various fresh water fish. Rainbow trout, golden orfe, goldfish, bream, tench, roach, perch, carp, raffe, pace, chib, 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 David (1967) with oysters (Crassostrea virginica). Although not a fish study, the results are appropriately considered in this discussion. Effluents from treatment of sewage without and with biodegraded LAS (5 mg/l) had approximately the same toxicities to oysters with respect to survival of larvae, development of eggs and increase in length of larvae. Thus, biodegraded LAS did not contribute to the toxicity of the sewage effluents.

f. Acute Toxicity to Invertebrates

Daphnia, a commonly tested invertebrate, showed no effect from exposure to LAS (44.7% LAS) at concentrations less than 1 mg/l. The 24 hr LC_{50} for this species was 3.46 (2.31-5.22) mg/l (Shell Chemical Company, unpublished data).

The effects of LAS (supplied by SDA, 60.8% LAS) 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.

Swedmark et al. (1971), in a broad-ranging study of marine organisms, have 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 1-H. Other than the cockle and scallop, adults of the species examined were markedly more sensitive. These data parallel the findings in fish with respect to increased sensitivity of early developmental stages.

TABLE 1-H

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

Swedmark et al. (1971)

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.

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.

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) reports 96-hour TLm values for the amphipod Gammarus pseudolimnaeus and for the snails Physa integra and 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 (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. In another report by these same authors (1968), brine shrimp (Artemia sp.) exhibited a 50% lethality at 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%).

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 (14.6-38.9) and 92 mg/l.

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 finds 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, 50 mg/l and 50 mg/l, respectively.

Thus, for invertebrates, toxicities of LAS vary widely due in some measure to the protective morphological characteristics (exoskeletons, closure mechanisms) of many organisms. These traits allow organisms in the adult stage to resist exposure to toxicants from any source. On the other hand, early developmental forms of many bivalves, crustaceans and lower forms are susceptible to LAS at concentrations found toxic for sac-fry of fish. In some cases, toxic effects of LAS were noted on larval stages at concentrations as low as 0.05 mg/l.

2. Chronic Toxicity

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 (SDA Interim Reference Sample, LAS Lot No. 1-1, LAS-60.8%) to fish remains to the present the only effort 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 96-hour 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.

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.

The effect of 30 days' exposure of 4 species of fresh water fish to the same formulation described by Arthur (1970) above was examined by McKim et al. (1975). Statistically significant reductions in the 30-day standing crop were noted (Table 1-I).

These few studies which only 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. However, before any definitive evaluation of the chronic effects of LAS can be attempted, further work is necessary using test samples containing well characterized LAS as the only surfactant and on typical environmental degradation samples. Moreover, further chronic studies with fish and organisms of lower trophic levels would also be required.

3. Effects of Environmental Conditions on Toxicity

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.

TABLE 1-I

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.

McKim *et al.*, 1975

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 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 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 higher 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.

Gafa (1974) reported a decrease of LC_{50} of a C_{12} LAS to goldfish (Carassius auratus) of 15.0 mg/l at 0 degrees of hardness to 5.7 mg/l at 50 degrees of hardness.

Brown et al. (1968) have examined the effects of zinc on the toxicity of LAS to rainbow trout (Salmo gairdnerii). In either control fish or in fish exposed to zinc (as zinc sulphate) for 100 days at 0.8 mg/l, the 72-hour LC_{50} for LAS was 0.5 mg/l tested in the absence of zinc. However, the administration of LAS with 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.

These studies showing the effects of temperature, dissolved oxygen levels, water hardness and heavy metals on the toxicity of LAS to various fish species re-emphasize the necessity for establishing rigorously reproducible environments for the conduct of acute and chronic aquatic toxicity studies.

4. Interactions with Other Chemicals

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 toxicity of 50 ng/ml of p,p'-DDT was substantially enhanced by the 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 did not. Hille (1970) found no synergism between LAS and dieldrin 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 and 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. 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.

B. Effects of LAS on Higher Plants

There are a few studies which have examined the effects of LAS on plants. Lichtenstein et al. (1967) have 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.

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).

These investigations on the effects of LAS on plants do not suggest any problem at environmental levels (<0.5 mg/l). However, the limited number of studies and the finding that 10 mg/l LAS does inhibit orchid growth to some degree after a 5-month interval would suggest that some additional long-term studies be performed to establish with more certainty the safety of LAS for plants, especially those which may be important in natural ecosystems or with the agricultural use of sludge wastes.

C. Effects on Birds and Wildlife

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

D. Mode of Action

The means by which LAS exerts a toxic effect on fish, and possibly 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." Long-term, sublethal stresses such as impaired feeding due to loss of chemoreceptor function, altered respiratory rates due to gill damage or altered uptake of other toxicants may play an important role in the chronic toxicity of LAS.

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 our information in this area. 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 the 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 μg/g in muscle to 1000 to 3000 μg/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.

While these studies on the accumulation of LAS and/or its metabolites by aquatic organisms represent a substantial beginning in understanding the nature of the toxic effects of LAS and its biodegradation residues, a considerable amount of additional work would be required to uncover the precise mode of action of LAS in acute and chronic exposures.

E. Aquatic Toxicity and Aquatic Safety

The setting of a water quality standard for any specific material has been based, in general, on the toxicity of the material to aquatic organisms. This issue has been considered by a number of investigators (Alabaster, 1970; Lloyd, 1972; Abel, 1974; Kimerle and Swisher, 1977) and several important factors have been enunciated. With respect to the toxicity of LAS in natural waters, the facile biodegradation of those isomers which would be expected to be most toxic must be weighed in establishing criteria. It is generally agreed that information from acute toxicity, sublethal chronic toxicity and field tests should be used to prepare water quality standards. For acute toxicity tests, the standardization of procedures, the use of a limited number of appropriate test species and the use of chemically well-defined LAS samples would be a major step in increasing the reliability of the data for use in setting standards. The limited evidence that LAS levels lower than 0.5 mg/l can exert a toxic effect on some aquatic organisms, especially at early life cycle stages, indicates the necessity for further work in this area of chronic toxicity. Finally, although a "maximum permissible concentration of 0.5 mg/l would probably be harmless under most conditions" (Abel, 1974), the long-term

quality of waterways remains the single most important consideration (Alabaster, 1970; Sprague, 1971; Lloyd, 1972).

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V. HUMAN SAFETY

The last extensive consideration of mammalian toxicity due to LAS was in a general review of the oral toxicity of surfactants (Swisher, 1968) based on data available up to the end of 1966. Since LAS had only been in large scale use from the middle of 1965, there was not an extensive documentation on either the animal toxicity or human safety of LAS. On the basis of the information available in 1966, Swisher, concluded 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.

Our evaluation of the data available up to the end of 1976 dealing with various measures of mammalian toxicity as indicators of human safety, as well as actual human exposure, agrees with the view that LAS does not represent a hazard to human health.

A. Animal Studies

Acute Toxicity - Oral. Earlier 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 1-J, 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 grew 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).

Acute toxicity tests on commercial detergent products possibly containing LAS have been carried out in mice, rats, dogs and pigs; however, it is not possible to ascribe the effects observed to any particular component of the products employed (Muggenberg et al., 1974). 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

TABLE 1-J

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	♂	125.6	873	(773-906)
	7	♂	151.9	659	(560-774)
	10	♂	235.7	404	(359-454)
	6	♀	99.3	760	(675-855)
	7	♀	111.1	670	(584-769)
	10	♀	145.5	409	(362-462)
Wistar Rats	8	♂	166.4	1525	(1317-1766)
	8	♀	123.6	1820 **	(1099-1491)
ddY/Mice	7	♂	27.2	1665	(1508-1838)
	8	♀	22.0	1950	(1540-2480)
	7	♂	27.4	1575	(1433-1731)
	7	♀	23.0	1850	(1674-2044)

Kobayashi et al., 1973.

*Specific pathogen free.

**This value is outside the reported confidence limits.

level of 100 ml/kg was without effect (Leuschner et al., 1969).

Acute Toxicity - Percutaneous. 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).

Acute Toxicity - Systemic Exposure. The intravenous injection in rabbits or the intraperitoneal injection in rats of a 2 mg dose of LAS (PERLAN ALB ^{TM}) 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).

Acute Irritation - Skin. 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 Comapny, 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). 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).

Acute Irritation - Ocular. 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).

Subacute Toxicity - 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 increase, 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.

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, exhibited 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 this 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.

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 (Leuschner et al., 1969).

Subacute Toxicity - Percutaneous. The administration of 2.0 ml LAS concentrations up to 10% applied daily to abraded rabbit skin for 28 days did not result in any systemic toxicity as determined by a histological evaluation of 14 organs and blood values. A severe skin irritation at the site of application was the only remarkable finding. With a 10% solution of a hand dishwashing liquid containing 19% LAS and 19% tallow alkyl ethoxylate sulfate only a moderate skin irritation was found following a 91-day exposure of intact rabbit skin or a 28-day exposure of abraded rabbit skin (Procter and Gamble Company, unpublished data).

No severe skin damage was noted after a 15-day exposure 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 (Brown, 1969).

Chronic Toxicity - Oral. The exposure of rats to 100 ppm (0.01%) of sodium dodecylbenzenesulfonate 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.

Acute, Subacute and Chronic Toxicity-Summary. The acute oral toxicity of LAS in rodents ranges from about 0.5 to over 2.0 grams per kilogram body weight. Materials having acute oral LD_{50} values in this range are designated as moderately toxic (Gleason et al., 1969) and include other household materials such as sodium chloride and sodium bicarbonate. An LAS solution at a concentration of approximately 10% will be an acute irritant for rabbit skin, while at 1 percent no skin irritation is observed. At concentrations above 0.1%, LAS will cause irritation in rabbit eyes. Below this concentration there is little or no reaction.

A subacute oral toxicity 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 contrast 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%. The percutaneous exposure of rabbits to LAS at concentrations up to 20% for several weeks did not result in systemic toxicity although a 30% concentration applied for 15 days did result in weight loss.

Following chronic exposure of rats to LAS at doses up to 0.5% of the diet for two years, no pathological responses were noted.

These toxicity studies indicate that under normal use conditions there appear to be no hazards from direct percutaneous exposure to solutions of LAS at the concentrations commonly employed (usually 0.1-1 percent).

The studies concerned with ocular toxicity showed substantial irritation occurred in rabbits at ten to fifty times normal use levels. Direct ocular exposure of rabbits using the Draize procedure to undiluted LAS induced severe ocular congestion and edema which was reversible. The chronic studies indicate that a very large factor of safety exists in long-term low-level exposure. Estimates of surfactant intake (0.3-3 mg/day/person, Swisher, 1968) are orders of magnitude below doses which result in no observable detrimental changes in experimental animals.

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 Swiss ICR mice three times weekly for 18 months of aqueous solutions of a detergent containing 15.6% LAS and 18.6% tallow alkylethoxylate sulfate at concentrations of 0.1, 1.0 or 10.0% 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 enhance the cellular uptake of NQO and thereby increase the tumorigenicity. 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).

Mutagenicity. 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 dosages up to 1000 mg/kg of detergent and at 200 mg/kg for 5 consecutive days in a subacute protocol. 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 with a detergent containing LAS (Procter and Gamble Company, unpublished data).

Reproduction Studies. Buehler et al. (1971) have reported on a three-generation study (two litters per generation) in rats following an 84-day exposure to a dietary level of LAS at 0.5%. General reproduction parameters, including fertility, gestation, parturition, neonatal viability, lactation and post-weanling growth, were similar in control and treated groups. Body weight gains of parental animals, organ to body weight ratios and hematology were unaffected by the treatment with LAS. Similarly, Bornmann et al. (1961) found no detrimental effects in a two-generation study in rats given 0.1% LAS in their drinking water for 26 weeks.

A detergent containing 15% LAS and 13% ammonium fatty alcohol polyglycol ether sulfate was given to male and female rats in their drinking water at levels of 0.5 and 1.0 ml per kg from 12 to 7 days before copulation. Females were also treated during gestation. No differences were found between control and treated groups in the number of fetuses per litter, fetal weight or fetal pathology (Leuschner et al., 1969).

Teratogenesis. Chiba et al. (1976) recently examined the teratogenesis of LAS in pregnant SD-JCL rats. The dams (16/group) were given 0, 0.1 or 1.0% LAS in the diet day 0 through day 20 of gestation. Daily food intake averaged 24-26 gm/day - the equivalent of approximately 780 mg LAS/kg for the 1% LAS group. No visible signs of toxicity were evident throughout gestation. On day 21, 10 dams/treatment were killed and the fetuses recovered; the remaining dams were allowed to deliver normally.

No significant differences in maternal histopathology, uterine weight, placental weight, number of corpora lutea or in the number of implantations were noted in dams killed on day 21. Embryonic development and litter parameters were normal except for two cases of resorbed fetuses and a single case of external malformation (Ectopia viscerum) in the 1% LAS group. These were not statistically significant.

In pups delivered at term, a slightly smaller litter size (8.3 vs. 9.2 for controls) was recorded for the 1% LAS group and a slight difference in weanling rate (based on survivors at 5 weeks) was also noted: 100, 94.9, and 78.3%, respectively, for the 0, 0.1 and 1.0% LAS groups. Surviving pups were maintained for a period of 35 days after birth. No noticeable effects on development were seen in pups exposed to 1% LAS in utero. Body and organ weights were comparable to controls and no increase in visceral or skeletal anomalies attributable to LAS exposure were reported.

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 for mice (6-15 days gestation) and rabbits (6-18 days gestation), and 600 mg/kg/day for rats (6-15 days gestation). Toxic doses for the commercial detergents ranged from 1200 to 3200 mg/kg. 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.

In a companion study (Palmer et al., 1975b), percutaneous administration of LAS to pregnant mice, rats and rabbits was examined. The test solutions were applied as 0.03%, 0.3% and 3.0% solutions to all three test species during the first two-thirds of gestation. The highest dosage (3%) was equivalent to 60 mg/kg in rats, 500 mg/kg in mice and 90 mg/kg in rabbits based on the amount applied and skin area. The findings were similar to those obtained following oral administration of LAS. Reduction

in litter size and post-implantation embryo loss were again associated with pronounced maternal toxicity. No increase in the incidence of any teratogenic anomalies was found following treatment with LAS, even at a dose of 3% that caused some toxic manifestations in the pregnant dams.

In a similar study using LAS and a commercial hand dishwashing detergent containing 17% LAS, as well as other unknown components, Sato et al. (1973) found that 2.2% LAS applied to the skins of mice of a ddY/S strain from day 0 to 13 of gestation did not have any influence on rate of insemination, number of implantations or mean of fetal body weight.

A mixture of 55% tallow alkylethoxylate sulfate and 45% LAS was examined for teratogenic effects by oral administration in rabbits at doses up to 300mg/kg on days 2 through 16 of gestation or in rats at doses up to 800 mg/kg on days 6 through 15 of gestation. In addition, another group of rats was fed the detergent mixture for two generations. In no case was any evidence of teratogenicity or embryo toxicity observed (Nolen et al. 1975).

In another study, mice of ICR/JCL strain were treated percutaneously from day 1 to day 13 of gestation with a 15% or 20% aqueous solution of a kitchen detergent containing 17% LAS and other unknown materials. The treatment had no effect on body weight, individual organ weights, or hematological and clinical chemistry parameters of the pregnant dams. The litters of the treated groups showed no increase in teratogenic

abnormalities in internal organs or ossification as compared to untreated and water-treated control groups (Iimori et al. 1973).

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. 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 cases 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 1-K. 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.

A summary of the results of a study to examine the effects of percutaneous administration of a detergent containing 17% LAS as well as other

TABLE 1-K

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.

surfactants in ICR/JCL strain mice and Wistar rats is also 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 relation 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).

Carcinogenicity, Mutagenicity and Teratogenicity-Summary. The two-year feeding studies in rats and the chronic skin painting experiments in mice show no evidence of any systemic or cutaneous carcinogenic potency for LAS. In a single study, repeated concomitant oral administration of LAS at 80 mg with the carcinogen 4-nitrosoguanidine-N-oxide resulted in an increase in number and severity of gastric tumors as compared to carcinogen treatment alone. The mechanisms for this phenomenon are not apparent since no co-carcinogenic effect of LAS was found with another potent carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine.

The few preliminary mutagenicity studies that have been carried out would suggest no problems of genetic effects from LAS exposure. However, these preliminary results should be confirmed by a complete battery of studies.

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.

Pharmacology-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) pentanoate 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 administerd 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).

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.

Pharmacology-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 ($\sim$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).

Pharmacology-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.

Pharmacology - 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.

Pharmacology-Summary. 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.

Several studies have examined a few specific effects of subacute administration of LAS to rats. In two abstract reports, changes in clotting time were noted following administration of LAS (0.2%) with butylhydroxytoluene (0.8%). In another study, fasting blood glucose levels were increased 10 to 20 percent following oral exposure of rats to LAS at 250 mg/kg/day for several weeks. In contrast, evidence from a number of chronic oral studies with LAS have shown no unusual alteration in blood chemistry values. Further work should be performed to determine whether LAS together with other agents, such as BHT, may result in physiological changes.

B. Human Studies

Skin Irritation. Studies of the adsorption of LAS and a number of other anionic detergents to callus obtained from the instep

showed that the degree of adsorption of detergent roughly paralleled skin roughness caused by the surfactant (Imokawa, 1974). A correlation between desquamative skin changes 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).

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) examined surfactants for their ability to increase the titratable thiol groups in isolated human epidermis. They found a rapid increase in free thiol group following treatment with an 0.04% solution of LAS: however, there was little correlation with epidermal penetration of the surfactant.

In a comprehensive examination of skin responses of humans, rabbits and guinea pigs to a number of materials, including several detergents containing from 4 to 17% LAS, Nixon et al. (1975) found that only direct testing on human skin accurately assesses the degree of irritancy hazard to humans. 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) examined a number of surfactants, including LAS, for their effects on human skin and various animal skins. The results showed 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.

Skin Sensitization. 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), an 0.1% aqueous LAS preparation caused no sensitization

in 86 subjects, whereas a 0.1% solution in 50% ethanol induced a sensitization response in 6 of 86 subjects. Further studies have demonstrated that the positive response was due to the 50% ethanol alone.

Pharmacology. 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 of surfactant on human skin was pH dependent. In the range of pH 7.0 to 11.0, adsorption of LAS decreased with increasing alkalinity of a post-treatment rise. 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. Exposure of human hair to LAS, even at concentrations many times normal use levels, did not alter either tensile strength or moisture retention in comparison to untreated hair even though as much as 50% of the added LAS could not be removed by rinsing.

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). In contrast, 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).

Human Studies-Summary. 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.

C. Epidemiology

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 Center 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.

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.

Occupational Exposure. Only one published account of the effects of occupational exposure to LAS was found (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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ALKYL SULFATES

Synopsis

From the time of their development, alkyl sulfates have been used principally as wool-washing agents and as active ingredients in heavy duty laundry formulations. More recently, alkyl sulfates have been incorporated into a wide variety of specialty products such as shampoos, dentrifrices, antacids as well as into certain foods with no indications of hazard to human health.

There are presently no environmental standards of water quality with respect to alkyl sulfates. Levels of AS, as such, in streams and waterways are not presently being monitored, but MBAS levels would include AS, if present. Linear primary AS are quite readily biodegraded within a few days under both laboratory and field conditions. Slightly branched and secondary AS are also easily degraded but at a somewhat slower rate.

With respect to aquatic toxicity, the acute toxicity values for AS in fish generally range from 5 to 20 mg/l. No correlation was noted between the chemical structure of AS and toxicity; however, uptake as well as toxicity increased with increased water hardness. With invertebrate species, toxicity occurred in 2 to >200 mg AS/l range. Growth of phytoplankton was inhibited at AS concentrations of 10 mg/l or greater while soil bacteria were inhibited at a concentration of 100 ppm.

Although AS toxicity in fish is characterized by gill damage, fish exposed to radiolabelled AS were found to concentrate the label primarily in the gall bladder, the gut and the liver; the label was rapidly excreted with butyric acid-4-sulfate being the major metabolite.

In terms of human safety, AS are relatively non-toxic. The acute oral LD_{50} values in the rat range from 2000 to 20,000 mg/kg and no deleterious effects have been noted in long-term feeding studies with that species at AS levels up to 1% of the diet. Metabolic studies in rats indicate that greater than 80% of the ^{35}S-label is excreted in the urine within 48 hours, regardless of the route of administration, with butyric acid-4-sulfate as a major metabolite.

Occluded patch tests with 1% AS solutions produced dermatis and histological changes in the skin of rabbits and guinea pigs but no effect was observed at a concentration of 0.1 percent. Little or no ocular irritation was noted in rabbits at a concentration of 1% but concentrations greater than 10% were classified as primary eye irritants according to the Draize procedure.

No detrimental effects on litter parameters nor increased incidence of abnormalities have been linked to AS except at doses which were severely toxic to the dams. There are no indications from long-term feeding or skin-painting studies that alkyl sulfates exhibit any carcinogenic activity.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

ALKYL SULFATES

I. INTRODUCTION

Until the mid-1960's, anionic alkyl sulfate (AS) surfactants were predominantly used in household and industrial wool-washing applications (Tomiyama et al., 1969). At present, however, alkyl sulfates are widely used in heavy duty laundry products (Kerfoot and Flammer, 1975) and in a large variety of specialty products such as shampoos, cosmetics, dentifrices, antacids, depilatories, etc. with no evidence of hazard to human health (Gleason et al., 1969).

The bulk of alkyl sulfates incorporated into these products are linear primary alkyl sulfates but some linear and branched secondary AS are also utilized. The total amount of alkyl sulfates manufactured and used in commercial products ranges from 150 to 200 million tons (Soap and Detergent Association estimate).

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

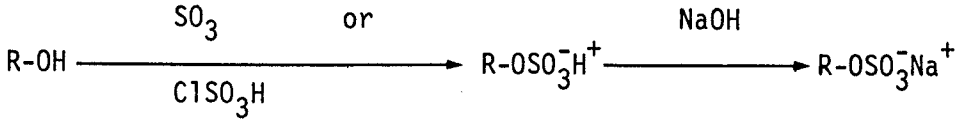
(1) environmental fate and distribution, including biodegradation,

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

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

Synthesis of Alkyl Sulfates

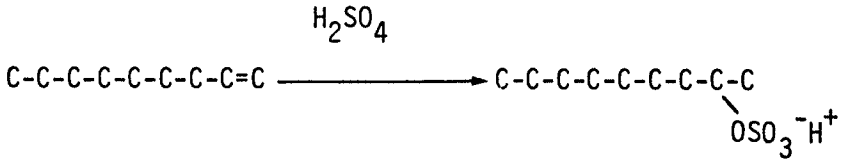
Primary AS are usually manufactured by conventional sulfation of the parent alcohol with either sulfur trioxide or chlorosulfonic acid. The product of this reaction is then neutralized with an appropriate base.



(R usually averages between 12-18 carbons)

The carbon chain precursors for these sulfates can be either linear or highly branched. Among the linear alcohols are included those which are completely linear as well as those containing some secondary methyl branching (slightly branched). In contrast, the highly branched AS are derived from tetrapropylene stock.

Secondary AS are more readily prepared by reacting the parent alkene rather than the corresponding alcohol with sulfuric acid.



The sulfate ester group does not necessarily add at the double bond position, but rather at any position along the chain except the terminal carbon atoms. A complex mixture of isomers can thus occur (Higgins and Burns, 1975; Kerfoot and Flammer, 1975; Swisher, p. 36, 1970).

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

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II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alkyl sulfates are one of several chemical entities classified as anionic surfactants and thus can be detected with many of the procedures utilized in the detection of LAS (See Chapter 1, pp.19-26). The MBAS analytical procedure measures AS along with other anionic surfactants but does not distinguish among them.

B. Water Quality Standards

There are presently no standards in the United States or Europe specifically restricting alkyl sulfates (AS). These anionic surfactants are included among those measured in the environment using the MBAS method. The restrictions applying to MBAS levels were discussed in Chapter 1, pp. 26-29.

C. AS in Natural Water Bodies

AS are not presently being monitored, as such, in the United States or Europe. MBAS measurements in water bodies include AS surfactants as well as other anionics. Levels of anionic surfactants detected in natural water bodies were discussed in Chapter 1, p. 30.

III. BIODEGRADATION

As a class, alkyl sulfates are biodegraded quite readily. Linear, primary AS generally undergo complete primary biodegradation within a few days and secondary and slightly branched AS surfactants are also biodegraded quite readily. In contrast, highly branched AS might be expected to degrade at a considerably slower rate.

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

A. Laboratory Test Systems

1. Oxygen Uptake - Biochemical Oxygen Demand

Alkyl sulfates are readily biodegraded in standard 5 or 20 days BOD tests. Neither slight branching nor increments in the length of the carbon chain appear to exert a significant effect on the rate of degradation (see Table 2-A). For example, after 5 days, slightly branched C_{11}AS and linear C_{15}AS had utilized 73% and 67%, respectively, of their theoretical oxygen demand (Procter & Gamble Co., unpublished data). Glucose generally utilizes about 70% of its theoretical oxygen demand in this time period (Swisher, p. 142, 1970).

2. CO_2 Evolution

All of the AS surfactants cited above were also examined using Sturm's evolved CO_2 procedure (1973). All were readily degraded (see Table 2-A) with the percent of evolved CO_2 ranging between 74% and 95%. Under these conditions, glucose evolved 80-85% CO_2 (Procter & Gamble Co., unpublished data).

TABLE 2-A

Biodegradability of Alkyl Sulfates

Carbon Chain Length	% BOD_5*	% BOD_{20}**	% CO_2†
C_{11}	72.9	T.D.††	93.2
C_{11}^{∞}	72.9	T.D.	89.1
C_{12}	63.1	T.D.	85.0
C_{13}^{∞}	65.7	93.9	85.8
C_{12-13}	61.0	96.2	74.4
C_{12-14}	57.0	70.1	95.0
C_{15}	67.0	T.D.	94.7
C_{15}^{∞}	62.5	87.1	80.7
C_{16-18}	60.3	T.D.	83.2

* Percent biochemical oxygen demand at 5 days.

** Percent biochemical oxygen demand at 20 days.

† Percent of theoretical CO_2 production.

†† Total depletion of oxygen.

$^{\infty}$ Slight methyl branching.

(Procter & Gamble Co., unpublished data)

3. Die-Away Tests

a. River Water Test

Maurer et al. (1971) reported that 5 mg/l of C_{16} AS underwent complete primary biodegradation (MBAS) after one day in a river water die-away test. Similarly, greater than 95% of n-$C_{12-14-16}$ AS had been biodegraded (MBAS) in one day (Huddleston and Allred, 1967) and complete removal (MBAS) of 10 mg/l of n-pri-$C_{12-14-16}$ (64:25:11) AS was achieved by 2 days (Continental Oil Company, unpublished data).

After 3 days, Sekiguchi et al. (1975) could detect (as MBAS) none of the 20 mg/l C_{12} AS added to samples to Tama River water. However, it took 20 days for the total organic carbon (TOC) to dissipate completely as compared to 13 days for the same concentration of glucose. The extent of degradation of 5 mg/l of C_{12} AS in seawater was also examined; no MBAS activity could be detected at 5 days. In 4 separate tests with Chesapeake Bay water, Cook and Goldman (1974) found that it took an average of 1 to 3 days to achieve a 75% decrease in azure-A-reactive substances for $C_{12ave.}$ AS.

b. Fortified and Inoculated Waters

Cordon et al. (1972) found that 40 mg/l C_{16} AS was completely degraded (MBAS) after 2 days while Crauland et al. (1967) noted that it took unacclimated bacteria 3 days to completely biodegrade (MBAS) C_{18} AS but acclimated bacteria required only one day to achieve the same results.

In a static die-away test (EMPA method), Gafa and Lattanzi (1974) found that 3 commercial AS were all biodegraded (MBAS) greater than 95% within 3 days. The materials tested included: 100% linear $C_{12-14-16}$ AS (ALFOL 1216/P™, mol. wt. 206); 85% linear C_{12}-C_{15} AS (DOBANOL 25™, mol. wt. 206); and 55% linear $C_{11-13-15}$ AS (DIADOL HA 115™, mol. wt. 203). Similar findings were reported for n-$C_{12ave.}$ AS by Arpino (1969) and Lundahl et al. (1972).

c. Shake Culture Test

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

4. Simulated Treatment Processes

a. Activated Sludge

Janicke (1971) observed that n-C_{12} AS was completely biodegraded (>99% MBAS; >94% TOC) up to a surfactant loading level of 100 mg/l in a laboratory-scale, activated sludge unit. At concentrations greater than 100 mg/l, the following signs of surfactant overloading were evident; reduction in nitrification; a 20-25% reduction in the degradation of organic nitrogen; high turbidity of the effluent and the disappearance of protozoa from the sludge.

Fisher and Gerike (1975) reported 99% removal (MBAS) of n-C_{12} AS in the OECD confirmatory test (1971) after 1 day. Similar results were reported by Sakaguchi et al. (1975) and Allred and Huddleston (1967) in a semi-continuous activated sludge unit. Linear pri-$C_{12-14-16}$ (64:25:11) AS was also completely biodegraded (MBAS) in a single 24-hour cycle in a semicontinuous activated sludge unit (Continental Oil Co., unpublished data).

Borstlap (1967) examined the intermediates obtained in batch activated sludge units containing 7.5 liters of unacclimated activated sludge and 50 liters of 500 mg/l AS surfactant as the sole carbon source. After one week, no MBAS activity could be detected for n-C_{14} AS but intermediates (7-10%) were found. After 3 weeks, MBAS activity for n-oxoC_{14} AS, and tetrapropylene-derived oxoC_{13} AS was zero and 18%, respectively, with 13-18% and 51%, respectively, of intermediate products remaining.

b. Trickling Filters

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

c. Anaerobic Systems

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

In another study, 25 mg/l of a coconut-alcohol-derived AS were fed into an anaerobic digester tank system over a 3 1/2 month period. The tank had a capacity of 336 gallons of sewage per day. Average retention time was 68.5 hours. Surfactant removal averaged 66% (measured as the number of ^{35}S counts in a chloroform extract) with BOD and COD removals of 36% and 41%, respectively (Procter & Gamble Co., unpublished data).

Under microaerophilic conditions ($\leq$ 1 ppm O_2 level), Maurer et al. (1971) found that 5 mg/l C_{16} AS completely biodegraded (MBAS) within 3-6 days at 25°C in a river water test. At a concentration of 10 mg/l, it took 9-10

days to completely biodegrade at 25°C and 34 days at 35°C. In another study conducted under microaerophilic conditions, Cordon et al. (1972) found that 97% of C_{16} AS had biodegraded (MBAS) in a static die-away test at 35°C after 7 days. However, it took approximately 20 days for the TOC to be utilized.

B. Influence of Test System Variables

1. Inoculum

Goodnow and Harrison (1972) examined the degradation (MBAS) of a tallow alkyl sulfate ($\sim C_{17}$) at concentrations of 0.01, 0.05 and 0.5 g/l by 45 strains of 34 species of bacteria representing 19 genera found in water, soil and sewage. Of the bacteria tested, all except two degraded the surfactant between 19 and 100% within 72 hours; no degradation (0%) of the surfactant had occurred with either Acetobacter peroxydans ATCC838 and Escherichia coli B/r at 72 hours.

2. Temperature

Mann and Reid (1971) found that DOBANOL 25 and a coconut-alcohol-derived AS biodegraded 97-98% (MBAS) even during the winter months in a trickling filter sewage treatment plant.

C. Field Studies

Oba et al. (1976) analyzed raw municipal sewage entering two Japanese

sewage treatment plants over a one-year period. Of the total surfactant content entering the sewage treatment plants, 16% consisted of AS and AES surfactants which were completely removed (far infrared analytical method) during passage through the plants.

In field trials in a trickling filter sewage treatment plant, Mann and Reid (1971) found that pri-AS derived from either coconut alcohols or DOBANOL-25 displayed a high order of biodegradability (96-98% removal of MBAS).

D. Metabolic Pathways of Biodegradation

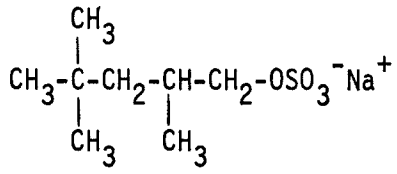
Linear primary alkyl sulfates readily undergo primary biodegradation via sulfatase enzymes which split off the sulfate ester group to form inorganic sulfate and the corresponding alcohol. The alcohol is then oxidized to the corresponding aldehyde and subsequently to the carboxylic acid which is degraded by β-oxidation (Higgins and Burns, 1975; Swisher, 1976).

$$R\text{-}CH_2OSO_3^-Na^+ \xrightarrow{enz} R\text{-}CH_2OH + Na_2SO_4$$

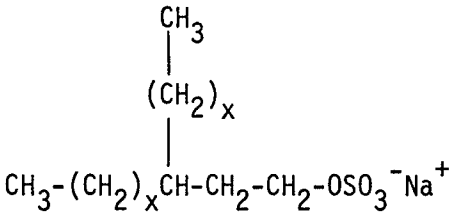
$$R\text{-}CH_2OH \rightarrow R\text{-}CHO \rightarrow R\text{-}COOH \rightarrow CO_2 + H_2O$$

The rapid breakdown of linear primary alkyl sulfates does not necessarily apply to secondary or branched AS. The work of Huyser (1961) and Hammerton (1955, 1956) indicated that some, but not all branched primary and secondary AS were resistant to biodegradation. For example, during an 18-day river water

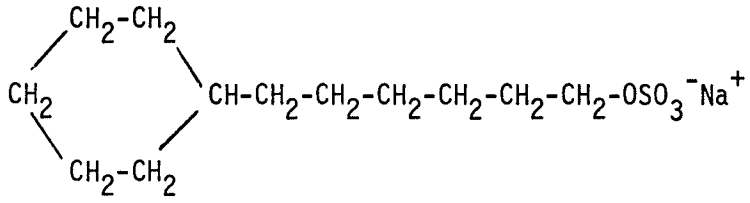
test, the following compound did not degrade (MBAS) at all:



while branched chain primary alkyl sulfates such as:



and cyclic alkyl sulfates such as:



were completely degraded in 14 and 7 days, respectively (Huyser, 1961). The microbial sulfatase enzymes which hydrolyze n-pri-AS appear to be inactive against secondary and branched analogues although some microbes are able to produce sulfatases that are specific for these compounds (Higgins and Burns, 1975). For example, Fitzgerald and Payne (1972) found that Pseudomonas $C_{12}B$ was induced by secondary AS to form pri- and sec- alkylsulfatases. Furthermore, they found that alkyl sulfatase synthesis was essentially unaffected by the presence in the culture medium of sulfate or cysteine but sulfatase synthesis was repressed by the presence of a number of carbon sources including some primary and secondary alcohols, acetate, propionate, etc.

Additional work in this area was reviewed extensively by Swisher (p. 302, 1970).

It thus appears that linear primary alkyl sulfates and secondary and slightly branched AS are readily biodegraded but that the biodegradability of certain highly branched compounds cannot be predicted with any great assurance from the information that is presently available.

E. Summary

Linear primary alkyl sulfates very readily undergo primary biodegradation in the field as well as in a wide variety of laboratory tests. Increments in carbon chain length and reduced ambient temperature exert no significant influence on the degradative rate. Biodegradation is also extensive under anaerobic conditions. The degradative pathway appears to be initiated by sulfatase enzymes which hydrolyze the sulfate ester group producing inorganic sulfate and an alcohol which eventually undergoes β-oxidation.

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IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. Fish

The available acute toxicity data for fish are summarized in Table 2-B. The LC_{50} values range from about 2-1,000 mg/l, although most values range from 5-20 mg/l. These values show no particular correlation with carbon chain length or molecular weight among these surfactants.

Tovell *et al.* (1974) showed that Na-$C_{12\,ave.}$ AS was more toxic to goldfish and rainbow trout in hard water (300 mg/l $CaCO_3$) than in soft water (60 mg/l $CaCO_3$) or distilled water. It was observed that uptake increased with hardness as shown in Table 2-C. These authors also showed that absorption of alkyl sulfate by fish is a "function of the hardness of the water in which the fish have become acclimatized as well as the water in which the fish are treated." Toxicity increased with the hardness of the acclimatization water in treatment waters of the same hardness.

TABLE 2-B

The Acute Toxicity of Alkyl Sulfates to Fish

Species	Surfactant	Experimental Conditions	Toxicity (LC_{50} 6 hr) (mg/l)	Source
Goldfish (Carassius auratus)	n-C_{12} AS, 93% AI*, MW-186	Static, 20°C, hardness - 10°, 10 fish/conc.	60.0	Gafa (1974)
	n-C_{14} AS, 92.4% AI, MW-214		5.0	
	n-C_{16} AS, 95.3% AI, MW-242		>300	
	n-C_{12-16} AS, 94.3% AI, MW-206		12.0	
	n-C_{13} AS, 94.8% AI, MW-200		18.3	
	n-C_{14} AS, 94.3% AI, MW-214		6.3	
	n-C_{12-15} AS, 95.8% AI, MW-206		7.8	
	C_{14} AS, branched, 98% AI, MW-214		49.1	
	C_{14} AS, branched, 94% AI, MW-242		7.8	
	ALFOL 1216-P (n-C_{12-16}) MW-206	Static, fish - 6-7 cm, hardness - 10°C, 10 fish/conc	12.0	Gafa and Lattanzi (1974)
	DOBANOL 25 ($C_{12,15}$ AS) MW-203		7.8	

*Active ingredient

TABLE 2-B

(Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Goldfish (continued)	DIADOL HA 115 (C_{11-15} AS) MW-203	Static, fish - 6-7 cm, hardness - 10°C, 10 fish/ conc.	LC_{50} 6 hr - 8.1	Gafa and Lattanzi (1974)
	$Na\text{-}C_{12ave.}$ AS	Static, fish - 6 cm, pH - 6.8-7.2 hardness - 300 mg/l $CaCO_3$	LT_{100}* at 70 mg/l AS 90-110 minutes	Tovell et al. (1974)
		distilled water	>24 hrs	
Rainbow trout (Salmo gairdneri)	$Na\text{-}C_{12ave.}$ AS	Static, fish 10 cm, pH - 6.8-7.2 hardness - 300 mg/l $CaCO_3$	LT_{100} at 70 mg/l AS 40-45 minutes	"
		hardness - 60 mg/l $CaCO_3$	3 hrs	
	$Na\text{-}C_{12\ ave.}$ AS	Static, fish - 27 g., hardness - 25 mg/l $CaCO_3$	LT_{50} at 100 mg/l AS 4.9 hrs (3.9-6.1) 95% CL	Abel and Skidmore (1975)

*Lethal time for 100% of the population

TABLE 2-B

(Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Bluegill (Lepomis macrochirus)	$Na\text{-}C_1$ AS	Static, 20°C, pH 7.1, hardness-35 mg/l $CaCO_3$	LC_{50} 96 hr - 1000	Procter and Gamble Company, unpublished data
	$NH_4\text{-}C_{11}$ AS		96 hr - 26.0 (19.0-35.4)	
	$NH_4\text{-}C_{11}$ AS, branched		96 hr - 16.5 (13.1-21.0)	
	$Na\text{-}C_{12}$ AS		96 hr - 4.83 (4.06-5.75)	
	$NH_4\text{-}C_{12}$ AS		96 hr - 20.3 (16.0-25.7)	
	$NH_4\text{-}C_{13}$ AS, branched		96 hr - 18.4 (15.2-22.2)	
	$NH_4\text{-}C_{15}$ AS		96 hr - 5.19 (3.97-6.77)	
	$NH_4\text{-}C_{15}$ AS		96 hr - 3.39 (2.59-4.43)	
	$NH_4\text{-}C_{15}$ AS, branched		96 hr - 2.13 (1.37-3.31)	
	$NH_4\text{-}C_{16}$ AS		96 hr - 21.7 (16.7-28.1)	
	$NH_4\text{-}C_{12\text{-}14}$ AS		96 hr - 3.2 (2.8-3.7)	
	$C_{16\text{-}18}$ AS		96 hr - 76.0 (50-116)	

TABLE 2-B

(Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Guppy (*Lebistes reticulatus*)	$C_{12ave.}$ AS	ASTM - D 1345-59	Minimum concentration with lethal effect - 10 mg/l	Borstlap (1967)

TABLE 2-C

Effect of Water Hardness on Uptake of Na-C_{12}AS by Fish*

Water Hardness (mg/l as $CaCO_3$)	Concentration in Tissue ($\mu g/g \pm SE$)	
	Rainbow Trout	Goldfish
0 (distilled)	-----	9.6 ± 0.4
60	6.8 ± 1.2	24.2 ± 1.9
200	-----	73.9 ± 5.2
300	42.0 ± 4.8	85.7 ± 7.1

*Fish exposed to 70 mg/l Na-$C_{12ave.}$AS. Rainbow trout died after 35 minutes exposure, goldfish after 112 minutes.

Source: Tovell et al. (1974)

2. Invertebrates

The available acute toxicity studies for invertebrates are summarized in Table 2-D. The range of toxicity for these limited data is about 2 to >200 mg/l.

Hidu (1965) studied the effects of AS (27-31.4% active) on the development of fertilized eggs of clams (Mercenaria mercenaria) and oysters (Crassotrea virginica) into free swimming larvae. There was a significant reduction of fertilized egg development (62% and 61% of control) for clams and oysters at 1 mg/l. No development occurred at 2.5 mg/l. Larval survival was not reduced significantly at the 95% level until 5 mg/l. Clam survival was 68% of the control after a 10 day exposure; oyster survival was 19% of the control after a 12 day exposure. Larval growth was also reduced after these exposures (clams, 70% of control; oyster, 17% of control).

B. Toxicity of AS to Algae and Microorganisms

Ukeles (1965) studied the effect of Mg-$C_{12ave.}$ AS (CONCO SULFATE M^{TM}) on 12 species of marine phytoplankton (Chlorophyceae) at 1, 10, 100, and 1,000 mg/l. No growth of any species occurred at the two highest

TABLE 2-D

The Acute Toxicity of Alkyl Sulfates to Invertebrates

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Brine shrimp (*Artemia salina*)	$Na\text{-}C_{12ave}AS$	Static, 24.5°C, artificial seawater	LC_{50} 24 hr - 3.6	Price *et al.* (1974)
Daphnia magna	$Na\text{-}C_{12\ ave}AS$ (Ziegler derivative)	Static, 20° ± 1°C, *Daphnia* 72 hrs. old, synthetic river water	LC_{50} 24 hr - 13.5 48 hr - 6.3	Lundahl *et al.* (1972)
	$Na\text{-}C_{12\text{-}14}AS$ (natural alcohol derivative)		24 hr - 6.3 48 hr - 2.8	
Mosquito (*C. p. quinquefasciatus*)	$Na\text{-}C_{12}$	Static, 24 hr. old pupae	LC_{50}* 78	Piper and Maxwell (1971)
	$NH_4\text{-}C_{12ave.}AS$		55	
	Triethanolamine - $C_{12ave.}AS$		102	
	$Na\text{-}C_{10}$ AS		44	
	Na-2 ethylhexyl sulfate		>200	

*Time not given.

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

The MAC-5 day concentration for a marine flagellate (Dunaliella sp.) was found to be >1 but <10.0 mg/l. (Procter & Gamble COmpany, unpublished data).

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

The effect of AS on bacteria has been studied by several authors. Kopp and Müller (1965) found that the motility of Proteus mirabilis (bacteria isolated from human urine) was completely inhibited by Na-AS at varying concentrations, depending on the carbon chain length. No motility was found at the following concentrations: C_6 - 50 mmoles/l, C_8 - 20 mmoles/l, C_{10} - 5 mmoles/l, C_{12} - 0.5 mmoles/l, and C_{14} - 0.2 mmoles/l. In this case, toxicity

seems to increase with increasing carbon chain length. Growth was also impaired at similar concentrations. At 10 mmoles/l, C_8AS, C_{10}AS, and C_{12}AS were bacteriostatic, but C_6AS had no effect at this concentration.

Lundahl et al. (1972) studied the growth of E. coli on a gelatin medium containing Na-C_{12}AS (Ziegler derived) or Na-C_{12-14}AS (natural alcohol derived). The concentrations which did not allow the development of more than 5 colonies per plate were 50 g/l and >200 g/l, respectively.

The natural composition of the bacteria population in soils can be affected by AS. Hartman (1966) examined the growth of soil bacteria from an oak forest and a grassy field, and from two samples of surface water. The results are shown in Table 2-E. The reduction of colonies counted compared to control was the measure of inhibition. The soil bacteria were more sensitive to AS than the water bacteria, and the percent of surfactant-sensitive bacteria increased with soil depth.

C. Effects of AS on Higher Plants

The only study found examining the effects of AS on higher plants shows a stimulatory effect. Corn seeds that had been watered with 0.01, 0.1, and 1.0 g/l $C_{12ave.}$AS weighed 97, 130, and 136% of the control, respectively. Since the length and dry weight of corn plants were also stimulated at these concentrations the effects were due to actual increase in growth rather than imbibition of water. (Nadasy et al., 1972).

TABLE 2-E

The Effect of 100 ppm AS on Soil Bacteria

Source of Inoculum	Depth	% Reduction of Bacterial Colonies (compared to control)
Soil forest	Surface	14
	4"	23
	8"	46
	15"	67
Grassy field	Surface	0
	24"	11
Brook	Surface	11
Well	Surface	7

Source: Hartman (1966)

D. Effects of AS on Birds and Wildlife

No studies were found which examined the effects of AS on birds or wildlife.

E. Interactions with Other Chemicals

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

F. Mode of Action

The mode of action of AS has been most commonly investigated in fish. The behavioral response involves an increase in swimming activity and an increase in respiratory activity. Later signs of poisoning include surfacing, loss of balance, overturn with loss of mobility, and death (Abel and Skidmore, 1975; Lang, 1967).

These symptoms are accompanied by progressive gill damage. Abel and Skidmore found that after a 3 hr exposure (60% LT_{50}) to 100 mg/l Na-$C_{12ave.}$AS, 6.1% of the secondary lamellae were affected in rainbow trout, while at death 86.9% were affected.

Although damage is primarily observed in the gills, studies by Tovell et al. (1975) showed that after a 24 hr exposure of goldfish (Carassius auratus) to 50 mg/l [^{14}C] or [^{35}S] Na-$C_{12ave.}$AS, the radioactivity was most concentrated in the gall bladder. If the surfactant was administered internally, about 50% of the radioactivity was found in the gall bladder. The gut and the liver concentrated the surfactant to a lesser extent. The principal route of entry was across the skin surface. Excretion was fairly rapid, with levels of radioactivity in unfed fish falling 38%, compared with a reduction of 68% in fed fish in 24 hrs. An examination of the metabolites revealed one principal metabolite, identified as butric acid 4-sulfate, and 4 minor ones. The authors suggested that the surfactant was absorbed and metabolized by the liver, and the metabolites were returned to circulation or secreted into the gall bladder.

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

Tovell et al. (1974) related toxicity to cell permeability. These authors observed that the presence of bivalent ions greatly increased the toxicity of Na-$C_{12ave.}$AS to goldfish and rainbow trout (see Table 2-B). These authors

proposed that the different rate of absorption could be due to a change in permeability of certain tissues, or to a change in the availability of the surfactant.

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

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V. HUMAN SAFETY

A general consideration of the safety of surfactants to humans was presented in Chapter 1 (LAS, p. 134).

The safety of the alkyl sulfates to humans is well recognized by the Food and Drug Administration who have approved the use of broadcut sodium dodecyl sulfate for use in food as (1) an emulsifier in or with egg whites provided it does not exceed 1000 ppm in egg white solids and 125 ppm in frozen or liquid egg whites; (2) as a whipping agent at a level not to exceed 5000 ppm by weight of gelatin used in the preparation of marshmallows; and (3) limited use in fruit drinks up to a level of 25 ppm (GRAS List, as cited in : "The Food Chemical News Guide").

Broadcut dodecyl sulfate salts (NH_4, Mg, K, Na) have also been cleared for use in adhesives (121.2520), cellophane (121.2507) and in paper and paper board for use with dry, aqueous and fatty foods (121.2571, 121.2526) (GRAS List, as cited in: "The Food Chemical News Guide").

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

A. Animal Studies

Acute Toxicity-Oral. The acute oral toxicity of AS to rats generally ranges from above 1000 mg/kg to 4000 mg/kg depending on the nature of the material tested (Table 2-F). For AS used commerically, the LD_{50} values are 5000 to 15,000 mg/kg. The only comparison of identical compounds in two species revealed that certain slightly branched chain AS were about 3 times as toxic to guinea pigs as to rats (Smyth et al., 1941).

Acute Toxicity-Intraperitoneal. The acute intraperitoneal LD_{50} in mice of a series of AS of carbon chain length from C_8 to C_{18} (2 carbon increments) ranged from 284 to 477 mg/kg. There appeared to be no trend relating toxicity to chemical structure of the surfactant (Gale and Scott, 1953).

Acute Toxicity-Percutaneous. The treatment of guinea pigs with a dose of slightly branched C_{8-}, C_{14}- or C_{17}AS equivalent to the oral LD_{50} by holding the surfactant in contact with the skin of the test animals for 4 days resulted in some deaths. The authors indicated that quantitative statements were not justifiable because of uncertainties in the procedures (Smyth et al., 1941).

Acute Irritation-Skin. At concentrations of 25 to 30 percent, AS of all carbon chain length tested (C_{10}-C_{15}) were primary irritants for rabbit skin. At concentrations of 1 percent, little or no skin irritation occurred (Brown and Muir, 1970; Olson et al. 1962; Continental Oil Company, Ethyl Corporation, Procter and Gamble Company, unpublished data).

TABLE 2-F

Acute Oral Toxicity of Alkyl Sulfates

Carbon Chain	LD_{50} (mg/kg) RATS	References
$C_{12ave.}$ (linear)	1288	Walker et al., 1967
C_8 (slightly branched)	4120	Smyth et al., 1941
C_{14} (slightly branched)	1250	"
C_{17} (slightly branched)	1425	"
C_{12} (linear)	2730	"
DOBANOL 23	1000-2000	Brown & Muir, 1970
$C_{12,14}$ (coconut oil)	1000-2000	
$C_{12,14,16}$(64% C_{12})	2330	Continental Oil Company
	1783	(unpublished data)
C_{16}(66%)+C_{18}(32%)	13100	Procter & Gamble Company (unpublished data)
C_{16}(27%)+C_{18}(62%)	7640	
C_{16}(57%)+C_{18}(33%) +C_{20}(9%)	~19600	
C_8	3200	Gale and Scott (1953)
C_{10}	1950	
C_{12}	2640	
C_{14}	>3500	
C_{16}	>3000	
C_{18}	>3000	

TABLE 2-F

(continued)

Acute Oral Toxicity of Alkyl Sulfates

Carbon Chain	LD_{50} (mg/kg)	References
	MICE	
$C_{12ave.}$ (linear)	1500	Olson et al., 1962
$C_{12ave.}$ (linear)	1460	Tomiyama et al., 1969
	GUINEA PIGS	
C_8 (slightly branched)	1520	Smyth et al., 1941
C_{14} (slightly branched)	650	
C_{17} (slightly branched)	425	

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

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

Brown (1971) investigated a number of animal tests for assessment of irritation due to surfactants. With a sodium salt of a broad cut coconut alcohol sulfate (C_{12} and C_{14}), he found that a 7 day occluded exposure to a 1 percent solution was highly irritating to rabbits, although a 0.1% solution was not. In rats, a 5 percent solution of the AS did not elicit a response after occlusion for 16 hours. In uncovered exposures on rabbits and guinea pigs, a 1 percent solution of surfactant was not irritating. Similarly, repeated percutaneous exposure to

hairless mice for 28 days showed no histochemical skin changes.

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

In a similar histological study with AS (C_{12} - C_{15}, 70 percent linear, 30 percent branched), Iimori et al. (1971) found hypertrophy and edema of the epidermis in guinea pigs following a 2 hr exposure (unoccluded, but restrained) with 10 and 30 percent solutions of the surfactant. Recovery was not complete in 168 hours with either concentration of test solution.

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

Skin Sensitization. The data from several studies indicate that AS are not skin sensitizing agents. Brown and Muir (1970) found that topical application of various ethanolamine and ammonium salts of alcohol sulfates

TABLE 2-G

Extraction of Proteins and Amino Acids from Guinea Pig Skin by Alkyl Sulfates

Carbon Chain Length	Soluble Protein	Total Amino Acids
9	50.8	62.7
10	166.1	84.2
11	119.5	100.4
12	238.9	194.8
13	198.5	141.7
14	163.9	110.3
15	77.9	41.3

Prottey and Ferguson, 1975

did not sensitize guinea pigs. Intradermal treatment with mono- or triethanolamine salts of the surfactant did sensitize, whereas the ammonium salt did not. Thus, the authors suggest that sensitization may be due to the presence of free amines rather than the surfactant.

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

Acute Irritation-Ocular. The administration of slightly branched chain AS at 4 or 8 percent concentrations gave rise to corneal necrosis in rabbits as determined by fluorescein staining (Smyth et al., 1941).

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

Subacute Toxicity-Oral. Several branched chain AS were included in the drinking water of rats for a thirty day interval. From the amount of water consumed the daily doses in several test groups were estimated to range from 230 to 1510 mg/kg/day. At the end of the study, the major pathology observed was seen in the kidneys with light cloudy

swelling and secretion in the tubules. Blood counts were normal in all treatment groups. The doses at which no toxic effects were seen ranged from 230 to 440 mg/kg/day (Smyth et al., 1941).

A commercial AS (IRIUM^{TM}) was fed to rats in their diet for 5 weeks at levels of 30 or 60 mg/rat/day. At the end of five weeks, the weight gain was reduced in both treated groups and histological examination of the liver revealed swollen liver cells and prominant hepatocyte nuclei in the treated groups. Kidneys were normal in all groups. (Hatton et al., 1940).

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

Walker et al. (1967) administered a $C_{12ave.}$ and a C_{12}-C_{15} mixture of AS to rats in the diet for 13 weeks. At a dose of 5000 ppm (0.5 percent) some increases in organ weights were noted along with changes in serum urea levels. No histopathological changes were found. At 1000 ppm (0.1 percent), no changes were observed.

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

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

Subacute Toxicity-Percutaneous. The repeated application of $C_{12ave.}$ AS at a 1 percent concentration to abraded rabbit skin gave rise to exfoliation and edema. On unabraded skin, no response was observed (Ethyl Corporation, unpublished data).

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

Acute, Subacute and Chronic Toxicity-Summary. The acute oral toxicity of AS for rats generally in the range of 1000 to 4000 mg/kg and thus, these surfactants can be considered to be relatively non-toxic. With respect to their effects on skin, concentrations of 1 percent in occluded exposures give rise to dermatitis and histological changes in the skin of rabbits and guinea pigs. At 0.1 percent, no effect from AS exposure has been reported. Concentrations of AS above 10 percent result in primary irritation to rabbit eyes tested according to the Draize procedure, while little or no irritation was found with 1 percent solutions. The subacute and chronic oral toxicity studies indicate that the inclusion of sodium $C_{12ave.}$ AS in the diet of rats at 1 percent for up to one year produces no significant toxic effects.

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

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

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

Fukushima et al. (1974) exposed male Wistar rats to N-methyl-N-nitro-N'-nitroso-guanidine (MNNG) (50mg/l) with and without sodium dodecyl sulfate (0.25 percent) in their drinking water for 26 to 30 weeks. With MNNG alone, 3 of 6 animals had stomach adenocarcinomas, while the inclusion of the surfactant with MNNG gave rise to adenocarcinomas in 8 of 10 rats. In addition the group treated with both MNNG and Na C_{12} ave. AS had 3 stomach sarcomas, while none was present in rats given MNNG alone. The authors suggest that the surfactant may have increased the

absorption of the carcinogen in the stomach tissue and this could be the cause of the increased tumor yield. However, the small number of animals involved in this study does not allow definitive conclusions to be drawn.

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

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

Pharmacology-Metabolism. The administration of potassium dodecyl ^{35}S-sulfate orally or intrapertioneally to rats at a level of 1mg per rat (no weight of rats was given) was followed by a rapid excretion of ^{35}S-label in the urine. More than 80 percent of the label is excreted in 24 hours, with over 90 percent eliminated in 48 hours. Less than 1 percent of the label was found in the feces and 0.4 percent in the carcass at 48 hours. A major metabolite was the sulfate of 4-hydroxybutyric acid which was excreted unchanged when given orally to rats indicating that the liver may be the primary site of oxidative degradation of the C_{12} AS (Denner et al., 1969).

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

Recently, Burke et al. (1976) examined the metabolic pathway of ^{35}S-labelled C_{11} AS. Similar to the even-numbered carbon chain AS, over 80% of the ^{35}S-label was excreted in the urine within 48 hours after

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

Merits (1975) administered [1-^{14}C] C_{16} AS (4.4mg/kg) and [^{35}S] C_{16} AS (2.9 mg/kg) intravenously as the sodium and trimethyl ammonium salts to dogs and orally as the erythromycin salt (14.4mg/kg) to dogs and rats. In rats, both labeled materials were well absorbed and rapidly excreted in the urine: 87-94% in urine, 4-5% in the feces. A similar picture was seen in dogs following intravenous administration. Oral administration of either ^{14}C or ^{35}S-labeled C_{16} AS in dogs, however, resulted in the excretion of considerable amounts of unmetabolized C_{16} AS in the feces by 72 hours. With the ^{35}S - C_{16} AS, 50-54% of the label was recovered in urine, 33-41% in the feces; with the ^{14}C-labelled material, 50-79% of the label was found in the urine, 12 to 40% in the feces. The main metabolite in both dogs and rats was the sulfate ester of 4-hydroxybutyric acid; δ-[^{14}C]-butyrolactone was also isolated as a minor metabolite present in dog and rat urine in animals give ^{14}C-labelled C_{16} AS. An additional metabolite,

the sulfate ester of glycollic acid, made up about 20% of the urinary radioactivity in dogs but not in rats.

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

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

Pharmacology-Gastrointestinal. Lish and Weikel (1959) examined the influence of Na $C_{12\ ave.}$ AS on intestinal absorption of the anionic dye

phenol red and the cationic dye methyl violet through the colon of rats. These solutions were injected directly into colonic sacs isolated by ligation. The absorption of phenol red was markedly enhanced from little or none absorbed in the absence of surfactant to 77 percent absorption with Na $C_{12\ ave.}$ AS. Absorption of methyl violet was unaffected.

Using a surgical procedure in cats to allow direct irrigation of the esophagus and stomach, Berenson and Temple (1975) found that a 10 percent solution of Na $C_{12\ ave.}$ AS gave rise to edema, congestion and inflammation within 15 minutes. A 20 percent solution of the surfactant caused extensive mucosal disruption, ulceration and necrosis. A detergent preparation containing 24 percent Na $C_{12\ ave.}$ AS, 60 percent sodium tripolyphosphate and 16 percent soap produced only slight changes in the esophagus but produced lesions in the stomach comparable to those elicited by 20 percent Na $C_{12\ ave.}$ AS alone.

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

Pharmacology-Neuromuscular. The effects in cats on pulmonary arterial pressure after intravenous injection of a series of sodium salts at alkyl sulfates (C_8, C_{10}, C_{12}, C_{14}) was examined by Schumacher et al. (1972). The C_8 and C_{14} surfactants were not effective, while

the C_{10} and C_{12} compounds caused a dramatic increase in pulmonary arterial pressure with infusions of 1 mg/kg/min. The inhibition of this increase by papaverine indicated that the response was due to a direct effect on smooth muscles of blood vessels and bronchi.

In a series of *in vitro* studies examining the effects of AS on neural transmission and on smooth and striated muscle, Gale and Scott (1953) reported no effect on nerve impluse conduction in sciatic nerve of the frog at dilutions of 1:100 of AS with carbon chain lengths of C_8 to C_{18}. On frog gastrocnemius muscle, a potassium-like effect was elicited by the surfactants at 1:100 dilutions. Experiments with isolated rat intestinal segments showed that the AS induced an increase in contractile activity at dilutions ranging from 1:100 for C_{18} AS to 1:20,000 for C_{12} AS. No effects on isolated turtle heart were found with 1:100 dilutions of the C_8 to C_{18} AS tested.

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

Pharmacology-Hormone Effects. The intramuscular injection into rats of sodium alkyl sulfates (C_8, C_{10}, or C_{12}) enhanced the reduction of blood

sugar caused by treatment with insulin, although the surfactant alone had no effect on blood sugar levels. The doses administered (1 ml of 3.44 mM C_8, 0.76 mM C_{10}, 0.17 mM C_{12}) indicated increasing potency with longer chain length. The surfactants did not influence the binding of insulin to cells, but may have enhanced cellular penetration of the hormone (Stausser et al., 1968).

Pharmacology-Cellular Effects. Hemolysis of erythrocytes from dogs, sheep and rabbits by alkyl sulfates was studied by Tomizawa and Kondo (1971). The hemolytic capabilities of the surfactants increased with increasing carbon chain length, differing by an order of magnitude as the carbon chain length increased by two unit increments (Table 2-H).

Prottey and Ferguson (1975) found that sodium $C_{12\ ave.}$ AS released histamine from rat mast cells at a concentration of 0.3 mM.

B. Human Studies

Skin Irritation. In a patch test using a 1 percent solution of Na $C_{12\ ave.}$ AS, Oba et al. (1968) report erythema and fissure of the treated skin after 24 hours.

With a C_{14} - C_{15} AS, moderate irritation was observed with application of 0.5 ml of an 0.05 percent solution. Under the same conditions a coconut oil derived material exhibited mild irritation (Procter and Gamble Company, unpublished data).

Table 2-H

Hemolytic Concentrations of Sodium Alcohol Sulfates

Carbon Chain Length	Dog	Rabbit	Sheep
8	3.91×10^{-2}M	3.58×10^{-2}M	3.41×10^{-2}M
10	2.31×10^{-3}M	2.70×10^{-3}M	2.29×10^{-3}M
12	2.15×10^{-4}M	4.70×10^{-4}M	2.13×10^{-5}M

Tomiyama and Kondo (1971)

Smeenk (1969) and Brown (1971) have reported several in vitro and in vivo studies with human skin to evaluate the effects of surfactants including sodium dodecyl sulfate. The tests employed were (1) degree of liberation of thiol groups from callus powder, (2) epidermal permability to potassium ion, (3) extraction of amino acids from intact skin, (4) patch tests, (5) arm-immersion tests. In each of the tests, 1 percent solutions of a broad cut dodecyl alkyl sulfate gave rise to increases in response as compared to controls. In the patch tests with 0.25 ml of 1 percent solution of the surfactant, 37 of 50 volunteers exhibited little or no response. The arm immersion test was carried out with 2 groups of 15 subjects, each at 0.1 percent concentrations. With 30 minute exposures on each of 5 consecutive days, scaling and/or erythema were observed.

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

Valer (1968a, 1968b, 1969) examined the effects of commercial surfactants used in detergents in Hungary, among them sodium $C_{12\ ave.}$ AS. Using a patch test and a stripping procedure, concentrations of 1 percent of the surfactants gave rise to little or no response in the patch test. On stripped skin, however, slight to moderate reactions occurred including localized hyperemia and exudative infiltration.

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

Ito et al. (1969) studied the evaluation of skin roughness induced by various surfactants including sodium $C_{12\ ave.}$ AS using three separate exposure procedures, i.e., immersion, drip and a cup technique. The cup procedure was found to be not appropriate for skin testing of surfactants. At concentrations of 0.05 to 0.35 percent, sodium $C_{12\ ave.}$ AS induced skin roughness which was roughly correlated to defatting rate in lanolin.

The investigations of Tronnier et al. (1970) also showed an increase in skin roughness with exposure to 2 percent sodium $C_{12\ ave.}$ AS. This concentration of surfactant also resulted in water removal from the skin.

Blank and Gould (1959) investigated the penetration of broad cut sodium dodecyl sulfate into human abdominal skin using an in vitro procedure with skin taken from autopsy specimens. Electrical conductivity measurements established the integrity of skin samples used in the test. From unbuffered mildly alkaline solutions, sodium dodecyl sulfate penetrated skin only slightly and was retained by the stratium corneum. At pH 10.5, the surfactant penetrated to the dermis.

With an in vitro procedure, Bettley (1965) found that exposure of human epidermis of 0.04M Na $C_{12\ ave.}$ AS induced a 50 percent penetration of potassium ion. In a later study, Wood and Bettley (1971) reported that exposure of isolated epidermis to sodium $C_{12\ ave.}$ AS (0.04M) produced an increase in titratable thiol groups after incubation for several hours.

Howes (1975) examined the penetration of sodium ^{14}C-dodecyl sulfate (7.3 mg/ml - 0.73 percent) through human epidermis in vitro. For 24 hours, little penetration occurred, but during the second 24 hours of exposure, penetration increased rapidly accompanied by marked swelling of the test tissue.

Prottey and Ferguson (1975) reported that 10mM sodium $C_{12\ ave.}$ AS caused a denaturation of human callus keratin as measured by an increase in titratable thiol groups. Under the same conditions, a 1mM solution had no effect.

In another study with callus from human skin, Imokawa et al., (1974) found that the binding of AS increased as the alkyl chain increased from C_8 to C_{12} in two-carbon increments. The intensities of skin roughness observed after exposure to 1 percent solutions of AS for 20 minutes at 40°C correlated well with its binding to callus.

Mucosal Irritation. A patch test was conducted to determine the sensory irritation of AS to oral mucosa. At 7.5 percent concentration, irritation was noted in 4 minutes, while at 1 percent, only a slight

sensory perception of irritation was noted after 20 minutes (Hatton et al., 1940).

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

Pharmacology - Effect on Peptic Activity. Fogelson and Shoch (1944) reported that administration of 200 mg of an AS mixture (C_{12} ave. C_{16}, C_{17} and C_{18} AS) every 2 hours effectively inhibited pepsin secretion in vivo. Kirsner and Wolff (1944) also found an appreciable, but temporary decrease in peptic activity following administration of 780 mg/hour of this mixture to ulcer patients on a low fat diet (lipids interfere with the inhibitory effects of AS on peptic activity). Pepsin levels, however, returned to pre-test levels within 30 minutes after the administration of AS was halted. Contrary to Fogelson and Shoch, Kirsner and Wolff (1944) found that treatment with alkyl sulfates provided no apparent beneficial effect on the healing of gastric ulcers. They did note, however, the absence of toxic effects in humans despite ingestion of exaggerated levels of AS; e.g., administration of AS levels as high as 8.4 g/day for a total comsumption of 258 g in 38 days produced only slight nausea, decreased appetite and mild to moderate diarrhea.

C. Epidemiology

Accidential Exposure. Berenson and Temple (1974) report an oral exposure to a detergent preparation that contained broad cut $C_{12}AS$ - 24 percent, soap - 16 percent and sodium tripolyphosphate - 60 percent. Members of a family that had ingested this detergent on two occasions within a 24 hours interval had oropharyngeal burns, esophogeal lesions and hematemesis. One month later, barium contrast diagnostic procedures revealed no gastrointestinal strictures.

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APPENDIX A - AS NOMENCLATURE AND ABBREVIATIONS

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

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

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

In Section III, the phase "complete biodegradation" refers to complete primary biodegradation. The complete conversion of a surfactant to carbon dioxide, water and other inorganic compounds is referred to as ultimate biodegradation.

ALCOHOL ETHOXYLATES

Synopsis

Surfactants of the alcohol ethoxylate type have found wide use in a large number of detergent formulations, in part, due to their tolerance of water hardness and low foaming characteristics.

Since in most instances analytical methods have not distinguished between alcohol ethoxylates and alkylphenol ethoxylates, it is not possible to ascertain which type of surfactant contributes to levels of nonionics reported in certain waterways. With respect to polyethylene glycol levels a single study reported increases in several English rivers over an eight year interval. The absence of data on levels of alcohol ethoxylates does not permit an adequate analysis of aquatic safety in terms of actual environmental concentrations.

Alcohol ethoxylates are in general rapidly degraded with a loss of surfactant properties. The factors that result in a reduction of biodegradability of these materials are the degree of alkyl chain branching, length of ethoxylate moiety and the presence of secondary rather than primary alkyl ether linkages. The study of the biodegradation of alcohol ethoxylates is hampered by the lack of definitive analytical methods for the surfactants and their primary degradation products, alkyl chains and polyethylene glycols of varying chain length. With respect to the mechanisms of biodegradation, the primary attack appears to be at the alkyl-polyethylene glycol ether linkage. Increases in ethoxy chain length retard the rate of degradation, but this retardation is significant only outside the range generally used in commercial detergent formulations (i.e., $\bar{5}$ 20 EO units/mole).

The acute toxicity values of alcohol ethoxylates for fish and invertebrates generally range from 1-6 mg/l. The aquatic toxicity of this class of surfactants tends to decrease as ethoxylate chain length increases. These findings are in contrast to acute toxicity values in rodents which increase with increasing ethoxylate length.

The results from other mammalian toxicity tests have shown that these surfactants are well tolerated by systemic and dermal exposure. The use of certain of these alcohol ethoxylates in human therapy as an analgesic has not revealed any untoward reactions after exposure for periods of several months at doses many times those expected from environmental sources. Thus, although adequate chronic animal tests are not available, the human experience to date would suggest that the continued use of the alcohol ethoxylates poses no threat to environmental quality or to human safety.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

ALCOHOL ETHOXYLATES

I. INTRODUCTION

With the trend toward low phosphate detergents and the increased demand for synthetic rather than natural fiber fabrics, the household and institutional use of nonionic surfactants has increased dramatically in recent years. Nonionic surfactants, however, have been used industrially in significant amounts since the early 1930s. Nonionic surfactants are reportedly superior to other surfactants in their ability to clean manmade fibers, and to perform well in cold water (Hatch, 1975; Davis, 1975). Nonionics also have a wide variety of applications including cosmetics, agriculture, chemicals, textiles, and they are used in processes where their emulsifying and wetting properties are needed.

Alcohol ethoxylates (AE), one of several groups of chemical entities classified as nonionic surfactants, comprise a major portion of the growing nonionic surfactant market. Because of their enhanced biodegradability, primary and secondary alcohol ethoxylates have now largely replaced alkylphenol ethoxylates (see Chapter 5, APE) in household and institutional cleaning products. Additionally, their relative insensitivity to water hardness and their low foaming properties have promoted their use as components in the newer detergent formulations. With the ever-increasing use of AE surfactants, it is appropriate to review the available data on AE with respect to:

(1) environmental fate and distribution, including biodegradation,

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

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

AE surfactants are derived from both linear and branched-chain alcohols. Commercially, AE are prepared by reaction of an alcohol and ethylene oxide (Satkowski et al., 1967). The majority of AE currently on the market are primary branched AE. Typical AE are depected below:

primary AE: $CH_3-(CH_2)_x\ CH_2-O-(CH_2-CH_2-O)_y\ H$

secondary AE:

$$CH_3-(CH_2)_z-\underset{\displaystyle O-(CH_2-CH_2-O)_y\ H}{\underset{|}{CH}}-(CH_2)_{z'}-CH_3$$

where: x usually equals C_6 to C_{16}

y usually equals EO_3 to EO_{20}

z + z' usually equals C_6 to C_{16}

U.S. International Trade Commission (1975) figures indicate that of the 834 million pounds of nonionic surfactants sold during 1973, 501 million pounds consisted of alcohol ethoxylates and other nonbenzenoid ethers of which 377 million pounds were mixed linear alcohol ethoxylates. Kerfoot and Flammer (1975) report that approximately 300 million pounds of linear alcohol ethoxylates are currently consumed each year in the U.S. for the manufacture

of household products; an additional 100 million pounds per year are utilized in industrial applications.

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

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II. ENVIRONMENTAL LEVELS

A. Analytical Methods

The methods and problems associated with the analytical determination of surface active agents were reviewed in detail in Chapter 1 (LAS, pp. 19-26). The following is a brief examination of some of the specific procedures not covered earlier which are employed in the determination of the presumptive levels of nonionic surfactants in the environment and in biodegradation studies.

One of the major problems in the study of nonionic surfactants has been the lack of a broadly applicable method of analysis. This is due, in part, to the fact that nonionic surfactants as a class encompass a heterogeneous group of compounds. Thus, establishment of a method which is both reliable and applicable to all nonionics and/or their degradation products has proved to be a formidable task. Indeed, due to the similarity of the responses of intact surfactant and its degradation products, it is often difficult to distinguish between them analytically (Swisher, p. 55, 1970). To overcome this problem, a prepurification step (e.g., foam stripping) is frequently incorporated into the analytical procedure; but even here, the conditions under which prepurification is accomplished can have a considerable effect (e.g., rejection of biodegradation intermediates) on the experimental results (Swisher, p. 55, 1970).

The majority of analytical methods currently employed in biodegradation and environmental studies to detect nonionic surfactants are based on the reactions of the ether oxygens in the ethoxylate portion of the surfactant molecule.

1. Physical Methods

Due to the lack of specific chemical methods, physical procedures, particularly determination of foaming potential and measurement of changes in surface tension, have been extensively employed to monitor the degradation of nonionic surfactants. A discussion of these procedures and their drawbacks can be found in Chapter 1 (LAS, pp. 20-21).

2. Specific Chemical Techniques

Heinerth (1966), Schonborn (1966), and Swisher (1970) have reviewed in depth the analytical chemical techniques utilized in the determination of nonionic surfactants. The majority of these techniques are based on the reaction of the polyethoxylate chain to form complexes with cobalt, bismuth, etc. A brief discussion of the major methods follows:

a. Bismuth Iodide Active Substances (BIAS)

The OECD expert group on the biodegradability of nonionic synthetic detergents (1975) has recently recommended the adoption of Wickbold's (1972) iodobismuthate method as the official procedure for the determination of

nonionic surfactants (specifically water-soluble ethoxylates) in water and sewage. Although not an ideal procedure, Wickbold's method can be applied to both alkylphenol ethoxylates (APE) and alcohol ethoxylates (AE) with hydrophilic chain lengths between 6 and 30 ethylene oxide (EO) units.

In Wickbold's procedure, the nonionic surfactant is first concentrated and isolated by gas stripping. After several intermediate steps, an aqueous solution of the stripped, nonionic surfactant is precipitated with a modified Dragendorff reagent ($KBiI_4$ + $BaCl_2$ + glacial acetic acid). The precipitate is filtered, washed with glacial acetic acid, and dissolved in an ammonium tartrate solution. The bismuth in solution is titrated potentiometrically with pryyolidinedithiocarbamate solution at pH 4 to 5. The nonionic surfactant is expressed as bismuth iodide active substances (BIAS). The method has a sensitivity of approximately 0.01 mg/l (OECD, 1975).

A major advantage of Wickbold's procedure is the capability to detect unchanged nonionic surfactants without interference from biodegradation products (Hoefman et al., 1976). Using Patterson's TLC procedures (1966, 1967a, 1968), Hoefman and co-workers found that biodegradation products capable of reacting with the Dragendorff reagent remain in the aqueous phase after sublation. Comparison by TLC of the precipitated nonionic surfactant-Dragendorff complex and the original material indicated no change in composition had occurred.

The major disadvantage of Wickbold's procedure is the necessity of multiplying the titration results by an empirical factor which varies according to the length of the ethoxylate chain. The method is also subject to interference

by cationic surfactants which combine with the precipitating mixture and thus add to the apparent nonionic detergent content. Cationics, if present, must therefore be removed by running the solution through an ion-exchange column. (OECD, 1975)

b. Cobalt Thiocyanate Active Substances (CTAS)

Many variants of the cobalt thiocyanate analysis have been used to study alcohol ethoxylate and alkylphenol ethoxylate surfactants (Crabb and Persinger, 1964, 1968; Greff et al., 1965; Lashen et al., 1966; Sebban, 1968). The basis for these procedures is the formation of a blue complex between the ammonium cobaltothiocyanate reagent and the hydrophilic polyethoxylate chain of the nonionic surfactant. The complex is extracted into an organic solvent and the nonionic content determined spectrophotometrically by comparing the absorbance of the complex with standard curves for the individual ethoxylates. The sensitivity of these procedures ranges from 0.1 mg/l to 20 mg/l (Allred and Huddleston, 1967; Heinerth, 1966). The cobalt thiocyanate analysis has several drawbacks. The method is subject to a large number of interferences (e.g., strongly acidic or basic solutions, cationic surfactants, solid particles), it lacks sensitivity above 15 and below 3 EO units, and, depending on EO content, the method exhibits large differences in molar absorptivity (Bergeron, 1973; Heinerth, 1966).

The EO chain length at which the color intensity of the cobalt thiocyanate complex diminished was clarified by Crabb and Persinger (1968).

Using individual, pure EO adducts, they found that for low molecular weight adducts, the efficiency of color development and extraction into the organic phase was dependent upon the concentration of the cobaltothiocyanate reagent. Additionally, the apparent molar absorption of the cobalt complex did not vary linearly with EO chain length for low molecular weight compounds. Crabb and Persinger found that at least 5 EO units were required in the usual procedure to produce a significant extractable color, but that an absolute minimum of 3 EO units could be detected provided that a saturated reagent was utilized.

Swisher (pp. 56-57, 1970) suggests that this decline in CTAS response (cobalt thiocyanate active substances) as the ethoxylate chain is shortened may be a factor in the large discrepancies reported concerning the extent of biodegradation of nonionic surfactants. Another source of potential error in the evaluation of samples with unknown molecular weight distribution was pointed out by Crabb and Persinger (1968). The absorption coefficients of higher molecular weight complexes are 10-15 times greater than those of lower molecular weight. Therefore, the presence of a small percentage of a high molecular weight adduct in a lower molecular weight sample would result in values out of proportion to the actual concentration.

An improved CTAS procedure (Boyer, 1976) for the determination of non-ionic surfactants in biodegradation and environmental samples was recently adopted by the Soap and Detergent Association. This method utilizes improved sublation (isolation by foaming) and ion exchange procedures prior to quantitation with cobalt thiocyanate and is applicable to both linear and branched

alcohol ethoxylates and alkylphenol ethoxylates with alkyl chain lengths between C_8 and C_{18} and ethoxylate chain lengths between 4 and 20 EO units. This procedure consistently yields greater than 90 ± 7% recoveries from environmental samples spiked with either alkylphenol ethoxylate or alcohol ethoxylate surfactants in the 0.5 to 0.8 mg/l CTAS concentration range. The method has a 0.1 mg/l limit of detection.

Results comparable to those achieved with Wickbold's method were found when Boyer's procedure was tested in a variety of biodegradation studies (e.g., shake flask, semi-continuous and continuous activated sludge tests) as well as with environmental samples. Analyses by six different laboratories of the same sewage treatment plant influent, effluent, and receiving water samples indicated good intra-lab reproducibility (±10%) but significant variability was noted between laboratories:

Sample	CTAS (mg/l)		Coefficient of Variation
	Range	Mean ± S.D.	
Influent	1.1 - 4.7	2.9 ± 1.1	± 38%
Effluent	0.8 - 2.3	1.5 ± 0.4	± 27%
Receiving water	0.3 - 0.8	0.5 ± 0.1	± 20%

However, by carefully standardizing the sampling techniques and increasing the number of sublation steps from two (as required in Wickbold's procedure) to four, the coefficient of variability was reduced to ± 11% for "light solids" solutions and ± 16% for "heavy solids" solutions. In summary, Boyer's procedure is simple, quick and easy to automate and is considered to be an acceptable alternative to Wickbold's (1972) method for the analysis of nonionics in biodegradation and environmental samples.

c. Picrate Analysis

Favretto and Tunis (1976) have recently published a spectrophotometric procedure for the analysis of 0.1 mg/l levels of APE in water and waste waters. Their procedure is based on the determination of the polyether complex with the sodium cation after extraction as the picrate into 1,2-dichloroethane. The degree of polymerisation is determined by GC, TLC, or a combination of these two techniques.

This method does offer a more absolute nonionic surfactant measurement (±14%) than CTAS methods in the range of 6.5 to 21 moles of EO; however, major differences in absorptivity are seen between 3 and 6.5 moles of EO. To compensate for differences in absorptivity, a factor derived from the average EO content of the sample is utilized in the calculation of the results. The EO content must be determined by time-consuming GC and TLC techniques, thus making this method impractical for any monitoring program involving numerous samples.

d. Other Methods

Other chemical techniques which have been applied to the determination of nonionic surfactants include the phosphomolybdic acid procedure (Stevenson, 1954), the phosphotungstic acid method (Pitter, 1966), and the chlorosulfonic acid procedure of Han (1967), cited in Swisher, p. 55, 1970). The first two methods are time-consuming and are subject to many interferences; e.g., by proteins. Han's approach involved the conversion of the polyethoxylate chain to an ethoxy sulfate. Determinations were based on methylene blue

activity.

Another newly developed procedure for the determination of either AE or their major degradation intermediates, the polyethylene glycols (PEG), is the method of Huber and Frohlke (1975). Their procedure involves the hydrolytic removal of the ethoxylate portion of the AE molecule to yield formaldehyde. The formaldehyde is steam distilled. Next, the distillate is reacted with a dinitrium salt and sulfuric acid; the resulting solution is then measured spectrophotometrically. Although subject to many interferences, particularly with sugars, the use of cleanup procedures and spectrophotometric measurements against an appropriate blank solution allows the detection of 1-10 ppm AE with a standard deviation of 2-4 percent.

3. Physicochemical Analyses

Thin layer, paper and gas chromatography have all been utilized to some extent to study the biodegradation of nonionic surfactants. The most widely used of these procedures is Patterson's et al. (1966, 1967a, 1968) thin layer analysis for the determination of alkylphenol ethoxylates and alcohol ethoxylates. The ethoxylates separate (according to their degree of ethoxylation) into individual spots; these spots are then visually matched against adjacent standards. The method has a sensitivity of 0.1 mg ethoxylate/liter and can be used with river, sewage and activated sludge samples without prepurification. However, the method is complicated, contains an element of subjective analysis (visual matching of the developed spots), is time-consuming, and therefore not applicable for routine analyses (Heinerth, 1966).

Sawyer et al. (1970a, b) have automated Patterson's procedure. Although automation has reduced the analysis time/sample, the required equipment is cumbersome and relatively expensive. Moreover, the accuracy (±10%) of the procedure is still limited by the subjective determination of the Patterson method.

McCoy and Bullock (1969) applied circular thin-layer chromatography to the determination of the oxyethylene distribution in mixed carbon-number, primary AE containing up to 14-18EO units/mole. Circular thin-layer chromatographic processes were employed to separate the 3,5-dinitrobenzoate ester derivatives of the mixed-carbon-number-AE into groups possessing the same number of EO units/mole. The recovered esters were then determined spectrophotometrically. The process is slow due to the many steps involved, but has an accuracy of ±5% and can be applied to the determination of PEG, secondary AE and APE as well as primary AE.

Fischesser and Seymour (1976) recently published a procedure for determining EO distribution using programmed multiple development thin-layer chromatography. This procedure allows the resolution of acetate esters of materials containing 0-12EO groups using conventional TLC plates. The Rf of the separated materials depends on the number of EO groups and is independent of alkyl chain length. The separated materials are visualized by H_2SO_4 charring with the absorbance of the charred spots measured by spectrodensitometry. Accuracy is stated to be better than 7% with a relative standard deviation of less than 7 parts per hundred.

Due to the insufficient volatility of nonionic surfactants, gas chromatography (GC) has not been used to any meaningful extent. However, Tsuji and Konishi (1974) did report using GC in combination with reagent induced cleavage of ether linkages in their analyses of hydrophilic and hydrophobic groups of surface active EO adducts, and recent papers have reported the use of high speed liquid chromatography (Nakamura and Matsomoto, 1975) and gas chromatography/hydrogen bromide techniques (Tobin et al., 1976) in the analysis of AE. Nakamura and Matsumoto's procedure (1975) is applicable to PEG and AE up to 30 EO units/mole and is based on the direct proportionality between the number of moles of EO and the retention time. To reduce adsorption onto the column, the test sample is acetylated prior to passing it through the column. The composition of the alkyl chain is determined with gas chromatographic techniques. In Tobin's procedure (1976), the ether linkages of the surfactant are cleaved with hydrogen bromide, and the cleavage products are subsequently analyzed by gas chromatography. This technique allows determination of the removal of both hydrophobic and hydrophilic moieties of the surfactant molecule. The results obtained by Tobin and co-workers with their GC/HBr technique were in general agreement with several currently utilized analytical procedures (Wickbold, 1972; Evans and Dennis, 1973; Sturm, 1973).

Although the alkylphenol portion of APE is characterized by a marked ultraviolet (UV) absorption, UV is rarely used in the determination of ethoxylates due to the presence of many interfering substances which necessitate complicated purification procedures (Heinerth, 1966). Infrared

spectroscopy has also been applied to the study of nonionic surfactant biodegradation (Puttnam, 1972; Frazee et al., 1964; Osburn and Benedict, 1966), but this method also suffers from a high susceptibility to interference.

4. Degradation Products

Polyethylene glycols, the major degradation intermediates of alcohol ethoxylates, can be separated from the parent compound by thin-layer chromatography with proper selection of extraction conditions or of the developing solvent. The polyethylene glycol (PEG) content of AE surfactant can be isolated via selective extraction procedures; the PEG concentration can then be determined spectrophotometrically following treatment with Dragendorff reagent. (Swisher, p. 57, 1970).

Another spectrophotometric method for the determination of low levels (0.5-1 mg/l) of mono- , di- and triethylene glycols in surface waters was developed by Evans and Dennis (1973). Their procedure is based on the oxidation of glycols with acidified permanganate to the corresponding aldehydes. The aldehydes are reacted with 3-methylbenzothiazol-2-one hydrazone hydrochloride and the resulting solution measured spectrophotometrically. Recoveries of added samples (1-5 mg/l) averaged 100.5% with a coefficient of variation of ±7%; at the 1 mg/l level the coefficient of variation was 9% while at the 5 mg/l level, it was 4%.

B. Water Quality Standards

1. National Regulations

At present, there are no national criteria for limiting nonionic surfactants in waters in the United States. Certain European countries, including France, Italy and Germany, have been considering the enactment of legislation concerning nonionics since 1972, although no action has been taken as yet (OECD, 1975). Nonionics, however, must conform to the directive requiring average primary biodegradability of not less than 90% (Official Journal of the European Communities, No. L 347/51, Directive 73/404/EEC, 1973).

2. State and Local Regulations

Regulations affecting nonionics were enacted in Suffolk County, New York and in New Shoreham, Rhode Island where the sale of detergents containing nonionic and methylene blue active surfactants is prohibited. The sale of detergents that do not biodegrade in a secondary sewage treatment plant is prohibited in Oregon. In addition, the sale, possession or use of non-degradable detergents is unlawful in Dade County, Florida (Soap and Detergent Association, 1975).

C. Nonionic Surfactants in Natural Water Bodies

The use of nonionic surfactants has increased in recent years, replacing to some extent the linear alkylbenzenesulfonates in domestic and industrial detergent formulations (Cain, 1974). Nonionics, however, are used in detergent formulations at lower concentrations than anionics, hence, the levels of

nonionics found in natural waterways, as reported in England, are generally lower (Offiler, 1973). Monitoring for nonionics has, in general, not been for a specific type. Therefore, the following section generally discusses nonionics as a whole, including alkylphenol ethoxylates and alcohol ethoxylates.

1. Pathways for Nonionic Surfactants into the Environment

a. Sewage Treatment Plants

As was discussed earlier, sewage treatment plants receive large amounts of surfactants (see Chapter 1, pp. 31-33). It has, however, been shown that most nonionics are degraded during treatment.

Bock (1973) measured the concentrations of nonionics using the Wickbold (1972) method at the Marl-West trickling filter sewage treatment plant of the Lippe River Authority (total population of 38,000 connected to this system). He reported 2.3 mg/l (nonionic load of 14.03 kg/day) at the inlet and 0.5 mg/l (nonionic load of 3.69 kg/day) at the outlet following the biological purification stage, showing a 74% reduction. The Standing Committee on Synthetic Detergents (Dept. of the Environment, 13th Progress Report, HMS Office, London, 1972) as cited in Oba and Sugiyama (1974) showed similar concentrations of nonionics in discharges from sewage treatment plants in England. The range for 15 sampling locations was 0.1-2.7 mg/l, with most values between 0.1 and 1.0 mg/l. The 16th Progress Report of the Standing Committee on Synthetic Detergents reported nonionic concentrations (as LISSAPOL NX^{TM}) in effluents from four treatment plants in England. These concentrations ranged from 0.09-0.47 mg/l.

b. Septic Tank Systems

Although septic tank systems are a possible source of leaching of nonionics into the environment, little information was found regarding this pathway or the fate of nonionics in the soil

c. Agricultural Runoff

Nonionic surfactants are often used as wetting agents in pesticide formulations (Parr and Norman, 1965). However, no information was found concerning residues in agricultural runoff.

2. Nonionic Concentrations in the Aquatic Environment

Although no levels have been reported for a specific class of nonionics, a summary of concentrations of nonionic surfactants in European rivers is shown in Table 3-A. Several different methods were used in these analyses, so the values may not be directly comparable. The levels reported are almost always lower than anionics at the same sampling point. Patterson et al. (1967b) showed a range of 0.2-1.0 mg/l for nonionics in the Calder and Aire Rivers of England; the range for anionics was 0.3-1.4 mg/l. In fact, Gerike and Schmid (1973) report that the levels of nonionics in the Rhine near Düsseldorf are about 10% of those for anionic surfactants.

No reports of nonionic surfactant levels in the United States were found.

Table 3-A

Concentrations of Nonionic Surfactants in Rivers

Location	Analytical Method	Sampling Time	Concentration* mg/l	Source
Calder River Wakefield, England	Patterson	1967-1974	0.24 - 0.69**	Dept. of the Environment Standing Technical Committee on Synthetic Detergents (1975)
Calder River various locations	-	1965	0.5-0.8	Patterson et al. (1967b)
Calder River Kirkthorpe	-	1966-1967	0.3-0.7	Patterson et al.(1967b) (taken from Mr. M. Lovett of the Yorkshire Ouse and Hull River Authority)
Aire River above Castleford	-	1966	0.3	Patterson et al. (1967b)
Aire River above Castleford	Patterson	1967-1974	0.17 - 0.49	Dept. of the Environment (1975)
Aire River at Castleford	-	1965-1966	0.2-1.0	Patterson et al. (1967b)
Aire River below Castleford	Patterson	1967-1974	0.18 - 0.42	Dept. of the Environment (1975)
Aire River below Castleford	-	1966	0.4-1.0	Patterson et al. (1967b)
Aire River Swillington	-	1966-1967	0.2-0.65	Patterson et al. (1967b) (taken from M. Lovett of the Yorkshire Ouse and Hull River Authority)
Aire River Beale	-	1966-1967	0.2-0.55	

Table 3-A (continued)

Location	Analytical Method	Sampling Time	Concentration* mg/l	Source
Thames River Laleham	Patterson	1966-1973	0.01-0.05**	Dept.of the Environment (1975)
Lee River				
Cingfold Mill	"	1966-1973	0.01-0.05**	"
Lemsford Mill	"	1968-1974	0.06-0.22**	"
Lippe River				
Wasserwerke CWH	Wickbold	-	0.019-0.019	Wickbold (1972) as cited in: Oba and Sugiyama (1974)
Oelder Brücke	"	-	0.020-0.025	"
Hervest	"	-	0.022-0.025	"
Friedrichsfeld	"	-	0.014-0.017	"
"	"	quarterly samples	0.02-0.1 in most cases 0.02-0.06	Bock (1973)
above Marl	"	-	usually approx. 0.03, high annual mean of 0.06 in 1969	"
Rhine River				
Wesel	"	-	0.019-0.023	Wickbold (1972) as cited in: Oba and Sugiyama (1974)
Rees	"	-	0.015-0.017	"
Emmerich	"	-	0.021-0.026	"
"	"	-	0.02-0.11 mostly from 0.02-0.05	Bock (1973)

* range
** as LISSAPOL^{TM} (C_9APE)

Since monitoring for nonionics has only begun in the last ten years, few overall generalizations can be made from the data. However, levels are varied and do not appear to be increasing with time in Europe.

In general, aquatic levels of nonionics fall in the range of 0.01 to 1.0 mg/l. The higher values (TLC method) were found in the Yorkshire wool-treatment district of England, the Aire and Calder Rivers (Patterson et al., 1967b) and the lower values as measured by Wickbold's (1972) method in the Lippe and Rhine rivers of Germany (Bock, 1973).

a. Degradation Products

Levels of polyethylene glycols (PEG), the major degradation intermediates of AE, were determined in the Yorkshire-wool-treatment-district of England over an eight-year period (1967-1974). Generally, PEG levels increased during this time although individual determinations varied widely. PEG levels ranged from 0.22-0.81 mg/l in the Calder River at Wakefield; 0.01-0.19 mg/l in the Aire River above Castleford; and 0.09-0.52 mg/l below Castleford. (STCSD Report, 1975). Similar levels for these same waters were reported by Patterson et al. (1967b).

PEG levels of 0, 0.03, and 0.30 mg/l as ORITEX-40^{TM} were found in discharges from Dalmur, Sheildhall and Glascow sewage treatment works. (STCSD Report, 1975).

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III. BIODEGRADATION

This section will consider the biodegradability of both primary (pri) and secondary (sec) alcohol ethoxylate surfactants (AE) and, to a lesser extent, their major degradative products, the polyethylene glycols (PEG). A detailed examination of the analytical procedures employed to study the biodegradation of nonionic surfactants under both laboratory and field conditions was provided in Section II.A. of this report; a review of the influence that a variety of factors exert on the rate of degradation of surfactants in general can be found in Chapter 1 (LAS,III).

A. Laboratory Test Systems Used in AE Studies

1. Oxygen Uptake - Biochemical Oxygen Demand

In tests with a Warburg respirometer, Myerly et al. (1964) reported complete primary degradation of n-pri-$C_{12-14}AE_9$ and n-sec-$C_{11-15}AE_9$ and Vath (1964a,b) found that n-pri-$C_{12}AE_{7.4}$, sec-$C_{13-15}AE_{9.5}$ and $PE_{8.5}G$ 400 also underwent complete primary biodegradation.

2. CO_2 Evolution

In a study with a series of AE_3 in which the alkyl chain length varied (C_8 to C_{20}, in two carbon increments), Sturm (1973) noted substantial CO_2

evolution (>65% of theoretical CO_2) with all surfactants examined. Glucose generally yields in excess of 80% of theoretical CO_2 under the conditions of this test. Vath (1964b) reported similar results with n-sec-$C_{11-13}AE_6$ containing a randomly labelled ^{14}C-ethoxylate chain and Tobin et al. (1976a) found that n-$C_{12-15}AE_9$ had evolved greater than 70% of the theoretical CO_2 by 22 days.

Sturm (1973) also tested the degradability of a diverse group of PEG (molecular weight range, 300-4000). PEG 300, PEG 400, and PEG 600 were all readily degraded (87%, 80%, and 90% of theor. CO_2, respectively), while PEG 1000, PEG 1540, and PEG 4000 exhibited some resistance to biodegradation. These data correlated with his findings that $C_{16-18}AE_{11}$ (molecular weight of ethoxy chain ~500) was degraded readily while $C_{16-18}AE_{30}$ (molecular weight of ethoxy chain ~1300) was somewhat resistant to degradation.

3. Die-Away Tests

a. River Water Test

In a series of river water degradation runs, Blankenship and Piccolini (1963) observed no difference in the primary degradative rate of $C_{13}AE_6$, $C_{12}AE_8$ and $C_{12}AE_{10}$; all degraded greater than 95% (surface tension measurements) in 6 days. $C_{12}AE_{20}$ and $C_{12}AE_{30}$, however, were slower to degrade (95% and ~80%, respectively, in a 20-day period). Vath (1964b) found that

both n-pri-$C_{10-12}AE_{7.4}$ and n-sec-$C_{11-13}AE_{8.3}$ lost 95% of their surface activity within 8 days, and Huddleston and Allred (1965) observed complete primary degradation (foam loss) of n-pri-$C_{10-12}AE_6$ in river water within 4 days.

Kurata and Koshida (1975) report that both pri-$C_{12}AE_9$ and sec-$C_{12-14}AE_9$ were completely degraded (CTAS) in both "clean" and "highly polluted" Tama river water samples.

Working with secondary AE, however, Booman (unpublished data) noted that an increase in EO chain length decreased the rate of degradation (foam loss). In a 20-day period, n-sec-$C_{11-15}AE_9$ degraded 95% while n-sec-$C_{11-15}AE_{12.5}$ degraded only 72% during this time. By 40 days, however, both surfactants had completely degraded.

Branched-chain AE surfactants also appear to be biodegraded at a slower rate than linear chain AE. Allred and Huddleston (1967) found that $C_8AE_{65\%}$, $C_{10}AE_{65\%}$, $C_{12}AE_{65\%}$ (equivalent to an average of 5.5, 6.7 and 7.9 EO units/mole, respectively) and branched-chain $C_{13}AE_{76\%}$ (equivalent to ~14.4 EO units/mole) were degraded (CTAS) 83%, 100%, 100% and 45%, respectively, within 5 days. By 30 days, the branched-chain compound had achieved 86% primary degradation.

b. Shake Culture Test

Allred and Huddleston (1967) found that $C_8AE_{65\%}$, $C_{10}AE_{65\%}$ and $C_{12}AE_{65\%}$ (equivalent to an average of 5.5, 6.7 and 7.9 EO units/mole, respectively, were degraded completely (CTAS) within 3 days, while the branched-chain

$C_{13}AE_{76\%}$ (equivalent to ~14.4 EO units/mole) was degraded only 30% in 8 days. Similarly, Asahara et al. (1972) found $C_{12}AE_{8.5}$ was degraded 98% (CTAS) within one day for the same compound. Sekiguchi also noted that it took one month for the organic carbon (TOC) to completely dissipate.

In a comparison of Wickbold's method (1972) and the gas chromatographic/HBr technique of Tobin et al. (1976), Tobin and co-workers (Tobin et al., 1976a,b) reported that Wickbold's method indicated greater than 96% removal of $C_{12-15}AE_9$ in a shake culture test after approximately 4 days while their GC/HBr analysis indicated that the polyethoxylate moiety was less extensively changed (25% degraded). A more recent study, however, using both Tobin's HBr technique and evolved CO_2 procedures indicates that approximately 80% of the polyethoxylate portion of ^{14}C-labelled, single-cut $C_{14}AE^*_9$ biodegrades (data to be published, Shell Chemical Co.). Other investigators (Fischer and Gerike, 1975; Sturm, 1973; Wickbold, 1974) have also found that the PEG moiety readily undergoes biodegradation.

Using fresh sludge from a municipal sewage treatment plant as the inoculum, Booman (unpublished data) found that n-pri-$C_{12}AE_8$ was degraded (foam loss) completely in 7 days, but n-sec-$C_{12-15}AE_9$ was degraded only 12-50% during this time period. Based on these findings, since secondary AE have been established to be biodegradable in the field, the shake culture test may not accurately predict removal of secondary AE.

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

In studies with n-pri-$C_{12-15}AE_6$ and (randomly attached) n-sec-C_{11-15} AE_9, Stead et al. (1972) found that the primary compound was degraded completely (TLC method of Patterson et al., 1966) in about 6 days while the secondary surfactant required approximately 11 days. Patterson et al. (1967) also reported slower degradation of secondary AE when compared to primary AE with this procedure.

4. Simulated Treatment Processes

a. Activated Sludge

In a bench-scale, continuous activated-sludge test, Lashen et al. (1966) observed a 92% degradation (CTAS) of n-sec-$C_{11-15}AE_9$ (TERGITOL 15-S-9™) and after a 4-day acclimation period, Fisher and Gerike (1975) reported 98% removal of n-$C_{16}AE_{10}$ (Wickbold's 1972 method) in the OECD confirmatory test (1971). In a coupled units test, however, these investigators found that the COD removal rate was only 54% for this compound. In the same manner, n-$C_{16}AE_{30.7}$ yielded only a 33% COD removal rate versus a 98% value with Wickbold's method. A COD removal of 69 ± 9% was recorded for PEG 600 in the coupled units test.

In a semicontinuous activated-sludge process, Allred and Huddleston (1967) reported $C_8AE_{65\%}$, $C_{10}AE_{65\%}$, and $C_{12}AE_{65\%}$ (equivalent to an average of 5.5, 6.7 and 7.9 EO units/mole, respectively) all were degraded (CTAS) completely in 24 hours. Asahara et al. (1972) obtained similar results with $C_{12}AE_{8.5}$ while Mausner et al. (1969) found that n-pri-$C_{12-14}AE_{10}$ and methyl branched pri-$C_{12-15}AE_9$ were both completely degraded (foam loss) with a 17 hr retention period.

Han (1967) using his sulfation-MBAS method, reported degradation values of 100% and 86%, respectively, for n-pri-$C_{14-16}AE_8$ and n-pri-$C_{14-16}AE_{16}$ in a 24 hr semicontinuous activated-sludge test. Slower degradation rates were found with secondary AE - 92% for n-sec-$C_{11-15}AE_8$ and 57% for n-sec-$C_{11-15}AE_{15}$.

Lashen and Booman (1967) found that sludge acclimated to C_8APE_{10} was simultaneously acclimated to linear secondary AE having similar EO chains. Normally, n-sec-$C_{12-15}AE_9$ requires an acclimation period of 5 to 15 days to achieve 90% degradation (foam loss) with fresh domestic sludge, but less than 2 days were needed to achieve this level with C_8APE_{10}-acclimated sludge.

b. Trickling Filters

The only available data on the extent of AE degradation in a trickling filter process are the field trial findings of Mann and Reid (1971). See Section III.B. of this chapter for details.

c. Anaerobic Systems

The only information found on AE in anaerobic systems is a report by Vath (1964b) that n-sec-C_{11-15} $AE_{9.2}$ was rapidly degraded (CTAS, foam loss) in an anaerobic environment. The extent of degradation, however, was not specified.

5. Influence of Test System Variables

a. Inoculum

Using an inoculum obtained from settled domestic wastewater, Price et al. (1974) found that hexylene glycol, tetraethylene glycol, triethylene glycol and diethylene glycol were all relatively resistant to biodegradation. However, if an acclimated microbial seed was used, the glycols were degraded 90%, 88%, 86% and 67%, respectively.

b. Temperature

Stiff et al. (1973) observed greater than 94% BOD removal for both n-pri-$C_{9-11}AE_8$ (NONIDET LG™) and n-pri-$C_{12-15}AE_9$ (NONIDET SH™) in a porous-pot activated-sludge unit at 8°, 11° and 15°C and Mann and Reid (1971) reported that even in winter, approximately 90% of $C_{12-15}AE_9$ degraded in a trickling filter plant.

In another study, Evans and David (1974) examined the extent of biodegradation (method of Evans and Dennis, 1973) of mono- , di- and triethylene

glycols in river water at 8° and 20°C. Monoethylene glycol was degraded completely at both temperatures, although a greater length of time (14 vs 3 days) was needed at the lower temperature. The degradative rates of di- and triethylene glycols varied at 20°C, depending on the origin of river water; complete breakdown generally occurred within 7 to 11 days. Degradation was minimal for both glycols by 7 days at 8°C.

B. Field Tests

The available data on the performance of AE in the field are quite limited, but indicate extensive degradation of AE.

Conway et al. (1965) reported that after 4 weeks on test, greater than 94% of n-sec-$C_{11-15}AE_9$ (TERGITOL 15-S-9) was removed (CTAS) from an activated-sludge plant which served approximately 110 mobile homes, and in a field trial with n-pri-$C_{12-15}AE_9$ (DOBANOL 25-9^{TM}) and $C_{9-11}AE_8$ (DOBANOL 91-8^{TM}), Mann and Reid (1971) found that even during the winter months both surfactants degraded 89% and 84%, respectively, in a trickling filter sewage treatment plant. Degradation was determined by the TLC method of Patterson et al. (1966).

Mann and Schoberl (1976) reported that within 34 days, 96% of sec-$C_{13-14}AE_{10}$ was removed (method of Wickbold, 1972) from a pond containing brackish water while Tobin et al. (1976a) found that 90% of n-pri-$C_{12-15}AE_9$ had been removed (Wickbold's method) from ambient bay water by 15 days during October and November. GC/HBr analysis also indicated rapid degradation of the alkyl

chain, but revealed a slower degradation of the polyethoxylate moiety (only 21% had been removed at 3 weeks).

C. Effect of Chemical Structure

Variations in alkyl chain length appear to have no significant effect on the rate of AE degradation. Sturm (1973) studied the rate of CO_2 evolution with a series of n-pri-AE_3 in which the length of the hydrophobic chain was increased in increments of two carbon atoms from C_8 to C_{20}. No notable differences in the rates of degradation were observed. Surfactants $C_{12}AE$ through $C_{20}AE_3$ exhibited some variability in CO_2 production, but all compounds yielded in excess of 65% of the theoretical CO_2 concentration.

Gledhill (1975) reports that modifications in either the length of the alkyl chain (C_8-C_{20}) or in the number of EO units (EO_3-EO_{11}) of alcohol ethoxylates had little effect on the rate and extent of CO_2 evolution. In other investigations, ethoxylate chain length was seen to influence biodegradation. Gerbil and Naim (1969a,b) noted that an increase in the number of EO units above 20 units had adverse effects on the rate of degradation and Sturm (1973), in tests with a series of C_{17} AE in which the EO chain length varied from 3 to 30 units, found no significant effect on degradation up to 11 EO units. The presence of more than 20 EO units appeared to markedly reduce biodegradability.

Similar results were reported in a river water die-away test by Blankenship and Piccolini (1963). They found no difference in the degradation rates of C_{12} AE_6, C_{12} AE_8 and C_{12} AE_{10} (>95% degradation in $\sim$6 days as detected by surface tension) but observed that the rates for higher homologs, C_{12} AE_{20} and C_{12} AE_{30}, were much lower (>20 days).

Pitter (1968) observed that the extent of biodegradation (phosphotungstic acid method) for n-C_{10-16} AE was linear (but in a divergent direction) between 3 and 20 EO units (e.g., 8 EO, 65%; 15 EO, 32.5%; and 20 EO, 7.5% degradation). Pitter also found that oxygen consumption decreased with an increase in the length of the ethoxylate chain.

Another study (Dai Ichi Kogyo Pharmaceutical Co., 1974) confirmed that increases in the length of the ethoxylate chain of n-pri-C_{12-13} AE reduced the degradation rate (BOD, 5 days):

EO_{10}	~35% degradation
EO_{20}	~20% degradation
EO_{30}	~15% degradation
EO_{40}	~12% degradation

1. Degradation Products

Pitter (1972) examined the biodegradability (via COD, IR, TLC) of a variety of polyethylene glycols within a molecular weight range of 300 to 3500. He found, with one exception, that as the molecular weight of PEG increased, the degradation rate decreased. Furthermore, the degradation rate was dependent upon both the type of microbial acclimation and the duration of that acclimation. The single exception to Pitter's generalization was noted with diethylene glycol which had a slower (rather than faster) degradation rate than triethylene glycol.

In Pitter's experiments, all PEG with molecular weights less than 600 degraded completely within 15 days (PEG 300 - 4 days; PEG 400 - 9 days; PEG 500 - 12 days; PEG 600 - 14 days) while the higher molecular weight materials persisted; at 15 days, PEG 1000 had only degraded about 16% while PEG 1500 had not degraded at all. Based on his findings, Pitter believes that the biological breakdown of PEG most probably occurs via a stepwise depolymerization.

Patterson et al. (1967) have also reported rapid degradation of lower molecular weight PEG as well as the resistance of higher molecular weight PEG to biodegradation.

D. Metabolic Pathways of Biodegradation

Although alcohol ethoxylates have been shown to undergo rapid biodegradation with subsequent release of the ethoxylate chain as polyethylene glycols, the exact mechanism of action remains unclear. The principal points of metabolic attack on the AE molecule are the hydrophobic alkyl chain, the hydrophilic ethoxylate chain, and their connecting ether bridge.

The extent of alkyl chain metabolism has proved difficult to assess with nonionic surfactants due to the unavailability of unequivocal analytical methods. However, Nooi et al. (1970) have proposed a mechanism for alkyl chain degradation based on their findings with six long- and short-chained ^{14}C-labelled $C_{18}AE$ condensates in a batch activated-sludge system. The radioactive label was located either at random in the alkyl chain, at random

in the ethoxylate chain, or at the 1-carbon position of the alkyl chain. By measuring the amount of $^{14}CO_2$ produced, the ^{14}C-activity of the solution, and the amount of ^{14}C bound to the sludge at 7 days, Nooi and associates observed the rapid formation of $^{14}CO_2$ and the accumulation of acidic products which were shown (IR, NMR) to contain both carboxyl and ethoxy groups.

Based on these results Nooi and co-workers (1970) proposed a mechanism for AE biodegradation. Initially, a rapid uptake of substrate occurs with little or no $^{14}CO_2$ produced. Next, the alkyl chain is degraded, most probably by oxidation of the terminal methyl group; β-oxidation follows. After the major portion of the alkyl chain has degraded, the resulting hydrophilic intermediates are largely desorbed from the sludge. After some period of acclimation, these hydrophilic intermediates are also decomposed. Increasing the length of the ethoxylate chain retards the degradation of the alkyl chain as well as that of the EO chain itself, with the degradation of the 1-carbon atom and the ethoxylate chain being more retarded than that of the alkyl chain. Based on their findings, Tobin et al. (1976a) recently proposed a similar model of AE degradative pathways.

Patterson et al. (1970), however, believe that two degradative mechanisms proceed simultaneously. These are dissociation of the AE molecule into hydrophobic and hydrophilic entities along with a rapid oxidation of the hydrophobic group. They state that the somewhat slower rate of degradation of secondary AE (such as that seen by Vath, 1964b) results from a slower rate of hydrolytic cleavage of the molecule due to the presence of a secondary alcohol grouping.

If both routes do occur simultaneously, an accumulation of polyethylene glycols would be anticipated (Cain, 1974). Supporting data have been reported by Frazee et al. (1964). They found that if 19 mg/l of n-$C_{14}^{AE}{}_{8.3}$ was allowed to decompose in river water for 5 days, subsequent analysis (IR) revealed no AE or alkyl chain residues were present, but approximately 5.7 mg/l PEG remained. In an earlier study, Patterson et al. (1967) also observed a buildup of PEG-type residues as the parent AE disappeared (TLC analysis). Additionally, they noted that surfactants with longer EO chains (e.g., EO_{15}, EO_{20}, EO_{30}) degraded more slowly, yielding greater and more persistent quantities of PEG residues. Secondary AE, although degraded more slowly than primary AE, appeared to follow the same metabolic pathways.

Unfortunately, although this information does elucidate the mechanisms involved in the degradation of alcohol ethoxylates, many areas of uncertainty remain. For example, the data currently available do not clearly delineate whether the initial stage of biodegradation is hydrolytic and/or oxidative in nature.

E. Summary

Although secondary and slightly branched primary alcohol ethoxylates appear to be degraded somewhat more slowly than linear primary AE, alcohol ethoxylates as a class undergo extensive, relatively rapid primary biodegradation both in the laboratory and under field conditions. Linear primary AE also show rapid ultimate biodegradation to CO_2 and H_2O.

Neither variations in the alkyl chain length nor increments in the length of the ethoxylate portion of the molecule (within the range utilized in detergent formulations) was found to affect the rate of AE degradation. However, increments beyond 20 EO units definitely retard the degradation of the molecule.

The major degradative pathway of alcohol ethoxylates appears to be hydrolysis of the ether linkage and subsequent oxidation of the alkyl chain. The polyethoxylate moiety of the AE molecule readily degrades to form lower molecular weight polyethylene glycols and ultimately, to CO_2 and H_2O.

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IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

The toxicity of AE has been evaluated using various species and test methods. The results show that fish are generally more susceptible to alcohol ethoxylates than are invertebrates. In addition, toxicity of AE decreases with increasing ethylene oxide chain length while decreasing the alkyl chain length may decrease toxicity.

The various methodologies and factors influencing these acute toxicity tests have been discussed in Chapter 1, (LAS, pp. 99-100).

1. Acute Toxicity - Fish

Table 3-B summarizes the acute toxicity data available for fish. It appears that the LC_{50} values for alcohol ethoxylates generally range from 1-6 mg/l, however, exceptions to this range can be found. For example, Gloxhuber and Fischer (1968) found decreasing toxicity with increasing EO numbers, up to a 60 minute LC_{50} of 150 mg/l for $C_{12}AE_{20}$. Similar results were reported by Macek and Krzeminski (1975) and Knauf (1973) who showed that decreasing the carbon numbers, and keeping the EO value constant decreased the toxicity to fish. On the other hand, Unilever Research Laboratories (unpublished data) found the most toxic AE_3 of those examined had an alkyl chain length of C_{10}, and that a decrease in toxicity occurred when the alkyl chain was shorter than C_{10} or longer than C_{12}.

TABLE 3-B. Acute Toxicity of Alcohol Ethoxylates to Fish

Species	Surfactant*	Experimental Conditions	Toxicity**	Source
Rainbow trout (Salmo gairdneri)	$C_{12}AE3.25_{TM}$ (ALFOL 12^{TM})	Fish - 2.5", static, 18°C, pH 7.2-7.8		Tovell et al. (1975)
		Trout acclimated in hard water	Average survival time in 5 mg/l AE (Minutes ± SE):	
			In hard water - (300 mg/l $CaCO_3$) 129 ± 22	
			In soft water - (60 mg/l $CaCO_3$) 66 ± 3	
		Trout acclimated in soft water	In hard water - 152 ± 8	
			In soft water - 107 ± 10	
	$C_{12-15}AE_7$ (DOBANOL 25^{TM})	Fish - 7-11 cm, 24 or 48 hr. water change-over, 11° ± 2°C, pH 7.6-8.4	LC_{50} 96 hr - 2.0-2.7	Unilever Research Laboratories, unpublished data

* n-linear; pri-primary; sec-secondary

** mg/l unless stated otherwise

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Rainbow trout (continued)	$C_{16-18}AE_{14}$	Fish - 8-10 cm, static, 15°+ 0.5°C, hardness 20 mg/l $CaCO_3$	LC_{50} 24 hr - 0.8 (0.6-1.2) 48 hr - 0.7 (0.6-1.4)	Unilever Research Laboratories, unpublished data
	$C_{12-14}AE_{11}$	"	LC_{50} 24 hr - 6.2 (5.4-7.7) 48 hr - 6.2 (5.4-7.7)	
	$C_{14}AE_{8}$	"	LC_{50} 24 hr - 2.5 (2.3-3.1) 48 hr - 2.4 (2.2-3.0)	
Brown trout (Salmo trutta)	$C_{16-18}AE_{14}$	Fish - 2.0-4.0 cm, continuous flow, 15°C, hardness 250 mg/l $CaCO_3$	LC_{50} 24 hr - 1.0 (0.8-1.3)	
Salmon (Salmo salar)	$C_{12}AE_{4}$	Fish - parr 8.2-11.7 cm, static, 15°C, flow through 10-11°C, 10 fish/concentration	LC_{50} static 24 hr - 2.2 flow through 24 hr - 3.5 48 hr - 2.7 96 hr - 1.5	Wildish (1974)

* mg/l unless stated otherwise

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Salmon (continued)	Polyoxyethylene glycol (PEG)	Fish - parr, 8.2-11.7 cm, flow through 10-11°C, 10 fish/concentration	LC_{50} 96 hr >1,000	Wildish (1974)
Bluegill sunfish (Lepomis macrochirus)	pri-$C_{12-15}AE_3$TM (NEODOL 25-3TM)	Fish - 1.0 g, static, 18°± 0.5°C, pH 7.1, hardness 35 mg/l $CaCO_3$	LC_{50} 24 hr - 1.8 (1.2-2.4) 96 hr - 1.5 (1.2-1.8)	Macek and Krzeminski (1975)
	pri-$C_{12-15}AE_9$TM (NEODOL 25-9TM)	"	LC_{50} 24 hr - 2.1 (1.6-2.9) 96 hr - 2.1 (1.5-2.8)	
	"	Fish - 1.0 g, flow-through, 21°± 1°C, pH 7.1, hardness - 38 mg/l $CaCO_3$	LC_{50} 24 hr - 2.7 (2.2-3.3) 96 hr - 2.1 (1.7-2.6)	
	pri-$C_{10-12}AE_6$ (ALFONIC 1012-60TM)	Fish - 1.0 g, static, 18°± 0.5°C, pH 7.1, hardness 35 mg/l $CaCO_3$	LC_{50} 24 hr - 6.4 (4.2-9.6)	

* mg/l unless stated otherwise

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Bluegill sunfish (continued)	pri-$C_{13}AE_9$ (SURFONIC TD-90™)	Fish - 1.0 g, static, 18°± 0.5°C, pH 7.1, hardness 35 mg/l $CaCO_3$	LC_{50} 24 hr - 7.8 (6.2-9.9)	Macek and Krzeminski (1975)
"	sec-$C_{11-15}AE_9$ (TERGITOL 15-S-9)	"	LC_{50} 24 hr - 4.7 (3.7-5.9)	
	"	Fish - 1.0 g, flow-through 21°± 1°C, pH 7.1, hardness - 38 mg/l $CaCO_3$	LC_{50} 24 hr >4.0 <5.6	
"	pri-$C_{12-15}AE$, 60% EO by weight	Fish - 5.6 g, static 18-19°C pH 7.6-7.7, hardness - 45-52 mg/l $CaCO_3$	LC_{50} 24 hr - 2.8 (2.62-3.00)	Hendricks et al. (1974)
"	n-pri-$C_{13-15}AE_9$ (NEODOL 25-9)	Fish - 1.1 g, static, 21°C, pH 7.1	24 hr - 1.87 (1.36-2.56) no effect - 1.4	Shell Chemical Co., unpublished data
"	n-pri-$C_{12-13}AE_{6.5}$ (NEODOL 23-6.5 (S))	"	24 hr - 2.45 (2.00-3.01) no effect - 1.6	"

*mg/l unless stated otherwise

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity	Source
Bluegill sunfish (continued)	n-pri-$C_{12-13}AE_{6.5}$ (NEODOL 23-6.5 (P))	Fish - 1.1 g, static, 21°C, pH 7.1	24 hr - 2.36 (1.90 - 2.93)	Shell Chemical Co., unpublished data
"	NH_4^+-sec-$C_{11-15}AE_3$ (TERGITOL 15-S-3A)	Fish - 2 - 3 1/2", static, 10-25°C	24 hr - >32 Biodegraded** 24 hr - 86	"
"	n-$C_{12-15}AE_9$ (NEODOL 25-9(75n))	"	24 hr - 8.0 Biodegraded 24 hr - >100	
"	n-$C_{12-15}AE_9$ (NEODOL 25-9 (98n)	"	24 hr - 11.0 Biodegraded 24 hr - >100	"
"	sec-$C_{11-15}AE_9$ (TERGITOL 15-S-9)	"	24 hr - 9.4 Biodegraded 24 hr - 87.5	"
"	Coconut alcohol, 7.5 EO	"	24 hr - 12.3 Biodegraded 24 hr - >100	"
Golden orfe (Idus melanotus)	$C_{12}AE$ various EO chain length	Fish - 5 cm, static, 18-20°C, 10.4°d total hardness	LC_{50} 60 min EO_2 - 1.9 EO_{20} - 150	Gloxhuber and Fischer, (1968)
	$C_{12-14}AE_{11}$	Fish - 5-7 cm, static, 20°C	LC_0 48 hr - 2.3 LC_{50} 48 hr - 2.7 LC_{100} 48 hr - 3.5	Fischer, personal communication as cited in Gloxhuber (1974)

*mg/l unless stated otherwise

**Biodegraded to the loss of CoSCN active substance

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Golden orfe (continued)	$C_{16-18}AE_{14}$	Fish - 5.5 cm, static, 20°+ 1°C, hardness 150 mg/l $CaCO_3$	LC_{50} 24 hr - 1.8 (1.7-2.0)	Unilever Research Laboratories, unpublished data
	"	Fish - 5-7 cm, continuous flow, 20°C, hardness 268 mg/l $CaCO_3$	LC_{50} 24 hr - 2.8 (2.4-3.5)	
	$C_{12-14}AE_{11}$	Fish - 5.5 cm, static, 20°+ 1°C, hardness, 150 mg/l $CaCO_3$	LC_{50} 24 hr - 2.9 (2.8-3.0)	
	$C_{14}AE_8$	"	LC_{50} 24 hr - 1.0 (1.0-1.1)	
Goldfish (Carassius auratus)	$C_{12}AE_{3.25}$ (ALFOL 12)	Fish - 2.5", static, 18°C, pH 7.2-7.8		Tovell et al. (1975)
		Goldfish acclimated in hard water	Average survival time in 5 mg/l AE (Minutes ± SE):	
			In hard water - (300 mg/l $CaCO_3$) 252 ± 44	
			In soft water - (60 mg/l $CaCO_3$) 161 ± 23	

* mg/l unless stated otherwise

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Goldfish (continued)	$C_{12}AE_{3.25}$ (ALFOL 12)	Fish - 2.5", static, 18°C, pH 7.2-7.8 Goldfish acclimated in soft water	Average survival time (Minutes ± SE): In hard water 300 mg/l $CaCO_3$ 267 ± 39 In soft water 60 mg/l $CaCO_3$ 170 ± 23	Tovell et al. (1975,
	n-pri-$C_{12-14}AE_{6.3}$	--	LC_{50} 24 hr - 1.4	Monsanto Company, unpublished data
	n-pri-$C_{12-14}AE_{7.4}$	--	LC_{50} 24 hr - 1.4	
	n-sec-$C_{11-15}AE_{7}$	--	LC_{50} 24 hr - 2.1-2.5	
	n-sec-$C_{11-15}AE_{9}$	--	LC_{50} 24 hr - 2.1	

* mg/l unless stated otherwise

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Goldfish (continued)	$C_{16-18}AE_{14}$	Fish - 6 cm, static, 20°C, hardness, 200 mg/l $CaCO_3$	10.0-12.5**	Unilever Research Laboratories, unpublished data
	$C_{12-14}AE_{11}$	"	0.0-5.0**	
	$C_{14}AE_{14}$	"	0.0-5.0**	
Fathead minnow (Pimephales promelas)	n- pri-$C_{12-14}AE_{6.3}$	--	LC_{50} 24 hr - 1.8	Monsanto Company, unpublished data
	n- pri-$C_{12-14}AE_{7.4}$	--	LC_{50} 24 hr - 1.8	
	n- sec-$C_{11-15}AE_{7}$	--	LC_{50} 24 hr - 2.9	
	n-sec-$C_{11-15}AE_{9}$	--	LC_{50} 24 hr - 2.8	

* mg/l unless stated otherwise
** LC_0- LC_{100}; lowest dose tested was not stated.

TABLE 3-B Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Guppy (*Lebistes reticulatus*)	pri-$C_{12-15}AE_9$	Fish - male and females, 2°cm, young 0.7 cm, 25°C	LC_{100} 24 hr males - 11 females - 9 young - 6	Van Emden *et al.* (1974)
Guppy (*Poecilia reticulatus*)	$C_{16-18}AE_{14}$	Fish - 3 weeks old, static, 22°+ 1°C, hardness 20 mg/l $CaCO_3$	LC_{50} 24 hr - 0.7 (0.6-0.8)	Unilever Research Laboratories, unpublished data
Harlequin (*Rasbora heteromorpha*)	"	Fish - 1.3-3.0 cm, continuous flow, 20°C, hardness 20 mg/l $CaCO_3$	LC_{50} 24 hr - 1.5 (1.2-1.8)	
Minnow (*Phoximus phoximus*)	"	Fish - 5 + 1 cm, static, 10°+ 1°C, hardness 210 mg/l $CaCO_3$	LC_{50} 24 hr - 3.4 (3.3-3.6)	
Cod (*Gadus morrhua*)	AE_{10}	Fish - 30 cm, continuous flow, 6-8°C	LC_{50} 96 hr - >0.5 <1.0	Swedmark *et al.* (1971)
Flounder (*Pleuronectes flesus*)	"	"	"	

* mg/l unless stated otherwise

Most of the mortality due to alcohol ethoxylates occurs in the first 24 hours. The reduced toxicity with time is probably due to degradation of the surfactant (Macek and Krzeminski, 1975; Unilever Research Laboratories, unpublished data). The degradation of alcohol ethoxylates has been found to reduce their toxicity to fish. Unilever Research Laboratories (unpublished data) showed that biodegradation products of $C_{12-15}AE_7$ (DOBANOL-25) were not acutely toxic to rainbow trout and Reiff (1975) found that $C_{14-15}AE_7$ (DOBANOL 45-7^{TM}) was no longer acutely toxic to rainbow trout after 11 days' storage of a 20 mg/l solution. Similar results were found by Shell Chemical Company (unpublished data). In addition, Wildish (1974) found that polyethylene glycol (PEG), a degradation product of alcohol ethoxylates, was not acutely toxic to salmon (LC_{50}>1000 mg/l).

Test conditions may also affect the toxicity of alcohol ethoxylates to fish, although Macek and Krzeminski (1975) found that the toxicity of some alcohol ethoxylates (see Table 3-B) to bluegill was similar in static and continuous flow conditions.

Tovell et al. (1975) found that rainbow trout and goldfish were less susceptible to $C_{12}AE_{3.25}$ (ALFOL 12) in hard water than in soft water, even when acclimated in soft water. For example, rainbow trout acclimated in soft water showed a survival time in 5 mg/l AE of 152 minutes in hard water and 107 minutes in soft water.

It appears that alcohol ethoxylates are slightly more toxic to fish than

alkylphenol ethoxylates which show a range of toxicity from 4-12 mg/l. LC_{50} (24 hour) values for AE generally fall in the range of 1-6 mg/l, while compounds with high EO numbers are less toxic.

2. Acute Toxicity - Invertebrate

A summary of the acute toxicity values found for invertebrates is shown in Table 3-C. Wide variations are seen between species, even of the same class, and no patterns are readily discerned. Daphnia magna appears to be one of the more susceptible species, with 24-hour LC_{50} values ranging from 1-5 mg/l. Other crustaceans are more tolerant of alcohol ethoxylates. Brine shrimp as well as fish are tolerant of ethylene glycol (Price et al., 1974).

Maxwell and Piper (1968) found that increasing the EO numbers of pri-$C_{13}AE_x$ decreased their toxicity to mosquito pupae. The LC_{50} for the AE_3 was 13 mg/l, while the LC_{50} for the AE_{12} was 64 mg/l.

Saski et al. (1971) tested 4 alcohol ethoxylates (see Table 3-C) and found that the compound with the highest degree of hydrophilicity was the least toxic to planaria. No correlations were found with surface tension or critical micelle concentration.

Degradation products of $C_{12-15}AE_7$ (DOBANOL 25/7EO) were found not to be acutely toxic to fish, as discussed above (Unilever Research Laboratories, unpublished data). However, Foret-Montardo (1971) found that the toxicity of PLURAFAC RA 43™ (a modified AE) to the annelid, Scolelepis fuliginosa was decreased only slightly after storage of various solutions for 28 days under nonsterile conditions. The amount of degradation after this time was not determined.

Czyzewska (1976) tested the effect of a commercial surfactant mixture on larvae development of a crab (Rhithropanopeus harrisi subsp. tridentatus). The mixture contained: 19.4% APE, 4.85% AE, and 7.25% ABS. Larvae in eggshells were highly tolerant of the mixture, and normal hatching occurred at 10,000 mg/l. Older larvae were more sensitive, with total mortality of the population occurring by 6 hr at 10,000 mg/l. At 1 mg/l, the survival time was 336 hours. Increased mortality of larvae was observed just prior to- and post-moulting. However, this susceptibility decreased with the age of the larvae.

The reported LC_{50} values of alcohol ethoxylates normally used as surfactants range from 1-100 mg/l for invertebrates. The polyethylene glycols are less toxic than AE, with LC_{50} values from 1000-20,000 mg/l.

Table 3-C Acute Toxicity of AE to Aquatic Invertebrates

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Arthropoda - Crustacea				
Daphnia magna	$C_{12-18}AE_{14}$	Static, 20°+1°C Hardness - 202 mg/l $CaCO_3$	LC_{50} 24 hr - 1.1 (1.0-1.3)	Unilever Research Laboratories, unpublished data.
	$C_{12-14}AE_{11}$	"	24 hr - 5.1 (4.8-5.5)	
	$C_{14}AE_8$	"	24 hr - 2.0 (1.9-2.2)	
Daphnia, sp.	n-pri-$C_{12-14}AE_{6.3}$	--	LC_{50} 24 hr - 2.5 48 hr - 2.4 96 hr - 1.5	Monsanto Company, unpublished data.
	n-pri-$C_{12-14}AE_{7.4}$	--	LC_{50} 24 hr - 2.3	
	n-sec-$C_{11-15}AE_7$	--	LC_{50} 24 hr - >5	

*mg/l unless stated otherwise

Table 3-C Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Arthropoda-Crustacea				
Daphnia, sp. (cont'd)	n-sec-$C_{11-15}AE_9$	--	LC_{50} 48 hr - 13	Monsanto Company, unpublished data
Daphnia magna	$C_{12-18}AE_{7.4}$ (ALFONIC 1218-60™)	--	LC_{50} 24 hr - 3.3	Continental Oil Co., unpublished data
Daphnia sp.	n-pri-$C_{12-15}AE_9$ (NEODOL 25-9)	Daphnia-24 hrs old, static, 21°C	LC_{50} 24 hr - 1.71 (1.10 - 2.51)	Shell Chemical Co., unpublished data
"	n-pri-$C_{12-13}AE_{6.5}$** (NEODOL 23-6.5 (S))	"	24 hr - 1.05 (0.63 - 1.26)	"
"	n-pri-$C_{12-13}AE_{6.5}$** (NEODOL 23 - 6.5 (P))	"	24 hr - 0.57 (0.35 - 0.93)	"
Leander adspersus	AE_{10}	Continuous flow, 6-8°C, at least 5 organisms/conc.	LC_{50} 96 hr - >100	Swedmark et al. (1971)
Leander squilla	"	"	"	"
Eupagurus bernhardus (hermit crab)	"	"	"	"

*mg/l unless stated otherwise

**The two products are derived from different feedstocks.

Table 3-C Continued

Species	Surfactant	Experimental Conditions	Toxicity	Source
Arthropoda-Crustacea				
Hyas araneus (spider crab)	AE_{10}	Continuous flow, 6-8°C at least 5 organisms/conc.	LC_{50} 96 hr - >100	Swedmark et al. (1971)
Carcinus meanas (shore crab)	"	"	LC_{50} 96 hr - >100	
Balanus balanoides	"	Static, 6-8° at least 5 organisms/conc	LC_{50} 96 hr - Stage II nauplius larvae - 1.2	
Artemia salina (brine shrimp)	ethylene glycol†	Static, 24.5°C	LC_{50} 24 hr - >20,000	Price et al. (1974)
	diethylene glycol†	"	24 hr - >10,000	
	triethylene glycol†	"	24 hr - >10,000	
	ethylene glycol† monobutyl ether	"	24 hr - 1,000	
	diethylene glycol† monoethyl ether	"	24 hr - >10,000	
	ethylene glycol† monoethyl ether	"	24 hr - >10,000	

*mg/l unless stated otherwise

†These materials are not normal components of AE surfactants, but are potential biodegradation products of AE.

Table 3-C Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Arthropoda - Insecta				
Aedes aegypti (mosquito)	pri-$C_{12-15}AE_9$	2nd and 3rd stage larvae, 25°C	LC_{100} 24 hr - 200	Van Emden et al. (1974)
Culex pipiens quinquefasciatus (mosquito)	$C_{8-10}AE_{15}$	Pupae, static, 25°C, pH 7.5-8.0, failure of adult to emerge criteria for effectiveness	LC_{50} >180	Maxwell and Piper (1968)
	$C_{13}AE_3$	"	LC_{50} - 13	
	$C_{13}AE_6$	"	LC_{50} - 29	
	$C_{13}AE_9$	"	LC_{50} - 44	
	$C_{13}AE_{12}$	"	LC_{50} - 64	
Isonychia sp. (mayfly)	nonionic blend of 2 primary alcohol ethoxylates - C_{12-15}, 60% ethoxylation	Organisms - larvae 10.5 mm, static, 10.5°±5°C, pH 7.8, hardness 60 mg/l $CaCO_3$	LC_{50} 24 hr - 5.96 (4.73-7.52)	Dolan et al. (1974)

*mg/l unless stated otherwise

Table 3-C Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Mollusca Gastropoda				
Biomphalaria glabrata (snail)	pri-$C_{12-15}AE_9$ (Polyglycol ether of primary alcohol)	Snails, 1.5-2 cm, static, 25°C	LC_{100} 24 hr - 11	Van Emden et al. (1974)
Goniobasis sp. (snail)	pri-$C_{12-15}AE$ (60% of EO by weight)	Static, 19-20°C pH-7.6-7.9, hardness 58-60 mg/l $CaCO_3$	LC_{50} 24 hr - 15.9 (13.3 - 20.5)	Hendricks et al. (1974)
Mollusca Pelecypoda				
Mytilus edulis (common mussel)	AE_{10}	Adult, 6-8°C, continuous flow, at least 5 organisms/ conc.	LC_{50} 96 hr - 50	Swedmark et al. (1971)
Cardium edule (cockle)	"	"	LC_{50} 96 hr - <5	
Mya arenaria (clam)	"	"	LC_{50} 96 hr - 100	

*mg/l unless stated otherwise

Table 3-C Continued

Species	Surfactant	Experimental Conditions	Toxicity*	Source
Platyhelminthes - Turbellaria				
Dugesia lugubris (planaria)	Polyoxyethylene 23 lauryl ether	planaria - 1.0 cm	LT_{100}** 500 mg/l 6.20 hrs	Saski et al. (1971)
	Polyoxyethylene 20 cetyl ether	"	1.13 hrs	
	Polyoxyethylene 20 stearyl ether	"	1.43 hrs	
	Polyoxyethylene 20 oleyl ether	"	1.54 hrs	

* mg/l unless stated otherwise

** LT_{100} - Lethal time to 100% of the population.

3. Acute Toxicity - Algae

Some studies have discussed the toxicity of alcohol ethoxylates to algae. Monsanto Company (unpublished data) found that linear primary alcohol ethoxylates ($C_{12-16}AE_{6.3}$) stimulated the growth of Selenastrum sp. 50% greater than the control at 0.7 mg/l after a 7 day exposure. This surfactant inhibited growth 50% at 5 mg/l. Another linear primary AE ($C_{12-14}AE_{7.4}$) inhibited the growth of this algae 50% at 3.8 mg/l. Two linear secondary AE ($C_{11-15}AE_7$ and AE_9) inhibited growth 50% at 56 and 52 mg/l, respectively.

Hall (1973) reported that a diatom (Navicula seminulum) was more sensitive to linear AE (C_{12} AE_6) than a green alga (Selenastrum capricornutum), or a blue-green alga (Microcystis aeroginosa). The minimum algistatic (inhibition of cell division) concentration for 5 days (MAC-5 day) for S. capricornutum was 50 mg/l and recovery as indicated by growth was rapid upon addition of the cells to fresh growth medium. An algicidal response (cell death) was observed at 1000 mg/l. The MAC-5 day was 5-10 mg/l for Navicula sp., while the algicidal level was found to be 100 mg/l. The blue-green alga was not severely affected by any treatment up to 1000 mg/l.

Davis and Gloyna (1967) tested 12 species of blue-green algae and 6 species of green algae for their susceptibility to 20 mg/l $AE_{7.0}$ and $AE_{7.4}$. The results indicate that some species are more susceptible than others. Batch additions (50 mg/l) of both compounds to pond water had stimulatory effects on Cyanophyta, but depressed Euglenophyta and Chlorophyta.

4. Sublethal Effects

The sublethal effects of alcohol ethoxylates include erratic and exaggerated swimming movements in rainbow trout at concentrations of $C_{12-15}AE_7$ (DOBANOL 25) less than 2 mg/l (96 hour LC_{50} -2.0 - 2.7). The fish then showed loss of balance and an inability to maintain station in mid-water. A passive stage followed in which the fish lay at the bottom with very slow ventilation of the gill chamber. Recovery occurred with removal to clean water (Unilever Research Laboratories, unpublished data). Swedmark et al. (1971) reported that cod exhibited these effects at concentrations of 0.5 mg/l $C_{16-18}AE$, although recovery occurred after transfer to clean water.

Swedmark et al. (1971) tested invertebrates for sublethal reactions to AE_{10}. They found that the reaction time (median time required for the larvae to lose their swimming activity) was less than 6 hours for Balanus balanoides at 1 ppm and about 10 hours at 10 ppm for Zoea larvae of Hyas araneus. The 96 hour LC_{50} values for these species are greater than 100 mg/l.

Hidu (1965) studied the effect of alkyl polyether alcohol on the larvae of clams (Mercenaria mercenaria) and oysters (Crassostrea virginica). He found a 50% reduction in the percentage of fertilized clam and oyster eggs developing to normal 48 hour straight-hinge larvae at 1.75 and 1.60 mg/l, respectively. The growth of clam larvae (2-12 days) and oyster larvae (2-14 days) was reduced at 2.5 mg/l.

Sublethal effects of alcohol ethoyxlates have been observed at concentrations of 1-2 mg/l. In most cases, especially the invertebrates, these concentrations are much lower than the LC_{50} concentrations discussed above.

5. Interaction with Other Chemicals

No studies were found which described the interactions of alcohol ethoylates with other chemicals.

B. Effects of AE on Soil Microorganisms

A few reports of the effect of alcohol ethoxylates on soil microorganisms were found. Vandoni and Goldberg-Federico (1973) reported that $C_{12}AE$ (0.1% by weight) had a slight inhibitory effect on the nitrification process in two soils they tested.

In an examination of the effect of nonionics on soil microfungi, Lee (1970) found that pots of soil watered daily with 50 ml of 1000 mg/l C_{11-15} AE_9 (TERGITOL 15-S-9) showed a 16% reduction in number of microfungi (as percent of control). No reduction in number of species was found. Further examination of the sensitivity of isolates *in vitro* showed that some species were stimulated at 10 ppm, including *Penicilliumstoloniferum*, *P. simplicissimum*, *P. steckii*, *Aspergillus* sp., *Aspergillus ustus* (isolates I and II), *Chaetophoma* sp. and *Sporonema* sp. The author suggested that these organisms were utilizing the surfactant as a carbon source. At 100 ppm, growth ranged from 14-97% of the control for various species; and at 1000 ppm growth ranged from 0-63% of the control values. Even at this high concentration, 11/22 species achieved 50% or better growth. Other effects, such as reduced sporulation, pigment diffusion and reduced exudate production were observed at concentrations less than 1000 ppm. The concentrations tested are higher, however, than concentrations expected in soil.

In studies to determine the degradation of SOIL PENETRANT 3685^{TM} (alkyl polyoxyethylene ether), Valoras et al. (1976) found that microbial activity was not inhibited or stimulated by treatments to various soils with concentrations as high as 2000 ppm. About 50% degradation occurred within 60 days at concentrations of less than 1000 ppm.

C. Effects of AE on Higher Plants

Few studies have examined the effects of surfactants on higher plants. Ernst et al. (1971) studied the effect of some polyethoxyethanols on orchid seedlings (Phalaenopsis) or seeds of Epidendrum. They found no mortality of Phalaenopsis in vitro at 10 mg/l. Survival of this species was 40% after 5 months in 1000 mg/l $C_{12-14}AE_6$ (ALFONIC 1214-6^{TM}) and n-pri-$C_{12-15}AE_9$ (NEODOL 25-9^{TM}); survival was 70% in the same concentration of n-sec-$C_{11-15}AE_9$ (TERGITOL 15-S-9), and $C_{13}AE_9$ (SURFONIC TD-90). Fresh weight of the seedlings was 64.9-80.1% of the control at 10 mg/l, 67.4-105.1% at 100 mg/l, and 34.7-55.7% of the control at 1000 mg/l. Little effect on germination and growth of Epidendrum was found at 10 or 100 mg/l. The authors found no correlation between growth and surface tension, since little or no further reduction of surface tension occurred between 100 and 1000 mg/l. Based on the results which suggest a coincidence between phytotoxicity and reduced interfacial tension, the authors theorize that the site of surfactant action is the cytomembrane.

Hartmann (1966) found that AE was growth stimulating to oats and barley at concentrations of 4-8 mg/l. At these concentrations, however, growth of root hairs was inhibited.

D. Effects of AE on Birds and Wildlife

The U.S. Department of Interior, Fish and Wildlife Service (1976) has tested sec-$C_{11-15}AE_9$ (PA-14) for use as an avian stressing agent for control of blackbirds and starlings. This compound breaks down oil in the feathers of these birds, removing their natural waterproofing. If feathers become water-soaked, the birds' body temperature drops to lethal levels. The oral toxicity of PA-14 to sparrow hawks is 6300 mg/kg.

E. Mode of Action

The mode of action of AE to aquatic organisms is not completely understood. Gloxhuber and Fischer (1968) described the effects of alkylpolyglycol ether on golden orfe:

> "A short time after the fish are put into the solutions, they become excited and try to escape quickly when touched with a glass rod. Somewhat later, they float in the lateral position and can no longer be correctly spatially oriented, finally they show a very shallow and irregular breathing and generally die after 30 minutes. Changes in the mucous coatings of their surface are not observed."

Similar effects were observed by Swedmark et al. (1971). However, these authors reported an accumulation of mucus on the gills as well as a swelling of gill epithelium in cod.

Further examination of this question showed that goldfish placed in water containing pri-$C_{12}AE_3$ absorbed this surfactant with the highest concentration being found in the gall bladder. The excretion rates (48 hours) were 71% and 88% from unfed and freely fed fish, respectively. No unmetabolized $C_{12}AE_3$ was excreted, and 6 metabolites were isolated but not identified (Unilever Research Laboratories, unpublished data).

Some authors ascribe the toxic effects of alcohol ethoxylates to changes in cell membranes. Wildish (1974) gave two possible explanations for the effect of polyoxyethylene chain length on lethality to salmon parr (Salmo salar). First, he suggested that the uptake rates across cell membranes are limiting to longer EO chain, less lipid soluble surfactants. Secondly, he theorized that increasing the chain length results in less efficient binding to proteins in the animal, causing a slower poisoning process. The author felt this possibility was contradicted by tests showing unchanged esterase activity.

Gloxhuber and Fischer (1968) suggested that the toxic effects of alkylpolyglycol ether were not due exclusively to the gill injury caused by lower surface tension, although the surface tension at various LC_{50} values was similar. These authors found that the intoxication symptoms of the surfactants were very similar to those found for tetracaine, a local anesthetic.

The mode of action of alcohol ethoxylates appears to be related to changes in surface tension and the critical micelle concentration (Swedmark et al. (1971). However, Saski et al. (1971) found that changes in surface tension and the CMC were not major determinants of the toxicity of some AE to planaria.

In summary, the mode action of AE and other nonionic surfactants has not been specifically elucidated at this time.

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V. HUMAN SAFETY

A general consideration of the human safety of surfactants was presented in Chapter 1 (LAS, p. 134).

The data reviewed below on AE surfactants indicate they exhibit a low order of acute toxicity in experimental animals, either orally or after ocular or skin exposure. There is insufficient data on chronic animal exposure studies for an evaluation of the long-term effects of ingesting of alcohol ethoxylates, but the use of these surfactants as analgesics or anesthetics in humans has not produced deleterious effects even when used at doses far in excess of those to be expected from environmental sources.

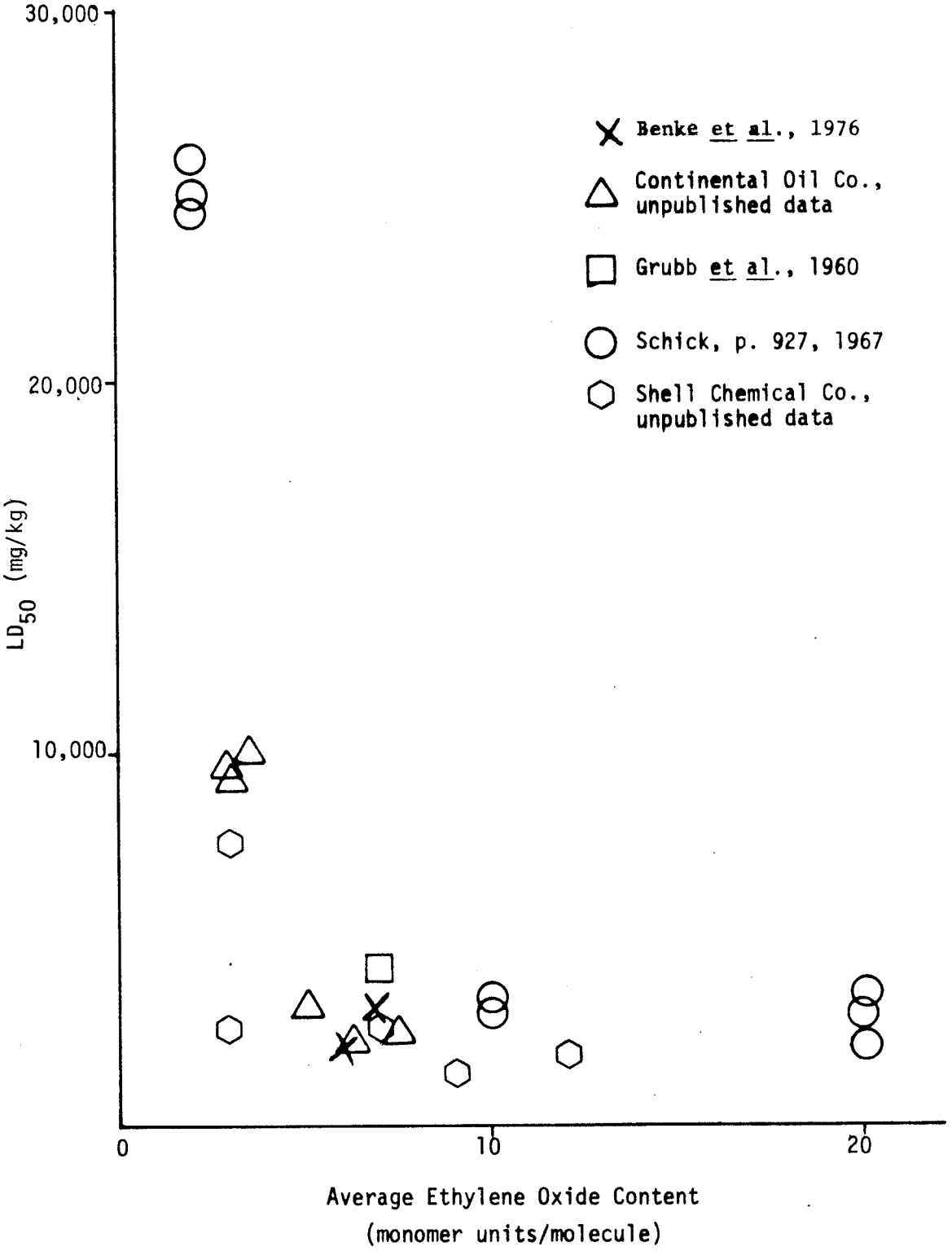
A. Animal Studies

Acute Toxicity - Oral. Alcohol ethoxylates have a low order of acute oral toxicity in the rat. LD_{50} values ranged from 1600 to >25,000 mg/kg, depending on the length of the ethoxylate chain. Toxicity appears to increase rapidly as the length of the ethoxylate chain increases (see Figure 3-1) up to maximum toxic levels at about 10 EO units/surfactant molecule. Further increments in the length of the ethoxylate portion of the molecule do not appear to influence the degree of toxicity. The length of the alkyl chain exerts a negligible effect on toxicity.

The guinea pig seems to be particularly sensitive to the acute toxic effects of n-pri-$C_{12}AE_{11.9}$. The oral LD_{50} value for n-pri-$C_{12}AE_{11.9}$ in guinea pigs was 384 mg/kg compared to 1,170 mg/kg in mice (Zipf et al., 1957).

Figure 3-1

Acute Oral LD_{50} of Alcohol Ethoxylates in the Rat



Emesis and diarrhea were the only effects observed in beagle dogs or juvenile rhesus monkeys (Macaca mulatta) dosed by gavage with 1650 and 1500 mg/kg, respectively, of $C_{12-13}AE_{6.5}$. Similar results were seen with $C_{14-15}AE_7$ at levels of 3300 and 6700 mg/kg, respectively, for rhesus and cynomolgus (M. fasicularis) monkeys. (Benke et al., 1976).

Polyethylene glycols, the intermediate degradation products of AE, are practically nontoxic if given by the oral route. The oral LD_{50} values for PEG 200-600 range between 16,000 and 44,000 mg/kg in rats, mice, guinea pigs and rabbits (Patty, 1966).

Acute Toxicity - Subcutaneous. Soehring et al. (1951) studied the acute subcutaneous toxicity in mice of a series of primary $C_{12}AE$ with molecular weights of about 600. LD_{50} values were determined at two and 24 hours (Table 3-D). As noted with acute oral administration, toxicity increased with increasing EO chain length. A latent toxicity accounts for the difference between the two-hour and 24-hour determinations.

TABLE 3-D

SUBCUTANEOUS LD_{50} OF SEVERAL LAURYL AE IN MICE

	LD_{50} (mg/kg)	
	2 hr	24 hr
n-pri-$C_{12}AE_9$	1,800	1,050
n-pri-$C_{12}AE_{7.13}$	1,200	640
n-pri-$C_{12}AE_{11.9}$	620	400

Soehring et al. (1951)

Acute Toxicity - Intravenous. Both Grubb et al. (1960) and Berberian (1965a) found that AE were approximately 10 times more toxic when administered intravenously rather than orally. In rats the LD_{50} for n-pri-$C_{12}AE_7$ was 390 mg/kg (Grubb et al., 1960) while Berberian (1965a) reported an LD_{50} value for n-pri-$C_{12}AE_9$ of 100 mg/kg in male Swiss Webster mice. Similar findings were observed by Benke et al. (1976); i.e., the acute LD_{50} values for $C_{14-15}AE_7$ in weanling and adult rats, and adult guinea pigs were 104, 68 and 40 mg/kg, respectively.

Soehring et al. (1951) described the effect of intravenous administration of n-pri-$C_{12}AE_{11.9}$ in rabbits. Seven mg/kg n-pri-$C_{12}AE_{11.9}$ produced slight "numbness" in about five minutes. Ten mg/kg elicited spasms of approximately 15 minutes duration with deficient muscle coordination noted for several minutes thereafter. Two out of four rabbits tested at this level died. Twelve mg/kg in rabbits caused extension spasms of the extremities, opisthotonus, increased respiratory and heart rates, and reduction in corneal reflex. Deficient muscle coordination was evident for about six hours. Soehring et al. (1951) also reported that dogs showed good tolerance (only slight drowsiness and increased salivation) following 10 mg/kg of n-pri-$C_{12}AE_{7.13}$ intravenously. Doses of 20 mg/kg and above in dogs produced "cramping" and a narcosis-like state similar to that seen in mice after either s.c. or i.v. administration. However, all dogs recovered.

Acute Toxicity - Intrapleural. Soehring et al. (1951) reported that 10 mg/kg n-pri-$C_{12}AE_{7.13}$ administered intrapleurally in dogs caused only increased salivation and slight drowsiness. Doses of 20 mg/kg and above

produced severe toxicity (cramping, narcosis, etc.) after a five-minute latency, but full recovery was observed.

Acute Toxicity - Intraperitoneal. Soehring et al. (1951) reported an acute LD_{50} value of 200 mg/kg in mice with n-pri-$C_{12}AE_9$. All deaths occurred within 30 minutes of dosing. Soehring et al. (1951) also reported that single sublethal doses of n-pri-$C_{12}AE_9$ (approximately 1/2 LD_{50} value) in mice produced a similar picture to that seen following i.v. and s.c. administration (cramps, narcosis-like state, etc.) after a latent period of about three minutes.

In a series of tests with several species, Benke et al. (1976) found LD_{50} values of 177 (154-205) mg/kg and >100<200 mg/kg, respectively, for $C_{12-13}AE_{6.5}$ and $C_{14-15}AE_7$ when given intraperitoneally to mice. In rats, the LD_{50} value was 209 (184-223) mg/kg for both surfactants. Ptosis and depression were observed in guinea pigs following intraperitoneal dosing with 50 to 100 mg/kg $C_{14-15}AE_7$, but all animals appeared normal by 4 hours. Doses of 300, 400 and 600 mg/kg of $C_{14-15}AE_7$ given i.p. to cynomolgus monkeys (1 animal/dose) produced ataxia, depression, reduced touch response, hypothermia and death. Convulsions were seen at 400 and 600 mg/kg. Dosage levels of 200 and 250 mg/kg produced no toxic manifestations while a dose of 330 mg/kg $C_{12-13}AE_{6.5}$ given to two rhesus monkey produced one death.

Acute Toxicity - Inhalation. The approximate LD_{50} for either $C_{12-13}AE_{6.5}$ or $C_{14-15}AE_7$ was between 1.5 and 3.0 mg/l in male rats for a four hour aerosol exposure to either material. Labored breathing, rales, corneal opacities and decreased activity were noted with the majority of deaths occurring 2 to 5

days after exposure. Atelectasis was indicated as the cause of death and vacuolation and hyperplasia of the corneal epithelium was seen in about 50% of the test animals (Benke et al., 1976).

Acute Toxicity - Dermal. The dermal LD_{50} values in rabbits for a series of n-pri-AE ($C_{10-14}AE_{2.8}$; $C_{13-18}AE_{3.5}$; $C_{10-14}AE_{6.3}$; $C_{12-18}AE_{7.4}$) were found to be 1000-4000 mg/kg when applied undiluted (Continental Oil Co., unpublished data). Similarly, application of n-pri-$C_{12-15}AE_3$, n-pri-$C_{12-15}AE_9$, n-pri-$C_{12-13}AE_3$, or n-pri-$C_{12-13}AE_{6.5}$ undiluted to rabbit skin resulted in dermal LD_{50} values between 2020-3300 mg/kg (Shell Chemical Co., unpublished data).

Acute Irritation - Skin. Acute skin irritation studies with AE and their derivatives have been performed by several investigators. Grubb et al. (1960) using undiluted n-pri-$C_{12}AE_7$, found marked skin irritation in rabbits at doses ranging from 100 to 5,120 mg/kg. However, these reactions occurred at doses considerably greater than exposure under actual use conditions. Berberian (1965a) noted slight irritation on abraded and intact rabbit skin with 15% or 20% aqueous solutions of n-pri-$C_{12}AE_9$; undiluted, this compound produced slight irritation on intact skin and moderate irritation on abraded skin. Skin tests with several undiluted, linear primary AE produced minimal to extremely irritating reactions in rabbits according to the Draize criteria. The materials examined included $C_{12-13}AE_3$ (severe), $C_{12-13}AE_{6.5}$ (mild), $C_{12-15}AE_3$ (extreme irritation), $C_{12-15}AE_7$ (mild), $C_{12-15}AE_9$ (severe) and $C_{12-15}AE_{12}$ (minimal irritation). (Shell Chemical Co., unpublished data).

Acute Irritation - Ocular. Eye irritation studies in rabbits with several undiluted n-pri-AE formulations ($C_{10-14}AE_{2.8}$; $C_{12-18}AE_{3.5}$; $C_{10-14}AE_{6.3}$; $C_{12-18}AE_{7.4}$) produced maximum average scores ranging from 28-33 (of a possible 110 points) by 72 hours (Continental Oil Co., unpublished data). Grubb et al. (1960) found that higher concentrations (10-100%) of $C_{12}AE_7$ produced varying degrees of irritation but only transient irritation (~1 hr) was noted with a 1% solution. Several undiluted linear primary AE ($C_{12-13}AE_3$, $C_{12-13}AE_{6.5}$, $C_{12-15}AE_3$, $C_{12-15}AE_9$, $C_{12-15}AE_{12}$) were severe to extremely irritating eye irritants in rabbits according to the Draize criteria. $C_{12-15}AE_7$ was only moderately irritating (Shell Chemical Co., unpublished data). In another study, n-pri-$C_{12-15}AE_9$, n-pri-$C_{12-13}AE_{6.5}$-1, and n-pripC$_{12-13}AE_{6.5}$-2 were all classified as corrosive according to the Draize procedure with respective scores of 73, 51 and 73 of a possible 110 points after 72 hours (Shell Chemical Co., unpublished data).

Acute Irritation - Mucosal. Berberian (1965a) applied 5 ml of undiluted n-pri-$C_{12}AE_9$ to the vaginal and cervical mucosa of two dogs. No irritation was noted.

Subacute Toxicity - Oral. Oral feeding of AE produced little or no effect on test animals. Grubb et al. (1960) fed rats diets containing 0.0471, 0.2355 or 1.1775% of a n-pri-$C_{12}AE_7$ formulation for four weeks with no adverse effects. Berberian (1965a,b) also using rats (Sprague Dawley) found n-pri-$C_{12}AE_9$ to be well tolerated (inactivity, dyspnea and excess salivation were noted), with high doses of 195 and 290 mg/kg/day over 22 days.

He estimated a 5-day LD_{50} of 1,200 mg/kg/day in rats. An aerosol preparation containing 20% n-pri-$C_{12}AE_9$ dosed orally had a 5-day LD_{50} in rats of 6.4 ml/kg/day (1,280 mg/kg); a dose of 1 ml/kg/day (200 mg/kg) was well tolerated. Rats administered up to 500 ppm $C_{12-13}AE_{6.5}$ in the diet for 91 days showed no adverse effects. Rats fed 1000, 5000 or 10,000 ppm of a commerical formulation containing $C_{12-13}AE_{6.5}$ had reduced final body weights, growth values and food consumption at 91 days. These findings may have been due to a dose-related palatability of the surfactant. Rats fed diets containing 1000, 5000 or 10,000 ppm of a commerical formulation containing $C_{14-15}AE_7$ for 91 days exhibited no adverse effects (Brown and Benke, 1976).

Subacute Toxicity - Inhalation. In an inhalation system, Grubb et al. (1960) exposed rats to steam generated from a 20% aqueous solution of n-pri-$C_{12}AE_8$, two hours/day for five days. Seven of nine rats showed no change; two had mild laryngeal irritation, of which one also had diffuse peripheral hemorrhages on the lungs. Grubb concluded that, in rats, exposure to therapeutic levels of n-pri-$C_{12}AE_7$ produced no significant toxic effects.

Subacute Toxicity -Intramuscular. The local irritating action to AE (C_{16}, C_{12}) and polyoxypropylene ether on muscle tissue was investigated by Mima et al. (1962). Rabbits were injected in the femoral region with 1 ml of test solution (1 and 5% in normal saline). Each test material had a distinguishing alkyl group and molecular weight. Reactions were observed at 1 and 5 hours and 1, 3 and 5-7 days. The severity of the reaction was graded on a visual observation scale of -- (no change) to ++++ (site cystic with induration and aseptic suppuration). Mima concluded that the severity of the local

irritating reaction was directly proportional to concentration of material and the number of injections. Increasing the degree of EO polymerization caused a decrease in reaction severity. Molecular shape also influenced the local irritating reaction; i.e., broad molecules were less irritating than long and narrow molecules.

Subacute Toxicity - Intradermal. Delacretaz et al. (1971) used a 25% C_{18} polyethylene glycol ether gel in intradermal studies. Injection of 0.05 ml (12.5 mg) of this gel daily for five days resulted in severe inflammation upon biopsy three days post injection in both epidermis and dermis.

Subacute Skin Irritation and Sensitization. The studies of Grubb et al. (1960) and Berberian (1965a) show little or no irritation in rabbits following repeated skin exposure to n-pri-$C_{12}AE_7$ and n-pri-$C_{12}AE_9$, respectively. Grubb et al. (1960) also showed that no skin sensitization occurred when a challenge dose was applied. Delacretaz et al. (1971) found severe irritation after five days of application of a gel with 25% C_{18} polyethyleneglycol ether. Skin biopsy showed morphological changes to the dermis layer in addition to surface erythema. The skin was described as "stiff".

No systemic toxicity was produced in 4- or 13-week percutaneous tests of $C_{12-13}AE_{6.5}$ or $C_{14-15}AE_7$ in rabbits. The skin was abraded for the 4 week test but not for the 13-week exposure. Application of 50 mg/kg/day (5 days/week) for 4 weeks produced slight to moderate skin irritation with $C_{12-13}AE_{6.5}$ and moderate to pronounced irritation with papular eruption with $C_{14-15}AE_7$.

The same results were seen after 13 weeks on unabraded rabbit skin (Brown and Benke, 1976).

Fissured skin was noted in ten guinea pigs immersed in 10% aqueous solutions of $C_{14-15}AE_7$ four hours per day for five consecutive days. Microscopically, mild to moderate subacute dermatologic changes characterized by hyperkeratosis, acanthosis and infiltration of the superficial dermis were seen at necropsy (Brown and Benke, 1976).

Nine topical applications over a 3-week period of either n-pri-$C_{12-15}AE_9$ (NEODOL 25-9) or n-pri-C_{12-13} $AE_{6.5}$ (NEODOL 23-6.5-1™) to guinea pig skin followed by a challenge application two weeks later were non-sensitizing. Identical treatment with n-pri-$C_{12-13}AE_{6.5-2}$ (NEODOL 23-6.5-2™), however, produced slight erythema in one of eight test animals and questionable erythema in two other animals following a challenge application (Shell Chemical Company, unpublished data).

Subacute Mucosal Irritation. In studies using n-pri-$C_{12}AE_9$, Berberian 1965a,b) found that no vaginal or cervical irritation occurred in dogs after repeated applications.

In one group, 5 ml of a 15% aqueous solution of n-pri-$C_{12}AE_9$ was introduced into the vagina once daily, five days a week for two consecutive weeks. No irritation was noted. Similarly, another group was treated with 10 ml of

a 15% n-pri-$C_{12}AE_9$ aerosol cream formulation three times weekly for six months (79 vaginal exposures/dog). No changes were observed in test animals when compared to controls treated with the aerosol formulation without n-pri-$C_{12}AE_9$.

Chronic Toxicity. There are no studies available concerned with the long-term effects of alcohol ethoxylates in laboratory animals. However, Smyth et al. (1950, 1955) observed no adverse effects in either dogs after one year or rats after two years exposure to PEG 400 at levels of 2% of their diets.

Mutagenicity. In a series of studies with AE_6, no significant differences from control values were noted in a dominant lethal study or in in vivo and in vitro cytogenetic studies. In the dominant lethal assay, male mice were orally administered either 20, 100 or 200 mg/kg AE_6 subacutely or 100, 500 or 1000 mg/kg acutely. No significant variations in the mutagenic indices were seen. Similarly, no significant differences in chromosomal anomalies were noted in bone marrow cells of hamsters given 80, 400 or 800 mg/kg AE_6 orally, then killed at 6, 24 or 48 hours post-dosing; nor were there any significant chromosomal anomalies observed in human leucocytes incubated for 18, 24, or 48 hours with 2, 20 or 100 µg/ml AE_6. (Procter & Gamble Co., unpublished data).

Carcinogenicity and Co-carcinogenicity. No studies were found in this area.

Reproduction Studies. In a two generation reproduction study, rats (25♂, 25♀/group) were fed $C_{14-15}AE_7$ in the diet at levels of 0, 0.05, 0.1 and 0.5%. In three treatment groups, the females received the compound only during the 6th through the 15th day of gestation; the males were untreated in these groups. Three additional groups received the compound continuously during the study. There was also one control group.

No treatment related changes were noted for the parental rats or pups with respect to general behavior and appearance or survival. The fertility, gestation and viability indices were comparable for the control and treated groups. Parental female rats and pups continuously treated at the 0.5% dosage level did not gain as much body weight as did control rats. Hematological studies for the P_1 and P_2 generations did not show any marked or consistent differences between control and continuously treated rats.

Although several maternal and fetal parameters had 1 or 2 group values which were significantly different from control group values, none of these differences appeared to be biologically significant or related to $C_{14-15}AE_7$ feeding.

No indication of $C_{14-15}AE_7$ related teratogenesis was observed in any feeding group from either generation.

No compound related gross or microscopic pathologic lesions were observed in any parental rats examined from either generation. Compound related organ

weight effects were limited to increased group mean relative liver weights of male and female P_1 generation rats from the 0.5% continuous feeding group at the 91 day sacrifice and increases in group mean relative liver weights of males from the 0.5% continuous feeding group of the P_2 generation at the 60 day and cesarean section sacrifices (Procter & Gamble Co., unpublished data).

Similar findings were reported for $C_{12}AE_6$ in another two generation reproduction study utilizing an identical protocol. No changes considered to be related to $C_{12}AE_6$ were seen for the parental rats or pups with respect to general behavior or appearance or survival. The fertility, gestation and viability indices were comparable for the control and treated groups. Parental rats and pups given 0.5% $C_{12}AE_6$ continuously in the diet did not gain as much body weight as control rats. Hematological studies for the P_1 and P_2 generation did not show any marked or consistent differences between control and treated rats.

A statistically significant increase in embryolethality occurred in the F_{2c} generation in the group fed 0.5% $C_{12}AE_6$ continuously. A statistically significant decrease in mean liver fetal weight occurred in the F_{2c} generation in the group fed 0.1% continuously and a statistically significant increase in soft tissue anomalies was seen in the F_{2c} generation in the group fed 0.5% continuously. These observations were not considered compound related. No other signs of apparent toxicity were detected in this investigation in all other groups of either generation regarding the number of gravid females at

the 13 or 21 day cesarean sections, the mean number of corpora lutea, implantation sites, live fetuses, live fetal weight, number of females exhibiting dead or resorbed fetuses, number of dead or resorbed fetuses and fetal development.

No compound-related gross or microscopic pathologic lesions or organ weight variations were observed in any P_1 or P_2 generation rats which were sacrificed and necropsied at any of the several sacrifice intervals (Procter & Gamble Co., unpublished data).

Teratogenesis. No adverse effects on fetal parameters (mean body weight; sex; external, visceral or skeletal anomalies) were observed following oral administration of $C_{12}AE_6$ to pregnant rabbits (25/group) at levels of 0, 50, 100 or 200 mg/kg/day from day 2 to day 16 of gestation. Cesarean sections were performed on the 28th day of pregnancy.

A definite increase in maternal toxicity at 100 and 200 mg/kg/day was observed, but no effects upon other parameters (body weight, corpora lutea, implantations, live fetuses and abortions) were reported at any treatment level.

Ataxia, or ataxia and loss of righting reflex were noted for 4 rabbits at the 100 mg/kg/day level and for 10 rabbits at the 200 mg/kg/day level. Nine control rabbits and 31 treated rabbits died during the study. Early deliveries were noted for 2 control rabbits and 7 treated rabbits. Surviving

rabbits at the 200 mg/kg/day dosage level generally showed slight losses of body weight. (Procter & Gamble Co., unpublished data).

Acute, Subacute, and Chronic Toxicity - Summary. The alcohol ethoxylates, like the alkylphenolethoxylates, exhibit a low order of toxicity (oral LD_{50} >1600 mg/kg) to rats. Alkyl chain length does not appear to be a significant factor in oral toxicity, while increasing ethoxylate length results in increasing toxicity. In contrast, acute dermal toxicity of several AE to rabbits was remarkably similar and apparently independent of chemical structure.

In general the acute ocular and dermal responses to aqueous solutions of AE are mild to moderate. Other mucosal tissues and the respiratory tract also seem to tolerate aqueous solutions of these surfactants well in both acute and subacute tests. Undiluted AE, however, are capable of causing moderate to marked dermal and ocular irritation.

No evidence was found that AE are mutagens in either in vitro or host-mediated mutagenicity tests and no teratogenic or adverse reproductive effects were attributable to the oral administration of AE in either rats or rabbits. No data on the carcinogenicity or effects of chronic oral or dermal exposure of test animals to this class of surfactants were found.

Pharmacology - Absorption and Metabolism. Rapid absorption and excretion of ^{14}C-labelled AE was seen following oral administration to rats; i.e., greater than 70% of the recovered radioacivity was excreted with 24 hours. Excretion values for urine, feces and expired air ($^{14}CO_2$) were 54%, 26%, and 3%, respectively. Less than 4% of the administered radioactivity remained in tissues, organs and carcass at 72 hours. Rapid absorption was also seen after topical application of 12.5 mg/kg ^{14}C-labelled AE to the shaved backs of rats. By 72 hours, 29% of the administered radioactivity had been excreted in the urine, 8% in the feces, 11% as $^{14}CO_2$ and 40% remained in tissues, organs and carcass (Procter and Gamble Co., unpublished data).

Drotman and Dupre (1976) reported in a meeting abstract that following an orally administered dose of ^{14}C-labelled $C_{16}AE_9$, significantly less of the label (no amount given) was recovered in the 0-8 hour urine collection of rats chronically fed 1% $C_{16-18}AE_9$ in the diet for 13 weeks compared to naive rats. No differences in the distribution of ^{14}C-excretory products were distinguishable by 72 hours. Respired $^{14}CO_2$ represented the major route of elimination with lesser amounts in urine and feces, respectively. Approximately 60% of the total $^{14}CO_2$ expired, was eliminated within the first 8 hours.

In an examination of the extent of dermal penetration of AE surfactants, weanling and adult rats and guinea pigs were bathed in solutions of ^{14}C-labelled AE_x. No differences in the blood concentration of the surfactant

during and after bathing were observed in weanling and adult rats. However, weanling guinea pigs had twofold more ^{14}C-label in their blood than adults of this species. (Benke, 1976).

Pharmacology - Anesthetic/Analgesic Activity. The most outstanding pharmacological characteristics of alcohol ethoxylates are their surface anesthetic and local analgesic activities. In an extensive series of experiments, Soehring, Schultz and associates have examined these properties in several laboratory species under a variety of experimental conditions (Soehring et al. 1952b, 1954; Schultz, 1952; Siems and Soehring, 1952; Schultz et al., 1952, 1953; Stellmach et al., 1952; and Soehring and Frahm, 1955). Additional work, particularly with respect to the endoanesthetic activity of these compounds, has been reported by Zipf and Dittman (1964) and Zipf and Kreppel (1955).

Pharmacology - Surface Anesthesia. When applied to the rabbit cornea, Soehring et al. (1952b) found that aqueous solutions of 0.5% n-pri-$C_{12}AE_9$, 1% n-pri-$C_{12}AE_{7.13}$, and 1% n-pri-$C_{12}AE_{11.9}$ were all very effective surface anesthetics. No irritation or delayed effects were seen following treatment.

Zipf and Dittman (1964), working with a series of n-pri-AE_9 homologs in which the length of the alkyl chain varied (C_1, C_2, C_3, C_4, C_6, C_8, C_{10}, C_{12}, C_{14}), found n-pri-$C_4AE_9$and lower homologs had no surface anesthetic effect when applied to rabbit cornea. Linear pri-C_8AE_9 and above all exhibited good anesthetic effect with increasing activity as the alkyl chain was lengthened. No irritating effects were seen.

Pharmacology - Local Analgesia. In a study with two dogs whose canine teeth contained surgically implanted silver electrodes, Stellmach et al. (1952) found that infiltration of a 2% solution of n-pri-C_{12} AE_9 into the oral infraorbital nerve canal increased the animals' pain threshold values to electrical stimulation by 1600 to 2100%.

Pharmacology - Endoanesthetic Activity. Zipf and Kreppel (1955) found that n-pri-C_{12} $AE_{7.13}$; n-pri-C_{12} AE_9; and n-pri-C_{12} $AE_{11.9}$, if given intravenously to guinea pigs under urethane anesthesia, displayed long-lasting endoanesthetic activity. Depending on the dose, the impulse activity of normally energized lung stretch receptors could be partially or totally inhibited. Zipf and Dittman (1964), working with a series of n-pri-AE_9 homologs in which the length of the alkyl chain varied from C_1 to C_{14}, found that the endoanesthetic effects on the stretch receptors of guinea pig lung increased as the alkyl chain of n-pri-AE_9 increased.

Pharmacology - Anticonvulsant Activity. Weak anticonvulsant activity was seen in mice following subcutaneous administration of C_{12} AE, but only within a dosage range (75-110 mg/kg) which approached neurotoxic levels. The compound was inactive following oral administration (Frey, 1962).

Pharmacology - Hemolysis. A series of alcohol ethoxylates have been shown to exert a hemolytic effect on animal and human erythrocytes in vitro (Kondo and Tomizawa, 1968; Grubb et al., 1960; Schultz et al., 1951; Soehring and Nasemann, 1952). A single report by Soehring and Nasemann (1952) indicates an apparent lack of effect on coagulation time in vivo.

Working with canine blood and a series of pri-$C_{12}AE$ homologs ($C_{12}AE_6$, $C_{12}AE_7$, $C_{12}AE_8$), Konda and Tomizawa (1968) found that the hemolytic action of these compounds in vitro was influenced by a variety of factors. The concentration of pri-$C_{12}AE$ needed to produce lysis was found to be linearly related to the red blood cell RBC concentration; hemolytic activity decreased as the length of the ethoxylate chain increased; and hemolysis increased as the temperature rose due to greater adsorption of AE to the RBC surface and an increased release of lipids from the RBC membrane. Similar results were reported by Mima et al. (1962) who found that the hemolytic activity of $C_{12}AE$ decreased as the number of EO units increased; additionally, Mima noted that the hemolytic activity was proportional to the local irritating action of each homolog.

An indication of the absence of effect on in vivo coagulation time was reported by Soehring and Nasemann (1952) following treatment with n-pri-$C_{12}AE$. They found that multiple injections of n-pri-$C_{12}AE_{11.9}$ (up to 20 mg/kg s.c. for five days or 14 mg/kg i.v. for three days) produced no apparent effect on clotting time in four rabbits.

Pharmacology - In Vitro Ciliary Activity. Using an in vitro test system, Grubb et al. (1960) measured the extent of ciliary inhibition produced in rat tracheal ring preparations exposed to n-pri-$C_{12}AE_7$ for 10 minutes. No inhibition was noted at an aqueous concentration of 20 mg/l; activity was reduced in four of five preparations at 50 mg/l, and a concentration of 500 mg/l completely inhibited the five tracheal specimens tested at this level.

Pharmacology - In Vitro - Enzymes. Furuchi (1974) observed a strong inhibition (~90%) of hog pancreatic amylases occurred when a concentration of 0.6% polyethylene glycol monolauryl ether was added to the incubation medium.

B. Human Studies

Human Safety Aspects of AE

Acute Inhalation Exposure. No toxic manifestations were noted in 16 test subjects exposed continuously for 8 hours to the steam emitted from a water vaporizer containing 4, 4-8, and 5-20 tablets/quart of commercial formulation containing n-pri-$C_{12}AE_7$. No reference to the AE concentration per tablet was given (Larkin, 1957).

Skin Irritation and Sensitization. A repeated patch test in 53 subjects, 13 males and 40 females, was performed by Berberian (1965a) with aerosol creams containing 10, 15, and 20% n-pri-$C_{12}AE_9$. In a series of pre-challenge applications, test patches were placed on the anterolateral surface of the upper arms 3 times weekly for 3 weeks. Pre-challenge doses were 0.1 ml (10, 15, and 20 mgs). Challenging doses of 0.05 mls (5. 7.5, and 10 mgs) were applied 16 days after the ninth pre-challenge patch. Both the test and control patches showed mild erythema after repeated skin application. However, none of the 51 subjects challenged showed an edematous reaction or eczematous flare. Eighteen of the subjects had mild (1+) erythema reaction to one or another of the test or control substances, which was concluded as not indicative of sensitization.

In another repeated patch test, twelve subjects were exposed to a series of nine patches containing 1% aqueous solution of either n-pri-$C_{12-15}AE_9$ (5% active NEODOL 25-9) or n-pri-$C_{12-13}AE_{6.5}$ (5% active NEODOL 23-6.5) over a three week period. Challenged two weeks later with a challenge patch, no irritation occurred with the $C_{12-15}AE_9$ material while very slight skin irritating properties were observed (1/12 had very slight erythema) with the $C_{12-13}AE_{6.5}$ material (Shell Chemical Co., unpublished data).

Slight to negligible skin irritation was noted in ten human volunteers exposed 4 hours/day on 3 alternate days (occluded patch) to undiluted or a 25% aqueous solution of $C_{14-15}AE_7$. Slight irritation was reported in 8 subjects exposed for 24 hours to an occluded patch containing a 10% aqueous solution of $C_{12-13}AE_{6.5}$ (Benke et al., 1976).

Mucosal Irritation. Berberian (1965b) examined the irritancy of a $C_{12}AE_9$ aerosol cream formulation intended for use as a human contraceptive. The formulation was applied to the penile mucosal surface of 15 male subjects for a period of 6-8 hours for 4 consecutive days; treatment was halted for the next 4 days, then repeated for 4 additional days. Thirteen of the 15 males observed no irritation or unusual effects, while 2 individuals observed slight erythema after 2 of the 8 applications.

Spermicidal Activity - In Vitro. Holzaepfel et al. (1959) reported that a 0.25% aqueous solution of alkoxypolyoxyethylene ethanol was highly spermicidal, requiring 5 minutes or less to kill a sample of human spermatozoa while Harvey and Stuckey (1962) found that exposure to a 0.5% aqueous solution of pri-C_6AE_4 for 30 minutes was not lethal to human sperm cells.

However, aqueous solutions of pri-C_8AE_7, pri-$C_{12}AE_{19-23}$ and pri-$C_{16}AE_{19-23}$ were spermicidally active at concentrations of 0.06-0.125%, 0.03-0.06% and 0.03-0.06%, respectively. No spermicidal activity was seen with PEG 1000. Berberian (1965a) similarly found that n-pri-$C_{12}AE_9$ was lethal (sperm immobilization) to human spermatozoa within 20 seconds at concentrations of 1:1200 to 1:3000. However, the sperm cells did not lyse nor disintegrate even at concentrations of 1:200.

Use of AE as Therapeutic Agents

Alcohol ethoxylates and their derivatives have been studied by several investigators seeking to define the safety of formulations containing such products for human therapeutic use. These surfactants have been investigated for use as: (1) a local surface anesthetic; (2) an antipruritic; (3) a mucosal anesthetic; and (4) a respiratory inhalant for respiratory congestion where thick mucosal secretions are presented.

Local Surface Anesthetic/Antipruritic. Lutzenkirchen (1952), Blasiu (1953), and Heyman (1954), investigated AE for use as surface anesthetics and antipruritics. Lutzenkirchen (1952) observed no intolerance of hypersensitivity to n-pri-$C_{12}AE_9$ when used in a variety of mediums (oils, lotions, pastes) at concentrations of 10, 5, and 1%. In one instance, equal parts of n-pri-$C_{12}AE_9$ and olive oil were tolerated for several days. Over the longer term, a 10% concentration did cause drying of the skin. Blasiu (1953) performed clinical studies with "THESIT™" (n-pri-$C_{12}AE_{7.13}$ and n-pri-$C_{12}AE_{11.9}$)

in 89 carcinoma patients treated with X-ray or radium irradiation therapy. Side effects of such treatment (erythema, skin eruptions and skin ulcers) were treated for dermatological itching and pain with ointment (3%) and vaginal or rectal suppositories (5%). All formulations were well tolerated in two patients who had severely damaged and poorly nourished skin. Schoog (1953) also reported the use of "THESIT" as an antipruritic. Heyman (1954) treated 103 burns (5-1°, 86-2°, 12-3°) with 1-2% and 5% aqueous solutions of "THESIT" to alleviate the associated pain. The pain of first degree burns did not subside with "THESIT" because "THESIT" requires contact with open epidermis and exposed corium. Heyman observed no skin irritation or hypersensitivity. However, "THESIT" worked well on second and third degree burns where such criteria were satisfied.

Schultz (1952) also reported that good local analgesic effects were achieved with "THESIT" in 63 individuals following treatment for a variety of skin diseases (e.g., painful surgical wounds, topical ulcers, second degree burns, interdigital mycoses). Generally, prompt cessation of pain followed application of a 1% or 2% aqueous solution, however, equivocal results were observed in the treatment of 28 patients with pruritis.

In a total of 63 cases, there were 5 reports of skin reactions - all involved irritation of already inflamed tissue. No irritation developed on intact skin. No adverse effects on wound healing or irritation or surrounding tissue was seen. Daily application of a 2% aqueous solution of n-pri-C_{12}^{AE} "THESIT" preparation to the oral mucosa for periods up to 8 weeks also produced no sensitization or irritation.

Schultz et al. (1952, 1953) demonstrated that the duration of analgesia produced by n-pri-$C_{12}AE_9$ could be significantly extended by the addition of 2.5 mg% of either adrenalin or arterenol. Subcutaneous injections with 0.4% n-pri-$C_{12}AE_9$ in physiological saline or intracutaneous injections with 0.1% n-pri-$C_{12}AE_9$ in physiological saline resulted in complete localized analgesia for 20-25 minutes in ten human volunteers. The addition of either adrenalin or arterenol increased the duration of analgesia to 4 to 5 hours. Some pain was reported upon subcutaneous injection and hyperemia of varying degrees was noted at the injection site after either subcutaneous or intracutaneous injections. No additional side effects were reported following repeated exposure over a 2-month period.

Hartung and Rudolph (1970) treated 2557 eczema patients with a n-pri-$C_{12}AE$ ("THESIT") preparation. Of this number, 38 cases of allergic response to the skin application were recorded. Two of these cases were clearly due to contact dermatitis from the "THESIT" ointment.

Mucosal Analgesics. Both Strack (1950) and Hochrein and Schleicher (1951) have studied the use of n-pri-$C_{12}AE_9$. n-pri-$C_{12}AE_{7.13}$ and n-pri-$C_{12}AE_{11.9}$ as mucosal analgesics to alleviate pain caused by peptic ulcers and gastritis. Strack (1950) established an oral dose of 20 ml of 0.25% solution of n-pri-$C_{12}AE_9$ taken before meals 3-4 times daily. Treatment of 44 patients over a period of 4 months produced no ill effects. Moreover, promising therapeutic results were reported in addition to the alleviation of pain. Hochrein and Schleicher (1951) found treatment of 50 patients with peptic ulcer or gastritis was of shorter duration with n-pri-$C_{12}AE_9$ than with

other treatment methods. These investigators made no mention of intolerance. Hochrein and Strack (colleagues, at times working together) determined that n-pri-$C_{12}AE_9$ had no influence on gastric circulation or secretion (acid values remained unchanged; stomach temperature did not increase) and no histamine-like effect occurred. A reduction of bile production and reflex evacuation and a reduction of normal stomach peristalsis did occur. Strack (1950) concluded that n-pri-$C_{12}AE_9$ was well tolerated and offered therapeutic advantages.

Respiratory Inhalant. Larkin (1957) undertook a clinical study with infants and children to assess the therapeutical benefits of an AE-containing formulation in the treatment of respiratory tract disease. The "standard dose" (usually 4 tablets/quart) of a commercial formulation containing n-pri-$C_{12}AE_7$ was added to a water vaporizer; 92 test subjects were then exposed to this vapor continuously for a period of 8 hours. Other children served as controls. Good therapeutic results were noted and no intolerance or irritation was reported.

C. Epidemiology

Accidental Exposure. The issue of accidental ingestion of detergents was considered in Chapter I (LAS, p. 158). No reports of human exposure to alcohol ethoxylates in use or manufacturing situations have been found.

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APPENDIX B - AE NOMENCLATURE AND ABBREVIATIONS

Throughout this chapter the designation of AE has been used to indicate alcohol ethoxylates. The number of carbon atoms in the alkyl chain has been numerically designated via a subscript. If the information was available, the following designations for linear (n), primary (pri) and secondary (sec) AE were also specified.

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

$C_9AE_{9.5}$ - nonyl alcohol ethoxylate (average 9.5 ethylene oxide units).
n-pri-$C_{12}AE_{8-12}$ - linear, primary dodecyl alcohol ethoxylate (8-12 ethylene oxide units).

In Section III, the phrase "complete biodegradation" refers to complete primary biodegradation. The complete conversion of a surfactant to carbon dioxide, water and other inorganic compounds is referred to as ultimate biodegradation.

ALCOHOL ETHOXY SULFATES

Synopsis

The anionic alcohol ethoxy sulfates are principally used as components of light duty liquid dishwashing products, shampoos and other household specialty products.

There are presently no environmental standards of water quality with respect to AES. Levels of AES, as such, in streams and waterways of the United States are not presently being monitored separately, but MBAS levels would include AES, if present. The limited information available on the biodegradability of AES indicates that AES surfactants are readily biodegraded under both aerobic and anaerobic conditions in the field as well as in laboratory tests.

The toxicity of AES to aquatic organisms as measured by the 24-96 hr LC_{50} values is in the 1-10 mg/l range. AES toxicity to fish appears to be affected both by changes in the number of EO units in the ethoxylate portion of the molecule as well as alkyl chain length.

Alcohol ethoxy sulfates exhibit a low order of oral and dermal toxicity in laboratory animals. In terms of human safety, chronic oral exposure of rats to 0.5% AES in the diet for two years, a level far in excess of any expected human intake, produced no deleterious effects. Ocular and skin irritation

studies in rabbits indicate care should be exercised against direct eye contact and excessive dermal exposure to concentrations of AES greater than 1 to 2 percent; however, the severity of these animal test procedures in relation to actual human exposure raises problems with respect to human safety evaluations.

There are no indications from chronic feeding studies or long-term cutaneous exposure that AES exhibit carcinogenic activity. No evidence that AES are mutagens was found in either in vitro or host-mediated mutagenicity tests and no detrimental effects on reproductive parameters or increased incidence of teratogenic occurrences have been linked to AES exposure.

It appears that normal use levels of AES do not pose either an environmental hazard or a significant risk to human health.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

ALCOHOL ETHOXY SULFATES

I. INTRODUCTION

The alcohol ethoxy sulfates (AES) are known for their reduced sensitivity to water hardness, their high foaming capabilities and their "softness" to the skin (Kerfoot and Flammer, 1975). AES have principally been used as components in light duty liquid dishwashing products and laundry detergent formulations, but are also utilized in shampoos and other household speciality products.

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

(1) environmental fate and distribution, including biodegradation,

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

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

U.S. International Trade Commission (1975) figures indicate that a total of 220 million pounds of anionic sulfated ethers were produced in 1973. Of this total output, 96 million pounds were ethoxylated, sulfated salts of mixed linear alcohols; an additional 10 million pounds were ethoxylated, sulfated salts of lauryl alcohol.

Production of AES involves three major steps:

(1) ethoxylation of a fatty alcohol (prepared from either vegetable oil or petroleum hydrocarbons)

$$R\text{-}OH + \left(\begin{array}{c} CH_2\text{-}CH_2 \\ \diagdown\ \diagup \\ O \end{array}\right)_x \xrightarrow[\text{catalyst}]{KOH} R\text{-}O\text{-}(CH_2CH_2O)_x\ H \quad (\text{usually } x = 2\text{-}4)$$

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

$$R\text{-}O\text{-}(CH_2CH_2O)_x\ H \xrightarrow{SO_3} R\text{-}O\text{-}(CH_2CH_2O)_x\ SO_3\ H$$

(3) and neutralization to form either the sodium or ammonium salt (Walker et al., 1973).

$$R\text{-}O\text{-}(CH_2CH_2O)_x\ SO_3\ H \xrightarrow{OH^-} R\text{-}O\text{-}(CH_2CH_2O)_x\ SO_3^-\ Na^+ \ (\text{or } NH_4^+)$$

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

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II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alcohol ethoxy sulfates, one of several chemical entities classified as anionic surfactants, can be detected with many of the procedures utilized in the detection of LAS (see Chapter 1, pp. 19-26). Presumptive levels of AES in the environment are generally detected with methylene blue procedures.

B. Water Quality Standards

There are presently no standards in the United States or Europe specifically restricting alcohol ethoxy sulfates (AES). These anionic surfactants are included among those measured in the environment using the MBAS method. The restrictions applying to MBAS levels were discussed in Chapter 1, pp. 26-29.

C. AES in Natural Water Bodies

AES *per se* are not presently being monitored in the United States or Europe; however, MBAS measurements in water bodies include AES surfactants as well as other anionics that may be present. Levels of anionic surfactants detected in natural water bodies were discussed in Chapter 1, p. 30.

III. BIODEGRADATION

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

A. Laboratory Test Systems

1. Oxygen Uptake - Biochemical Oxygen Demand

Alcohol ethoxy sulfates, as measured by their biochemical oxygen demand at 5 (BOD_5) or 20 (BOD_{20}) days, appear to be substantially biodegraded. Neither the length of the alkyl chain (see Table 4-A) nor the length of the ethoxylate portion of the molecule, at least within the range normally used in detergent formulations (i.e., 2 to 4 EO units/mole), appear to significantly influence the rate of degradation. A noticeable early difference in degradation rates can be seen, however, with AES containing six vs twelve EO units/mole (i.e., 68% vs 35% BOD_5), respectively. By 20 days, all AES tested had achieved a BOD greater than 70% of TOD.

2. CO_2 Evolution

The degradation of the above cited AES surfactants was also monitored (Table 4-A) using Sturm's evolved CO_2 procedure (1973). All were readily biodegraded; the percentage of evolved CO_2 ranged from 71 to 100 percent of theoretical (Procter & Gamble Co., unpublished data).

TABLE 4-A

AES BIODEGRADABILITY SUMMARY

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

*Percent biochemical oxygen demand at 5 days.

**Percent biochemical oxygen demand at 20 days.

†Percent of theoretical CO_2 production.

‡Total depletion of oxygen.

●Results from two separate experiments

(Procter & Gamble Co., unpublished data)

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

3. Die-Away Tests

a. River Water Test

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

b. Fortified and Inoculated Waters

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

c. Shake Culture Test

Linear C_{12-14} (40:60) AE_3S and n-$C_{12}AE_{40\%}S$ (equivalent to an average of 4 EO units/mole) were both reported to be degraded (MBAS) 100% within 2-3 days

(Continental Oil Co., unpublished data; Allred and Huddleston, 1967, respectively).

d. Bunch - Chambers Test

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

4. Simulated Treatment Processes

a. Activated Sludge

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

b. Trickling Filters

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

c. Anaerobic Systems

$C_{14-16-18}$ (14:32:54) AE_3S at levels of 26 and 52 mg/l were fed into two laboratory-scale septic tank systems for eight months. AES removals (MBAS) of 81% and 72%, respectively, were reported. The effluent from each vault was then passed through an aerobic seepage bed; overall removal was 98.5% and 99%, respectively (Procter & Gamble Co., unpublished data). Removal of $C_{14-16-18}$ (14:32:54) AE_3S added at levels of 20, 50 or 100 mg/l was greater than 95% (method of detection unspecified) in a six-month anaerobic sludge digester study (Procter & Gamble Co., unpublished data).

5. Influence of Test System Variables

a. Inoculum

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

b. Temperature

A single study (Procter & Gamble Co., unpublished data) examined the rate of AES degradation as a function of temperature. Although the rate of degradation was reduced at lower temperatures (5°C), as might be expected,

biodegradation did occur (see III.A.2. of this chapter for details.)

B. Field Studies

Household detergent products containing 10-13% AE_3S were exclusively used for laundering purposes in 14 homes for approximately a one-year period. Ten homes had septic tank-type sewage treatment while the remaining four homes had aerobic cavitette-type sewage treatment systems. No adverse effects were observed with respect to operation of the units, and AE_3S removal (MBAS) ranged from 46-66 percent (Procter & Gamble Co., unpublished data).

Utilizing their far infrared method, Oba et al. (1976) found 16% of the surfactant content present in raw municipal sewage entering two Japanese sewage treatment plants consisted of AES plus alkyl sulfates. These surfactants were completely removed during passage through the two treatment plants.

C. Summary

The data available on biodegradation indicate that AES surfactants as a class readily undergo primary biodegradation in the laboratory and under field conditions in both aerobic and anaerobic systems. Within the range utilized in detergent formulations, neither increments in length of the alkyl chain nor the length of the ethoxylate portion of the molecule appear to significantly influence the rate of biodegradation. Based upon BOD and evolved CO_2 data, AES would appear to readily undergo ultimate biodegradation to CO_2 and H_2O.

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IV. ENVIRONMENTAL SAFETY

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

A. Aquatic Toxicity

1. Fish

The available acute fish toxicity data for AES are summarized in Table 4-B. In general, the 24 hr. LC_{50} values range from 1-10 mg/l. Some AES, however, are less toxic; e.g., Gafa (1974) reported an LC_{50} value of 55 mg/l for $C_{12}AE_{2.6}S$ and Procter & Gamble Co. (unpublished data) recorded a value of 375 mg/l for $C_{10}AE_{2.1}S$.

There is some evidence to suggest that toxicity tends to increase with increasing alkyl chain length (see Procter & Gamble Co. and Monsanto Co., data, Table 4-B). In tests with 14 different AES with carbon chains ranging from 8 to 19.6 carbons and 1 to 3 EO units Procter & Gamble Co. (unpublished data) found that $C_{15.9}AE_{2.1}S$ was the most toxic to bluegill with a 96 hr LC_{50} of 0.3 mg/l and toxicity generally increased with increments in the carbon chain.

TABLE 4-B Acute Toxicity of AES to Fish

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Goldfish (*Carrassius auratus*)	C_{12} $AE_{2.6}S$ MW-300	20°C, static, hardness - 10°	LC_{50} 6 hr - 55.0	Gafa (1974)
	C_{14} $AE_{3.0}S$ MW-346		- 6.0	
	C_{16} $AE_{3.4}S$ MW-392		- 41.0	
	C_{12} $AE_{2.6}S$ MW-300, 5% branched		- 66.5	
	C_{14} $AE_{3.0}S$ MW-346, 5% branched		- 8.1	
	C_{15} $AE_{3.2}S$ MW-368.5, 5% branched		- 3.7	
	C_{11-16} AE_3S	Fish - 6 cm, 20°C, static, hardness - 200 mg/l $CaCO_3$	LC_{50}-LC_{100} 24 hr - 10.0-15.0 48 hr - 10.0-15.0	Unilever Research Laboratories, unpublished data

TABLE 4-B (Continued)

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

TABLE 4-B (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Fathead minnow (cont'd)	C_{18} AE_2S	Static, 21°C, pH - 7.0-7.2, hardness - 100 mg/l $CaCO_3$	LC_{50} 24 hr - 80	Monsanto Co., unpublished data
	C_{18} AE_4S		LC_{50} 24 hr - 15	
	C_{18} AE_6S		LC_{50} 24 hr - 2.1	
Guppy (Lebistes reticulatus)	A sulfated polyglycol ether of a primary alcohol with 3 EO groups/molecule, 59.5% active material	Static, 25°C, males - 0.05 g - 0.08 g females - 0.12 g - 0.38 g young (14 days old) - 0.007 g	LC_{50} 24 hr - male - 8 female - 5 young - 4	Van Emden et al. (1974)
Guppy (Poecilia reticulatus)	C_{11-16} AE_3S	Static, 20°C, fish - 1.0 cm, hardness - 150 mg/l $CaCO_3$	LC_{50} 24 hr - 4.7 (2.7-5.8)*	Unilever Research Laboratories, unpublished data

* 95% confidence limits

TABLE 4-B (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Golden orfe (*Idus melanotus*)	C_{11-16} AE_3^S	Static, 20°C, Fish - 7 cm, hardness - 150 mg/l $CaCO_3$	LC_{50} 24 hr - 4.3 (4.0-4.6)	Unilever Research Laboratories, unpublished data
Harlequin (*Rasbora heteromorpha*)		Continuous flow, 20°C, Fish - 1.3-3.0 cm, hardness - 20 mg/l $CaCO_3$	LC_{50} 24 hr - 4.4 (3.9-5.0) 48 hr - 3.9 (3.4-4.5)	
Minnow (*Phoximus phoxinus*)		Static, 10°C, fish - 5 cm, hardness - 210 mg/l $CaCO_3$	LC_{50} 24 hr - 5.8 (5.5-6.2)	
Brown trout (*Salmo trutta*)		Continuous flow, 15°C, fish - 2.8 or 5.8 cm, hardness - 26-36 mg/l $CaCO_3$	LC_{50} 24 hr - 5.7 (4.3-7.8) 48 hr - 2.6 (1.7-3.7)	
			LC_{0-100} 96 hr - 1.0-2.5	

TABLE 4-B (Continued)

Source	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Rainbow trout (Salmo gairdneri)	C_{11-16} AE_3S	Static, 15°C, Fish - 8-10 cm, hardness - 20 mg/l $CaCO_3$	LC_{50} 24 hr - 2.4 (2.0-2.9) 48 hr - 1.9 (1.5-2.3)	Unilever Research Laboratories, unpublished data
		Continuous flow, 15°C, fish - 3.0 cm, hardness - 300 mg/l $CaCO_3$	LC_{0-100} 24 hr - 3.0->4.6	
			LC_{50} 48 hr - 2.8 (2.3-3.4) 96 hr - 2.2 (1.8-2.2)	
Bluegill (Lepomis macrochirus)	$C_{14.7}$ AE_1S	Static, 20°C, Fish - 1.0 g, pH - 7.1, hardness - 35 mg/l $CaCO_3$	LC_{50} 24 hr - 2.79 (2.22-3.52) 48 hr - 2.14 (1.78-2.58) 96 hr - 1.90 (1.5502.31)	Procter and Gamble Co., unpublished data

TABLE 4-B (Continued)

Source	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Bluegill (Lepomis macrochirus)	C_8AE_3S	Static, 21°C, pH 7.1, hardness-35 mg/l $CaCO_3$	LC_{50} 24 hr - >250	Procter & Gamble Co. unpublished data
	$C_{10}AE_{2.1}S$		375	
	$C_{12}AE_{2.1}S$		87	
	$C_{12}AE_{3.0}S$		73**	
	$C_{12}AE_{3.0}S$		37**	
	$C_{13}AE_{3.0}S$		24	
	$C_{14}AE_{1.9}S$		4.3	
	$C_{14}AE_{2.6}S$		>5.7<7.5	
	$C_{14}AE_{3.0}S$		7.1	
	$C_{14.7}AE_1S$		1.9	
	$C_{15}AE_{2.6}S$		>2.1<2.4	
	$C_{15.9}AE_{2.1}S$		0.3	
	$C_{17.9}AE_{1.9}S$		10.8	
	$C_{19.6}AE_{1.1}S$		15	

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

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

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

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

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

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

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

2. Invertebrates

Several studies have been conducted to determine the toxicity of AES to the water flea (Daphnia magna). A 24 hr LC_{50} value was reported for $C_{12-14}AE_3S$ (ammonium salt) of 16.3 mg/l. The sodium salt of this compound showed a LC_{50} value of 18.9 mg/l (Continental Oil Co., unpublished data).

Another study found that the 24 hr LC_{50} for the same species was 19.6 (18.1-21.4) mg/l using $C_{11-16}AE_3S$ (Unilever Research Laboratories, unpublished data).

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

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

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

B. Toxicity of AES to Microorganisms

The effect of AES on microorganisms in relation to biodegradation was discussed in Section III.A.5.a. Lundahl et al. (1972) examined the

bactericidal effect of $C_{12}AE_3S$, $C_{12}AE_3S$ (Ziegler-derived) and $C_{12-14}AE_{2.2}S$ (natural-alcohol-derived) on E. coli. The lowest concentrations which prohibited the development of more than 5 colonies per plate (for 5 days at 37°C) were 18, 4, and 2 g/l, respectively.

The MAC_5 (minimum algistatic concentration for a 5-day exposure) for three species of algae is as follows:

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

(Procter & Gamble Co. unpublished data)

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V. HUMAN SAFETY

A general consideration of the human safety of surfactants was presented in Chapter 1 (LAS, p. 134).

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

A. Animal Studies

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

Signs of acute oral toxicity noted in rats following AES administration at concentrations approaching LD_{50} values include an immediate decrease in motor activity, a decreased respiratory rate, ruffed fur and abdominal griping and diarrhea which often persists up to 6 days post-dosing. Nearly all deaths

occur within 24-48 hours of dosing with loss of coordination, prostration, and deep breathing evident prior to death (unpublished data: Ethyl Corporation; Procter & Gamble Company).

Acute Toxicity - Dermal. In the rabbit, the dermal LD_{50} values reported for AES on both intact and abraded skin ranged from 4700 to 12,900 mg/kg (unpublished data: Continental Oil Co.; Ethyl Corp.; Procter & Gamble Co.). With concentrations approaching LD_{50} values, moderate to severe erythema and edema are generally noted at 24 hours with severe desquamation and fissuring evident at the end of one week. Death generally occurred 3-4 days after treatment (unpublished data: Ethyl Corp.; Procter & Gamble Co.).

Acute Toxicity - Inhalation. Rats (number unspecified) survived a one-hour inhalation exposure to a 60 mg/l concentration of a 59% active solution of n-NH_4C_{12-14} (40:60) AE_3S delivered at a flow rate of 7ℓ/min. (Continental Oil Co., unpublished data). No additional information was available.

Acute Irritation - Ocular. Undiluted AES were determined to be positive eye irritants and were classified as corrosive in tests done according to the Draize procedure. Eye irritation studies in rabbits with several undiluted AES (e.g., $C_{10-12}AE_3S$, $C_{12-13}AE_3S$, $C_{12-13}AE_6S$, $C_{14-16}AE_{11}S$) resulted in extensive corneal damage, inflammation and hemorrhage of the iris and maximal conjunctival irritation with no significant improvement seen over a 7-day period (unpublished data: Continental Oil Co.; Ethyl Corp.; Procter & Gamble Co.).

A 10% aqueous solution of $NaC_{12-14-16}AES$ (STEOL-CS-125™) was found to be moderately irritating according to the Draize procedure (Stepan Chemical Co., unpublished data). In another study, a 10% aqueous solution of $C_{12-13}AE_6S$ (26.7% active) produced iritis and slight to moderate conjunctivitis which cleared by 2 days (Procter & Gamble Co., unpublished data.). One to two percent aqueous AES solutions produced only minimal conjunctival irritation (Brown and Muir, 1970; unpublished data: Ethyl Corp.; Procter & Gamble Co.).

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

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

skin irritants according to the Draize procedure.

In a comparison of the skin irritancy of natural-alcohol-derived versus synthetic-alcohol-derived AES, mild to moderate erythema with slight scaliness was seen in rabbits 14 days after application of a 40% aqueous solution of synthetic-alcohol-derived $NaTE_3S$ (54.4% active). With a 40% aqueous solution of the natural-alcohol-derived $NaTE_3S$ (40.6% active), mild erythema but with spotted necrosis leading to permanent scar tissue was seen in two or three rabbits and involved approximately 25% of the test site (Procter & Gamble Co., unpublished data). The natural-alcohol-derived product appears to be more acutely irritating to rabbit skin than the synthetic product.

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

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

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

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

Connor et al. (1975, 1976) eventually identified the sensitizers in AES (LES 13-2035) to be 1-dodecene-1, 3-sultone, 1-tetradecene-1, 3 sultone,

2-chloro-1, 3 dodecene sultone and 2-chloro-1, 3-tetradecene sultone. The authors speculated that perhaps small quantities of dodecene and tetradecene were present during the sulfonation reaction and were carried throughout the manufacturing process. Ritz et al. (1975) have recently tested the above sultones in guinea pigs and found them to be very potent sensitizers.

Subacute Toxicity - Oral. No adverse effects were observed in rats (12/sex/group) fed diets containing 40, 200 or 1000 ppm of either $C_{12-15}AE_3S$ or $C_{12}AE_3S$ for 91 days. At a 5000 ppm level, however, increased organ weights were seen in both treatment groups. Both male and female rats fed 5000 ppm $C_{12-15}AE_3S$ had increased liver weights. In the 5000 ppm $C_{12}AE_3S$ group, increased kidney weights were noted in males and increased kidney, liver and heart weights in females. Histopathological examinations revealed no tissue abnormalities. The 5000 ppm $C_{12-15}AE_3S$-treated males also exhibited a significant increase in serum total protein concentration (6.2 g/100 ml vs 5.9 for controls) but the ratios of serum protein fractions were unaffected (Walker et al., 1967).

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

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

In a similar study, rats (20/sex/group/treatment) were given 0.01, 0.1 or 1% of either natural- or synthetic-alcohol-derived $NaTE_3S$ in the diet for 91 days. The only parameters which were significantly different from control values were red blood cell counts in females and food consumption and body weight gain in males on the 1% natural-alcohol-derived diets and the liver to body weight ratios for females given either the 0.1% natural- or 1% synthetic-alcohol-derived diets.

The red blood cell counts in females on the 1% natural-alcohol-derived diet were significantly lower (although within established ranges) than

controls (7.31 million/mm^3 vs 7.79 for controls). The males in this group showed significantly lower total food consumption (1902g vs 2051g for controls) and body weight gain (364g vs 393g for controls) when compared to control values. The liver to body weight ratios for females on the 0.1% natural- and 1% synthetic-alcohol-derived diets were significantly higher (3.63×10^{-2} and 3.61×10^{-2}, respectively) than control values (3.15×10^{-2}) (Procter & Gamble Company, unpublished data).

Subacute Skin Irritation. Sixty-five percutaneous applications of a 5% aqueous solution of $C_{12-13}AE_6S$ (26.7% active) to the intact skin of 6 restrained rabbits (2 ml/kg) over a 91-day period produced minimal to pronounced dermal irritation (erythema, drying, fissuring, desquamation). Histopathological examination of other tissues was unremarkable (Procter & Gamble Co., unpublished data). Similarly, 20 applications of this surfactant at the same concentration to the abraded skin of 6 restrained rabbits in a 28-day period resulted in localized dermal irritation (erythema, drying and desquamation) with papular eruptions seen by week 2 through week 4. Histopathology was normal (Procter & Gamble Co., unpublished data).

In two other 28-day skin irritation studies, unrestrained rabbits with abraded skin were treated with aqueous solutions of either $NaTE_3S$ or NH_4AE_3S. The first topical application contained 200 mg/kg of active ingredient; all applications thereafter contained 50 mg/kg of active ingredient. Mild skin irritation was observed with both treatments. Histological examination of

the tissues revealed mild skin irritation with $NaTE_3S$ and moderate to severe skin irritation with the NH_4AE_3S preparation. One of 12 rabbits treated with the latter preparation died from unknown causes on day 5 of the study; the tissues were too autolyzed to examine (Procter & Gamble Co., unpublished data).

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

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

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

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

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

Acute, Subacute and Chronic Toxicity - Summary. The alcohol ethoxy sulfates exhibit a low order of oral and dermal toxicity in test animals. Acute ocular and skin irritancy responses in rabbits to aqueous solutions of AES ranged from slight to moderate. Undiluted AES, however, are moderate to severe dermal irritants and positive eye irritants in rabbits.

No significant adverse effects were noted in rats administered AES concentrations up to 0.5% of the diet for periods of up to two years, nor were there any indications of a direct carcinogenic effect resulting from the ingestion of AES surfactants in two chronic feeding studies.

Mutagenicity. In a series of studies with a 55% AES:45% LAS mixture, no significant differences from control values were noted in a dominant lethal study or in vivo or in vitro cytogenetic studies. In the dominant lethal assay, male mice were orally administered either 100, 150 or 200 mg/kg subacutely or 500, 750, or 1000 mg/kg acutely of the surfactant mixture. No significant differences from water-dosed controls were observed in the mutagenic parameters (not specified) examined. Similarly, no significant differences in chromosomal anomalies were found in bone marrow cells of male rats given 40, 500 or 1000 mg/kg of the surfactant mixture orally, then killed 18, 24 or 48 hours post-dosing. Likewise, human leucocytes incubated for 18, 24 or 48 hours with 4, 40 or 200 µg/l of the surfactant mixture exhibited no increased incidence of chromosomal anomalies above the water control group (Procter & Gamble Co., unpublished data).

Carcinogenicity. No papillomas or other skin tumors were observed in 30 female Swiss mice following twice weekly percutaneous applications with a 5% aqueous solution (0.1 ml) of $C_{12}AE_3S$ for two years (Tusing et al., 1962). Further, thrice weekly percutaneous applications of a 10% aqueous solution of an 18.6% tallow alcohol ethoxy sulfate (TES) and 15.6% LAS formulation to 50 Swiss ICR mice for 18 months did not induce any carcinogenic response either on the skin or systemically (Procter & Gamble Co., unpublished data).

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

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

Similarly, no adverse parental toxicity or significant differences in either litter parameters or liability of offspring were noted in two generations of rats fed diets containing either 0.1%, $C_{12}AE_3S$ (Tusing et al., 1962)

or 1% (800 mg/kg/day) of a detergent formulation containing 55% TE_3S and 45% LAS (Nolen et al., 1975).

Teratogenesis. There are no studies available in which the potential teratogenicity of AES was specifically examined; however, several investigators have studied the effects of administering a commercial liquid detergent formulation containing both AES and LAS to pregnant mice, rats and rabbits (Iimori et al., 1973; Iseki, 1972; Nolen et al., 1975; Palmer et al., 1975). Except at dosage levels which were toxic to the dams, no significant differences in the litter parameters of laboratory animals compared to control values were noted in these studies. Levels up to 300 mg/kg of a mixture containing 55% TE_3S and 45% LAS given orally to rabbits on days 2 through 16 of gestation up to 800 mg/kg given to rats on days 6 through 15 of gestation gave no indications of any embryotoxic or teratogenic effects attributable to AES (Nolen et al., 1975). Details of the studies cited above have been previously reported (Chapter 1, LAS, pp. 145-150). There are no indications from the available data that detergent formulations containing AES at doses which are several orders of magnitude above possible human exposure levels posed any teratogenic hazard to laboratory test animals.

Pharmacology - Absorption and Excretion. In both man and rat, an oral dose of $C_{16}AE_3S$ (labeled with ^{14}C in the 1-position of alkyl group) was readily absorbed from the gastrointestinal tract and excreted principally via the urine (see Table 4-C) with lesser amounts found in feces and expired air. Conversely, an oral dose of $C_{16}AE_9S$ (also labeled with ^{14}C in the 1-position

of the alkyl group) was poorly absorbed by both species. Most of the recovered radioactivity was in the feces; less than 2% of the label appeared in rat bile within 72 hours. Small amounts of radioactivity were found in urine and expired air with less than 2% of the radioactivity remaining in the carcass, tissues and organs at 72 hours.

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

TABLE 4-C

EXCRETION OF RADIOLABELLED AES IN RAT AND MAN

% of Dosed Activity

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

McDermott et al., 1975

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

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

B. Human Studies

Ocular Irritation. Ten and 20% concentrations of a liquid formulation containing 9% active NH_4^+AES as the only surfactant were found to be non-irritating following instillation into the eyes of 20 human volunteers (Witco Chemical Corporation, unpublished data).

Mucosal Irritation. A 25% concentration of the above product was reported to be non-irritating to male and female genitalia when applied once daily for a two week period (Witco Chemical Corporation, unpublished data).

Skin Irritation. Patch tests with 0.25 ml of a 1% aqueous solution of $NaC_{12}AES$ in 50 human volunteers produced no reaction in 45 individuals and only a slight irritation in five subjects (Smeenk, 1969).

No irritation was observed in a 24 hr patch test on humans (average of 10 subjects) with 10% concentrations of 20% active ALFONIC 1412-S™, NEODOL-25-1S™, and NEODOL-25-3S™. Moderate irritation was seen, however, with 25% concentrations of these products in a 10-day occlusive patch test with ten test subjects. The completely linear alcohol-based material was somewhat less irritating than the two slightly branched derivatives; i.e., irritation scores at the end of 10 days were 0.15, 1.1, and 1.0, respectively, of a possible maximum score of 4 (Witco Chemical Corporation, unpublished data).

Skin Sensitization. Walker et al. (1973) have reported that clinical trials with more than 1500 batch of AES in 70,000 women gave no evidence of allergic response. Further, no sensitization occurred in humans exposed to either a 0.25% aqueous solution of $NaTE_3S$ (71 subjects) or a 0.1% aqueous solution of AE_6S (61 subjects) (Procter & Gamble Co., unpublished data).

A unique outbreak of severe allergic contact dermatis occurred in Norway in 1966. Associated with the use of a liquid dishwashing product containing 18.7% $C_{12}AES$, this outbreak was eventually traced to a particular batch of $C_{12}AES$ (LES 13-2035). Patch tests with a 30% concentration of the dishwashing product (6.5% AES) were performed on individuals who had developed dermatitis after use of the detergent. Positive responses occurred in all 23 individuals tested as well as in 3 of 29 controls. Similar tests with $C_{12}AES$ (LES 13-2035)

produced 18 of 18 possible positive responses and 5 positive responses in 29 control subjects (Magnusson and Gilje, 1973).

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

C. Epidemiology

Accidental Exposure. A 45-year-old factory worker employed in a plant which manufactured AES (LES 13-2035) developed dermatitis of the hands and other parts of the body some weeks after emptying this material from barrels into a mixer. Several of the barrels contained AES (LES 13-2035) contaminated with sultone sensitizers (1-dodecene-1,3-sultone, 1-tetradecene-1,3-sultone, 2-chloro-1,3-dodecene sultone and 2-chloro-1,3-tetradecene sultone). In patch tests with 1% aqueous solutions of batch LES 13-2035 of AES, another batch of AES, the extracted unsulfated matter present in batch LES 13-2035 and a distillate of the C_{12} alcohol raw material, the subject responded positively to batch LES 13-2035 which contained sultone sensitizers, to AES, and to the extracted unsulfated LES 13-2035, but not to the lauryl alcohol extract (Magnusson and Gilje, 1973).

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

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APPENDIX C - AES NOMENCLATURE AND ABBREVIATIONS

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

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

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

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

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

In Section III, the phrase "complete biodegradation" refers to complete primary biodegradation. The complete conversion of a surfactant to carbon dioxide, water and other inorganic compounds is referred to as ultimate biodegradation.

ALKYLPHENOL ETHOXYLATES

Synopsis

The alkylphenolethoxylates, although not presently used to any significant extent in household detergent products, still find considerable use in industrial and agricultural applications. As such, these surfactants find their way into the environment with the resulting possibility of human exposure.

At this time, there are no national water quality standards established in the United States for nonionic surfactants, and no information was found concerning the levels of nonionic surfactants present in U.S. waterways. However, concentrations of 0.01 mg/l to 1.0 mg/l have been detected in various natural waterways of Europe.

Although the data presently available on environmental levels of nonionic surfactants are sparse, the reported levels do not appear to adversely affect aquatic species. Laboratory studies with concentrations of APE which exceed environmental levels seem to indicate that these surfactants are slightly more toxic to fish than to mollusks, while crustaceans are less susceptible than fish. Immature members of both vertebrate and invertebrate aquatic species appear to be more sensitive than adult members of the same species, and for most aquatic organisms, the initial 24 hours of surfactant exposure appear to be the most critical period with respect to toxicity.

Until recently, considerable controversy existed as to what extent APE are biodegraded, if at all. It is now generally accepted that APE undergo primary biodegradation provided sufficient acclimation time is allowed. Ultimate degradation of APE, however, has not been demonstrated.

An assessment of the fate of APE and the effects directly attributable to APE in the environment is difficult due to several factors. First, the alkyl chain attached to the phenyl ring can be either linear or branched in nature. Second, the industrially important APE consist of a complex mixture of materials with a range of ethoxylate chain lengths, usually from four to twenty ethylene oxide units. Finally, APE as a complex mixture of chemical entities exhibit wide variations in biodegradation and aquatic toxicity.

In relation to human safety, animal studies with chronic oral exposure to APE (1000 mg/kg/day for 2 yr) a level which exceeds estimated human intake by several orders of magnitude resulted in no deleterious effects on test animals. However, oral adiminstration of APE surfactants in a narrow molecular weight range (APE_{15} - APE_{25}) has been linked to an increased incidence of cardiotoxicity in certain laboratory animals. Acute irritation studies indicate that APE present no substantial problem from accidental cutaneous or ocular exposure. There was no indication from chronic feeding studies that APE exhibited carcinogenic activity in experimental animals. No studies were found concerning mutagenic, teratogenic or reproductive effects.

From the information available at this time, it appears that the use of APE does not pose either an environmental hazard or a significant risk to

human health. However, some areas of uncertainty such as the findings of cardiotoxicity of certain types of APE should be actively pursued along with mutagenesis, teratogenesis and reproduction studies.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

ALKYLPHENOL ETHOXYLATES

I. INTRODUCTION

Alkylphenol ethoxylates (APE) are one of several groups of chemical entities classified as nonionic surfactants. The use of APE has diminished considerably with the introduction of newer, more readily degraded nonionics (e.g. alcohol ethoxylates) so that, at the present time, there is little APE in domestic household products. This review was prepared to evaluate information on APE with respect to:

(1) environmental fate and distribution, including biodegradation,

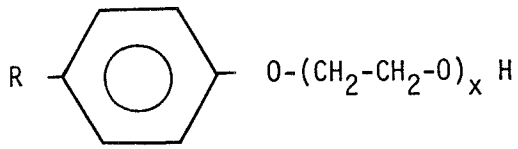
(2) effects on wild and domestic flora and fauna,

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

Toxicity studies with a variety of APE in laboratory animals and aquatic species at concentrations that exceed levels detected in the environment give no indication that the use of APE poses either an environmental hazard or a significant risk to human health.

Commercially, alkylphenols are manufactured by the addition of phenol to the double bond of an olefin in the presence of a catalyst. Reaction catalysts include sulfuric, boric, oxalic, tetraphosphoric and toluene sulfonic acids, ferric chloride or boron trifluoride. Of these, boron trifluoride is the most widely used for the production of surfactant-grade alkylphenols. (Enyeart, 1967) In general, the boron trifluoride catalyzed reaction yields a

monoalkylphenol with approximately 90% of the product substituted in the para position. If an excess of olefin is present, dialkyl derivatives are found in increasing amounts, with substitution occurring mainly in the ortho and para positions. (Enyeart, 1967) The alkyl phenol is purified by distillation, then reacted with several moles of ethylene oxide to produce APE. The addition of varying amounts of ethylene oxide to the alkylphenol enables the production of a diverse number of products which exhibit a wide range of solubility and performance characteristics.



Typical APE Surfactant

With respect to the amounts of material produced on an annual basis, the U.S. International Trade Commission (1975) figures indicate a total of 1.124 billion pounds of nonionics of all types were produced in 1973. The actual use of nonionic surfactants in household cleaning products cannot be determined from these Trade Commission figures. In their broad range of applications, nonionics are frequently intermediates which are chemically modified so that they are no longer nonionic. A survey by the Soap and Detergent Association of detergent manufacturers in the United States revealed that over 80% of the nonionics used as such in household and institutional cleaning products during 1965 fell into 4 categories: primary alcohol ethoxylates, secondary alcohol

ethoxylates, alkyl ethanolamides, and alkyl amine oxides. The remaining types of nonionics, including APE, made up less than 4% of the total surfactants used in household cleaning applications. (Mausner et al., 1969)

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

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II. ENVIRONMENTAL LEVELS

A. Analytical Methods

1. Nonionic Surfactants

A review of the analytical techniques employed in the determination of the presumptive levels of nonionic surfactants in the environment and in biodegradation studies can be found in Chapter 3 (AE, II.A.). Briefly, these methods include foaming potential, changes in surface tension, detection of either cobalt thiocyanate or bismuth iodide active substances and thin-layer chromatography.

B. Water Quality Standards

The nonionic surfactants are not usually differentiated for standard and regulatory purposes. The discussion of this area can be found in Chapter 3 (AE, II.B.).

C. Alkylphenol Ethoxylates (APE) in Natural Water Bodies

Levels of nonionics, including alkylphenol ethoxylates, in the environment have been discussed in Chapter 3 (AE, II.C.). APE are not presently being monitored, as such, in the United States or Europe.

III. BIODEGRADATION

A detailed examination of the procedures employed to study the biodegradation of nonionic surfactants can be found in Chapter 3 (Section II.A.) while a discussion of the influence various factors exert on the rate of biodegradation of surfactants in general was provided in Chapter 1 (LAS, III). Based on that foundation, this section will discuss these topic areas as they specifically apply to the biodegradation of alkylphenol ethoxylates (APE).

A. Laboratory Test Systems Used in Nonionic Studies

1. CO_2 Evolution

Using a bottle test procedure, Sturm (1973) examined the biodegradation of linear C_9APE_9 and $C_{10}APE_9$ via foam reduction, removal of CTAS (cobalt thiocyanate active substances) and CO_2 production. He reported that although significant reductions in foam and CTAS occurred within 4 days, less than 20% of the theoretical CO_2 yield was evolved during this time period. Furthermore, CO_2 was found to evolve slowly, reaching a plateau of approximately 40% after some 26 days on test. A branched alkyl chain reduced biodegradability even further; at 26 days, CO_2 production was approximately 5% of theoretical with br-C_9APE_8.

Gledhill (1975) also reported a low degree of ultimate degradation (<20% CO_2 evolved) with br-C_8APE using his CO_2 evolution, shake flask procedure.

2. Die-Away Tests

a. River Water Test

Using a cobalt thiocyanate analysis, Allred and Huddleston (1967) reported that within 5 days, 53% of a sample of straight chain derived $C_9APE_{65\%}$ (equivalent to an average of 9.3 EO units per mole) had degraded compared to 20% of a branched-chain $C_9APE_{65\%}$. At 20 days, the 2 ethoxylates had undergone primary degradation (65% and 25%, respectively); at 30 days, the br-$C_9APE_{65\%}$ had degraded 54% while the extent of degradation for the straight chain surfactant had not changed.

Similar results were found by Frazee et al. (1964). Employing IR and UV analyses, these investigators found that a br-C_9APE_{10} degraded appreciably (10EO to 4EO) between the second and third weeks of the study.

The rate of degradation appears to be dependent upon the degree of acclimation. Lashen and Booman (1967) report that degradation (foam loss) of TRITON X-100™(C_8APE_{10}) in a river water die-away test was most rapid (4-7 days) in river water taken below heavily industrial areas where nonionic use was high; degradation was slower with water taken from areas in which nonionic use was low. Similar results were reported by Kurata and Koshida (1975).

b. Shake Culture Test

Using the shakeflask procedure, Asahara et al. (1972) compared the extent

of primary degradation of br-$C_9APE_{9.5}$. Results indicated a 90% to 97% reduction in foam volume and CTAS at 6 days.

Allred & Huddleston (1967), however, reported only 65% degradation (CTAS) in 8 days with straight chain derived $C_9APE_{65\%}$ and found only 30% degradation for its branched-chain equivalent under the same test conditions.

An even larger difference in results depending on methodology was reported by Sekiguchi et al. (1972). Using Bunch-Chambers media, these investigators found a 94% (CTAS) decrease in C_9APE concentration within 1 day. However, simultaneous analysis for total organic carbon revealed a rapid decrease for the first 3 days, but no significant decrease thereafter; 47% of the organic carbon still remained after 31 days.

Swisher (p. 56, 1970) believes that inherent limitations in the applicability and interpretation of the cobalt thiocyanate analysis are responsible for such diverse results. (See Chapter 3, Section II.A.2.b.).

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

Using standard STCSD procedures, Stead et al. (1972) observed that by 21 days, a linear (C_7-C_9), but randomly attached APE_9 was degraded significantly more (61%) than a C_9 propylene-trimer-derived APE_8 (14%) as determined by foam volume measurements. The 2-ortho attached C_9-C_{10} linear APE_9 underwent complete

primary degradation (98%) under the same test conditions.

3. Simulated Treatment Processes

a. Activated Sludge

Sato et al., 1973 (cited in Oba, 1974) obtained >90% removal in 24 hours (colorimetric analysis) by aerating br-C_9APE_{10} with activated sludge, and Allred and Huddleston (1967), using a semicontinuous activated sludge method reported 75-95% degradation (CTAS) in 24 hours with straight chain derived $C_9APE_{65\%}$ (equivalent to an average of 9.3 EO units per mole) and 60-80% degradation for its branched-chain equivalent in the same time period. Booman (unpublished data), however, found that if the activated sludge unit was maintained in operation beyond 24 hours, the extent of biodegradation, even for a branched-chain APE was considerably increased, and by 75 days, surfactant levels (foam analysis) were insignificant.

A comparison of the results obtained from the OECD (1971) confirmatory test and from a modification of this procedure, a coupled units test (see Chapter 1, Section II, LAS) was undertaken by Fischer and Gerike (1975) using n-C_9APE_9 and br-C_9APE_{10}. A 10 mg/l concentration of br-C_9APE_{10} showed greater than 90% reduction (method of Wickbold, 1972) in the uncoupled OECD procedure but with the coupled units test (mean retention time of 6 hours), removal of 68% COD was seen. Similar findings were noted with 20 mg/l of n-C_9APE_9, 94% loss with the OECD procedure and 49% COD with the coupled units test (3 hr retention time).

Believing that the variability of laboratory activated sludge tests was due to the state of acclimation of the sludge used, Lashen and Lamb (1967) measured the time required for samples of activated sludge obtained from domestic and industrial sewage treatment plants to acclimate to 10 mg/l of TRITON X-100 (C_8APE_{10}) by 90% (foam analysis) in a semi-continuous activated sludge process. They found that the degree of acclimation of microflora to TRITON X-100 was extremely varied; the acclimation time (i.e., the time needed to achieve 90% reduction in foam in 24 hours) ranged from less than 4 days to up to 90 days depending on whether the sewage treatment plant handled industrial or domestic wastes, respectively.

b. Trickling Filters

Stennett and Eden (1971) found that approximately 30% of C_8APE_8 had degraded (analytical method unspecified) in a percolating filter apparatus within 2 weeks. Once acclimated, greater than 70% of the original sample had degraded by 3 weeks, and by 10 weeks, better than 90-95% of the original sample was removed.

c. Anaerobic Systems

The only data currently available on anaerobic degradation were reported by Booman and co-workers (Booman et al., 1966; Lashen et al., 1966). Employing a bench-scale septic tank system, they found that a branched-chain C_8APE_{10} which was randomly tagged with ^{14}C in the ethoxylate chain and with ^{3}H in the

aromatic ring, degraded between 58% (CTAS) and 63% (foam loss). The loss of ^{14}C was only 7%. If the effluent was then passed through a percolation field, overall degradation values ranged from 84% (foam loss) to 93% (CTAS). The average total loss of ^{14}C was 46% with a maximum removal of 60% to 65% at the end of study. No loss of ^{3}H was seen, indicating that the loss in ^{14}C was not due to adsorption.

4. Influence of Temperature on the Test System

The rate of biodegradation of APE appears to be sensitive to variations in temperature (Mann and Reid, 1971; Rudling and Solyom, 1974; Stiff et al., 1973; Stiff and Rootham, 1973). In a field study with NONIDET P40™ (C_8APE_{8-9}) and NONIDET P100™ (C_8APE_{14-15}) in a trickling filter sewage treatment plant, Mann and Reid (1971) reported only 20% of the APE degraded (TLC method of Patterson et al., 1966) in the winter months while 80% degraded during the summer.

In another study, temperature was seen to affect the rate of degradation of br-C_9APE consisting of tripropylene phenol ethoxylates with 8, 10, 14, 16 and 30 EO units under the degradation conditions of the OECD (1971) screening test. The length of the ethoxylate chain produced no significant differences in C_9APE biodegradability (Wickbold's method, TLC and GC); all were found to undergo >90% primary degradation within 12 days of incubation at 20°C. GC analysis of C_9APE_8, C_9APE_{10} and C_9APE_{14} samples incubated 4 days at 20°C revealed that a C_9APE_2 derivative was the major degradation product. After 28 days at this temperature, approximately 50% of the C_9APE_2 derivative had been degraded.

No degradation of the C_9APE_2 derivative was observed at 15°C. Additionally, it was also determined via benzene extracts of activated sludge and effluent that surfactant removal was due to biodegradation and not to adsorption onto the activated sludge (Rudling and Solyom, 1974).

Stiff et al. (1973) examined the removal of NONIDET P40 (C_8APE_{8-9}) from an activated sludge treatment plant at 8°, 12° and 15°C. Removal of NONIDET P40 was extensive at 15°C. At lower temperatures, degradation was found to be dependent on surfactant concentration. At 5 mg/l, greater than 90% removal was observed regardless of temperature, but at 20 mg/l, the degree of removal varied considerably; 40-95% with a mean of 68% at 12°C and 20-80% with a mean of 50% at 8°C. The authors suggest that at 12°C or below and a surfactant concentration of 20 mg/l, a stable population of microorganisms adapted to NONIDET P40 could not be maintained. In a separate paper by these investigators (Stiff and Rootham, 1973), the biodegradation of ETHYLAN TU™ (br-$C_9APE_{8.5}$), NONIDET P40 (br-C_8APE_{8-9})and ETHYLAN MO-91™ (n-APE_9) was compared at 5°, 8°, 12° and ∿20°C at the same surfactant concentrations and under identical test conditions. No significant differences from the earlier study were found as to the degree of APE removal; all were well degraded (>90%) at 20°C, but exhibited large fluctuations below 12°C.

B. Field Tests

There is a noticeable shortage of available data that deals with the performance of APE in the field. However, the data that are currently available indicate that APE do undergo primary biodegradation under field conditions.

Lashen and Booman (1967) used the cobalt thiocyanate and thin-layer chromatography methods to determine the biodegradation of TRITON X-100 (C_8APE_{10}) in an extended aeration-activated sludge sewage treatment plant. They found that the removal of 5 and 10 mg/l of this surfactant was greater than 90% after 20-44 days.

The biological treatability of TRITON X-100 (C_8APE_{10}) was examined in a package-type, extended-aeration, activated sludge treatment plant which served the entire waste treatment needs of a small college. Flow proportional spiking was conducted to provide TRITON X-100 concentrations of 4 and 10 mg/l in 2 of 3 separate aerators; the third aerator served as a control. Surfactant concentrations (CTAS) were determined for raw sewage, clarifier effluents and mixed liquors. The surface tension and foam volume of effluent samples were also determined. The test, initiated at the end of August, was run for 44 days; a second phase of the study in which only the 10 mg/l concentration was examined, continued from day 45 to day 71.

The authors observed greater than 90% removal (CTAS) which was associated with a high loss (90-95%) in the ability to foam or lower surface tension. Negligible adsorption (1-3 mg nonionic detergent/gm suspended solids) was seen during the test (Lashen and Lamb, 1967; Rohm and Haas, 1967).

In a field trial with NONIDET P40 (C_8APE_{8-9}), Mann and Reid (1971) found that the rate of degradation in a trickling filter sewage treatment plant corresponded to the temperature of the sewage and the filter bed. Mann and Reid offer two possible explanations for this, beyond assuming merely a temperature effect on reaction rate: (1) bacteria in the sewage which are capable of

degrading APE in the summer are absent or dormant in the winter; (2) the rate of bacterial film growth on the filter bed is influenced by climatic conditions and alters the characteristics of the sewage treatment process. (See Section III. A.4. of this chapter for further details.)

C. Effect of Chemical Structure

Perhaps due to the lack of reliable methods of analysis, few investigators to date have studied the influence that the chemical structures of the hydrophilic and hydrophobic portions of an APE molecule exert on the rate of biodegradation.

A Gram-negative bacillus ("strain KW7")isolated from sewage by selective culture degraded CONOX J099^{TM} ($C_9APE_{9.5}$), and br-$C_9APE_{9.5}$ via stepwise shortening of the ethoxylate chain and accumulation of 1,2 and 3 ethoxylate residues. No significant degradation of the benzene ring or the branched alkyl chain was noted. Degradation was determined by Wickbold's (1972) method and the TCL procedure of Patterson et al. (1966) (Unilever Research Laboratories, unpublished data).

Osburn and Benedict (1966), using UV and IR analyses, reported that APE degradation proceeded by two routes: carboxylation of the alkyl group, and degradation of the ethylene oxide chain. Their results indicated that both branched and linear structures containing 10 or less EO units/mole degrade by both routes. Degradation of the EO chain (apparently via a hydrolytic mechanism) occurs only when the chain contains 10 or less EO units. Linear structures with more than 10 EO units/mole are preferentially degraded by carboxylation of the alkyl group while branched-chain structures containing more than 10 EO units/mole were found to be essentially non-biodegradable in their system (river water die-away test).

Utilizing a shake flask test with C_8APE_{10}-acclimated bacteria, Booman et al. (1965) found that $C_8A_{12.5}$ degraded (CTAS) completely in 4 days, C_8APE_{10} in 4-5 days and $C_8APE_{7.5}$ in 5-7 days, while Huyser (1961) reported that resistance to degradation of APE increased with the number of EO units and with the degree of branching of the alkyl chain. Similar findings have been reported by Garrison and Matson (1964), Blankenship and Piccolini (1963) and Steinle et al. (1964).

The work done in this area was summarized by Osburn and Benedict (1966), indicating that:

(1) the degree of branching of the alkyl chain influences the rate of degradation - less branching results in a faster rate of degradation;

(2) a decrease in the rate of biodegradation is observed with an increase in the number of EO units/mole;

(3) the position of attachment of the benzene ring to the straight alkyl chain has a considerable influence on the rate of degradation - primary attachment produces a faster rate than secondary attachment. (See Swisher, pp. 319-320, 1970.)

D. Metabolic Pathways of Biodegradation

A brief discussion of a few selected areas concerning the metabolic pathways of biodegradation of APE surfactants follows.

Much controversy has centered around the extent of APE biodegradation. In recent years, several investigators have established that APE do undergo primary biodegradation, and Gledhill (1975) and Sturm (1973) have shown some degree (but not 100%) of ultimate APE biodegradation. The three principal points of possible metabolic attack on an APE molecule are the hydrophobic alkyl chain, the hydrophilic ethylene oxide chain and the aromatic ring. It is now generally agreed that if allowed sufficient acclimation time, APE will undergo substantial primary biodegradation via shortening of the EO chain and some carboxylation of the alkyl chain, perhaps by ω-oxidation (Osburn and Benedict, 1966; Lashen et al., 1966; Swisher, pp. 320-21, 1970), but the extent of further metabolism of the alkyl chain or benzene ring is unknown. No evidence for formation of a free phenolic group in the course of degradation has been reported.

Patterson et al. (1970) found that the usual route of degradation (TLC) for C_9APE_9 during a 6-week study using a batch die-away activated-sludge procedure was slow oxidation and hydrolysis of the alkyl groups, the aromatic ring and the ethoxy chain, simultaneously. When the pH was raised from pH 7 to pH 9.2, the hydrolysis of the ethoxylate chain was found to proceed at an increased rate.

Rudling (1972) reported the formation of C_9APE_2 as the single major product from a br-C_9APE_{10} and Booman and co-workers (Booman et al., 1965; Lashen et al., 1966) found that when a br-C_8APE_{10} which was randomly tagged with ^{14}C in the ethoxylate chain and with ^{3}H in the aromatic ring was added to a septic tank-percolation field system, a maximum loss of 60-65% ^{14}C was observed. This

loss was attributed to conversion of the ethoxylate chain to $^{14}CO_2$ and/or bacterial protoplasm. (See III. A.3.c. of this chapter for additional details.)

Cain (1974) points out that such release of EO units, one at a time, may be effected by:

(1) hydrolysis,

(2) oxidative cleavage of the ether bridge, or

(3) oxidation of the α-carbon atom and hydrolysis of the resulting ester.

In conclusion, APE have been shown to undergo primary biodegradation but the rate of ultimate biodegradation is generally found to be slow. The major degradative pathway appears to be shortening of the ethoxylate chain. Carboxylation of the alkyl chain has been demonstrated but the extent of degradation of either the alkyl chain or the benzene ring is not known at this time.

E. Summary

Attempts to characterize the course of biodegradation of APE surfactants have produced variable results. Recent studies indicate that, given sufficient acclimation time, APE do undergo primary biodegradation in a variety of test systems and field studies. The course of ultimate biodegradability of APE, however, is slow. The influence of modifications in ambient temperature on the

rate of primary biodegradation, as well as the role played by a particular APE configuration were also noted.

The major degradative pathway for alkylphenol ethoxylates appears to be via shortening of the ethoxylate chain, although some degree of carboxylation of the alkyl chain has been demonstrated.

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IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

The toxicity of APE has been evaluated for a variety of species, under a wide range of conditions; however, little work has been done on the toxicity of intermediaries. The results suggest that levels presently reported in the aquatic environment do not approach LC_{50} values. However, sublethal effects occur at APE levels less than 1 mg/l.

The advantages and limitations of the various methodologies of acute toxicity tests have been discussed in Chapter 1 (LAS pp. 99-100).

1. Acute Toxicity - Fish

Table 5-A summarizes the acute toxicity data available for fish. Several points are evident from this compilation. First, although many different species and surfactants were tested using varying methods, most of the LC_{50} values fall between 4 and 12 mg/l. Some rough correlations between toxicity and age of test species, time, temperature and hardness can be made.

Table 5-A Acute Toxicity of Alkylphenol Ethoxylates to Fish

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Rainbow trout (Salmo gairdnerii)	C_9APE_8	fish - 12-16 cm, semicontinuous flow, 15-15.6°C, pH 7.3-7.4, hardness 290-310 mg/l $CaCO_3$	LC_{50} 24 hr - 5.50 (5.0-6.05)* 96 hr - 4.70 (4.12-5.35) 14 day - 4.25 (3.97-4.54)	Calamari and Marchetti (1973)
	$C_9APE_{9.5}$	fish - 7-11 cm, 24 and 48 hr. solution change over, 11° ± 2°C, pH 7.6-8.4, hardness 179 mg/l $CaCO_3$. 10 fish/conc.	LC_{50} 96hr - 10-12.5 48 hr. changeover 96 hr. - 7.5-10.0 24 hr. - change over	Unilever Research Laboratories, unpublished data
	$C_9APE_{9.5}$ biodegradation products		96 hr. - 4.5-5.9 (as concentration of parent nonionic)	"
	C_9APE_{10}	fish - 6 ± 1 cm, static, 10 ± 1°C, hardness 210 mg/l	LC_{50} 24 hr. - 7.5 (7.2-7.8)	Unilever Research Laboratories, unpublished data
		fish - 8-10 cm, static, 15 ± 0.5°C hardness 20 mg/l $CaCO_3$	LC_{50} 24 hr - 9.8 (7.8-11.1) 48 hr - 9.4 (7.4-10.9)	"
		fish - 3.0 cm, continuous flow, 15 ± 1°C, hardness 300 mg/l $CaCO_3$	LC_{50} 24 hr range (7.3-10.5)** 48 hr - 7.4 (6.3-9.0) 96 hr - 5.8 (4.6 - 7.4)	"

*95% Confidence limits.
**No LC_{50} could be calculated. This range represents the LC_0-LC_{100}.

Table 5-A (Continued)

Species	Surfactant	Experimental Conditions	Toxicity	Source
Rainbow trout (continued)	C_9APE_{10} (continued)	fish - 5 ± 1 cm, static 20°C hardness 62.5 mg/l $CaCO_3$	LC_{50} 24 hr - 11.7 (11.0-12.7)	Unilever Research Laboratories, unpublished data
		fish - average 7.5 cm, static 20°C hardness 150 mg/l $CaCO_3$	LC_{50} 24 hr - 6.0-10.0** 48 hr - 7.1 (6.2-8.0)	"
	C_9APE_{10}	static, pH 7.3-7.4, 30-40 alevins/conc. hardness 240-260 mg/l 10 fry and fingerlings/conc.		Marchetti (1965)
		Stages:	LC_{50}	
		1 alevin (immed. after hatching)	3 hr - 62	
		2 alevin - 6 days	3 hr - 30	
		3 alevin - 12 days	3 hr - 13.5	
		4 alevin - 19 days	3 hr - 13.5	
		5 alevin-fry - 23 days	3 hr - 2.5	
		6 fry - 25 days	3 hr - 4.4	
		7 fry - 40 days	3 hr - 7.0	
		8 fingerling-210 days	3 hr - 8.0	

**No LC_{50} could be calculated. This range represents the LC_0-LC_{100}.

TABLE 5-A (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Bluegill sunfish (Lepomis macrochirus)	SURFONIC N-40,™ C_9APE_4	fish mean weight - 1.0 gram, static and dynamic, 18 ± 0.5°C, pH 7.1, hardness - 35 mg/l $CaCO_3$	LC_{50} static 24 hr - 1.5 (1.3-1.8)	Macek and Krzeminski (1975)
	SURFONIC N-95,™ C_9APE_9		LC_{50} static 24 hr - 7.8 (6.2-9.9)	"
	IGEPAL CO-520,™ C_9APE_5		LC_{50} static 24 hr - 2.8 (2.4-3.2)	"
	IGEPAL CO-630,™ C_9APE_9		LC_{50} static 24 hr - 8.9 (5.9-13.6) dynamic 24 hr - > 10.0	"
	IGEPAL CO-880,™ C_9APE_{30}		LC_{50} static 24 hr - > 1000.0	"
	TRITON X-45,™ C_8APE_{10}		LC_{50} static 24 hr - 3.5 (3.1-4.0)	"
	TRITON X-100, C_8APE_{10}		LC_{50} static 24 hr - 16.2 (13.3-19.8) dynamic 24 hr - > 10.0	"
	TRITON X-305,™ C_8APE_{30}		LC_{50} static 24 hr - 1080.0 (663.0-1470.0)	"

TABLE 5-A (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Golden orfe (Idus melanotus)	C_9APE_{10}	Fish - 7 cm, static, 20 ± 1°C hardness - 150 mg/l $CaCO_3$	LC_{50} 24 hr - 9.5 (8.5-10.2) 48 hr - 9.5 (8.2-10.2)	Unilever Research Laboratories, unpublished data
		Fish - 5-7 cm continuous flow, 20°C, hardness - 268 mg/l $CaCO_3$	LC_{50} 24 hr - 7.4 (6.7-8.2) 48 hr - 7.4 (6.7-8.2) 96 hr - 7.0 (6.1-8.7)	"
	C_9APE_9	Fish - 5-7 cm, static, 20°C	LC_0 - 48 hr - 5 LC_{50} - 48 hr - 7 LC_{100} - 48 hr - 10	Fischer, personal communication, as cited in Gloxhuber (1974)
Harlequin (Rasbora heteromorpha)	C_9APE_{10}	Fish - 1.3-3.0 cm, continuous flow, 20°C, hardness - 20 mg/l $CaCO_3$	LC_{50} 24 hr - 12.3 (10.6-14.2) 48 hr - 11.3 (9.1-13.7) 96 hr - 8.6 (5.6-11.8)	Unilever Research Laboratories, unpublished data
Minnow (Phoximus phoxinus)	C_9APE_{10}	Fish - 6-9 cm, static, 17 ± 1.5°C hardness - 305 mg/l $CaCO_3$	LC_{50} 24 hr - 9.9 (9.3-11.0) 48 hr - 8.6 (8.3-9.0)	"

TABLE 5-A (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Golden Shiner (*Carassius auratus*)	C_9APE_{10}	Fish - 6 cm. static, 20°C hardness - 200 mg/l $CaCO_3$	LC_{50} 24 hr - 13.8 (9.7-17.8) 48 hr - 13.8 (9.7-17.8)	Unilever Research Laboratories, unpublished data
Guppy (*Lebistes reticulatus*)	C_9APE_{11} (br-)	Fish - 2 cm., young - 0.7 cm. 25°C.	LC_{100} 24 hr-female-57 24 hr-male-64 24 hr-young-52	Van Emden *et al.* (1974)
	LISSAPOL™ NXA (C_9APE)	--	Deleterious effects encountered above 7 mg/l*	Madai and An der Lan, (1964) as cited in: Gloxhuber (1974)
	HOSTAPAL™ (APE)	--	Deleterious effects encountered above 4-6 mg/l*	"

*No additional information was provided.

TABLE 5-A . (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Brown trout (*Salmo trutta*)	C_9APE_{10}	Fish - 2.8 or 5.8 cm continuous flow, 15°C, hardness - 26-36 mg/l $CaCO_3$	LC_{50} 24 hr - 1.3 (1.0-2.0) 48 hr - 2.7 (1.8-60.7)	Unilever Research Laboratories, unpublished data
Cod (*Gadus morrhua*)	C_9APE_{10}	Fish - 30 cm, continuous flow, 6-8°C, or 15-17°C, at least 5 fish/ conc.	LC_{50} 96 hr - 2.5, 15-17°C 6.1, 6-8°C	Swedmark *et al.* (1971)
Flounder (*Pleuronectes flesus*)	C_9APE_{10}	Fish - 30 cm., continuous flow, 15-17°C. At least 5 fish/conc.	LC_{50} 96 hr - 3.0	"

Marchetti (1965) tested rainbow trout of different ages. The alevin was most tolerant of C_9APE_{10} immediately after hatching, and could tolerate 2 and 5 times, respectively, the concentrations tolerated by alevins 6 and 19 days old. Alevins with completely absorbed yolk sac (stage 5) were 20 times more susceptible than newly hatched alevins, showing respective 3 hr LC_{50} values of 2.5 and 62 mg/l. Tolerance increased in the fry and the fingerlings.

Van Emden et al. (1974) found that young guppies were slightly more sensitive than adults. However, newly hatched guppies were not tested.

Experimental conditions affect the toxicity of surfactants to fish. Temperature may be an important factor in determining toxicity. Rainbow trout were tested at several different temperatures; however, other variables were not held constant (Unilever Research Laboratories, unpublished data). Tests with cod suggest that this species may be more susceptible to C_9APE at higher temperatures (Swedmark et al., 1971). No correlation between water hardness and toxicity of C_9APE was observed although this has been found for other surfactants (Unilever Research Laboratories, unpublished data).

A surfactant's configuration may also affect its toxicity to aquatic organisms. Tests with $C_9APE_{9.5}$, C_9APE_8 and C_9APE_3 showed that toxicity increased with decreasing EO chain length for Salmo gairdneri, Gammarus pulex and Daphnia; however, LC_{50} values were given only for $C_9APE_{9.5}$ (Unilever Research Laboratories, unpublished data). Similar results were found for bluegill sunfish (Macek and Krzeminski, 1975). These authors found that APE with EO numbers of 30 were not very toxic to bluegill, with LC_{50} values of greater than 1000 mg/l.

In tests where toxicity values for various times are given, a slight increase in the LC_{50} value is usually seen with increasing time. Apparently, the first 24 hours is the period of greatest toxicity (Calamari and Marchetti, 1973; Unilever Research Laboratories, unpublished data). In support of this conclusion, it has been found that more frequent changing of test solutions increased the toxicity of APE. Analyses by the Wickbold (1972) method did not show a significant decline in concentration over 48 hours (Unilever Research Laboratories, unpublished data). It may be that metabolites are formed which are less toxic than the parent compound. On the other hand, the surfactant may be adsorbed on the sides of the container or the fish. This report also showed that partial degradation of C_9APE by a bacterium isolated from sewage produced materials somewhat more toxic to rainbow trout than the parent compound. On the other hand, it has been suggested that bluegill were less susceptible to APE degraded in an activated sludge unit than to the parent compound (GAF, unpublished data as cited in Macek and Krzeminski, 1975). These differences may be resolved to some extent with improvements in analytical techniques and better identification methods for intermediates in the biodegradation process.

2. Acute Toxicity - Invertebrates

A summary of acute toxicity values for invertebrates is shown in Table 5-B. It is difficult to compare results for different species from different studies, but it appears that most invertebrates are less susceptible than fish. In general, crustaceans have been found to be more tolerant than the mollusks.

Swedmark et al. (1971) have done most of the work which appears in Table 5 - B. Larvae of both the spider crab and the sessile Balanus balanoides were more susceptible than the adult forms. Further, they found that the intermoult stage of the decapod Leander adspersus was more tolerant to C_9APE_{10} than the post-moult stage. They also reported that higher temperatures increased the toxicity of this surfactant to mussels and clams.

Tests by Van Emden et al. (1974) suggest that the mosquito (Aedes aegypti) is very tolerant of C_9APE_{11}. Maxwell and Piper (1968) tested several different surfactants on Culex pipiens quinquefasciatus and found LC_{50} values ranging from 1 to >400 mg/l. Dinonylphenol-EO was the most toxic, followed by tridecylphenol-EO, monononylphenol-EO, and C_8APE. However, the length of the ethylene oxide chain also influenced toxicity. Compounds with extremely short EO chain length (an average of 1 EO per mole) or those with an extremely long chain were the least toxic.

The work by Corner et al. (1968) points out a problem in evaluating toxicity tests with formulations. They found that C_9APE was the least toxic component of an oil-spill-remover detergent. The stabilizer (coconut-fatty acid diethanolamide) and the solvent (kerosene extract) had LT_{50} (time in which a given concentration is lethal to 50% of the population) values for barnacles of 18 and 2 minutes, respectively, at 50 ppm BP 1002™ (compared to 120 minutes for C_9APE). As a result, toxic effects of any detergent formulation cannot necessarily be attributed to the surfactant.

Table 5-B Acute Toxicity of APE to Aquatic Invertebrates

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Arthopoda-crustacea				
Gammarus pulex	LISSAPOL NXA(C_9APE)	--	Deleterious effects appear above 1-2 mg/l	Madai and An Der Lan (1964) as cited in: Gloxhuber (1974)
Daphnia magna	C_9APE_{10}	Static, 20°C, hardness 202 mg/l $CaCO_3$	LC_{50} 24 hr - 44.2 (40.2-48.4)*	Unilever Research Laboratories, unpublished data
Elminius modestus Stage II nauplii (barnacle)	C_9APE_{10}	16-20°C	LT_{50} (min.) 500 ppm - 50 50 ppm - 120 25 ppm - 200 5 ppm - non-toxic	Corner et al. (1968)
Leander adspersus	C_9APE_{10}	continuous flow 6-8°C, 15-17°C at least 5 organisms/conc.	LC_{50} 96 hr - >100, 6-8°C adult 96 hr, 15-17°C intermoult stage-50 postmoult stage-10	Swedmark et al. (1971)
Leander squilla	"	continuous flow, 6-8°C, at least 5 organisms/conc.	LC_{50} - 96 hr >100	"
Eupagurus bernhardus (hermit crab)	"	"	"	"
Hyas araneus (spider crab)	"	"	LC_{50} - 96 hr adult - >100 96 hr Stage I zoea larvae - 10**	"

*95% Confidence Limit

**static tests used for larvae

TABLE 5-B (Continued)

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Balanus balanoides	C_9APE_{10}	continuous flow, 6-8°C, at least 5 organisms/conc.	LC_{50} 96 hr - <25 (adult) 96 hr - 1.5 (Stage II nauplius larvae)**	Swedmark et al. (1971)
Insecta				
Aedes aegypti (mosquito)	C_9APE_{11}	2nd and 3rd stage larvae, 25°C	LC_{50} - 24 hr - 500 LC_{100} - 25 hr - 1000	Van Emden et al. (1974)
Culex pipiens quinquefasciatus (mosquito)	various nonionics including C_9APE dinonylphenol ethylene oxide, C_8APE and tridecylphenol ethylene oxide adducts	pupae, static 25°C, pH 7.5-8.0	Wide range of LC_{50} values from 1 - >400 (see text)	Maxwell and Piper (1968)
Mollusca (Pelecypoda)				
Mytilus edulis (mussels)	C_9APE_{10}	adult, 6-8°C, 15-17°C continuous flow, at least 5 organisms/conc.	LC_{50} 96 hr - 12 (6-8°C) <10 (15-17°C)	Swedmark et al. (1971)
Mya arenaria (clam)	"	"	LC_{50} 96 hr - 18 (6-8°C) 96 hr - <10 (15-17°C)	"
Cardium edule (cockle)	"	"	LC_{50} 96 hr - 5 (6-8°C) 96 hr - <<10 (juvenile 15-17°C)	"

TABLE 5- B (Continued)

Species	Surfactant	Experimental Conditions	Toxicity	Source
Pecten maximus (scallop)	C_9APE_{10}	adult 15-17°C, continuous flow, at least 5 organisms/conc.	LC_{50} 96 hr - <<5 (15-17°C)	Swedmark et al. (1971)
Pecten opercularis	"	"	LC_{50} 96 hr - <<10	"
Gastropoda				
Biomphalaria glabrata (snail)	C_9APE_{11}	1.5-2 cm, static, 25°C	LC_{100} - 24 hr - 23	Van Emden et al. (1974)

In studies with a marine annelid, Scolelepis fuliginosa, Foret-Montardo (1971) found that storage affected the toxicity of LENSEX TA01™ (APE). The LT_{100} for 10 mg/l was 20 hours. After 7 days storage, the LT_{100} had dropped to 9.25 hours, and after 28 days, to 7 hours. At higher concentrations, toxicity decreased with storage.

3. Acute Toxicity - Microorganisms

The inhibition of algae by nonionic surfactants has been studied by several authors. Ukeles (1965) tested the Igepal series (CO-610™, APE_9; CO-630™, $APE_{9.5}$; CO-710™, $APE_{10.5}$; CO-730™, APE_{15}; CO-850™, APE_{20}; CO-880™, APE_{30}) for toxicity to 12 species of marine phytoplankton. All species were inhibited at 10-1000 mg/l. Although there was some variability, all species showed less inhibition at higher IGEPAL numbers. The most sensitive species were Stichococcus sp. and Nannochloris sp. These organisms were completely inhibited at 100-1000 mg/l of all surfactants. The least sensitive species was Protococcus, with growth almost always equal to controls. The authors suggested that thickness of cell walls correlated with tolerance to surfactants. However, they pointed out some exceptions, showing that the chemical composition of the cell wall may be important.

Davis and Gloyna (1967) also found variability in the response of blue-green and green algae to $C_9APE_{9.5}$. Oscillatoria borneti, Oscillatoria chalybia, Oscillatoria formosa, Gloccapsa alpicola, Scenedesmus obliquus, and Ulothrix fimbriata showed appreciable inhibition to $C_9APE_{9.5}$ at 20 mg/l. Anabaena variabilis, Oscillatoria tenuis, Ankistrodesmus

braunii, Chlorella pyrenoidosa and Chlorella vulgaris showed marked inhibition.

Batch additions of 50 mg/l $C_9APE_{9.5}$ to pond water had a stimulatory effect on blue-green algae and an inhibitory effect on Euglena. In addition, the authors found that algae contributed only a small amount to the degradation of the surfactant.

Little research has been done on the effect of surfactants on bacteria. Calcott and Postgate (1971) used nonionic surfactant to protect Aerobacter aerogenes from freezing and thawing damage. In this study, they found that MACROCYCLON^{TM}, a polyethylene glycol ether of p-tertoctylphenol-formaldehyde cyclic tetramer with 12 ethylene oxide residues per molecule, was toxic to 25% of a population of A. aerogenes at concentrations between 200 and 100,000 mg/l.

Vandoni and Goldberg-Federico (1973) found that C_8APE (0.1% by weight) had a slight inhibitory effect on the nitrification process in two soils they tested.

Based on the studies cited above, there is insufficient information available to make generalization as to the toxicity of APE to microorganisms. The studies show that the susceptibility of algae to APE is variable.

4. Sublethal Effects and Chronic Toxicity

Various sublethal effects have been observed for fish. Unilever Research Laboratories (unpublished data) described erratic and exaggerated swimming movements in rainbow trout at concentrations of $C_9APE_{9.5}$ greater than 5-6 mg/l. The fish then showed loss of balance and an inability to maintain station in mid-water. A passive stage followed in which the fish lay on the bottom with very slow ventilation of the gill chamber. Recovery occurred with removal to clean water. For comparison, the LC_{50} value (96-hour) for this species was 10-12.5 mg/l. Similar effects were observed by Swedmark et al. (1971).

Swedmark et al. (1971) tested several invertebrates for sublethal effects due to C_9APE_{10}. Such functions as byssal activity, heart rate, siphon activity, and burrowing activity of bivalves were examined. New byssal threads in mussels were no longer formed after 17 hrs at concentrations above 10 mg/l. The heart rate of juveniles of this species was reduced and irregular at much lower concentrations (0.5 mg/l).

Siphon activity of Cardium edule (cockle) and Mya arenaria (clam) was affected after 120-hour exposures to 5 mg/l. Burrowing of the bivalves, Astarte montagui, A. sulcata, and C. edule, was inhibited at 4 mg/l and limited at 2 mg/l. No effect was seen at concentrations less than 1 mg/l.

Similar inhibitory effects were observed for crustaceans. The decapods, L. squilla and L. adspersus showed avoidance reactions (increased swimming, violent movements of abdomen and extremities) immediately after immersion

in 10-100 mg/l C_9APE_{10}. Impaired swimming continued for the 3-week exposure, more severely at higher concentrations. Locomotion of the hermit crab was affected after 6 days exposure to 20 mg/l C_9APE_{10}, but no similar effect was observed in the spider crab or the shore crab at concentrations less than 100 mg/l. Burrowing, response to food, and beating of cirri of various crustaceans was affected in 5-10 mg/l, and inhibited in concentrations of 15-25 mg/l.

It is apparent that both fish and invertebrates may be adversely affected at concentrations lower than those producing acute toxicity. Swedmark et al. (1971) suggest that sublethal effects may be lethal in the environment, by making a species more vulnerable to predation.

B. Interactions with Other Chemicals

Few investigations into synergistic toxicity have been conducted for nonionics, although some related studies have been reported. Patterson et al. (1967) reported that LISSAPOL NX™ (APE_{8-11}) and DOBANE JNX™ (an LAS) have a synergistic effect on foaming. The relationship of this effect to aquatic toxicity is unknown.

Since nonionics are often used in combination with pesticides, a possible synergistic effect was examined. The effects of the surfactant in these cases, however, is indirect. Woodham et al. (1974) found the use of an APE surfactant, ORTHO HDD™, resulted in more rapid initial penetration of insecticide into citrus leaves. Within 2 hours after application, a higher percentage (29.6 ppm) was found in internal portions

of the leaf, with smaller amounts at the surface and sub-surface (14.4 ppm). Without the use of surfactant, no significant penetration had occurred 1 day after application.

Similar results were observed by other authors. Sands and Bachelard (1973) found that KEMONIC 909^{TM}(C_9APE_9) doubled the uptake of picloram by eucalyptus leaf disks. Fisher and Walker (1955) as cited by Parr and Norman (1965) observed a 7-fold increase in the absorption of phosphorus by apple leaves with the addition of TRITON X-100(C_8APE_{10}).

C. Effects of APE on Higher Plants

Few studies have been conducted to examine the toxicity of APE to plants, although indirect effects have been discussed above. In 1956, Gast and Early tested many solvent and emulsifiers used in insecticide formulations for their effects on plants. Solutions (1% and 0.1% v/v were applied to the leaves of 4 inch plants of sweet corn, cucumber, cotton, lima bean, tobacco and tomato. After one week, the plants were rated for injury: 0 = no visible sign of injury, 1 = very slight injury, 2 = moderate injury, 3 = heavy injury, 4 = severe injury, usually a dead plant. Table 5-C. summarizes the results. TRITON X-45, TRITON X-100, and IGEPAL CA-710 were the most phytotoxic of the APE tested. Application of 1.0% solutions of these surfactants produced severe injury to tomato plants; however, 0.01% solutions had no effect.

TABLE 5 - C.

Summary of Some Injury Ratings for APE on Higher Plants*

Surfactant	Injury	
	Average Rating (for all plants)	
IGEPAL™	1.0%	0.1%
CO-430	1.42	0
CO-530	2.90	0.13
CO-630	2.77	0.52
CA-710	3.13	0.66
CO-710	2.95	0.50
TRITON™	1.0%	0.1%
X-45	3.13	0.63
X-100	3.18	1.25
X-120	1.13	0.08
X-155	0.19	0

Gast and Early, 1956

*Includes beans, corn, cotton, cucumber, tobacco and tomato.

Regupathy and Subramaniam (1974) found that pollen germination of the bitter-gourd (Momordica charantia) was significantly reduced by 25 ppm TRITON X-100(C_8APE_{10}) in the pollen culture medium. Pollen tube growth was reduced at 75 ppm.

Parr and Norman (1964) as cited in Parr and Norman (1965) found that 22 nonionic surfactants repressed the elongation of the primary root of cucumber seedlings at 100 mg/l; higher concentrations were more inhibitory.

Haapala (1970) found that TRITON X-100 affected beet cells in various ways, depending on the concentration. Above 70-100 mg/l plasmolysis with sucrose was impossible, protoplasmic streaming ceased, and leakage of

ions and sugars occurred. Below this concentration, retention of solutes was enhanced. The author suggested that the CMC (critical micelle concentration) was of prime importance in determining the response.

D. Effects on Birds and Wildlife

No studies were found that considered the effect of APE on birds or wildlife.

E. Mode of Action

The mode of action of APE varies depending on the organism, and is not completely understood. Swedmark et al. (1971) reported that C_9APE_{10} caused the swelling of the gill epithelium in fish and increase in mucous secretion of gill tissues. The respiratory function was affected and fish died as if by suffocation. A decrease in oxygen uptake was also observed in Mya arenaria. Preliminary results with bivalves suggest that surfactants affect ion balance and osmoregulation. These authors suggested that toxicity of this surfactant to aquatic organisms was related to decreased surface tension and the CMC. The effects on swimming, however, suggest a narcotic action (Swedmark et al., 1971).

Further investigations with fish showed that the gill tissue is the site of penetration of C_9APE_{10} in cod, and the blood is the transport vehicle. Exposure to 5 ppm at 11°C indicated that the liver took up large amounts of this surfactant (300 ppm at 30 min, ∿750 ppm at 8 hr) and transported it to the gall bladder (10 ppm at 30 min, ∿500 ppm at 2 hr; and reached a maximum of ∿5000 ppm at 8 hr). The high levels of surfactant in the gill (60 ppm at 30 min, 90 ppm at 2 hr), suggest this as a site of action. The authors stated that the

main cause of acute poisoning is the swelling of gill lamellae and changes in membrane permeability resulting in asphyxiation (Granmo and Kollberg, 1976).

Two modes of action have been described for the mosquito. Lowering the surface tension by APE caused drowning of pupae. Larvae are more able to use cuticular respiration (Van Emden et al., 1974; Piper and Maxwell, 1971). Piper and Maxwell (1971) found that a reduction in surface tension to 41 dynes/cm or less was required to give 50% mortality. A further reduction of 2-4 dynes/cm caused 90% mortality using C_9APE, C_8APE, dinonylphenol ethylene oxide, and tridecylphenol EO adducts. However, they found that dinonylphenol ethylene oxide had toxic effects before the surface tension was reduced sufficiently.

Bettley (1968) found that C_8APE affected the potassium permeability of yeast cells. The author suggested that this was evidence of the ability of surfactants to block certain enzyme systems.

Several possible modes of action have been suggested for APE. Two factors which appear to be important are the relation of toxicity to the surfactant's CMC and reduction in surface tension. APE also seems to have some effect on cell permeability.

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V. HUMAN SAFETY

A general consideration of the human safety of surfactants was presented in Chapter 1 (LAS, p. 134).

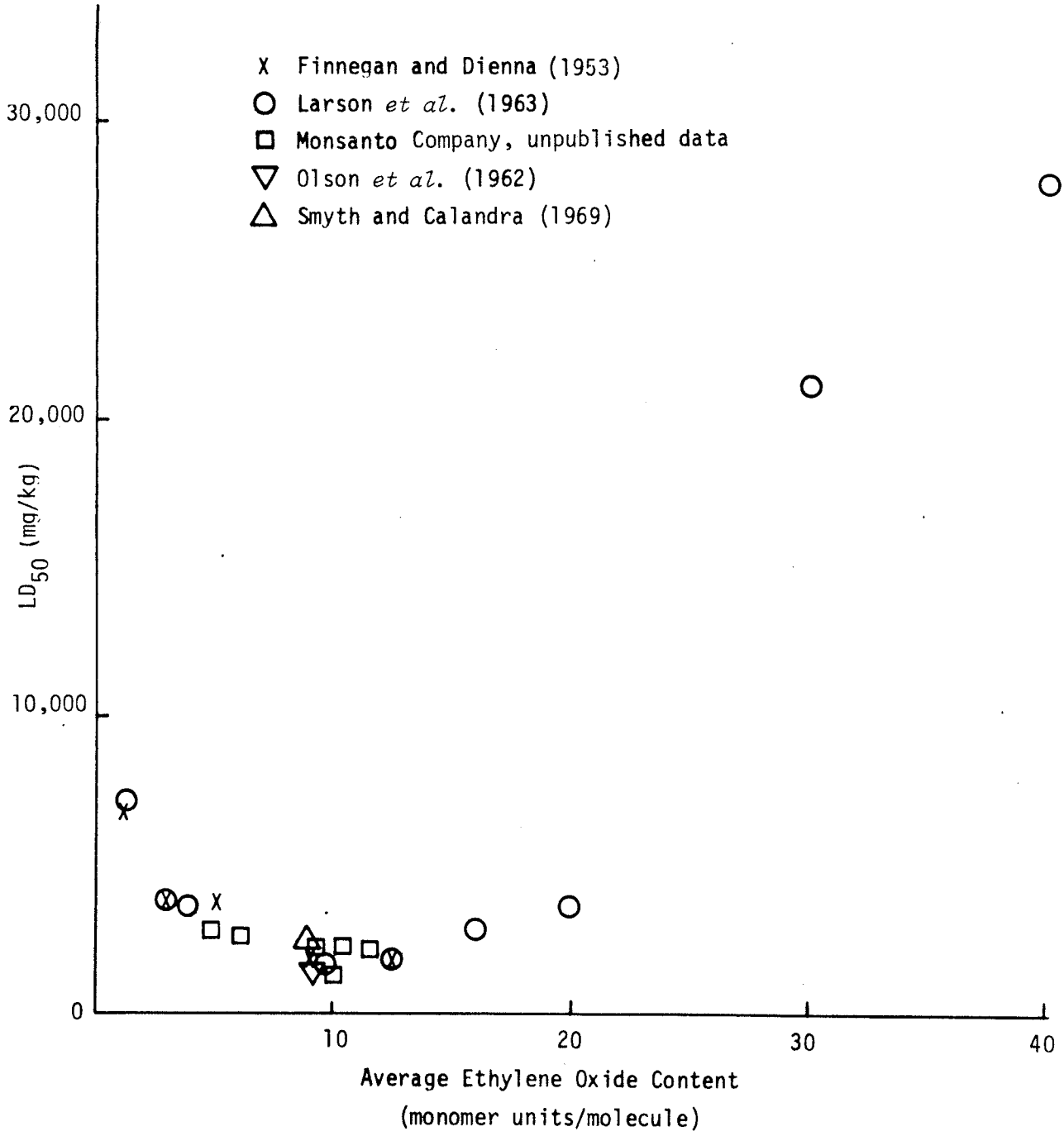
The data reviewed below on APE indicate a low order of acute and chronic oral toxicity to experimental animals of most surfactants in this group, with the exception of APE with 20 ethylene oxide units. These particular surfactants can give rise to cardiotoxicity in some mammalian species given orally in relatively short exposure intervals (5-14 days). No data with respect to mutagenesis, teratogenesis and effects on reproduction were found; however, the structural nature of these products would not predict adverse effects in these bioassays.

A. Animal Studies

Acute Toxicity-Oral. The acute oral toxicity for rats of alkylphenol ethoxylates depends on the ethylene oxide content. The materials exhibiting the greatest toxicity are those with average ethylene oxide monomer levels of about 10 units, with toxicity decreasing in severity as the ethylene oxide polymer either increases to 40 units or decreases to 1 unit (Larson et al., 1963). Chain length and degree of branching of the alkyl moiety do not appear to be factors in oral toxicity of APE. Figure 5-1 shows a compilation of the available rat oral LD_{50} data from a number of sources which agrees with this conclusion. The most toxic surfactants in this series have LD_{50} values for rats of between 1000 and 3000 mg/kg, a low degree of toxicity.

FIGURE 5-1

ACUTE ORAL LD_{50} OF ALKYLPHENOL ETHOXYLATES IN RATS



The minimum lethal oral dose for rabbits of a series of C_9 and C_{10}APE of 5 to 11.5 average ethylene oxide units ranged from 800 to 1,800 mg/kg, with no clear indication of the influence of ethylene oxide adduct composition and oral toxicity (Monsanto Company, unpublished data).

Acute Toxicity-Percutaneous. The minimum acute lethal doses for rabbits following percutaneous exposure to a series of C_9APE of 5 to 11.5 average units ethylene oxide content ranged from about 2,000 to 10,000 mg/kg. The toxicity decreased as the number of ethylene oxide units increased (Monsanto Company, unpublished data).

Acute Irritation-Skin. Skin irritation studies with several C_9- and C_{12}APE of 5 to 11.5 ethylene oxide units using the Draize procedure resulted in scores of 2.0 to 4.3 (mild to moderate) after 24 hours. However, 120 hours after application the animals no longer showed an irritant response for most of the materials tested (Monsanto Company, unpublished data).

Olson et al., (1962) examined the effects of a C_9APE_{9-10} on intact and abraded rabbit abdomen and on the rabbit ear epithelium. Application of 25 percent aqueous solutions resulted in slight irritation with Draize scores of 2.0 to 2.5.

Acute Irritation-Ocular. The irritant thresholds to rabbit eye mucosa was 15 percent for five APE surfactants with ethylene oxide content ranging from 1 to 13 units. APE compounds with 1 or 3 ethylene oxide units, among

a group of the most irritant of the series was C_8APE_{8-10} which exhibited an irritant threshold of 0.5 percent. A similar ranking was observed with this series of surfactants using the Draize procedure in rabbit eyes (Finnegan and Dienna, 1953).

The instillation of $C_9APE_{5.0-11.5}$ rabbit eyes and scored according to the Draize procedure resulted in moderate irritation within 24 hours. $C_{10}APE_6$ and $C_{10}APE_{10}$ produced moderately severe irritation. By 168 hours, the irritation was greatly reduced, with Draize scores of less than 10. Similar studies with a 25 percent aqueous solution of C_9APE_{9-10} resulted in severe corneal injury in unrinsed eyes. Rinsing the eye 30 seconds after application reduced the response to a slight effect (conjunctivitis) (Olson et al., 1962).

Subacute Toxicity-Oral. Fitzhugh and Nelson (1948) reported that the inclusion of polyethyleneglycol monoisooctylphenol of unidentified ethylene oxide content at levels of 2 or 4 percent in the diet of male Osborne-Mendel rats for 16 weeks resulted in significant reduction of body weight gain. At one percent surfactant in the diet, body weight gain was not significantly different from that of controls.

The consumption of a p-tertiary octylphenol ethoxylate$_{40}$ for three months by rats at 5 percent or by dogs at 0.35 or 5 percent of their diets had no significant effect either on urinary and hematological values or on any body tissues as revealed by histopathological evaluation (Larson et al., 1963).

In their detailed analysis of the toxicology of APE, Smyth and Calandra (1969) reported the effects observed after ninety days feeding of 13 different surfactants of this class to rats. In general, the APE containing 20 or more ethylene oxide units up to 40 exhibited little or no toxicity in rats at levels of 3 to 5 percent of their diets. Ingestion by rats of surfactants with fewer ethylene oxide units in the range of 4 to 15 resulted in reduced weight gains at a dose of one percent in the diet, and in the case of the $C_{10}APE_6$ retarded growth and increased liver weights were found at a dose of 0.04 percent in the diet. Increased liver weights were noted in the dose range of 0.2 to 1 percent in the diet for several alkylphenol ethoxylates of 4 to 9 ethylene oxide units.

A similar 90-day feeding study in dogs with 8 alkylphenol ethoxylates also was reported by Smyth and Calandra (1969). Of the 8 surfactants examined, which ranged from 4 to 30 ethylene oxide units, only C_9APE_{20} produced remarkable lesions. At a dose of 1 percent in the diet, focal myocardial necrosis was observed grossly while at 0.04 percent there was microscopic evidence of this lesion. These findings of severe toxic lesions in dogs were in marked contrast to the observations in rats with this specific surfactant which was among the least toxic in the 90-day feeding study. A more detailed analysis of the cardiotoxicity findings will be made below.

Subacute Toxicity-Percutaneous. The application to rabbit skin of several types of APE with ethylene oxide units ranging from 1 to 13 at concentrations up to 1 percent for 4 weeks (5 days per week) resulted in local effects but no systemic responses (Finnegan and Dienna, 1953).

Brown (1969) examined the effects of two isooctylphenol ethoxylates of 8-9 and 15 units in hairless mice and found that 2% aqueous solutions had no effect on oxygen consumption in skins taken from animals treated for 1 to 4 weeks.

In a later study on hairless mice, Brown (1971) reported an increase in activity of the enzymes phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, monoamine oxidase and succinic dehydrogenase in skin which had been painted with a 2% aqueous solution of isooctylphenol ethoxylate for 28 days. A similar material with 8 to 9 ethylene oxide units did not influence these enzymes after an identical treatment. Among the surfactants examined in this study, the alkylphenol ethoxylates were ranked as the least irritant in covered exposures in rabbits, guinea pigs and hairless mice.

Chronic Toxicity-Oral. Rats fed p-tertiary C_8APE_{40} at concentrations up to 1.4 percent in the diet for up to two years exhibited no adverse effects on growth, food consumption, hematology and urine parameters or pathological lesions (Larson et al., 1963).

No significant toxicological effects were observed in rats following feeding for two years of C_9APE_4 at doses up to 1,000 mg/kg/day and C_9APE_9 at doses of 0.27 percent in the diet. For the former surfactant, a slight elevation of liver weight was noted at a dose of 1,000 mg/kg/day in both sexes. However, the livers were normal when examined microscopically (Smyth and Calandra, 1969).

A similar two-year feeding study in dogs with these same two surfactants at doses identical to those used in the rat study did not result in any significant chronic toxicity as measured by a battery of clinical tests and by histopathological analysis (Smyth and Calandra, 1969).

Carcinogenicity and Cocarcinogenicity. The two-year feeding studies with several alkylphenol ethoxylates at levels of 1.4% of the diet in rats and 0.27% of the diet in dogs and rats gave no evidence of any carcinogenic effect (Larson et al., 1963; Smyth and Calandra, 1969).

With respect to possible cocarcinogenic activity, Takahashi et al. (1975) studied the effects of a C_9APE on the induction of gastric tumors in the rat by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Both the carcinogen MNNG (100 mg/l) and the surfactant (2000 mg/l) were given in the drinking water for 63 weeks after which the test animals received normal tap water. The inclusion of the surfactant with MNNG resulted in an overall tumor incidence of 80 percent compared to 52 percent in the group treated with MNNG only. In addition, the group receiving surfactant plus MNNG exhibited a marked increase in tumors of the small intestine (7 of 15) as compared to animals receiving MNNG alone (1 of 13).

Mutagenicity. No studies describing the examination of alkylphenol ethoxylates for mutagenic activity were found.

Reproduction Studies. No studies were found reporting on the effects of alkylphenol ethoxylates on reproductive performance of test animals.

Teratogenesis. There was no information available to us describing teratogenicity studies with alkylphenol ethoxylates.

Acute, Subacute and Chronic Toxicity-Summary. The alkylphenol ethoxylates exhibit a wide range of oral acute toxicities in rats ranging from about 1000 mg/kg to 30,000 mg/kg, depending on the length of the ethylene oxide adduct. Ocular effects also exhibited a structure dependence for irritant potency with highest toxicity in surfactants with 8-10 ethylene oxide units. Subacute and acute feeding studies showed that doses of APE up to 1% in the diet did not result in significant toxicity in rats or dogs. No indication of any direct carcinogenic effects were noted. A single report of a cocarcinogenic effect with N-methyl-N'-nitro-N-nitrosoguanidine in the induction of gastric tumors was found. Mutagenesis, reproduction and teratogenesis studies have not been reported for APE, and the acquisition of this information is highly desirable.

Pharmacology-Absorption and Metabolism. The metabolic fate of tritium-labeled p-tertiary C_8APE following a single oral dose in rats and dogs was reported by Larson et al. (1963). Both rats and dogs excreted about 90 percent or more of the tritium label in 72 hours. In dogs, the major portion of this radioactivity was excreted in the feces in 24 hours. Urinary excretion ranged from 1 to 2 percent in both species. In two rats examined for residual tritium label at 72 hours, about 2.3 and 4.0 percent of the administered radioactivity was found in the carcass.

Knaak et al. (1966) prepared two types of ^{14}C-labeled C_9APE (average 9 ethylene oxide units, range 8-15). One material was made from ^{14}C-labeled ethylene oxide and the other from ^{14}C-labeled nonylphenol. The fate and distribution of each of these materials was studied in rats following oral or intraperitoneal administration. In addition, isolated fractions of ^{14}C-labeled ethylene oxide surfactant, containing 7, 10, 12 and 15 ethylene oxide units also were studied.

Seven days following oral administration of ^{14}C-ethylene oxide or ^{14}C-nonylphenol labeled surfactant, 90 to 95 percent of the radioactivity was excreted in either feces or urine. The ^{14}C-ethylene oxide material was excreted in the ratio of 39 percent in the urine to 52 percent in the feces with 1.2 percent excreted as carbon dioxide. With ^{14}C-nonylphenol, the ratio was 20 percent in the urine, 78 percent in the feces with no label excreted as carbon dioxide. Excretion of the several purified ethoxylates followed the pattern observed with the mixture of ^{14}C-ethylene oxide surfactants described above with 85 to 90 percent of the label excreted by 7 days. Excretion of CO_2 was inversely proportional to the number of ethylene oxide units (3.3, 1.2, 0.78 and 0.20 percent ^{14}C-label excreted for ethylene oxide units of 7, 10, 12 and 15, respectively). A similar pattern of reduced urinary excretion with increasing ethylene oxide adduct length was found. In a reciprocal fashion, fecal excretion increased. These results provide good evidence that absorption of alkylphenol ethoxylates from the intestinal tract decreases with increasing ethoxylate chain length.

Among the metabolites found in urine, about 1 percent occurred as unchanged C_9APE. The principal metabolites were identified as the mono-

and dicarboxylic acids of polyethylene glycol and the glucuronic acid conjugate of nonylphenol.

Pharmacology - Lipid Metabolism and Experimental Atherosclerosis

One specific alkylphenol ethoxylate has been used as a tool over a period of several decades to study lipid absorption and metabolism in experimental animals in relation to the induction and prevention of atherosclerosis. This compound is identified as TRITON A-20™ or TRITON WR-1339™ and is a formaldehyde linked tetramer of t-isooctylphenol ethoxylate. Although this type of alkylphenol ethoxylate is not a component of detergents, a brief recapitulation of the significant findings in this area is appropriate.

In rodents, a number of investigators have observed that intravenous administration of TRITON A-20 induced hyperlipemia in the test animals with increases in both blood cholesterol and phospholipids (Kellner et al., 1951a; Rossi et al., 1964; Schurr et al., 1972). In several instances, this TRITON A-20 induced hyperlipemia was associated with a reduced severity of atherosclerotic lesions in animals on a high cholesterol diet (Kellner et al., 1951b; Still, 1962).

Weigensberg (1969) examined various chemical structure modifications of TRITON WR-1339 in an experimental atherosclerosis system in rabbits. The 20 and 30 ethylene oxide adducts of t-octylphenol and n-decylphenol formaldehyde polymers were tested. The t-octylphenol ethoxylates of 20 and 30 units were effective in reducing the severity of atherosclerotic lesions in rabbits on a cholesterol-olive oil diet as compared to untreated controls, even though serum lipid

levels were not reduced by surfactant treatment. The n-decylphenol ethoxylate$_{30}$ had no effect on the severity of the atherosclerosis while the 20 unit ethoxylate had only a slight effect. A subsequent abstract (Weigensberg and Stary, 1973) reported that t-octylphenol ethoxylate$_{30}$ formaldehyde polymer enhanced the regression of aortic atherosclerosis induced by dietary cholesterol following a shift to a normal diet.

In one study TRITON X-100 was found to inhibit and reverse the insoluble complex formed in vitro by interaction of low density lipoproteins with collagen, elastin and several sulfated polysaccharides at concentrations of 16 to 19 mg/ml of serum. TRITON WR-1339 required 3 to 25 times greater amounts to inhibit the interaction of sulfated polysaccharides depending on the specific biopolymer tested (Day and Powell, 1973).

In dogs, similar findings have been reported with respect to hyperlipemia resuling from treatment with TRITON WR-1339 (Scanu et al., 1961; Vidone et al., 1967; Butkus et al., 1968).

Pharmacology-Cardiotoxicity. The finding that C_9APE_{20} produced focal myocardial necrosis in dogs but not in rats after oral consumption of this surfactant for 90 days prompted studies to explore this phenomenon further (Smyth and Calandra 1969). Although it was found that exposures as brief as 5 days could produce the effect, subsequent studies were carried out for 14 days.

The oral administration of four alkylphenol (1 octyl, 2 nonyl, 1 decyl) ethoxylates$_{20}$ at 1000 mg/kg/day resulted in focal myocardial necrosis in all animals (1 male and 1 female per group). Six of the 8 dogs died in 4 to 14

days. Among the species of animals tested, only dogs and guinea pigs gave evidence of the cardiac lesions, while cats, rabbits and rats did not. The highly specific nature of this phenomenon is illustrated by the relatively low dose producing the lesion in dogs in 90 days (40 mg/kg/day), while in rats 5,000 mg/kg/day for the same treatment interval resulted in no cardiac pathology.

The length of the ethylene oxide chain appeared to be the determining factor in the induction of the myocardial necrosis. In a series of surfactants of 4 to 40 ethylene oxide units, only surfactants with 15, 17.5 or 20 units produced cardiotoxicity in dogs at 1000 mg/kg/day for 14 days. Alkylphenol ethoxylates with ethylene oxide chains shorter than 15 units or of 25 units and longer were not toxigenic for this lesion.

There appeared to be a correlation between the emetic potency and the ability to induce cardiac toxicity. However, blood electrolyte data did not support the view that the emesis was a significant contributory factor.

Studies with isolated cat hearts indicated that C_9APE (9, 20 and 40 ethylene oxide units) reduced the amplitude of contraction suggesting a direct effect on the heart rather than any secondary effect resulting from toxicity to another organ.

Pharmacology-Enzymes. Denk et al. (1971) have studied various alkylphenol ethoxylates of the TRITON™ series (X-15, X-100, X-305, N-10) for their interaction in vitro with the microsomal cytochrome P-450 system isolated from rat liver homogenates. They described a series of changes. Initially, the surfactants bind to P-450, possibly at a lipoprotein site, resulting in a spectral change of this heme enzyme. In the second stage, the blocking of a P-450 dependent

enzyme without a loss of P-450 suggested a solubilization of the proposed lipoprotein binding site. Finally, the P-450 is solubilized and degraded.

In another study, Furuichi (1974) found that TRITON X-100 did not inhibit pancreatic amylase at concentrations up to 1.6 percent in the incubation mixture.

Pharmacology - Effects on Infections. Several derivatives of TRITON A-20 were prepared by Cornforth et al. (1951) to examine their anti-tuberculosis effect in mice. Several oligomers of p-tertiary-octyl phenol were prepared by condensation with formaldehyde followed by addition of ethylene oxide adducts of an average of 20 units. At doses of 15 to 25 mg given intravenously twice weekly for two weeks, the survival of mice infected with Mycobacterium tuberculosis strain H37RV was significantly prolonged by TRITON A-20 and by all the oligomers from dimer to octamer. The monomer was inactive. Since all the products tested had similar bacteriostatic action in vitro, it is unlikely that the surfactants had a direct antibacterial effect in vivo.

Makin et al. (1973) investigated the irrigation of chronic Staphylococcus aureus infections in rabbits with solutions of the alkylphenol ethoxylate TRITON WR-1339 with and without penicillin. The results showed that the surfactant did not inhibit the penicillinase produced by the bacteria and that there was no advantage to irrigation of the wound with the surfactant-normal saline solution over that with saline solution alone.

Pharmacology - Summary. Absorption and metabolism studies in rats and dogs indicated that 90% of ingested APE was excreted within 72 hours. As the length of the ethylene oxide adduct increases, urinary excretion and conversion to CO_2 are decreased and fecal excretion increases.

A considerable number of studies have shown that TRITON WR-1339 will produce lipemia in rodents and dogs and will result in a reduction of atherosclerotic lesions in rodents. In contrast, this surfactant will induce atherosclerosis following repeated parental administration to dogs at nearly toxic doses (200-250 mg/kg/every four days).

At doses which were low (40 mg/kg/day) in relation to chronic toxicity studies which produced no effect in rats, a small group of ethoxylates ($\sim$20 ethylene oxide units) gave rise to focal cardiac necrosis in 90 days in dogs. At higher doses, lesions were seen in five days.

B. HUMAN STUDIES

Skin Irritation. Finnegan and Dienna (1953) found neither primary irritation after 48 hours in patch tests for five APE materials with ethylene oxide units of 1 to 13 units nor sensitization with the exception of 2 of 50 individuals who exhibited a mild positive response with C_8APE when challenged with an identical patch on the other arm two weeks after the initial irritation study.

Using an in vitro test system, Bettley (1965) reported that exposure of isolated human epidermis to isooctylphenol ethoxylate at 0.04M for two weeks resulted in a 25 percent increase in permeability to potassium ion. From surface tension measurements, he concluded that permeability was not directly related to this physical characteristic of the surfactants tested.

Wood and Bettley (1971) measured titratable groups on epidermal proteins after exposure for three hours to an 0.04M solution of isooctylphenol ethoxylate. They found only a very slight increase which was not correlated with epidermal permeability to potassium ions as measured earlier (Bettley, 1965).

Polano (1968) and Smeenk (1969) reported on the effects of two isooctylphenol ethoxylates (8-9 and 15 ethylene oxide units) on human skin. Of the five types of measurements made (immersion tests, patch test, amino acid extraction, titratable thiol groups, potassium ion permeability and patch tests,) only the immersion test and amino acid extraction analysis were weakly positive.

These same surfactants were examined by Brown (1971) to determine whether correlations could be made between in vitro and in vivo tests on human skin, and in vivo tests on the skins of rodents. While isooctylphenol ethoxylates were generally ranked among the least irritant in most test systems, the overall lack of correlation indicated that animal tests should be used with caution in predicting effects in humans.

C. EPIDEMIOLOGY

Accidental Exposure. The issue of accidental ingestion of detergents was considered in Chapter 1, p. 158. No reports of injury resulting from human exposure to alkylphenol ethoxylates in use or manufacturing situations have been found.

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APPENDIX D - APE NOMENCLATURE AND ABBREVIATIONS

Throughout this Chapter, the designation of APE has been used to indicate alkylphenol ethoxylates. Specific alkyl chain length is numerically designated via a subscript and all alkyl chains should be assumed to be branched unless otherwise specified.

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

$C_9APE_{9.5}$ - nonylphenol ethoxylate (average 9.5 ethylene oxide units)

C_8APE_{8-12} - octylphenol ethoxylate (8-12 ethylene oxide units).

ALPHA OLEFIN SULFONATES

Synopsis

Alpha olefin sulfonates, one of several groups of chemical entities classified as anionic surfactants, are relative newcomers to the domestic synthetic detergent industry. These readily biodegradable surfactants possess good water solubility and detergency characteristics, and, within the last decade, have been formulated into many types of detergent products.

At present, there are no environmental standards of water quality with respect to alpha olefin sulfonates and levels of AOS, as such , in streams and waterways of the United States are not presently being monitored. The levels of MBAS would include AOS, if present. Although the data are scant, AOS appear to be readily biodegraded under both laboratory and environmental conditions. Information with respect to the persistence and biodegradation of by-products of AOS manufacture, hydroxysulfonates, disulfonates and sultones, is not readily available.

Although the amount of information on the acute toxicity of AOS to aquatic organisms is also sparse, it appears that acute aquatic toxicity increases with increasing carbon chain length. Lethality generally occurs in the 0.5-15 mg/l range. There are insufficient data to establish the extent of AOS toxicity to juvenile life stages of aquatic organisms.

Information on the effects of AOS on plants is limited, but exposure to 40 mg/l AOS had no significant effect on germination and growth of tomato, barley and bean plants.

In terms of human safety, chronic oral exposure of rats to 5,000 ppm AOS for two years, a level far in excess of any expected human intake, produced no deleterious effects in rats. Studies of ocular irritation in rabbits indicate that concentrations of AOS greater than 1% induce mild to moderate irritation. Acute skin irritation studies in laboratory animals revealed AOS to be a skin irritant at 10% although AOS concentrations of 1% or greater produced negligible to mild irritancy in humans.

Long-term studies of the possible carcinogenicity of AOS by oral and percutaneous routes and of the higher molecular weight sultone by-products of AOS by percutaneous administration have been negative. Furthermore, a number of in vitro and host-mediated mutagenicity tests have not given any indications that AOS are mutagenic. A single set of experiments showed a positive response in a host-mediated microbial assay; however, the response may not be due to AOS or related materials.

With respect to teratogenic effects, an increase in cleft palates was found in offspring of mice given 300 mg/kg of AOS during gestation as well as an increased incidence of minor skeletal anomalies in both mice and rabbits at this dosage level. These responses generally occurred in groups where dams exhibited toxic responses.

From the information available at this time, it appears that normal use levels of AOS do not pose either an environmental hazard or a significant risk to human health. However, additional studies in certain areas, such as the teratogenicity, skin irritancy and effects on reproduction of AOS

and by-products of its manufacture would be beneficial in the evaluation of human safety aspects of AOS surfactants.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

BIBLIOGRAPHY (continued)

ALPHA OLEFIN SULFONATES

I. INTRODUCTION

Although alpha olefin sulfonates (AOS) have been available since the 1930s, their production for use in commercial surfactant formulations has been somewhat limited until recently, by the lack of suitable feedstock. However, development of continuous, short contact sulfur trioxide sulfonation processes and the increased availability of high purity, Ziegler-derived alpha olefin feedstock have made AOS surfactants competitive with other surfactants presently on the market.

One of several groups of chemical entities classified as anionic surfactants, AOS are reportedly efficient, readily biodegradable cleaning agents (Kerfoot and Flammer, 1975). Said to impart a "soft feeling" to washed fabrics (Tomiyama et al., 1969) AOS surfactants offer detergency and foam properties comparable to those of LAS and possibly possess a slight advantage over LAS in hard water (Marquis, 1968). Furthermore, AOS possess a high degree of stability, have good water solubility characteristics, and become rancid at a much slower rate than LAS (Marquis, 1968; Marquis et al., 1966; Tomiyama et al., 1969).

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

(1) environmental fate and distribution including biodegradation,

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

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

A. Chemical Characterization of AOS

Due to several factors (e.g., processing conditions, side reactions), alpha olefin sulfonates have proved difficult to characterize chemically. Although other sulfonation processes (SO_3-dioxane complexing, chlorinated solvent systems) have been used in the past, AOS are now commercially prepared by direct sulfonation of n,α-olefins with a dilute stream of vaporized sulfur trioxide (SO_3) in continuous thin film reactors. Depending on reaction conditions, this complex, highly exothermic reaction can follow several paths leading to a variety of reaction products (Shell Chemical Co., unpublished data). The major product mix of α-olefin sulfonation, neutralization and saponification is a mixture of alkene sulfonates, hydroxyalkane sulfonates and disulfonates; commercial AOS products contain all three of these components. Throughout this report the term "% active" has been used to indicate the sum of these components when referring to commercial AOS products. In addition, smaller amounts of alkene disulfonates, hydroxyalkane disulfonates, saturated sultones (which may be eliminated by thorough saponification) and unreacted α-olefins may also be present (Stepan Chemical Co., unpublished data).

1. Primary Product, AOS

There are three major stages in the production of AOS. These are:

(1) synthesis of n,α-olefins,

(2) sulfonation of these n,α-olefins, and

(3) neutralization/saponification of the resultant acid mixture.

a. Synthesis of n,α-olefins

Generally, the α-olefins utilized in the production of AOS possess an alkyl chain in the range of fourteen to eighteen carbon units; however, eleven through twenty carbon chains can be used (Marquis, 1968). Industrially, α-olefins are synthesized either by oligomerization of ethylene by Ziegler (triethyl aluminum) catalysts or via thermal cracking of paraffin wax. Even-numbered, Ziegler-derived α-olefins (C_4-C_{30}) are purer in α-olefin content and are reported not to contain the diolefinic and naphthenic components found in paraffin-derived α-olefins (Stepan Chemical Co., unpublished data). Although not of such high quality, paraffin-derived α-olefin are also used and usually have alkyl chains in the five to twenty-one carbon range, depending on feedstock employed (Marquis, 1968). Special processes, such as the Fischer-Tropsch synthesis with iron catalysts, the joint metathetic reaction of high molecular weight olefins with internal double bond and excess ethylene in the presence of a suitable metathesis catalyst or the "counterthermodynamic" double bond isomerization of olefins with internal double bonds to the α-olefin isomers are not yet utilized industrially to any appreciable extent (Fell, 1975).

b. Sulfonation

The direct sulfonation of α-olefin with SO_3, via continuous falling film techniques, is believed to follow two paths: the formulation of alkene sulfonic acid along with the concomitant migration of the double bond and the production of intermediate sultones (see Figure 6-1). Several side reactions can also occur; the products of these reactions can vary considerably, depending on experimental conditions. These side reactions are discussed under Section I. A. 2. of this chapter.

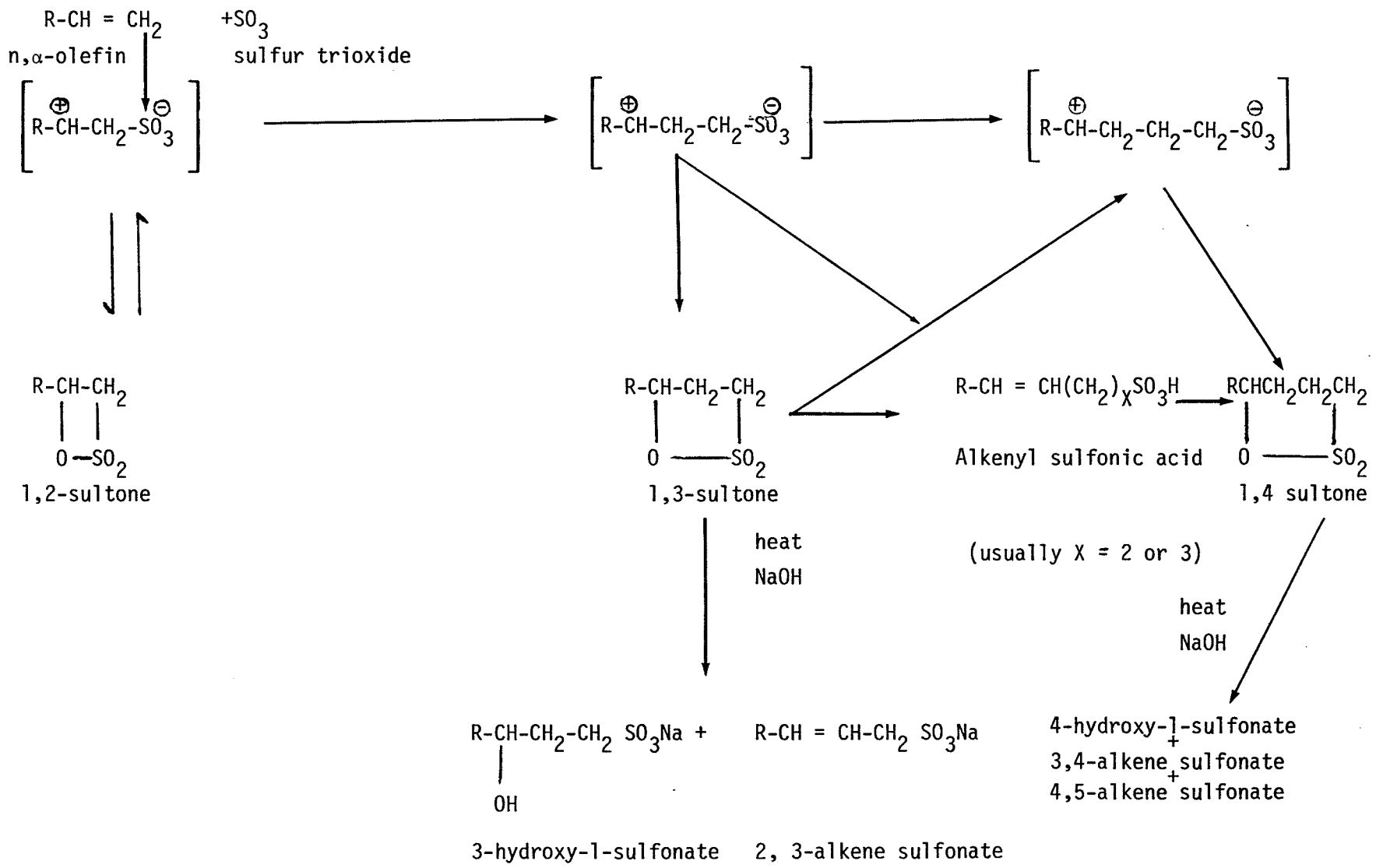
To obtain as high as possible conversion to AOS with wax-cracked base olefins, an SO_3 to α-olefin mole ratio of 1.15-1.20 is used; lower ratios result in large amounts of unreacted olefin, while higher ratios lead to color degradation (Marquis, 1968). With continuous thin film reactors, molar ratios in the range of 1.0 to 1.2 SO_3/olefin are typically employed (Fort et al., 1974).

c. Neutralization/Saponification

In order to convert the acid reaction mixture to water soluble surfactants, the reaction products are neutralized, then saponified with sodium hydroxide, thus hydrolyzing the intermediate sultones. This process yields AOS composed of approximately 60-65% alkenyl sulfonates, 35-40% hydroxyalkane sulfonates, plus several intermediates (Stepan Chemical Co., unpublished data).

FIGURE 6-1

Production of AOS by Direct Sulfonation of Linear Alpha Olefins



Due to the inherent tendency of olefins to form color bodies during sulfonation, AOS are generally subjected to a bleaching process. In the past, bleaching was a supplementary step involving a one-hour exposure to a 3% solution of sodium hypochlorite. However, bleaching and saponification are now done simultaneously by the addition of bleach and isopropyl alcohol to the saponification mixture (Marty et al., 1973).

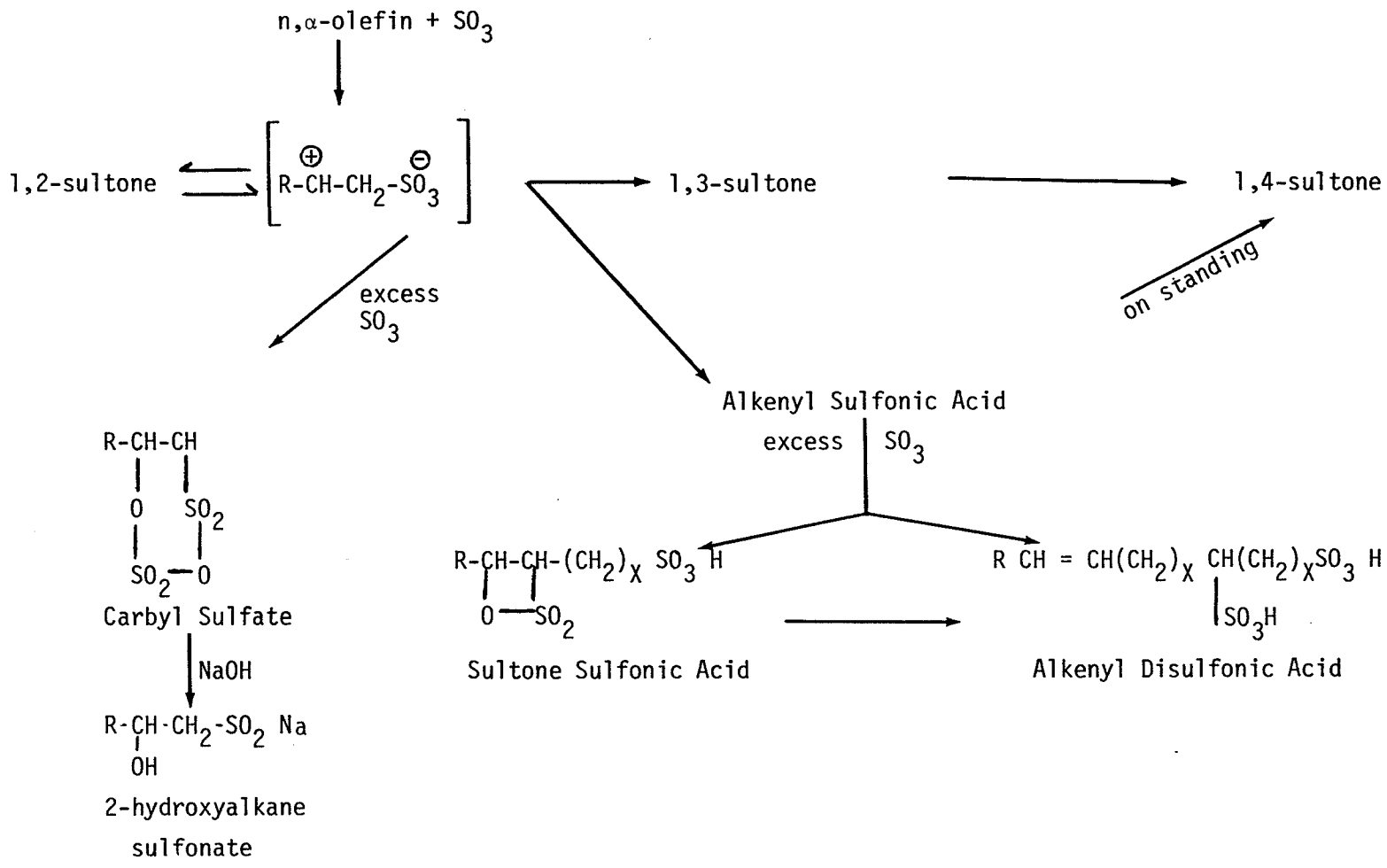
2. Secondary Products

At each stage in the production of AOS, variations in the molar ratios of the reactants or slight modifications in the experimental conditions can alter the composition of the reaction products and lead to the formation of a variety of secondary products. Additionally, the overall sultone content of the acid reaction mixture has been shown to increase on standing, at the expense of the alkenyl sulfonic acid (Shell Chemical Co., unpublished data). The known products of these side reactions are shown in Figure 6-2.

It is believed that the 1,2-sultone and/or its zwitterion is formed as an intermediate in the sulfonation of α-olefins (Mori et al., 1971). The 1,2-sultone, however, is rather unstable and upon standing, rapidly isomerizes to the 1,3-derivative. This reaction is advantageous in that

FIGURE 6-2

PRODUCTS RESULTING FROM SIDE REACTIONS IN THE MANUFACTURE OF ALPHA OLEFIN SULFONATES



it impedes the subsequent formation of the poorly soluble 2-hydroxysulfonate during hydrolysis. However, if the mixture is allowed to age too long, the 1,3-sultone slowly isomerizes to the relatively difficult to hydrolyze 1,4-sultone (Shell Chemical Co., unpublished data). Mori and co-workers (1971) found that upon warming, 1,2-sultone was converted to alkene sulfonic acid, 1,3-sultone, 1,4-sultone and disultone.

Hashimoto et al. (1973) analyzed α-olefin sulfonic acids with NMR spectroscopy. They found a mixture of alkene sulfonic acid, 1,3-sultone, 1,4-sultone, and unchanged olefin. Two unknown peaks, believed to be precursors to Δ'-alkene disulfonic acid, were assigned to 1,2-alkane disultone.

Alkyl carbyl sulfates, i.e., double sultones, are not usually formed under typical operating conditions of thin film sulfonation. However, under conditions of excess SO_3, the presence of alkenyl sulfonic acids can yield sultone sulfonic and disulfonic acids; mono substituted derivatives react to produce alkyl carbyl sulfates (Shell Chemical Co., unpublihsed data).

During hydrolysis, mildly alkaline conditions favor the formation of hydroxyalkane sulfonates while elevated temperatures and/or the presence of a limited supply of water favors the formation of alkenyl sulfonates. If sultone sulfonic acids are present during hydrolysis, they yield hydroxyalkane and alkenyl disulfonates, while alkylcarbyl sulfates, if present, readily hydrolyze to 2-hydroxyalkane sulfonate (Shell Chemical Co., unpublished data).

Thus, in summary, a number of compounds whose effects might be a part of "AOS effects" can be present in the complex mixtures generated during the manufacturing process. The influence processing conditions exert on product composition is not fully understood and the detailed nature of the products present at each stage of AOS production remains to be fully defined.

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II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alpha olefin sulfonates, one of several chemical entities classified as anionic surfactants, can be detected with many of the procedures utilized in the detection of LAS (see Chapter 1, pp. 19-26). As with LAS, the mainstay for the determination of presumptive levels of AOS in the environment has been the MBAS method and, therefore, subject to the inherent limitations of this procedure.

1. Specific Chemical Techniques

Oba et al. (1968) reported good reproducibility with the methylene blue colorimetric method in the determination of n,α-olefin sulfonates and their major components, the alkenyl sulfonates and hydroxyalkane sulfonates at concentrations above 0.8 μg/ml. However, disulfonate salts (impurities present in AOS in various proportions depending on reaction conditions) tend to reduce the methylene blue response. Oba and co-workers found that the initial MBAS response (expressed in mg/l) decreased as the disulfonate content increased, but the actual extent of biodegradation (expressed as % MBAS removed) was not affected by disulfonate content.

Sample	Disulfonate Content (%)	Initial MBAS Concentration (mg/l)	Biodegradability % MBAS Removed
AOS-C	<4	36	97
AOS-D	15	31	97
AOS-E	50	23	96

(Oba et al., 1968)

2. Physicochemical Analyses

With slight modifications in experimental procedures, many commonly used physicochemical analyses have been adapted to AOS determinations. For example, AOS are not sufficiently volatile to be analyzed with gas chromatography, but AOS can be hydrogenated, converted to their sulfonyl chloride derivatives and subsequently subjected to thermal decomposition gas chromatography (Nagai, 1970; Kirkland, 1960). Oba et al. (1976) recently utilized this technique of converting AOS to the sulfonyl chloride derivative in their FIR (Far Infrared Spectroscopy) procedure for the detection of AOS in sewage and river water. Analysis of the extent of primary biodegradation of AOS is accomplished with infrared procedures. Comparison of the amount of AOS detected in spiked samples revealed a standard deviation of ±0.8% in the ten samples examined.

Crilly and McGowan (1962) have also used IR procedures for sulfonate determinations, while Hashimoto et al. (1973) employed NMR spectroscopy, and Griffin and Albaugh (1966) combined ion-exchange chromatography with potentiometric titrimetry.

Fudano and Konishi (1971) have applied a salting-out chromatographic technique to the determination of mixtures of hydroxyalkane and alkene sulfonates, and Puschel and Prescher (1968) used paper chromatographic and ultraviolet procedures to identify and separate monosulfonates obtained during the sulfonation of α-olefins. Additionally, Allen and Martin (1971) have developed a technique for the separation and quantitation of alkene and

hydroxyalkane mono- and disulfonates. Separation is achieved via TLC techniques. Following separation, the material is charred with heat and SO_3 fumes, then quantitated by photodensitometric determinations. The method is linear in the 0-5 μg range, with decreased sensitivity at higher concentrations. This procedure can also be modified to detect unhydrolyzed sultones.

B. Water Quality Standards

There are presently no standards in the United States or Europe specifically restricting alpha olefin sulfonate (AOS). If present, these anionic surfactants are included among those measured in the environment using the MBAS method. The restrictions applying to MBAS levels were discussed in Chapter 1, pp. 26-29.

C. AOS in Natural Water Bodies

AOS is not presently being monitored in the United States or Europe. MBAS measurements in water bodies include these surfactants as well as other anionics. These levels have been discussed in Chapter 1, p. 30.

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III. BIODEGRADATION

A class of anionic surfactants, the alpha olefin sulfonates (AOS), became commercially available in the United States during 1965, but the extent of biodegradative studies to date with AOS is somewhat limited. In biodegradation studies, AOS surfactants are generally detected by methylene blue procedures. (See section II. A. 1. of this chapter).

A. Laboratory Test Systems

1. Oxygen Uptake - Biochemical Oxygen Demand

The biochemical oxygen demand for AOS in the C_{12}-C_{18} range and containing up to 40% hydroxyalkane sulfonates averaged 51.6% at 5 days. Glucose under the same conditions had a BOD_5 value of 69.6% (Procter & Gamble Company, unpublished data).

2. CO_2 Evolution

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

3. Die-Away Tests

a. River Water Test

Utilizing a river water die-away procedure, Sekiguchi et al. (1975a)

found that on the third day of the study, no AOS (20 mg/l added) could be detected (MBAS) in samples of Tama River water. The extent of degradation of 5 mg/l AOS in seawater was also examined; no MBAS activity could be detected in samples of seawater at 5 days. Similar findings were reported for C_{15-18} AOS by Marquis et al. (1966).

b. Fortified and Inoculated Waters

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

c. Shake Culture Test

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

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

4. Simulated Treatment Processes

a. Activated Sludge

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

b. Anaerobic Systems

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

B. Field Studies

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

C. Summary

Although scant, the data available indicate that AOS are quickly and readily degraded in both fresh and seawater under aerobic conditions. Some anaerobic degradation also occurs.

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IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

The information available on the toxicity of AOS to aquatic organisms indicates that the toxicity (LC_{50}) values fall in the range of about 0.5-15 mg/l.

1. Acute Toxicity

a. Fish

The available acute toxicity data for fish are summarized in Table 6-A. The 24-hour LC_{50} values for AOS range from 0.5-15 mg/l. The only toxicity values appearing outside of this range are the LC_{50} for dimer olefin sulfonate to bluegill (97 mg/l) (Colgate Palmolive Co., unpublished data) and the LC_{50} of vinylidene AOS (58 mg/kg) for the same species (Shell Chemical Co., unpublished data). The toxicity values show that for a given species the longer chain sulfonates are consistently more toxic than the shorter chain compounds.

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

TABLE 6-A Acute Toxicity of AOS to Fish

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

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

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Goldfish (continued)	AOS C_{16-18}, 90% (MW 340-344)	Fish - 5-6 cm, static, 20°C, hardness - 150 mg/l $CaCO_3$	LC_{0-100} 24 hr - 1.0-3.0	Unilever Research Laboratories, unpublished data
Golden orfe (Idus melanotus)	AOS C_{12-16}	Fish - 5-7 cm, static, 20°C tap water	LC_0 48 hr - 3.0 LC_{50} 48 hr - 4.2 LC_{100} 48 hr - 6.0	Fischer (personal communication) as cited in Gloxhuber (1974)
	AOS C_{14-16}, 90% (MW 312-316)	Fish - 5.5 cm, static, 20°C, hardness - 150 mg/l $CaCO_3$	LC_{50} 24 hr - 4.7 (4.5-5.0) 48 hr - 4.1 (3.9-4.3)	Unilever Research Laboratories, unpublished data
	AOS C_{16-18}, 90% (MW 340-344)		LC_{50} 24 hr - 1.2 (1.0-1.4) 48 hr - 1.1 (1.0-1.2)	

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Bluegill (Lepomis macrochirus)	AOS	Fish - 1.2 g, 18°C	LC_{50} 24 hr - 8.4 (7.2-9.9) 96 hr - >7.5 <8.7 no effect level - 7.5	Colgate-Palmolive Co., unpublished data
			LC_{50} 24 hr - 7.1 (4.8-10.6) 96 hr - 7.1 (4.8-10.4) no effect level - 4.9	
	Dimer olefin sulfonate	Fish - 1.2 g, 18°C	LC_{50} 24 hr - 97.1 (69.1-137.0)	
	AOS 28.9% active*	Fish - 1.1 g, 21°C pH 7.1	LC_{50} 24 hr - 1.61 (1.27-2.04) 96 hr - 1.40 (1.20-1.64) no effect level - 1.2	Shell Chemical Co., unpublished data.

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

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Bluegill (continued)	Vinylidene AOS 17.9% active*	Fish - 1.1 g, 21°C pH 7.1	LC_{50} 24 hr - 58.2 (47.1 - 71.9) 96 hr - 57.3 (46.5 - 70.6) no effect level - 37	Shell Chemical Co., unpublished data.
Fathead minnow (Pimephales promelas)	AOS C_{14-16}	Fish - 1.0 g, 20°C	LC_{50} 24 hr - 8.6 (5.3 - 14.0)	Colgate-Palmolive Co., unpublished data.
	AOS C_{16-18}	Fish - 1.0 g, 20°C	LC_{50} 24 hr - 1.8 (1.5 - 2.2)	

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

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Fathead minnow (continued)	AOS C_{14}	Fish - 1-2 cm, static, 21°C, pH 7.0-7.2, hardness - 100 mg/l $CaCO_3$	LC_{50} 24 hr - 15-21*	Monsanto Company, unpublished data
	AOS C_{16}		LC_{50} 24 hr - 3.2-6.9*	
	AOS C_{18}		LC_{50} 24 hr - 0.5-0.8*	
	BIOTERGE AS 40™ C_{14-16}		LC_{50} 24 hr - 8.2*	
Japanese killifish (Oryzias latipes)	AOS C_{15-18}		LT_0 5 hr - 0.5 LT_{100} 5 hr - 2	Tomiyama (1975)

*Range from three tests

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Carp (*Cyprinus carpio*)	AOS - technical grade	Fish - 3.5-5.5 cm, static, 21°C, pH 7.5-7.8, 10 fish/ conc, 4 conc tested	LC_{50} 24 hr - 3.2	Lopez-Zavala *et al.* (1975)
White tilapia (*Tilapia melanopleura*)		Fish - 5.0-7.0 cm, static, 21°C, pH 7.5-7.8, 10 fish/ conc, 2 conc tested	LC_{50} 24 hr - 2.0	
Guppy (*Poecilia reticulatus*)	Na-AOS, 90% C_{14-16}, (MW 312-316)	Fish - 1.0 cm, static, 20°C, hardness - 20 mg/l $CaCO_3$	LC_{50} 24 hr - 10.1 (9.3-11.0) 48 hr - 10.1 (9.3-11.0) 96 hr - 9.7 (8.9-10.6)	Unilever Research Laboratories, unpublished data
	Na-AOS, 90%, C_{16-18} (MW 340-344)		LC_{0-100} 24 hr - 1.0-2.0	

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Harlequin (Rasbora heteromorpha)	Na-AOS C_{14-16}	Fish - 1.3-3.0 cm, continuous flow, 20°C, hardness - 20 mg/l $CaCO_3$	LC_{50} 24 hr - 6.2 (5.5-7.1) 48 hr - 4.8 (4.1-5.6) 96 hr - 3.3 (2.3-4.2)	Unilever Research Laboratories, unpublished data
	Na-AOS C_{16-18}		LC_{50} 24 hr - 1.3 (1.2-1.4) 48 hr - 0.9 (0.7-1.1) 96 hr - 0.5 (0.3-0.7)	
Minnow (Phoximus phoximus)	Na-AOS C_{14-16}	Fish - 5 cm, static, 10°C, hardness - 210 mg/l $CaCO_3$	LC_{50} 24 hr - 5.3 (5.1-5.5)	
	Na-AOS C_{16-18}		LC_{50} 24 hr - 1.4 (1.3-1.5)	

Table 6-A Continued

Species	Surfactant	Experimental Conditions	Toxicity (mg/l)	Source
Brown trout (*Salmo trutta*)	Na-AOS C_{14-16}	Fish - 2.0-4.0 cm, continuous flow, 15°C, hardness - 250 mg/l $CaCO_3$	LC_{0-100} 24 hr - 2.7-4.3 LC_{50} 48 hr - 3.5 (3.2-3.9) 96 hr - 3.1 (2.7-3.5)	Unilever Research Laboratories, unpublished data
	Na-AOS C_{16-18}		LC_{0-100} 24 hr - 0.5-1.2 LC_{50} 48 hr - <0.3-0.5	
Rainbow trout (*Salmo gairdneri*)	Na-AOS C_{14-16}	Fish - 8-10 cm, static, 15°C, hardness - 20 mg/l $CaCO_3$	LC_{50} 24 hr - 5.1 (3.8-7.2) 48 hr - 3.5 (2.9-4.1)	
	Na-AOS C_{16-18}		LC_{50} 24 hr - 0.8 (0.6-0.9) 48 hr - 0.6 (0.5-0.8)	

b. Invertebrates

Daphnia magna, a cladoceran, is the only invertebrate for which toxicity information was found. A 24-hour LC_{50} of 15.6 mg/l (based on 100% activity) was reported for this species at 20°C for a 34.2% active Na-AOS with an equivalent weight of 299 (Continental Oil Co., unpublished data). In a static bioassay with this species at 20°C, an LC_{50} value of 14 mg/l (expressed in terms of mg/l of sodium dodecyl benzene sulfonate) was observed at 24 hours by Lundahl et al. (1972) while a slightly lower LC_{50} value (9.26 mg/l) was reported for a 28.9% active AOS (Shell Chemical Co., unpublished data). A 24 hr LC_{50} value of 2.47 mg/l was seen with this species with vinylidene AOS (Shell Chemical Co., unpublished data).

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

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

2. Subacute and Chronic Toxicity

Little information is available on the subacute or chronic effects of AOS on aquatic organisms. Hatching of fathead minnow (Pimephales promelas) eggs was 12-24% at 7.5 mg/l AOS-C_{14-16}, while lower concentrations and the

control showed 72-100% hatchability. No survival of hatched eggs was seen at AOS concentrations of 3.2 mg/l and greater. No effect on survival was observed at 1.8 mg/l. For comparison, the 24 hr LC_{50} for adults of this species was found to be 1.78 mg/l AOS (Colgate Palmolive Co., unpublished data).

In another study (Colgate Palmolive Co., unpublished data), midges (Chrionomus tentans) were exposed to AOS-C_{14-16} continuously through two generations. Survival in the first generation was reduced to 25% and 66% in two tests at 9.0 mg/l (87-90% in the control group). Survival of the second generation was also reduced (66% and 0%, respectively) at 9.0 mg/l. No effect on either generation was found at 4.5 mg/l.

3. Interactions with Other Chemicals

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

B. Effects of AOS on Higher Plants

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

surfactant and the control group.

C. Toxicity of AOS to Microorganisms

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

D. Effects of AOS on Birds and Wildlife

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

E. Mode of Action

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

Tomiyama (1975) felt that the biological effects of sulfonate-type surfactants were related to surfactant bonding with gill protein. In an examination of this question, he found that the addition of egg albumen to AOS minimized the toxic effect to Japanese killifish. "Therefore, it may be concluded that the biological effect of sulfonate-type surfactants is due to the formation of surfactant-fish protein complexes." However, this statement seems inconclusive at this time.

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V. HUMAN SAFETY

A general consideration of the human safety of surfactants was presented in Chapter 1 (LAS, p. 134).

The data reviewed below on AOS indicate they exhibit a moderately low order of acute toxicity in experimental animals. There is not, at present, a large body of data on chronic exposure studies for an evaluation of the long-term effects of ingestion of AOS, but the data available on AOS toxicity at doses far in excess of normal use levels and the relative ease of AOS biodegradation indicate that the use of these surfactants does not pose a significant hazard to human health.

A. Animal Studies

Acute Toxicity - Oral. Alpha olefin sulfonates have been shown to have a moderately low oral toxicity in rats and mice (Oba and Tamura, 1967; Oba et al., 1968; Webb, 1966; Ogura and Tamura, 1967; Tomiyama et al., 1969; unpublished data: American Cyanamid Co., Arco Chemical Co., Colgate Palmolive Co., Continental Oil Co., Ethyl Corp., Procter & Gamble Co., Shell Chemical Co., Stepan Chemical Co., Witco Chemical Corp.).

LD_{50} values ranged from 1300-2400 mg/kg in rats and 2500-4300 mg/kg in mice.(Note: all toxicity values in Section V of this report are based on 100% AOS activity unless specified otherwise.) Due to variations in AOS activity, purity, formulations, etc., correlations between α-olefin chain length and toxicity cannot be readily made. However, chain length does not appear to be a major determinant of acute oral toxicity in rodents.

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

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

LD_{50} Value (mg/kg) of C_{15-18} AOS in Mice

Method of Administration	LD_{50} Values (24 hrs)	(7 days)
Intravenous	90	90
Intraperitoneal	300	170
Subcutaneous	1,660	--

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

Acute Toxicity - Dermal. The acute dermal toxicity of C_{14-16} AOS (with 95% confidence limits) in rabbits was reported to be 1,130 (520-2,460) mg/kg and 2,150 (1,630-2,850) mg/kg, respectively (American Cyanamid Co., Ethyl Corp., unpublished data). In another study, the dermal LD_{50} values for undiluted AOS and a 36.8% solution of vinylidene AOS in rabbits were estimated to be 578 mg/kg and between 90 and 358 mg/kg, respectively (Shell Chemical Co., unpublished data).

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

Skin Sensitization. Eleven studies report on the skin sensitization potential in the guinea pig of 64 AOS samples distinguished by final product characteristics (unpublished data: Colgate Palmolive Co., Ethyl Corp., Gulf Research and Development Co., Lever Brothers Co., Procter & Gamble Co., Shell Chemical Co., Stepan Chemical Co.). No AOS formulations were tested. Most samples were

C_{14-16} AOS, a 20% aqueous solution of pure C_{16} AOS and a 10% aqueous solution of C_{16} vinylidene olefin sulfonate. The results indicated 3/20 animals cross-reacted to the C_{14-16} AOS material and 4/20 animals cross-reacted to the C_{16} vinylidene olefin sulfonate. These responses were consistent with those observed in the first study. However, in contrast to the results obtained in the first study, 7/20 animals cross-reacted to the pure C_{16} AOS material. It should be noted that in this study the assignation of cross-reactivity to C_{14-16} AOS was equivocal since 1/6 control animals yielded reactivity. No further attempt was made to investigate the discrepancy in cross-challenge data from the two laboratories (Procter & Gamble Co., unpublished data).

Because of the presence of 2-alkene sulfonate structures in AOS, sultones which have been shown to induce skin sensitization in guinea pigs (Connor et al., 1975, 1976; Ritz et al., 1975) may be formed during hypochlorite bleaching under conditions of low pH, i.e., pH $\leq$ 9.5. Thus, precise control of manufacturing conditions must be maintained to insure the elimination of these biologically reactive species. The sensitizing potential of these sultones was examined in a series of guinea pig sensitization experiments (Colgate Palmolive, unpublished data). A specially prepared AOS slurry which was bleached at pH 9.5 induced sensitization (10/10) when the challenge concentrations were 1% or 3% solutions of the slurry. The reactions were attributed to the presence of 1-tetradecene 1,3-sultone and the 2-chloro derivative. Rechallenge experiments with 3 µg of purified C_{14} and C_{16} sultones (either the 1-alkene or the 2-chloro) reaffirmed sensitization effects. Quantitatively, the lower thresholds of tolerance to induction of sensitization by 1-tetradecene 1,3-sultone and/or 2-chloro derivative in an especially prepared AOS slurry was 9 ppm or

less. However, guinea pigs already sensitized to the specially bleached AOS slurry reacted upon challenge to nanogram quantities of the sultones materials.

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

The major chemical components of AOS have also been examined for their skin sensitizing potential. Hydroxyalkane sulfonate (21% active) and alkene sulfonate (21% active) were both found to be non-sensitizers in the guinea pig (Shell Chemical Co., unpublished data).

Acute Irritation - Skin. The majority of data (unpublished data; American Cyanamid Co., Arco Chemical Co., Continental Oil Co., Ethyl Corp., Lever Brothers Co., Procter & Gamble Co., Stepan Chemical Co., Witco Chemical Corp.) concerned with the dermal irritation of AOS show it to be slightly to severely irritating to rabbit skin (see Table 6-B). Three of the AOS products tested were classified as primary irritants. The data, however, are inconsistent. In one study C_{14-16} AOS (10% active) was evaluated as a primary irritant (American Cyanamid Co., unpublished data), and only as a slight irritant in another study (Stepan Chemical Co., unpublished data). Such factors as AOS purity, method of production and/or variations in experimental technique may account for this inconsistency.

TABLE 6-B

ACUTE DERMAL IRRITATION OF AOS TO RABBITS

Carbon Chain Length	% AOS	Primary Irritation Score
C_{14}	10	0.6[g]
C_{14-16}	10	3.6[c]
C_{14-16}	1	0.2[d]
C_{14-16}	1	0.29[a]
C_{14-16}	10	1.0[g]
C_{14-16}	10	*6.2[a]
C_{14-16}	~18	4.8[b]
C_{14-16}	20	2.9[d]
C_{14-16} AOS slurry	40	4.8[h]
$C_{14-16-18}$	10	1.4[g]
C_{16}	10	1.1[g]
C_{16-18}	25.7	**6.6[f]
C_{16-18}	10	**8.0[f]
C_{17}	10	1.3[g]
C_{18}	27.5	2.3[f]
C_{18}	10	2.9[f]
NH_4 C_{15-18}	30	3.0[e]
NaAOS paste	44	3.9[e]
NaAOS spray-dried	80.5	3.1[e]

*Primary irritant according to Federal Hazardous Substances Act.
**Primary irritant according to Draize procedure.

Key: unpublished data:

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

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

There is general agreement that higher concentrations (10-40% of AOS are moderately to severely irritating to rabbits' eyes (unpublished data: Continental Oil Co., Ethyl Corp., Shell Chemical Co., Procter & Gamble Co., Stepan Chemical Co., Witco Chemical Corp.). In a typical example, 0.1 ml of 10% C_{14-16} AOS instilled in the conjunctival sac of rabbits produced slight to moderate erythema of the conjunctiva, moderate chemosis of the lids, slight discharge and discrete corneal opacity within 24 hours. Although some improvement was seen by 72 hours, corneal opacity remained (Continental Oil Co., unpublished data). At variance with the majority of data, 6%, 12%, and 20% AOS active solutions (BIO-TERGE AS-30™ formulation) were reported to be essentially non-irritating to rabbits' eyes (Stepan Chemical Co., unpublished data).

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

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

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

Incorporation of 50, 150 or 500 mg/kg of C_{14-16} (65:35) AOS (34% active) in the diet of rats for 91 days were reported to produce no treatment-related toxic or histopathological changes. "Apparent anomalies were observed in the

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

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

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

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

Subacute Skin Irritation. The subacute dermal irritancy of AOS and AOS-containing formulations has been evaluated by several investigators (Sadai et al., 1972; unpublished data: Ethyl Corp., Lever Brothers Co., Stepan Chemical Co.). In three separate studies, 10 applications (within 14 days) with either 0.5% or 1.0% AOS produced no skin irritancy or skin fatigue in rabbits (Stepan Chemical Co., unpublished data).

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

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

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

Dietary Level (ppm)	Mean Daily Intake (mg/kg/day)	
	♂	♀
1000	39	57
2500	96	132
5000	195	259

(Hunter and Benson, 1976)

Acute, Subacute, and Chronic Toxicity - Summary. The alpha olefin sulfonates exhibit a moderately low order of toxicity in rodents. Acute oral LD_{50} values in mice are larger (2,500 to >4,000 mg/kg) than intravenous, intraperitoneal and subcutaneous values ($\leq$1,600 mg/kg) indicating either a low rate or incomplete AOS absorption or rapid metabolism and elimination.

AOS is slightly to moderately irritating to rabbit skin. AOS concentrations of 1% are not ocular irritants in rabbits but concentrations greater than 5% are capable of producing severe ocular damage.

A subacute oral toxicity study has shown that rats fed 1,000 mg/kg/day AOS for 90 days suffered no adverse effects. Rats also appeared to tolerate well both repeated dermal and inhalation exposure to AOS.

Rats fed up to 5,000 ppm AOS for two years showed no signs of abnormality. However, there is a marked absence of additional data on the effects of chronic oral exposure to AOS in laboratory animals.

Carcinogenicity. Two carcinogenic studies evaluated AOS and indicated that AOS is not carcinogenic in rodents when administered either percutaneously or orally. In one study, no changes in skin or organ structures related to treatment, other than occasional dermatitis, were noted in Swiss-Webster mice (21/group) following 2 years of twice-weekly dermal applications of 5% aqueous solutions of either C_{15-18} AOS (90% active), hexadecane 1,4-sultone, or a sultone concentrate (64% active) extracted from the process stream during the sulfonation of C_{15-18} α-olefin (Chevron Chemical Co., unpublished data).

In another investigation Hunter and Benson (1976) observed no increased incidence of tumors in CFX rats fed up to 5000 ppm of C_{14-18} AOS for a period of two years.

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

Mutagenicity. Concentrations of 5000 mg/kg of four AOS products (21-38% active) were not mutagenic when tested with Salmonella typhimurium TA 1535 or Saccharomyces cerevisiae D 3 in a host-mediated assay with mice. Propane sultone (460 mg/kg), however, was mutagenic in both systems, while 1,4-butane sultone was found to be mutagenic for S. typhimurium but not mutagenic for S. cerevisiae (Colgate Palmolive Co., unpublished data).

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

derived from C_{14-16} α-olefins (the others were not identified). Product characteristics included: deoiled and bleached, bleached only, spray-dried, paste, sodium salts of AOS, and over- and under-saponified at high (169°C) and low (95-100°C) temperature and elevated and normal atmospheric pressure.

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

incomplete hydrolysis. The frequency of positive response was reduced (1/10) but not eliminated when the latter sample was rehydrolyzed (Ethyl Corp., unpublished data). A C_{14-16} (3:2) AOS paste (29.4% active) was found to be a skin sensitizer in 10/19 guinea pigs challenged with a 10% aqueous solution of the product; there were 5/10 positive responses with a 5% challenge concentration. Similar findings were reported for a C_{16-18} (55:45) AOS paste (25.7% active); positive responses were noted in 10/20, 8/20, and 2/20 animals challenged with 20%, 15% and 7.5% aqueous solutions, respectively, of the product (Procter & Gamble Co., unpublished data). In another study, repeated topical application of undiluted C_{16-18} AOS resulted in a 50% incidence of positive responses (10/20) in guinea pigs challenged with a 20% aqueous solution of the surfactant. When these animals were tested for cross-reactivity to other AOS materials, positive reactions were observed in 4/19 animals with a 10% aqueous solution of C_{14-16} AOS and in 4/19 animals challenged with a 10% aqueous solution of C_{16} vinylidene olefin sulfonate. No reactions (0/19 animals) were observed when these animals were cross-challenged with pure C_{16} AOS (20% aqueous solution). It should be noted that the interpretation of cross-reactivity was equivocal due to the presence of reactive control animals.

In order to clarify these findings, the primary sensitization study was repeated in a second laboratory, the only difference between the two tests being that a 15% aqueous solution was used as the challenge concentration. In this study a positive response was noted in 40% of the animals (8/20). These animals were then cross-challenged with a 10% aqueous solution of

Seven olefin sulfonate preparations tested at concentrations up to 2 mg/plate were found to be non-mutagenic in assays with Salmonella typhimurium bacterial strains TA 1535, TA 1536, TA 1537, or TA 1538. Five of these preparations tested at concentrations of 0.01, 0.1 and 1.0% were also non-mutagenic in the yeast assay with Saccharomyces cervesiae D3. The responses of acid or alkaline-bleached olefin sulfonate were confounded by poor survival of the yeast cells. When concentrations of test materials which did not decrease survival were used, there were no differences between control and treated groups.

The alkaline-bleached slurry gave only a 3% average survival. Without metabolic activation, the alkaline-bleached sample did not elicit positive responses at 0.01% and showed 88% average survival. The test was repeated at 0.02, 0.04, 0.06, 0.08, and 1% concentrations and no evidence of mutagenic effects was obtained. Survival of the cells was 12% and 21% for the acid-bleached and alkaline-bleached samples tested at 1% respectively. Both 1,3-propane sultone and 1,4-butane sultone served as the positive controls and were mutagenic in these systems (Colgate Palmolive Co., unpublished data).

In another study, a concentration of 283 mg/kg of a particular batch of C_{14-16} AOS (28.4% active) was found to be mutagenic in a host-mediated assay with rats when tested with Salmonella typhimurium TA-1530 (a point mutant), but not mutagenic with S. typhimurium TA-1534 (a frameshift mutant). In vitro plate assays with AOS concentrations up to 1% were negative for both

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

At a concentration of 1000 μg/ml AOS (36% active) was found to be 100% cytotoxic against actively dividing human diploid fibroblasts (WI38 cells) *in vitro*. Reduction in cell viability of 80 and 85% were noted for AOS which were 38% and 21% active, respectively. However, there are no indications that

AOS produce DNA damage in these mammalian cells in vitro. Although a generally low level of DNA repair, which in some cases exceeded control values, was seen in human diploid fibroblasts (WI38 cells) exposed to 0.1, 1. 10, 100 or 1000 μg/ml AOS in an unscheduled DNA synthesis assay, there was no indication that AOS induced unscheduled DNA synthesis (Colgate Palmolive Co., unpublished data).

Reproduction Studies. No reports were found of studies on the effects of alpha olefin sulfonates on reproductive performance of test animals.

Teratogenesis. Only a single study was found which assessed the possible teratogenic and embryopathic potential of AOS. Palmer et al. (1975) administered C_{14-18} AOS by gavage to pregnant rats (20/dose), mice (20/dose) and rabbits (13/dose) at dosage levels of 0.2, 2, 300 and 600 mg/kg/day. Mice and rats were treated on days 6-15 of pregnancy, rabbits on days 6-18 of pregnancy.

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

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

maternal mice and rabbits showed initial retardation in body weight gain at 0.2 and 2.0 mg/kg of AOS.

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

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

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

With respect to anomalies, four pups (3 litters) at 600 mg/kg and 2 pups (1 litter) at 300 mg/kg had cleft palates. There was also a significantly

higher incidence (98.5% vs 1% for controls) of skeletal anomalies (generally retarded ossification) seen at 600 mg/kg; a higher incidence (22-39%) of skeletal anomalies was also observed at the lower treatment levels.

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

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

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

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

Pharmacology - Antigenicity. Rabbits were immunized over a 2 1/2 month period with either C_{15-18} AOS, human serum albumin (HSA) or with a lipid-free, HSA-AOS complex. Serum from immunized rabbits was then tested via a precipitin reaction for the presence of antibodies for any of the above three antigens. AOS was found to possess no antigenic properties in the rabbit and immunization with the HSA-AOS complex did not provoke the formation of AOS antibodies in rabbit serum (Iimori and Ushiyama, 1971).

B. Human Studies

Skin Irritation. Two immersion and five patch tests were performed to assess the irritancy of AOS to humans (Oba et al., 1968, 1969; Webb, 1966; unpublished data: Lever Brothers Co., Procter & Gamble Co., Witco Chemical Corp.).

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

In immerison studies, an 0.3% AOS concentration caused negligible irritation after 30 one-minute hand immersions in a 1-hour period (Tomiyama et al. 1969) and a 0.25% concentration of a liquid detergent formulation containing

17% AOS was classified as a mild irritant in a test involving 15-minute immersions, three times daily for up to a maximum of 15 days. Fifty percent of the test subjects were able to complete 12 immersions before reaching a predetermined irritation level (a score of "2") at which point treatment was discontinued (Lever Brothers Co., unpublished data).

Skin Sensitization. One sensitization study involving approximately 200 human volunteers found 0.5% concentrations of AOS (3% aqueous solution of sample containing 16% AOS) to be nonsensitizing in a standard Draize patch procedure (Colgate Palmolive Co., unpublished data). AOS was applied to the back 3 times weekly for a total of 10 applications. Test subjects were challenged 72 hours after the last AOS application.

In a separate study using a standard Draize test, no evidence of contact sensitization (but severe irritation) was found in 88 men following application of an occlusive patch containing an 8% aqueous solution of AOS 3 times per week for a total of 10 applications. The men were challenged with a 4% AOS solution two weeks after the last application. (Colgate Palmolive Co., unpublished data).

C. Epidemiology

Accidental Exposure. The issue of accidental ingestion of detergents was considered in Chapter 1, p. 158. No reports of injury resulting from human exposure to alpha olefin sulfonates in use or manufacturing situations have been found.

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SECONDARY ALKANE SULFONATES

Synopsis

Currently, the anionic secondary alkane sulfonates are commercially available primarily in Europe and are principally used as components of liquid surfactant formulations.

There are presently no environmental standards of water quality with respect to SAS. Levels of SAS in streams and waterways are not presently being monitored, but MBAS levels would include SAS, if present. The limited information available on SAS surfactants indicates that they are readily biodegraded under both field and laboratory conditions.

There is a paucity of data available on the aquatic toxicity of SAS and the available information dealing with the human safety aspects of SAS is scant. The few studies available in these two areas do not allow any general conclusions to be drawn in terms of the environmental acceptability or human safety of SAS.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

SECONDARY ALKANE SULFONATES

I. INTRODUCTION

Available since the 1940's, commercial secondary alkane sulfonates (SAS) are predominantly linear (n) with the sulfonate group secondary and randomly positioned along the carbon chain. SAS have good detergency and foaming properties as well as high water solubility characteristics (Kerfoot and Flammer, 1975; Konecky and Fleming, 1963). Presently, the production and use of SAS surfactants, in a variety of detergent applications, are largely limited to the European continent.

Due to the relative inertness of n-paraffins to sulfuric acid, SAS cannot be produced by direct sulfonation. Therefore, commercial production of SAS is accomplished via a sulfoxidation reaction; i.e., n-paraffins in the C_{14}-C_{18} range are reacted with sulfur dioxide and oxygen. The reaction is catalyzed by ultraviolet radiation (Schneider, 1970). Process improvements have reduced the percentage of disulfonate by-products formed. The sulfonate group can be added at any position along the paraffin chain, yielding a mixture of isomeric sulfonic acids as a final product. Reaction by-products include di- and polysulfonates and may contain non-linear analogues (Higgins and Burns, 1975; Schneider, 1970; Swisher, 1970, p. 347). The sulfonic acids produced during the sulfoxidation process are subsequently base neutralized

and bleached prior to use. For example:

$$C_xH_{2x+2} \xrightarrow[UV]{SO_2/O_2} C_xH_{2x+1}SO_3^-H^+ \xrightarrow{NaOH} C_xH_{2x+1}SO_3^-Na^+$$

where x usually is between 14 and 18 carbons. The final product generally contains 85-87% alkane monosulfonate, 7-9% alkane disulfonate, <5% sodium sulfate and 1% unreacted paraffins (Schneider, 1970).

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II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alkane sulfonates, one of several chemical entities classified as anionic surfactants, can be detected with many of the procedures utilized in the detection of LAS (see Chapter 1, pp. 19-26). Normally, presumptive levels of alkane sulfonates in the environment are detected with methylene blue procedures. TLC and GC methods have also been used to detect SAS (Kupfer et al., 1969).

B. Water Quality Standards

There are presently no standards in the United States or Europe specifically restricting secondary alkane sulfonates (SAS). These anionic surfactants are included among those measured in the environment using the MBAS method. The restrictions applying to MBAS levels were discussed in Chapter 1, pp. 26-29.

C. SAS in Natural Water Bodies

SAS are not presently being monitored, as such, in the United States or Europe. MBAS measurements in water bodies include SAS surfactants as well as other anionics. Levels of anionic surfactants detected in natural water bodies were discussed in Chapter 1, p. 30. The contribution of SAS to MBAS levels in the United States would be minimal since these surfactants have not been extensively used in this country.

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III. BIODEGRADATION

This section will consider the limited information available on the biodegradability of secondary alkane sulfonates (SAS). A discussion of the procedures utilized to investigate the biodegradation of anionic surfactants both in the laboratory as well as under field conditions can be found in Chapter 1 (LAS, III).

A. Laboratory Test Systems

1. Oxygen Uptake - Biochemical Oxygen Demand

Only one study was found which examined the relationship between carbon chain length and biochemical oxygen demand in the standard 5 or 20 day BOD tests. The available data (see Table 7-A) indicate that the alkyl chain length does not appear to influence the rate of biodegradation of secondary alkane sulfonates. At 5 days, BOD values ranged from 20 to 56% for SAS with 13 to 18 carbon atoms, but by 20 days, all surfactants had exceeded 70% of the theoretical oxygen demand (Procter & Gamble Co., unpublished data). Similarly, using the Warburg test, Krone and Schneider (1968) reported an oxygen uptake of 35-40% of theoretical for a secondary alkane sulfonate at 7 days.

TABLE 7-A

BIODEGRADABILITY OF SECONDARY ALKANE SULFONATES

Carbon Chain Length	% BOD_5*	% BOD_{20}**	% CO_2†
C_{13}	42	T.D.‡	72
C_{14}	32	T.D.	70
C_{15}	40	80	70
C_{15}	21	T.D.	73
$C_{15.5}$	46	71	77
C_{16}	33	T.D.	68
C_{17}	20	T.D.	63
C_{18}	56	84	71

*Percent biochemical oxygen demand at 5 days.

**Percent biochemical oxygen demand at 20 days.

†Percent of theoretical carbon dioxide production.

‡‡Total depletion of oxygen.

(Procter & Gamble Co., unpublished data)

2. CO_2 Evolution

The degradation of several secondary alkane sulfonates possessing different carbon chain lengths was monitored using Sturm's evolved CO_2 procedure (1973). All were readily biodegraded (63 to 77% CO_2) with no apparent pattern of different biodegradative rates according to varying alkyl chain length (see Table 7-A). (Procter & Gamble Co., unpublished data).

3. Die-Away Tests

a. River Water Test

Krone and Schneider (1968) observed complete primary biodegradation (MBAS) of 10 mg/l SAS within 2-3 days in a river die-away test. Similar findings were also reported by Eden et al., 1968.

Working with an unacclimated inoculum, McAteer and Kinnard (1967) found that the biodegradative rates of a homologous series of commercial secondary alkane sulfonates were reduced with increasing alkyl chain length (see Table 7-B). Swisher (p. 233, 1970) points out that the additional time required to achieve complete removal of SAS with longer alkyl chains may reflect a longer acclimation time and not necessarily a slower rate of

biodegradation. Furthermore, Kölbel et al., 1964 (cited in Swisher, 1970, p. 233) found that pure, linear, primary alkane sulfonates (C_{10}, C_{12}, C_{14} C_{16}) remained completely undegraded for the first 3 days of the experiments, but were completely degraded (MBAS) by Escherichia coli within 6 days.

TABLE 7-B

CARBON CHAIN LENGTH vs SAS BIODEGRADABILITY

Carbon Chain Length	Days for Complete Removal (MBAS)	% COD Removal at 21 Days
13	4	26
15	4	24
16	4	28
17	7	27
19	10	14

(McAteer and Kinnard, 1967)

b. Fortified and Inoculated Waters

Lundahl et al. (1972) observed greater than 97% biodegradation (MBAS) of C_{14}, C_{14-15}, C_{13-17} and C_{14-17} alkane sulfonates within 3 days.

c. Shake Culture Test

A 20 mg/l sample of C_{14-18} SAS completely biodegraded (99% MBAS removed) within 7 days in a shake flask test. (Continental Oil Company, unpublished data).

4. Simulated Treatment Processes

a. Activated Sludge

Janicke (1971) found that C_{10-18} alkane sulfonate (90% C_{14-17}, predominantly with unbranched alkyl chain) was biodegraded greater than 98% (MBAS) up to a surfactant loading level of 100 mg/l in a laboratory-scale, activated-sludge unit (see Table 7-C). Even at a surfactant concentration of 200 mg/l, a removal of approximately 91% MBAS was achieved although a slight retardation in rate was noted. Janicke postulated that at higher concentrations of alkane sulfonates, the proportion of intermediates produced increased, thus accounting for the difference in MBAS and Total Organic Carbon (TOC) values presented in Table 7-C.

TABLE 7-C

SAS LOADING LEVEL vs BIODEGRADABILITY

Alkane Sulfonate Concentration (mg/l)	Percent Biodegradation	
	MBAS	TOC
20	99.5	96
50	99.5	91
100	98.3	84
200	90.7	--

(Janicke, 1971)

Eden et al. (1968) also found that 99% of SAS was removed (MBAS) from a laboratory-scale, activated sludge unit with a six hour retention period.

b. Trickling Filters

Removal of secondary alkane sulfonates averaged 97-99% (MBAS) two weeks after the first addition of SAS into a pilot-scale percolating filter sewage treatment system. The time needed for acclimation and the actual time required to biodegrade SAS once acclimation was achieved were not stated (Eden et al., 1968).

5. Influence of Temperature on the Test System

Using the official German activated sludge test, Krone and Schneider (1968) found that a reduced rate of SAS biodegradation occurred as the temperature was reduced. At 2 days, the extent of biodegradation (MBAS) was 30% at 5°, 50% at 15° and 90% at 25°C. By 11 days, the temperature differential was not as dramatic with greater than 80% MBAS removal at 5° and 90% removal at both 15° and 25°C. Acclimation appears to occur readily at low temperatures.

B. Field Studies

During winter operation (8°C), 85% of the SAS entering a trickling filter sewage treatment plant was removed (MBAS) during passage through the

plant (Krone and Schneider, 1968). Similarly, Kelly (1965; cited in Swisher, 1970, pp. 191, 362) reported a 70% removal of a spiked SAS sample added to the influent of a trickling filter sewage treatment plant which served a population of 1500 persons.

C. Metabolic Pathways of Biodegradation

Thysse and Wanders (1972) demonstrated that the initial attack of two strains of Pseudomonas (AJ1 and AJ2) on short chain n-alkane sulfonates consisted of a desulfonation reaction. The fatty acid formed after the sulfonate group is removed is subsequently degraded via β-oxidation. Terminal respiratory processes eventually lead to complete mineralization to CO_2 and water. Thysse and Wanders established that this pathway is the only metabolic pathway for the AJ1 strain growing on low molecular weight alkane sulfonates (C_4-C_6) but they were unable to exclude an additional pathway for the AJ2 strain growing on higher molecular weight (C_7-C_{12}) sulfonates. Although these studies were with primary alkane sulfonates, they are reported as a possible model for SAS biodegradation.

D. Summary

The information available on the biodegradability of secondary alkane sulfonates indicate that as a class, they are readily biodegraded under aerobic conditions. Alkyl chain length does not appear to influence the rate of biodegradation. Reduced environmental temperature does appear to retard the degradative rate. Metabolically, alkane sulfonates appear to be desulfonated and subsequently degraded via β-oxidation.

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IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity - Fish

Little information is available concerning the aquatic toxicity of secondary alkane sulfonates (SAS) to fish. Knauf (1973) reported that for the guppy, Lebistes reticulatus, toxicity increased with increasing alkyl chain length. A similar trend was noted in another study conducted with bluegill (see Table 7-D). The 96 hr LC_{50} values ranged from 1.3 mg/l (C_{18}SAS) to 144 mg/l (C_{13}SAS). (Procter & Gamble Company, unpublished data).

Schneider (1970) reported an LC_{50} for SAS to guppies (3.1-3.6 mg/l), trout (3.6-3.9 mg/l) and carp (23.6 mg/l). No experimental details were given.

B. Aquatic Toxicity - Invertebrates

Lundahl et al. (1972) found that the toxicity of SAS to Daphnia magna increased with increasing molecular weight (Table 7-E).

C. Aquatic Toxicity - Sewage Effluent

The sewage from a biologically active sludge treatment facility treated with distearyldimethylammonium chloride and an approximately threefold excess of SAS was studied over an eight-month period. A variety of organisms were examined including algae, higher water plants (Myriophyllum spicatum, Lemna minor), invertebrates (Daphnia magna, Planorbis corneus) and fish (Cyprinus

carpio). No indications of toxicity from this particular sewage effluent was observed (Neufahrt et al., 1976).

D. Toxicity of SAS to Microorganisms

Lundahl et al. (1972) studied the effect of SAS on Escherichia coli. This organism was incubated at 37°C on gelatin media containing various concentrations of surfactant. The authors found that the lowest concentration which did not allow the development of more than 5 colonies per plate was 20 g/l for C_{14-15} SAS, 200 g/l for C_{14} SAS, and greater than 200 g/l for C_{13-17} SAS and C_{14-17} SAS.

There are insufficient data to draw any definitive conclusions on the aquatic toxicity of SAS. However, the information available suggests that the aquatic toxicity of SAS increases with increasing alkyl chain length.

TABLE 7-D

Toxicity of SAS to Bluegill[†] (Lepomis macrochirus)

Chain Length	LC_{50} (mg/l)
13	144
14	42.9
15	5.6
15	13.5
15.5	8.2
16	4.6
17	1.6
18	1.3

[†]Static, 21°C, pH 7.1

Procter and Gamble Co. unpublished data

TABLE 7-E

Toxicity of SAS to Daphnia magna*

Surfactant	Molecular Weight	LC_{50}** (24 hr)
C_{12}SAS	272	282
C_{14}SAS	300	45
C_{14-15}SAS	307	21
C_{13-17}SAS	316	9.1
C_{14-17}SAS	---	10.5

* 20°C, in synthetic river water. (0.2g $NaHCO_3$, 0.35g NaCl, 0.026g K_2SO_4 in 1 liter of distilled water).

** expressed as mg/l Na-dodecyl benzene sulfonate. The method of calculation was not given.

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V. HUMAN SAFETY

A general consideration of the human safety of surfactants was presented in Chapter 1 (LAS, p. 134).

There are only a very limited number of studies describing the animal toxicity of secondary alkane sulfonates (SAS). Other than the usual battery of acute oral toxicity, skin and eye irritation studies, no information was found dealing with carcinogenic, mutagenic or teratogenic potential of this class of surfactants; these studies are now under way.

A. Animal Studies

Acute Toxicity - Oral. The acute oral LD_{50} in rats is reported to be in the range of about 1000 to 3000 mg/kg (Berth et al., 1972; Schneider 1970; Procter & Gamble Co., unpublished data). Quack and Reng (1976) reported oral LD_{50} values of 2000 mg/kg for the rat and between 500 and 1000 mg/kg for dogs. There are insufficient data to determine whether toxicity varies with length of the carbon chain.

Acute Toxicity - Dermal. The application of 2 ml/kg of 60 percent active paraffin sulfonate (1200 mg/kg) to the abraded skin of 3 rabbits resulted in the death of one. When applied to unabraded skin the same dose was not fatal (Procter & Gamble Co., unpublished data).

Acute Irritation - Ocular. The preparations of SAS for which data are available are positive eye irritants in the rabbit when tested by the Draize procedure. Both 20 percent and 60 percent active solutions of SAS produced severe ocular damage in one or more eyes of treated rabbits. Rinsing reduced the damage so that 2 of 3 rabbits exhibited normal eyes in seven days after treatment with 20 percent active SAS. Similarly, a C_{15-18} SAS, 40 percent active, gave rise to marked ocular irritation. (Unpublished data: Continental Oil Company, Procter & Gamble Company).

Acute Irritation - Skin. Severe irritation with scar tissue evident after seven days was produced by application of 20 and 60 percent active SAS. A 40 percent active preparation of C_{15-18} SAS also produced a marked skin response. (Unpublished data: Continental Oil Company, Procter & Gamble Company).

Skin Sensitization. In the guinea pig, topical application of 10% SAS to intact skin 3 times per week for 3 weeks gave no indication of sensitization. Similarly, guinea pigs challenged 2-3 weeks after the last intracutaneous injection of 0.02 ml of various concentrations (0.001-10%) of SAS, gave no indication of sensitization (Barail test), nor was sensitization noted (Magnusson-Kligman procedure) in guinea pigs induced intracutaneously with a 5% aqueous solution of SAS (Hoechst AG, unpublished data).

Subacute Toxicity - Oral. A single report has shown that SAS at a dose of 300 mg/kg/day in the food was not toxic to rats in a 45-day study. The toxic limit for rats in this time interval of treatment was 500 mg/kg/day (Schneider, 1970). A preliminary report by Quack and Reng (1976) also states that no signs of toxicity were observed in rats given 30 doses at concentrations less than 500 mg/kg SAS over a 44-day-period. No data were available for review.

Subacute Skin Irritation. In a 28-day study with New Zealand rabbits, the daily application to abraded skin of 2 ml/kg/day of a 10 percent active solution of SAS (200 mg/kg/day) did not result in any unusual systemic lesions. Severe skin irritation was noted in the treated rabbits (Procter & Gamble Co., unpublished data).

Chronic Toxicity - Oral. In a preliminary report, Quack and Reng (1976) reported no adverse effects in rats given 100 ppm SAS in their drinking water for one year. No data were available for review. A twelve-month oral feeding study mentioned by Schneider (1970) in his review, reportedly gave rise to no pathological findings. The absence of data on the doses of SAS used and on the specifics of the results does not permit evaluation of these two studies for safety.

Pharmacology - Enzyme Inhibition. The inhibitory action of secondary octane sulfonate and its C_{17} homolog on alkaline phosphatase, acid phosphatase, invertase and papain was examined by Czok et al. (1969). They found

that inhibitory action was greater for the longer chain compound. An inhibition of 50% occurred at a concentration of 25,000 to 50,000 mg/l for the C_8 compound, while only 25 to 600 mg/l were needed to achieve the same value with C_{17} SAS.

B. Human Studies

Skin Irritation. In a repeated patch test carried out according to the Draize procedure, 12 of 75 individuals who completed the study exhibited irritation of grade 2 or better using an 0.25 percent solution of a paraffin sulfonate. Little or no irritation occurred in the remaining members of the test panel. The panel consisted of persons ranging from 16 to 60 years of age with twice as many females as males (Procter & Gamble Co., unpublished data).

In another study, after one week of two 30-minute applications/day of 0.1% SAS (method of Polano, 1968), 5 of the 26 individuals who completed the study had no irritation, 15 had mild irritation, and 6 individuals had signs of distinct irritation (Hoechst AG, unpublished data).

Toxicity Summary. The few studies available for the evaluation of SAS do not allow any general conclusions to be drawn regarding the safety of this particular surfactant. Although SAS have a relatively low order of oral and dermal toxicities, the studies on skin and ocular irritation suggest that these sites may be susceptible to local effects from SAS exposure.

No information was found to assess the chronic oral effects of SAS or the potential for induction of carcinogenic, mutagenic or teratogenic lesions in experimental animals.

C. Epidemiology

Accidental Exposure. The issue of accidental ingestion of detergents was considered in Chapter 1, p. 158. No reports of injury resulting from human exposure to secondary alkane sulfonates in use or manufacturing situations have been found.

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