HUMAN SAFETY AND ENVIRONMENTAL ASPECTS OF MAJOR SURFACTANTS (SUPPLEMENT)

A REPORT

BY

MURIEL M. GOYER, JOANNE H. PERWAK, ANDREW SIVAK, PHILIP S. THAYER

ARTHUR D. LITTLE, INC.

TO

THE SOAP AND DETERGENT ASSOCIATION

ADL REFERENCE 84048

FEBRUARY 20, 1981

This report was prepared by Arthur D. Little, Inc., for the Soap and Detergent Association, Incorporated. The material in it reflects the best judgment of Arthur D. Little, Inc., in the light of the information available to it at the time of preparation. Any use which a third party makes of this report or any decisions made based on it are the responsibility of such third party.

PREFACE

This report was prepared as a supplement to our report to the Soap and Detergent Association, "Human Safety and Environmental Aspects of Major Surfactants" dated May 31, 1977, hereafter referred to as "the 1977 Report." The open literature published through mid-1980, as well as unpublished data supplied to us by the Association and its member companies on the human safety and environmental aspects of seven classes of surfactants have been evaluated and are presented in this supplement. We have also incorporated several relevant earlier papers which were not included in our initial review. For the purpose of continuity, we have provided highlights of data from our earlier report, presented in italicized, blocked modular format, as a preface to each major area of interest.

The surfactant classes for which data are reviewed include: (1) linear alkylbenzenesulfonates (LAS), (2) alkyl sulfates (AS), (3) alcohol ethoxylates (AE), (4) alcohol ethoxy sulfates (AES), (5) alkylphenol ethoxylates (APE), (6) alpha olefin sulfonates (AOS), and (7) secondary alkane sulfonates (SAS).

The areas of interest considered include those that were evaluated in our earlier report:

(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.

The new data presented in this supplemental report on the environmental and human health aspects of those seven, commercially important surfactants, re-affirm the view that present domestic and commercial use of these surfactants poses no threat to human health or environmental quality. The primary support for the human safety and environmental acceptability of these surfactants comes from the very low, residual concentrations of these surfactants in United States waterways, their facile biodegradation, the margin of safety between environmental levels and toxic concentrations for aquatic species, the low order of acute mammalian toxicity and the general absence of carcinogenic, mutagenic, teratogenic or other chronic toxic effects in mammalian test systems.

CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

The following abbreviations and symbols have been used throughout the text:

ABS	Branched alkylbenzene sulfonates, usually with alkyl groups in the detergent range; i.e. above C_6.
AE	Alcohol ethoxylates
AES	Alcohol ethoxy sulfates
AI	Active ingredient
AM	Active material
AOS	Alpha olefin sulfonates, α-olefin sulfonates
APE	Alkyl phenol ethoxylates
AS	Alkyl sulfates
AVE	Average; used to designate broad-cut-derived alkyl sulfates
BCF	Bioconcentration factor
BIAS	Bismuth iodide active substance
BOD	Biochemical oxygen demand
br-	Branched
b.w.	Body weight
C	Celsius
Ca	Calcium
CL	Confidence limits
cm	Centimeter
CMC	Critical micelle concentration
CO_2	Carbon dioxide
CO_3	Carbonate
COD	Chemical oxygen demand
conc	Concentration
CTAS	Cobalt thiocyanate active substance

DOC	Dissolved organic carbon
EC_{50}	The concentration required to produce an effect in 50% of the test individuals.
EEC	European Economic Community
Enz	Enzyme
EO	Ethylene oxide
EPN	A phenylphosphonothioate insecticide
E_x, E_n	Polyethylene glycol or ethoxylate nonionic averaging x (or n) EO units per molecule
F	Fahrenheit
F_1,F_2	Offspring of first, second, etc., filial generation
FIR	Far infrared spectroscopy
GC	Gas chromatography
GLC	Gas-liquid chromatography
g	Grams
HCl	Hydrochloric acid
hr	hours
HPLC	High-performance (high-pressure) liquid chromatography
IR	Infrared spectrosocopy
kg	Kilogram
L	Liter
LAB	Linear alkylbenzenes
LAS	Linear alkylbenzene sulfonates
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

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

MBAS	Methylene blue active substance
mg	Milligram
mgd	Million gallons per day
min	Minute
ml	Milliliter
mM	Millimole
mol. wt. M.W.	Molecular weight
MS	Mass spectrometry
n-	Linear
N	Normal
Na	Sodium
NH_3	Ammonia, ammonia liberation
NMR	Nuclear magnetic resonance
nm	nanometer (10^{-9} meters)
NOEC	No observed effect concentration
O_2	Oxygen, oxygen uptake
OECD	Organization for Economic Cooperation and Development
PC	Paper chromatography
PEG	Polyethylene glycol
pH	A measure of hydrogen ion concentration
POE	Polyoxyethylene residue
ppb	Parts per billion (equivalent to µg/L)
ppm	Parts per million (equivalent to mg/L)
pri-	Primary
Rf	In paper chromatography, the ratio of the movement of a given spot to that of solvent boundary.
SAS	Secondary alkane sulfonates

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

SCAS	Semi-continuous activated sludge system
SDA	Soap & Detergent Association
sec-	Secondary
SPC	Sulfophenylcarboxylic acids
STCSD	Standing Technical Committee on Synthetic Detergents
STORET	Storage and Retrieval of water quality data, U.S. Environmental Protection Agency computerized data base.
TAE	Tallow alcohol ethoxylates
tert-	Tertiary
TLC	Thin layer chromatography
TLm	The concentration that results in 50% survival over a specified time interval
TM	Registered manufacturer's trademark
TOC	Total organic carbon
TOD	Total oxygen demand
tp-	Tetrapropylene
UV	Ultraviolet spectroscopy
UVF	Ultraviolet fluorescence
μ	Micron
μCi	Microcurie
μg	Microgram
μM	Micromole
w/v	Weight in volume
w/w	Weight in weight
o	Degrees, as 37°
$^{o}/_{o}$	Percent
$^{o}/_{oo}$	Salinity in parts per thousand

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

The phrase "the 1977 Report" refers to an earlier Arthur D. Little, Inc., report to the Soap and Detergent Association entitled Human Safety and Environmental Aspects of Major Surfactants, NTIS Document PB 301 193, Springfield, Virginia. 1977.

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, 1971; or Inbred and Genetically Defined Strains of Laboratory Animals, Parts I and II, P.L. Altman and D.D. Katz, eds. Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1979.

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Linear Alkylbenzene Sulfonates

(LAS)

LINEAR ALKYLBENZENE SULFONATES

Synopsis

As in the mid-1970's, the linear alkylbenzene sulfonates (LAS) still represent a substantial portion of today's surfactant market. In 1978, approximately 640 million pounds of LAS were produced in the United States.

Although a number of analytical methods are available for the determination of LAS in water samples and in experimental studies, the assay for methylene blue active substances (MBAS) remains the predominant method used. Recently, attention has centered on improving the specificity for LAS directly (as opposed to the non-specific MBAS) as well as the sensitivity of various analytical methods.

With respect to environmental water quality, no national, state or local regulatory changes have been instituted since 1977, and a concentration of 0.5 mg MBAS/L remains the accepted maximum permissible level for drinking water. The United States Environmental Protection Agency reports mean MBAS levels for different bodies of water in the U.S. for the last decade that are generally well below the 0.5 mg/L value.

Studies carried out on biodegradation of LAS reconfirm the facile biodegradability (over 90%) of this class of surfactants in both laboratory and field situations.

Recent studies confirm that an increase in the carbon chain length of LAS is accompanied by an increase in toxicity for both fish and other aquatic organisms, reaching a maximum value between C_{16} and C_{20} and declining thereafter. Differences of two orders of magnitude in LC_{50} values have been observed between C_{10} LAS and C_{16} LAS. In general, LC_{50} values for LAS range from 0.1 - 10 mg/L for fish. Toxicity to invertebrates appears to occur in the same general concentration range. Sublethal effects, such as reduced swimming rates, may occur at slightly lower concentrations. Concentrations of 0.6 to 1.7 mg/L were lethal to minnows with chronic exposure, while invertebrate survival was reduced at 0.4 to 4.4 mg/L.

Toxicity appears to increase with the hardness of both culture and test water, but appears to decrease with the addition of suspended solids. Biodegradation reduces toxicity of LAS to aquatic organisms by at least two mechanisms: (1) preferential degradation of the 2-phenyl isomer and (2) oxidation of the structure, beginning with the alkyl chain. The mode of action of LAS has generally been related to its effect on gills, hypothesized to be a result of decreased selectivity of the cell membrane.

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

LAS, therefore, remain acceptable with respect to both human health and environmental safety for consumer and industrial use.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LINEAR ALKYLBENZENE SULFONATES

I. INTRODUCTION

The highly degradable, environmentally safe linear alkylbenzene sulfonates (LAS) were introduced as a prime component of detergents in 1965 to replace the more refractory tetrapropylene-derived alkylbenzene sulfonates (ABS) in use at that time. LAS are a complex mixture of isomers and homologues whose proportions are dependent on starting materials and reaction conditions. LAS in commercial use contain linear alkyl chains ranging from 10 to 14 carbons in length with phenyl groups placed at various internal carbon positions in the alkyl chain. LAS represent a substantial portion of the total surfactant market; e.g., in our 1977 Report, we noted a total of 678 million pounds of LAS were produced in the United States in 1973.

Due to their versatility, LAS surfactants remain a prime component of almost all types of household surfactant products (Matson, 1978).

Total United States production of surface-active agents in 1978 amounted to 4738 million pounds, 65% of which are in the anionic category. Total LAS production in 1978 was 640 million pounds (U.S. International Trade Commission, 1979).

BIBLIOGRAPHY

MATSON, T. P. 1978. The workhorse surfactants: LAS, alcohol sulfates and ether sulfates. J. Am. Oil Chemists' Soc. 55:66-69.

U.S. INTERNATIONAL TRADE COMMISSION. 1979. Synthetic Organic Chemicals. United States Production and Sales of Surface-Active Agents, 1979.

II. ENVIRONMENTAL LEVELS

Although a number of analytical methods are available for the determination of LAS in water samples and experimental studies, the primary method in use is the assay for methylene blue active substances (MBAS). The major limitation of this assay is the inability to discriminate between LAS and other anionic materials. A gradual decrease in MBAS levels was noted in most waterways during the change from tetrapropylene-derived ABS to LAS surfactants and by 1975, MBAS levels in waterways of the United States were well below the established standard for drinking water (0.5 mg MBAS/L).

A. Analytical Methods

The analytical methods available for studies of LAS and their biodegradation products included: physical methods (foaming and surface tension); chemical methods (MBAS and modifications thereof, azure A, two-phase titrations, and solvent extraction with iron chelates); and physicochemical methods (thin layer and paper chromatography, gas chromatography after desulfonation, infrared and ultra-violet spectroscopy, extraction of copper complex, and tracer labelling ^{35}S, ^{14}C, ^{3}H).

Recent papers have dealt with various aspects of these methods (described in the 1977 Report), particularly with respect to improving the specifity and sensitivity of assays for LAS (as opposed to MBAS).

By use of a derivative of methylene blue (notably the 3-N, N-dibutylamino derivative), Toei and Fujii (1977) developed a modified MBAS technique sensitive to "traces" of ABS, i.e., in the range of 1 to 5 x 10^{-7}M. Its application to water samples was not presented.

In another "trace" method, Silva (1977) developed a Parr bomb-based method for reduction of sulfate (derived from LAS, or other substances) to hydrogen sulfide, which is reacted with N,N-dimethylphenylene diamine and ferric chloride to form methylene blue for spectrophotometric measurement. The method is applicable in the range of 2-40 µgs (per sample), with good precision and a "slight negative bias" in accuracy, especially at higher levels.

Uchiyama (1977) determined LAS spectrophotometrically at 222 nm after extraction of methylene blue complexes with 1,2-dichloroethane, washing with HCl, and re-extraction of the solvent with water. Good separation from other surfactants is reported with recoveries of LAS (dodecylbenzene sulfonate) being 100 ± 5% in the presence of dodecyl sulfonate or di-2-ethyl hexyl sulfosuccinate.

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

A series of surface water samples have been analyzed by Waters (1976), using a modified MBAS method which more specifically determines LAS than the earlier methods, but is still not completely specific. The modification consists of preliminary treatments, involving particularly an extraction after complexing the surfactant with 1-methyl-heptylamine. Methylene blue is used for the final determination, in which levels of LAS as low as 5 µg/L can be detected. Recoveries from surface and distilled water samples range from 78-99+%. In a series of samples from Dutch and British sites, LAS values were found ranging from 3 to 70 µg/L and representing only 16 to 33% of total MBAS.

Gagnon (1979) developed a method for analysis of all anionic surfactants, using sodium dodecylsulfate as a standard. It involves complexing the surfactant with bis-(ethylene-diamine)-Cu(II) which is extracted into chloroform, with the copper determined by atomic absorption spectrophotometry. The method was demonstrated to be applicable in the range of 0-50 µg/L (ppb) with good sensitivity, precision, reproducibility and recovery (92-99%) from sea water. With environmental samples, some interference is possible from copper and natural organic chelators, requiring correction for background response.

Several papers have appeared on various physicochemical methods of analysis, all attempting to improve specificity and sensitivity for LAS. The most promising are based on high-performance liquid chromatography (HPLC).

Hashimoto et al. (1976, English abstract only) used HPLC for separation of the materials removed from an MBAS complex by cation exchange chromatography. Detection of 0.1 µg LAS was reported, and standard deviation (0.44%) and coef-

ficient of variation (3.6%) indicate good precision and accuracy. No large-scale application of the method was reported.

Further applications of HPLC include the development of a system for separation of sulfophenylcarboxylic acids. Swisher et al. (1978) demonstrated the formation of sulfophenylcarboxylic acids (SPC) from LAS and their further degradation, particularly in river water. The toxicity of SPC's to various aquatic organisms and the mouse was much less than that of the parent LAS.

HPLC analyses for both LAS components and SPC's were developed by Taylor and Nickless (1979), using cetyltrimethylammonium (CTMA) in a paired-ion technique. The use of CTMA in 87.5% methanol as the mobile phase increased resolution of LAS components enormously (theoretical plate number increasing from 100 or 200 to 3000). Reduction of methanol content to 75% gave similar resolution of SPC's, whether assembled mixtures of synthetic compounds, or resulting from biodegradation of LAS in river water. Some biodegradation products may not be SPC's, but most of the 13 products detected were. All of them in river water samples showed a rise in concentration, from 0 at zero-time to a peak at 2-5 days, followed by a decline, reaching 0 again at days 3-10.

The methods developed by Swisher et al. (1978) were further extended in laboratory studies of LAS biodegradation in lake microcosms (Eggert et al., 1979). The formation and eventual complete disappearance of SPC's were reported.

A gas-liquid chromatographic-mass spectrometric (GLC/MS) method was developed by Hon-Nami and Hanya (1978). Methylene blue is removed from MBAS-complexes and the LAS methylated to methyl sulfonate derivatives for analysis. Linear response to a mixture of linear dodecylbenzenesulfonates was obtained for total amounts between 5 and 25 µg, and MS fragmentography showed all components of the mixture, agreeing well with the results of fragmentography of the original alkylbenzene mixture. Analyses of river water samples showed good reproducibility at concentrations greater than 3 µg/L.

An earlier paper by Wang et al. (1975) presents the further development of the two-phase titration method for LAS and ABS in waters and commercial detergents. They further report the development of a simplified kit for field use. The essence of the method involves formation of a complex between anionic surfactant and (cationic) cetyldimethylbenzyl-ammonium chloride, and analysis by determination of residual (free) cation by titration in an acidified aqueous/chloroform mixture.

One special analytical problem is the determination of LAS in soils, sediments, and sewage sludge, in which much of the surfactant may be so firmly bound as to require special techniques for separation. For this purpose, Helmann (1979) has published a brief description of a method involving extraction of samples with methanolic HCl or methanolic ammonia followed by the "air-stripping" method of Wickbold (1971), formation of MBAS complexes, silica gel chromatography and spectrophotometric (IR) determination. This method is still under development and evaluation.

Holtzclaw and Sposito (1978) present a modified methyl green method for

the analysis of anionic surfactants in the fulvic acid fraction of sewage sludge. The method involves hydrolysis in 4N HCl, separation on an anion exchange resin, formation of a methyl-green-surfactant complex, its extraction into benzene, and colorimetric determination of the complex at 610 nm. Their results with two fulvic acid fractions indicate an anionic surfactant content of 4-6% (water and ash-free basis). The method is reported to be non-reactive to partially degraded LAS.

B. Water Quality Standards

The maximum permissible level of surfactants in drinking water involved in interstate commerce is 0.5 mg MBAS/L. Several states and Canadian provinces also set 0.5 mg MBAS/L as the maximum permitted level. The European Economic Community indirectly established a similar standard by prohibiting the marketing and use of detergents containing surfactants which have an average level of biodegradability less than 90% as MBAS. No information was found concerning regulatory controls on water quality in Japan.

No national, state or local regulatory changes have been issued with respect to permissible levels of surfactants (as MBAS) in drinking water since our 1977 Report (Soap and Oetergent Association, 1980).

C. LAS Levels in Natural Water Bodies

There are currently no direct water quality criteria for LAS or MBAS mandated for any body of water by the U.S. Environmental Protection Agency under the Federal Water Pollution Control Act Amendments of 1972 (P.L. 92-500). A considerable data base of MBAS levels for different bodies of water

in the United States indicates MBAS levels in natural waters generally well below the 0.5 mg/L standard for drinking water involved in interstate commerce. Where levels higher than 0.5 mg MBAS/L are found, these waterways usually receive untreated or inadequately treated sewage. The true level of LAS in natural water bodies is probably considerably less than MBAS levels indicate, due to the nonspecific nature of the MBAS assay.

1. Monitoring Data

In the 1977 Report, the STORET water quality data system indicated average MBAS concentrations at low levels. MBAS concentrations in surface waters ranged from 0.06 to 0.19 mg/L in the state of Minnesota from 1967 to 1974; from 0.00 to 0.20 mg/L in the Mississippi River Delta from 1964 to 1975; and from 0.01 to 0.27 mg/L in New York State from 1960 to 1975. No overall trend was observed in these intervals from year to year.

In the present analysis of EPA STORET data for 1970 through 1979, MBAS concentrations for both ambient surface waters and effluent levels at publicly owned sewage treatment works, industrial discharges etc., were examined on a national level, by major river basin and by four states and a local area, selected randomly as representative of various regions of the country. There was no attempt to relate the retrieved concentration values to a point source. Instead, the discussion is focused on MBAS values relative to the 0.5 mg/L level established for drinking water. Results of the retrieval are summarized below. It is important to reiterate, however, that MBAS values are not synonymous with LAS concentrations in the environment, but rather, reflect the presence of all anionic materials and/or any of a large number of inorganic and organic

substances that interfere with this analytical method. Furthermore, one must temper STORET data with the fact that the reported MBAS values are for surface waters, not drinking water, and secondly, that STORET consists of raw data entries with no mechanisms of quality assurance for such data.

a. National Level

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

Of the major river basins, less than half had annual mean concentrations which exceeded 0.5 mg MBAS/L in the ten year period. The North Atlantic Basin, comprised of the Delaware, Susquehanna, Potomac and James Rivers, along with the Maryland, Delaware and Virginia coastal areas, reported values which were higher than 0.5 mg MBAS/L for six of these - 1971 through 1975, and 1979. The Great Basin, consisting of the Northwestern Lahontan, Central Nevada, Great Salt Lake and the Colorado River basin region of California plus the Humboldt, Owens, Mojave and Sevier Rivers, followed with values exceeding 0.5 mg MBAS/L for five of the ten years. Other major river basins exceeded 0.5 mg/L for no more than two years, as shown in Figure 1-2.

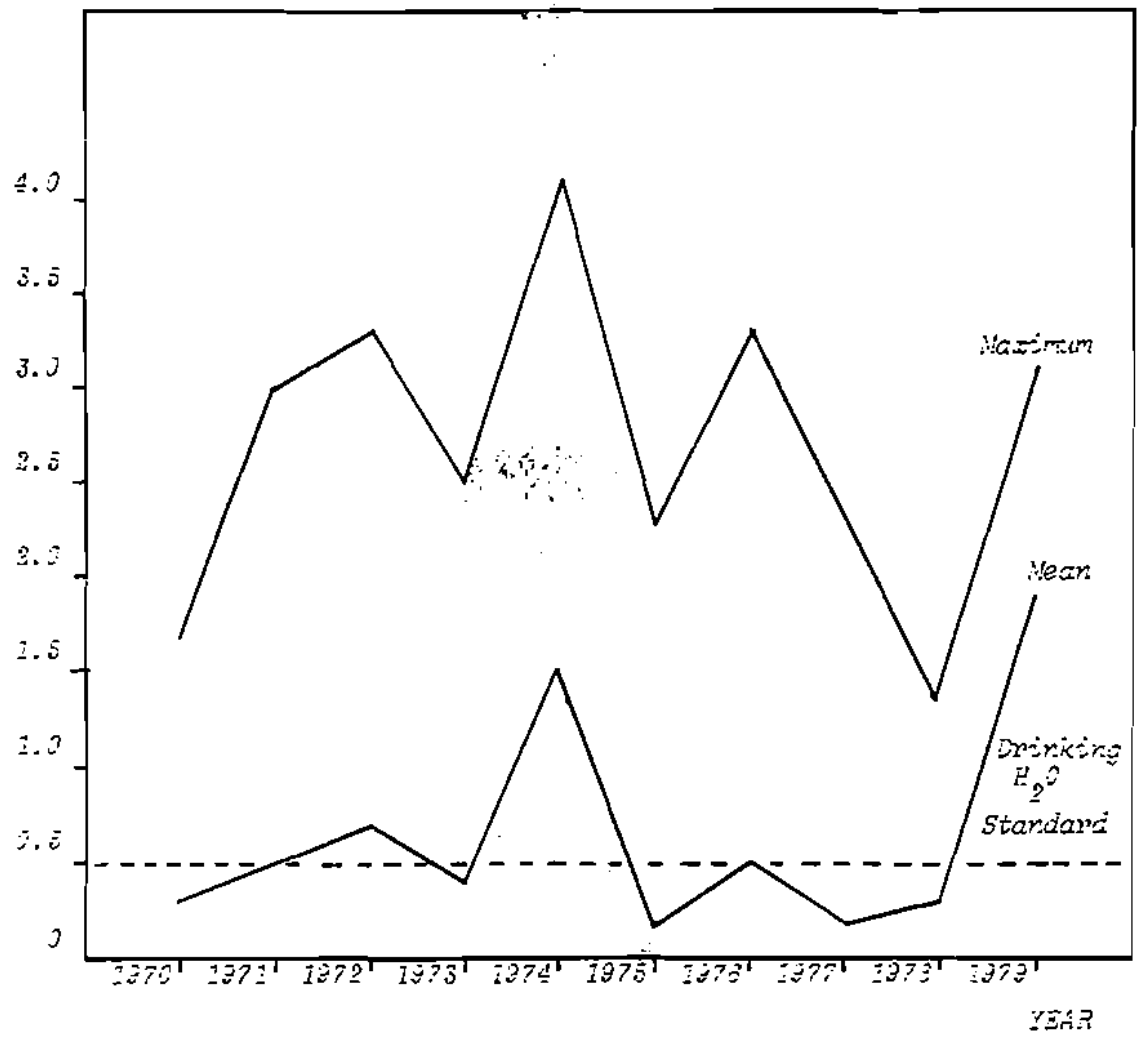
Effluent data from publicly owned sewage treatment works and industrial discharge sites are quite limited for the United States as a whole. Only the Northeast, North Atlantic and Southeast Basins presented data for 1970-1979. Mean MBAS values ranged from 0.5 to 10 mg/L.

METHYLENE BLUE ACTIVE SUBSTANCES

Maximum and Mean Ambient Concentrations

United States

1970-1979



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

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

Figure 1-1

MAJOR RIVER BASINS WITH MEAN CONCENTRATIONS
EXCEEDING 0.5 mg MBAS/L
(Annual Basis)

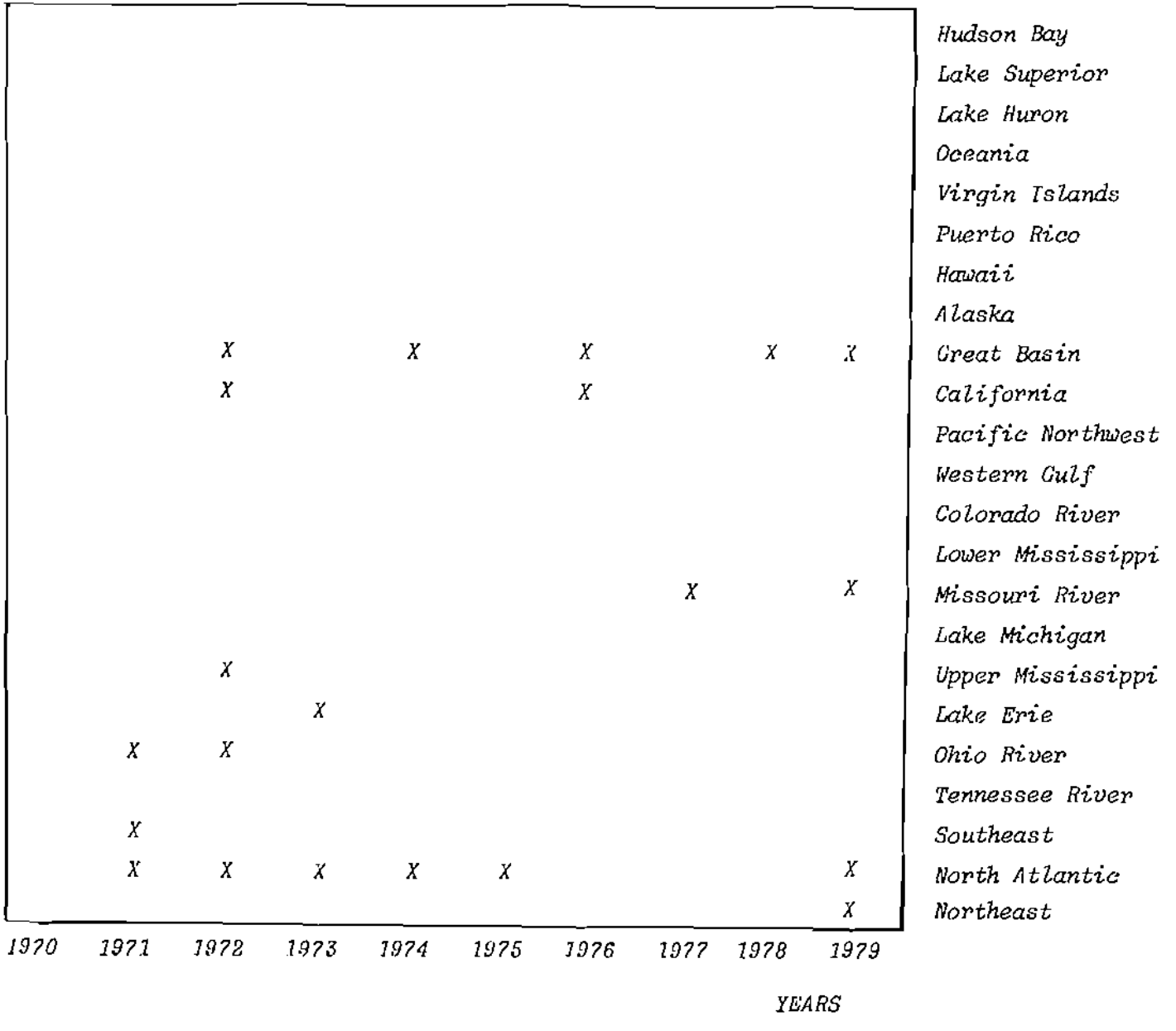


Figure 1-2

b. Other Retrievals

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

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

The New York City area was selected as a highly populated region to examine. A polygon was constructed around the metropolitan area to retrieve station data aggregated annually over the ten-year period. Of the 800 sampling stations along the New Jersey Coast, Lower Hudson River and Long Island, no more than 1 percent had a year with mean MBAS values higher than 0.5 mg MBAS/L during the time period*. At the 173 effluent stations within the same polygon, 1 or 2 observations had been recorded at or below 0.5 mg/L.

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

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

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

2. Summary

Water quality monitoring data from the STORET system indicate that:

1. In general, mean levels of MBAS in surface waters of the United States are below the 0.5 mg/L concentration considered acceptable for drinking water. In that actual LAS concentrations represent only a small fraction of MBAS levels, the actual margin of safety is even greater than MBAS values indicate.

2. No overall trend is evident for MBAS levels, but rather a continuing fluctuation from year to year is observed.

3. The North Atlantic and Great Basins reported the most instances of exceeding 0.5 mg/L in surface waters over the ten-year period, but these findings must be reconciled with rainfall, stream flow rate, and other climatic conditions. Furthermore, MBAS values retrieved from STORET are for surface waters, not drinking water.

BIBLIOGRAPHY

EGGERT, C. R., R. G. Kaley and W. E. Gledhill. 1979. "Application of a laboratory freshwater lake model in the study of linear alkylbenzene sulfonate (LAS) biodegradation." Presented at EPA Workshop on: Microbial degradation of pollutants in marine environments. EPA-600/9-79-012 pp. 451-461.

GAGNON, M. J. 1979. Note on a rapid and sensitive method for the determination of anionic detergents in natural waters at the ppb level. Water Res. 13: 53-56.

HASHIMOTO, S., K. Sakurai, and T. Nagai. 1976. Oetermination of trace amounts of alkylbenzene sulfonate in river water by high-performance liquid chromatography. Bunseki Kagaku 25(9):639-643.

HELMANN, H. 1979. Analytische bestimmung von aniontensiden in schweb- und sinkstoffen sowie klärschlämren. [Analytical determination of anion tensides in suspended and settling sediments and sewage sludge.] Fresenuis' Z. Anal. Chem. 295:393-397. (English translation)

HOLTZCLAW, K. M. and G. Sposito. 1978. Analytical properties of the soluble, metal-complexing fractions in sludge-soil mixtures: III. Unaltered anionic surfactants in fulvic acid. Soil Sci. Soc. Am. J. 42:607-611.

HON-NAMI, H. and T. Hanya. 1978. Gas-liquid chromatographic-mass spectrometric determination of alkylbenzenesulphonates in river water. J. Chromatog. 161:205-212.

IMANDEL, K., N. Razeghi and P. Samar. 1978. Tehran ground water pollution by detergents. Water, Air and Soil Pollution 9:119-122.

SILVA, H. 1977. Determination of sulfur in trace amounts. Analytical Letters 10(12):985-996.

SOAP AND DETERGENT ASSOCIATION. Personal communication, 1980.

SWISHER, R. D., W. E. Gledhill, R. A. Kimerle and T. A. Taulli. 1978. Carboxylated intermediates in the biodegradation of linear alkylbenzene sulfonates (LAS). VII International Congress on Surface Active Substances. Proceedings, Moscow, 1976. 4:218-230.

TAYLOR, P.W. and G. Nickless. 1979. Paired-ion high-performance liquid chromatography of partially biodegraded linear alkylbenzenesulphonate. J. of Chromatography 178:259-269.

TOEI, K. and H. Fujii. 1977. Short Communication. Spectrophotometric determination of traces of anionic surfactants with methylene blue derivatives. Analytica Chimica Acta 90:319-322.

UCHIYAMA, M. 1977. Determination of alkyl benzenesulfonate in water by U.V. method. Water Res. 11:205-207.

UNITED STATES ENVIRONMENTAL PRDTECTION AGENCY. STORET Data, 1980.

WANG, L. K., J. Y. Yank, R. G. Ross and M. H. Wang. 1975. Analysis of LAS, ABS and commercial detergents by two-phase titration. Water Resource Bull. 11(2):267-277.

WANG, L. K., S. F. Kao, M. H. Wang, J. F. Kao and A. L. Loshin. 1978. "Application and Determination of Surface-Active Agents Part II: Anionic Surfactants". In: Proceedings of the 33rd Industrial Waste Conference, Purdue University. pp. 918-934.

WATERS, J. 1976. The contribution of linear alkylbenzene sulphonate to the MBAS level of some UK and Dutch surface waters. Vom Wasser 47:131-140.

WICKBOLD, R. 1971. Anreicherung und Abtrenning der Tenside aud Oberflaechenwaessern durch Transport in der Grenzflaeche Gas/Wasser. Tenside 8(2): 61-63.

III. BIODEGRADATION

Biodegradation is defined by Swisher (1970) as "the destruction of chemical compounds by 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. Both laboratory investigations and field studies indicate that LAS are completely biodegradable, i.e., mineralizable, and that suggestions to the contrary are usually the result of some special test conditions.

A. Laboratory Investigations

There has been some development of new methodology in aspects of the study of surfactant biodegradation. Papers on fate and metabolism mostly deal with actual or simulated activated sludge systems, anaerobic systems, and, in a larger way, investigation of the fate of LAS in processes related to tertiary treatment of sewage.

In studying surfactant degradation in a simulated activated sludge unit (OECD/EEC), Painter and King (1978) found variations in surfactant reduction, largely attributable to the need for sufficient quantities of phosphate in the synthetic sewage. Reduction had varied because of differences in phosphate content in the peptone and meat extract used. Optimum phosphorus concentration was concluded to be 2-5 mg/L. Deficiency of phosphorus resulted

in low removal of both COD and surfactant, and in a dispersed, non-flocculant sludge which caused physical operating problems. The applicability of this finding to full-scale operating plants was not discussed.

One new paper on the degradation of LAS by anaerobic organisms has appeared (Citernesi et al., 1976). In the soil/water system investigated LAS showed a lower amount of residual adsorbed material on soil after 15 or 30 days than other surfactants tested, but there was still nearly 50% as much left (as MBAS) as was originally adsorbed. Analysis of the supernatant showed 90-100% removal, and the combined removal of adsorbed and supernatant material was 83-84%. The microbial population was considerably modified by 30 days of incubation, but the predominating survivors showed considerable ability to degrade LAS as measured by MBAS.

The abilities of a number of materials to adsorb LAS have been investigated, largely related to the design of (tertiary) removal processes. The substances involved are: calcium hydrosulfo-aluminate (Verzunova and Luk'yanova, 1974); iron in $Al_2(SO_4)_3$ and anionic polyamines (Okabe and Taga, 1976); Ouolites, i.e., phenol-formaldehyde anion exchange resins (Hinrichs and Snoeyink, 1976; Kim et al., 1976), and open-pore polyurethane (Smith and Navratil, 1979).

In studies somewhat related to the Citernesi study (1976) on anaerobic organisms, two groups have investigated the behavior of LAS in soil systems. The work of Archer and Yaron (1977) was done with reference to use of sewage effluent in agricultural irrigation. They expolored in detail the kinetics of

adsorption and release on two kinds of soils, but the apparent losses to soil were complicated by an unknown, but probably considerable, degree of biodegradation.

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

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

B. Field Studies

Field studies indicate LAS mixtures are readily biodegradable (over 90%). 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.

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

C. Effect of Chemical Structure

Four variations in chemical structure can influence the biodegradation of alkylbenzenesulfonates. They are: the position of the phenyl group; the length of the alkyl chain; the degree of branching and the presence of cyclic groups. However, all LAS isomers and homologs present in commercial surfactant formulations have been shown to degrade. The benzene ring is degraded as well as the alkyl chain.

D. Metabolic Pathways of Biodegradation

There are three demonstrated points of metabolic attack for LAS: the alkyl chain, the sulfonate group on the benzene ring, and the ring itself. Multiple degradative pathways are probably involved, but in general, the terminal methyl group on the alkyl chain is the first point of attack (ω-oxidation) followed by cleavage of the alkyl chain (β-oxidation), desulfonation, and finally, scission of the aromatic ring and its subsequent degradation.

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

(~13 mg/L), using a soil suspension as the bacterial inoculum. It is quite likely that LAS was toxic to soil microorganisms at this concentration, since toxic effects are observed between 10 and 20 mg/L in biodegradability screening studies. At concentrations greater than those measured in the environment, ultimate degradation of LAS is also less complete than at lower concentrations (Larson, 1979).

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

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

Further work on model substrates (Leidner et al., 1980), showed that very short chain LAS (C_1-C_4) were not degraded by unadapted soil organisms in the OECD screening test (mineral salts medium, containing the substrate as sole

carbon source at 8 mg organic carbon per L). On the other hand, the carboxylic acid analogs of these LAS chain lengths were readily degraded. The C_1 to C_4 LAS were still not degraded in the presence of the carboxylates.

Similar concerns about the degree of degradability of LAS were expressed by Pitter and Fuka (1979) based both on a review of the literature and their own experiments using adapted activated sludge. The experiments were conducted with extremely high levels of LAS (25-65 mg/L) as sole carbon source in an inorganic salts medium. Analyses for residual COD, DOC and UV absorption showed incomplete biodegradation (50-70%), although MBAS values indicated 95+% primary loss of LAS. They conclude that "the ultimate biodegradability of LAB's appears to be at least debatable." As pointed out in an "extension" of this subject (Swisher, 1980), this conclusion may be more related to the experimental conditions employed by Pitter and Fuka, than to the situation in the real world. The mineralizability of LAS has been demonstrated under close to real-life conditions; e.g., in semi-continuous activated sludge tests. Further, results from environmental sampling show low amounts of the expected intermediates, corroborating that LAS degrade in "real-world" conditions.

A number of papers discussed in the 1977 Report had demonstrated loss of the sulfonate group, and ring-opening of intermediates in the biodegradation of LAS. Three newer publications have used ^{14}C-ring labelling to demonstrate that the ring is oxidized with production of $^{14}CO_2$.

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

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

and biomass liquors from the SCAS system were submitted to a die-away test after supplementation with mineral salts, BOD water and yeast extract. In the SCAS units, conversions of both ring and alkyl carbons were similar: 62% to CO_2; 28-30% to biomass; and 8-10% as soluble residues. In the die-away tests with sludge solids (biomass) and supernatant, further conversion of both alkyl and ring carbons to CO_2 was demonstrated, with a final calculation that 95.9% of the original substrate fed to the SCAS units was converted to CO_2 and 3.8% recovered in biomass.

The mineralization of the benzene ring in natural waters has been well demonstrated by Larson and Payne (1980), using a C_{12} LAS uniformly labelled with ^{14}C in the benzene ring. The concentrations of LAS used, 50 and 500 µg/L, bracket the range found in water below the plant discharge. Water and sediments were collected from above (ASO) and below (BSO) the discharge of a trickling filter municipal waste water treatment plant on Rapid Creek, SD. Water from below the plant showed a higher degree of LAS-degrading activity, an indication of being already acclimated, while water from above the discharge showed a lag period and/or a slower rate of degradation. In both waters, the addition of the corresponding sediment increased the rate of CO_2 production significantly. Half-lives, and asymptotes for % $^{14}CO_2$ production were:

Water		Alone	+ sediment
ASO	$t_{½}$	~14 days	2.7 days
	% CO_2	70%	72%
BSO	$t_{½}$	1.4 days	0.7 days
	% CO_2	73%	80%

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

The intrinsic biodegradability of LAS is thus demonstrated, and it would appear that the results of Leidner et al. (1976) and Steber (1979) vs. those of Huddleston and Nielsen (1979a,b) are best explained by differences in experimental technique, especially in the inoculum used in die-away tests. The question raised by Leidner's detection of sulfophenylcarboxylic acids in environmental samples may need further examination. Even they, however, are ultimately biodegradable given the proper environment and microorganisms (Eggert et al., 1979).

The work of Miura et al. (1979) used oxygen uptake, reduction of MBAS and TOC (total organic carbon) to measure biodegradation of various surfactants (100 mg/L) in a system containing glucose, peptone and phosphate, and inoculated with sewage sludge. LAS was more slowly degraded than some of the others (C_{12ave}AS, AOS and various ethoxylates) in a 15-day test.

The apparent lower biodegradation of 5-phenyldecane sulfonate (as a minor component of Marlon A™, a C_{10}-C_{13} LAS) was investigated by Schöberl (1979) in the OECD confirmatory system, a simulation of a continuous flow activated sludge system. By itself, the compound is poorly degraded and is a poor stimulator of enzymes and organisms needed for its degradation. However, in the presence of Marlon A™, or after adaptation to Marlon A™, the compound is readily degraded (>90%) if present in sufficiently high concentration.

Other papers, difficult to evaluate without full translation, but apparently presenting little of new import, include the work of Itoh et al. (1979), Hrsak and Johanides (1975), Yakushev et al. (1978, abstract only) and Ohba et al. (1975, abstract only).

Finally, two recent papers deal with theoretical modelling aspects of biodegradation and mineralization (Larson, 1979, 1980). Data on LAS are limited but consistent with previous work, and indicate that the rate of LAS degradation is directly proportional to the concentration of LAS at sub-toxic levels. Half-lives for LAS mineralization in laboratory systems were comparable to those observed for glucose based on measured first order rate constants. Rates of LAS degradation by different sewage inocula were also in good agreement. Removal of LAS as measured by loss of DOC in SCAS systems was >90% after 24 hours.

BIBLIOGRAPHY

ARCHER, A. J. and B. Yaron. 1977. Behavior of anionic surfactants in a soil-sewage effluent system. J. Environ. Qual. 6(4):418-420.

CITERNESI, U., R. Materassi and F. Favilli. 1976. Interazioni dei detergent di sintesi con la microflora anaerobica del suolo. [Interaction of synthetic detergents with anaerobic microorganisms in the soil.] Nuovi Annali d'Igeieme e Microbiologia 27(1):39-49.

EGGERT, C. R., R. G. Kaley and W. E. Gledhill. 1979. "Application of a laboratory freshwater lake model in the study of linear alkylbenzene sulfonate (LAS) biodegradation." Presented at EPA workshop on: Microbial degradation of pollutants in marine environments. EPA-600/9-79-012 pp. 451-465.

HINRICHS, R. L. and V. L. Snoeyink. 1976. Sorption of benzenesulfonates by weak base anion exchange resins. Water Res. 10:79-87.

HOPPING, W. O. 1978. Activated sludge treatability of Type A. Zeolite. J. Wat. Pollut. Cont. Fed., 50(3-Part I): 433-441.

HRSAK, O. and V. Johanides. 1975. Biodegradation of linear alkylbenzene sulfonates. Hem. Ind. 29(8):359-363.

HUDOLESTON, R. L. and A. M. Nielsen. 1979a. LAS biodegradation: The fate of the aromatic ring. Household and Personal Products Ind. 82:72-74, 82.

HUDDLESTON, R.L. and A. M. Nielsen. 1979b. LAS biodegradation: The fate of the aromatic ring. Soap/Cosmetics/Chemical Specialties. 34-44.

INOUE, K. K. Kaneko and M. Yoshida. 1978. Adsorption of dodecylbenzenesulfonates by soil colloids and influence of soil colloids on their degradation. Soil Sci. Plant Nutr. 24(1):91-102.

ITOH, S., S. Setsuda, A. Utsunomiya and S. Naito. 1979. Studies on the biodegradation test method of chemical substances. II. Ultimate biodegradabilities of some anionic, nonionic, and cationic surfactants estimated by CO_2 production. Yakagaku 28(3):199-204.

KIM, B. R., V. L. Snoeyink and F. M. Saunders. 1976. Adsorption of organic compounds by synthetic resins. J. Wat. Pollut. Cont. Fed. 48(1):120-133.

LARSON, R. J. 1979. Estimation of biodegradation potential of xenobiotic organic chemicals. Appl. Environ. Microbiol. 38:1153-1161.

LARSON, R. J. 1980. The role of biodegradation kinetics in predicting environmental fate. In: Biotransformation and Fate of Chemicals in the Aquatic Environment. A. W. Maki, K.L. Dickson and J. Cairns, Jr., Editors. Am. Soc. Microbiol., Washington, DC, pp. 67-86.

LARSON, R. J. and A. G. Payne. 1980. Fate of the benzene ring of linear alkyl benzene sulfonate (LAS) in natural waters. Appl. Environ. Microbiol. (in press).

LARSON, R. J. and R. L. Perry. 1980. Use of the electrolytic respirometer to measure biodegradation in natural waters. Water Res. (in press).

LEIDNER, H., R. Gloor and K. Wuhrmann. 1976. Abbaukinetik linearer alkylbenzolsulfonate [kinetics of the degradation of linear alkylbenzenesulfonates]. Tenside Detergents 13(3):122-130. (English translation).

LEIDNER, H., R. Gloor, D. Wuest and K. Wuhrmann. 1980. The influence of the sulphonic group on the biodegradability of n-alkylbenzene sulphonates. Xenobiotica 10:47-56.

MIURA, K., K. Yamanaka, T. Sangai, K. Yoshimura and N. Hayashi. 1979. Application of biological oxygen consumption measurement technique to the biodegradation test of surfactants. Yukagaku 28(5):351-355 (English translation).

NIELSEN, A. M., J. D. Meyers, C. A. Bleckmann and R. L. Huddleston. 1980. LAS biodegradation: Ultimate fate of alkyl and ring carbon. Paper presented at the 1980 Annual SDA Meeting, January 31 through February 3 in Boca Raton, FL.

OHBA, K., R. Yagi and K. Miura. 1975. Conversion of detergents to biodegradable ones and their removal in municipal sewage treatment plants. Yosui to Haisui 17(11):1385-1391. (As cited in CA/085/009885E).

OKABE, A. and K. Taga, 1976. Coagulating and sedimentation for waste water. Japan. Kokai Patent No. 76 36757 03/27/76 (Lion Fat and Oil Co., Ltd.) (English abstract).

PAINTER, H. A. and E. F. King. 1978. The effect of phosphate and temperature on growth of activated sludge and on biodegradation of surfactants. Water Res. 12:909-915.

PITTER, P. and T. Fuka. 1979. The problem of ultimate biodegradability of linear alkylbenzene sulphonates. Tenside Detergents 16:299-302.

PROCTER AND GAMBLE COMPANY, unpublished data.

SCHOBERL, P. 1979. Der biologische 5-phenyldecansulfonat-abbau. [The biodegradation of 5-phenyldecanesulfonate.) Tenside Detergents 16(3):146-149 (English translation).

SMITH, C. M. and J. D. Navratil. 1979. Removal and preconcentration of surfactants from wastewater with open-pore polyurethane. Separation Science and Technol. 14(3):255-259.

STEBER, J. 1979. Untersuchungen zum biologischen abbau von ^{14}C-ringmarkiertem linearen alkylbenzolsulfonat in ober flächenwasser-und kläranlagenmodellen. [Investigation into the biodegradation of ^{14}C-labelled linear alkylbenzenesulfonate in models of surface waters and sewage purification plants.] Tenside Detergents 16(3):140-145.

SWISHER, R. D. 1970. Surfactant Biodegradation. Surfactant Sciences, Vol. 3, Marcel Dekker, Inc., New York.

SWISHER, R. D. 1980. The problem of ultimate biodegradability of linear alkylbenzene sulfonates: An extension. Tenside (manuscript submitted).

VERZUNOVA, L. I. and O. I. Luk'yanova. 1974. Adsorption of synthetic surfactants by calcium hydrosulfoaluminate. Kolloidn. Zh. 36(6):1196-1198.

YAKUSHEV, M. F., A. V. Plotnikov and A. P. Mochalov. 1978. Removal of synthetic surfactants from waste waters by radiation-induced degradation. Radiokhimiya 20(6):829-833. (As cited in CA/091/026666A).

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

The aquatic toxicity of LAS has been under continuous evaluation since their introduction in 1965. The 1977 Report reviewed the aquatic toxicity literature available up to that time, including acute and a few chronic studies. Recent information on the effects of LAS on aquatic organisms is presented below and provides some new insight in the use of toxicity studies for establishing environmental quality.

1. Acute Toxicity

a. Methodology

The 1977 Report summarized various methods used in the acute testing of surfactants in organisms. Procedures such as LC_{50} or EC_{50} determinations are still used. No new information will be added in this section.

b. Intact LAS Structure - Activity Relationship

An increase in the toxicity of LAS to various aquatic species has been associated with an increase in the length of the carbon chain. In addition, a decrease in toxicity was observed with chain lengths longer than 16 carbon units. Toxicity also appears to increase as the phenyl group occurs closer to the end of the alkyl chain. Side products such as dialkyltetralines, dialkylindanes, and alkylnaphthalenes appear to be considerably less

b. Intact LAS Structure - Activity Relationship (cont'd)

toxic than LAS isomers of comparable chain length.

A recent study by Maki (1979c) reported 96 hr LC_{50} values for $C_{11.8}$ LAS and C_{13} LAS of 3.94 and 2.19 mg/L for Daphnia magna. No observed effect concentrations (NOEC) for 21 day exposure to these two materials were 1.18 and 0.57 mg/L, respectively.

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

Maki and Bishop (1979) tested several LAS surfactants with alkyl chains ranging from C_{10} to C_{18}. The 48 hr LC_{50} to Daphnia magna ranged from 29.5 mg/L for C_{10} LAS to 0.11 mg/L for C_{16} LAS. There was a slight, statistically insignificant decrease in toxicity to 0.12mg/L with C_{18} LAS.

In summary, recent studies confirm that an increase in the carbon chain length of LAS surfactants is accompanied by an increase in toxicity for both fish and aquatic invertebrates, reaching a maximum value between C_{16} and C_{20}, and declining, thereafter.

c. Acute Toxicity to Fish - Intact LAS

LC_{50} values for LAS in various species of fish range from 0.5 to 10 mg/L. Early developmental stages are more sensitive to the acute toxic effects of LAS. Sublethal effects such as reduced swimming activity, breathing rates and opercular movements occur at slightly lower concentrations than those resulting in mortality.

Recent data confirm the range of toxicity noted in our 1977 Report. Lewis and Perry (1979) reported a 96 hr LC_{50} for bluegill (Lepomis macrochirus) of 1.67 (95% CL: 1.58 - 1.77) mg/L using $C_{11.8}$ LAS. Brown et al. (1978) reported a 96 hr LC_{50} of 0.36 (95% CL: 0.25 - 0.5) mg/L for juvenile rainbow trout (Salmo gairdneri) exposed to C_{10-15} LAS in sewage effluent. Tsai and McKee (1978) found a 96 hr LC_{50} of 6.2 mg/L for goldfish (Carassius auratus) exposed to LAS. The 48 hr LC_{50} values for Phoxinus phoxinus were 6.0 and 6.4 mg/L for two samples of C_{12}LAS (Lundahl and Cabridenc, 1978).

The fact that there appears to be little variation in the acute toxicity of LAS to fish was confirmed by Reiff et al. (1979). These authors tested several species of fish under varying conditions and found that the LC_{50} values for LAS ranged from 0.1 - 7.6 mg/L. Thus, regardless of method or species used, the results appeared to be consistent within an order of magnitude.

Vailati et al. (1975) found that toxicity varied with exposure time and developmental stage, as noted in the 1977 Report. However, these investigators found that eggs of rainbow trout were the most resistant, followed by adults, and then by fry with partially-absorbed yolk sacs. The mean 24 hr lethal concentrations for these respective stages were 10.8, 2.2 and 1.0 mg C_{12} LAS/L.

Over a longer exposure time, the eggs were the least resistant, with a 4-day mean lethal concentration of 0.12 mg/L.

The effect of LAS ($C_{11.8}$ and C_{13}) on ventilation rate in juvenile bluegills was investigated by Maki (1979a). This author found that the lowest significant effect levels for 24 hr mean ventilation rates were 2.19 mg/L for $C_{11.8}$ LAS and 0.39 mg/L for C_{13} LAS (as measured concentrations). A two day exposure to 2.0 mg/L $C_{11.8}$ LAS resulted in an increase in ventilation rate the first day which then decreased to the vicinity of control levels. Maki suggested that bluegill were exhibiting an acclimation response to LAS which is consistent with the work of Kimerle et al. (1979) demonstrating the facile metabolism of LAS by fathead minnows.

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

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

The authors suggested that toxicity manifested itself differentially on the various muscle groups used in locomotion, depending on concentration.

In summary, the results presented here confirm the conclusions drawn in the 1977 Report. Acute lethality is observed in the range of 0.5 - 10 mg/L LAS with a few exceptions. Sublethal effects are observed in a similar range. The finding of effects on swimming activity at concentrations as low as 0.2 mg/L is noteworthy. Also of interest is the observation that rainbow trout eggs were resistant over short exposure times. In addition, Maki's (1979a) report of acclimation bears some relevance to a consideration of aquatic risk and water quality criteria.

d. Acute Toxicity to Aquatic Organisms - Biodegraded LAS

Biodegradation products of LAS are considerably less toxic than intact LAS. Biodegradation resulting in 80-90% removal of MBAS may decrease LC_{50} values by a factor of ten. In addition, an examination of presumptive degradation products of LAS showed that they have very low toxicities.

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

Oolan and Hendricks (1976) reported similar reduction and eventual elimination

of toxicity after treatment of LAS in acclimated sludge. These authors found that the LC_{50} of intact LAS to bluegills was 0.85 mg/L for a 24-hr exposure. After the surfactant had degraded 76% (as MBAS), the 24-hr LC_{50} was estimated at 1.4 mg/L. No deaths occurred in 24 hours after 92% biodegradation (as MBAS), but one death (of 10) occurred after 96 hours. A similar pattern was observed for snails (Gonobasis sp.), but the increment in 24-hr LC_{50} values from 4.6 - 5.0 mg/L was not as distinctive. The authors suggested that the reduction in toxicity was associated with a change in homolog-isomer distribution.

Schöberl and Kunkel (1977) investigated the toxicity of various fractions of biodegraded LAS. Using 94-98% degraded C_{10-13} LAS (as measured by MBAS), the authors separated the residual surfactant from the non-surfactant metabolites (mainly sulfophenyl carboxylic acid). The surfactant fraction was tested on goldorfen (Leuciscus idus melanotus) at 10 mg/L and 20 mg/L; no mortality or other observable effects were seen by 48 hr. The non-surfactant metabolites were tested at 100 mg/L and 200 mg/L; no effects were observed at 48 hours. A combination of 20 mg/L and 200 mg/L of the respective fractions was also found to have no effect in this time period. The intact C_{10-13} LAS had an LC_{100} of 4-5 mg/L during the 48 hr test.

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

There has also been some further assessment of the toxicity of secondary products of LAS. The following LC_{50} values for Daphnia magna exposed to

alkyltetralin sulfonates for 24 hours were recorded by Conoco, Inc. (unpublished data):

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

These secondary products are considerably less toxic than the corresponding intact LAS. In addition, the longer carbon chain compounds are more toxic, paralleling findings for LAS.

Swisher et al. (1978) also examined the toxicity of intermediate biodegradation products of LAS and found them considerably less toxic than LAS. In addition, these investigators found the products to be less toxic than noted in previous tests cited in the 1977 Report and attributed this result to an increased purity of the samples tested. Table 1-A summarizes their results.

The data presented here, as well as the data presented in the 1977 Report suggest that there are two mechanisms which affect the toxicity of LAS. First, up to 98% reduction of LAS levels (as measured by M8AS) may be observed upon treatment in activated sludge, thus reducing LAS levels in effluents significantly. In addition, any LAS (as measured by MBAS) in the effluent is less toxic than intact LAS in the influent, perhaps by a factor of 3-10. This reduction in toxicity may be due to a change in the homolog-isomer distribution. In addition, intermediate biodegradation products of LAS (e.g., sulfophenyl carboxylic acid) are less toxic than intact LAS by a factor of 100 or more.

e. LAS-Related Acute Toxicity to Aquatic Organisms in Sewage Effluents

While no new information has been found in this area, several points should be restated from the 1977 Report:

- *MBAS degradation in the actual sewage treatment facilities may not reach levels observed in the laboratory. Levels of 1-12 mg/L MBAS have been reported in effluents from municipal sewage treatment plants.*

- *The toxicities of given levels of MBAS vary, due to differing degrees of secondary product formation.*

- *The toxicities of sewage treatment plant effluents are due to many factors, and in some cases, concentrations of MBAS may not be significant compared to toxic levels of other pollutants.*

TABLE 1-A. AQUATIC TOXICITY OF SULFOPHENYL CARBOXYLIC ACIDS

Effect	Concentration (mg/L)				
	LAS C_{10-13}	3-sulfophenylbutyric acid, disodium salt	4-sulfophenylvaleric acid, disodium salt	3-sulfophenylheptanoic acid, disodium salt	sulfophenylundecanoic acid, disodium salt
Acute LC_{50}					
Daphnia (24-hr)	6.9	> 22,000	~ 22,000	~ 12,000	2000
Fathead minnow (96-hr)	4.6	~ 28,000			1200
Chronic*					
Daphnia (4-wk)					
Survival*	>0.63	> 2000			> 200
Reproductive*	>0.63	> 2000			> 200
Fathead-egg-fry (30-day)					
Effect**	2	> 1400			> 52
No-effect**	1	> 1400			> 52

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

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

(Swisher et al., 1978)

f. Acute Toxicity to Invertebrates and Algae

Acute toxicity values of LAS for invertebrates range from 3 mg/L to more than 100 mg/L. Sublethal effects were reported between 0.025 mg/L (causing a retardation of the development of oyster eggs) and 5 mg/L which inhibited siphon retraction in the cockle. Earlier life stages, i.e. eggs and larvae, were found to be generally more susceptible than adults. Various species of algae exhibit mortality with LAS-concentrations of 50 mg/L.

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

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

Maciorowski et al. (1977) found that C_{13} LAS had no effects on the sphaeroid clam (Pisidium casertatum) at concentrations of 0.01 and 0.1 mg/L. Intestinal damage occurred at 1 and 10 mg/L with a loss of cilia, cell vacuolization, sloughing of column epithelia, and a marked reduction in cell size.

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

Although algae are not aquatic fauna, they are discussed at this point because of their critical role as the lowest trophic level of the aquatic food chain. Ohaliwal et al. (1977) exposed the blue-green alga, Plectonema boryanum, and the green alga, Chlamydomonas reinhardi, to $C_{11.2}$ LAS concentrations from 1 to 30 mg/L in short-term bioassays. The growth rate was reduced at 30 mg/L for blue-green algae, and at 20 mg/L for green algae. No morphological changes were observed.

Pybus (1973) used a mixture of C_{12} LAS and C_{12} AES in a study with the alga, Laminaria saccharina. A concentration of 50 mg/L of the surfactant mixture prevented the swimming of algal zoospores after 7 minutes; after 30 minutes exposure to 5 mg/L, the zoospores were swimming normally. A concentration of 10 mg/L resulted in reduced zoospore settlement and a reduction in growth rate of mature algae.

Surfactants have also been studied for their effects on Gymnodinium breve, a red tide dinoflagellate, in an effort to control its population. Hitchcock and Martin (1977) found that 0.025 mg/L of C_{13} LAS resulted in nearly 100% mortality of G. breve in 24 hours.

From the studies surveyed, aquatic invertebrate fauna appear to be sensitive to LAS concentrations from .025 mg/L, a sublethal level for barnacle larvae and dinoflagellates, to 29.9 mg/L, a 96 hr LC_{50} for blue crab. In studies in which different life stages were exposed to LAS, eggs and larvae were generally

more susceptible than adults. One experiment demonstrated that the LC_{50} of LAS for daphnids was halved by increasing the water hardness from 35 to 340 mg/L. Concentrations of .025 mg/L C_{13} LAS resulted in nearly 100% mortality in a red tide algae, however, most other types of algae exhibit sensitivity to LAS in the 10 to 50 mg/L range.

2. Chronic Toxicity

> *Experiments on chronic toxicity to aquatic life are uncommon, primarily because they require extended exposure periods in order to assess the effects of low concentrations of toxicants. The 1977 Report cited a five-week test on fathead minnows which found that fry (especially 7-14 days old) were far more sensitive to LAS than adults. Death occurred at 0.63 mg/L LAS in the fry, although concentrations of up to 2.7 mg/L did not affect growth, egg production, or hatchability during the test period. A six-week study with the scud (Gammarus lacustris) and two species of snails (Physa integra, Compelona decisum) found that survival was reduced at 0.4, 1.9 - 4.4, and 4.4 mg/L, respectively. Survival in the F_1 and F_2 generations of scud was reduced at levels of 0.2 mg/L LAS.*

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

A NOEC value of 0.9 mg/L $C_{11.8}$ LAS was calculated for fathead minnows by Maki (1979c) in a one-year toxicity test. For larval minnows, however, survival was impaired at 0.74 mg/L. In Daphnia magna exposed to either $C_{11.8}$ LAS or C_{13} LAS for 21 days, the 96 hr and 21-day LC_{50} values were 3.94 and 1.67 mg/L, respectively, for $C_{11.8}$ LAS; and 2.19 and 1.17 mg/L, respectively, for C_{13} LAS.

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

Thus far, fathead minnows have been the most frequently used test organisms in chronic toxicity tests with LAS. Concentrations of 0.63 to 1.67 mg/L were lethal to minnows in long-term experiments, while invertebrate survival was reduced at 0.4 to 4.4 mg/L. The NOEC value for fathead minnows was 0.9 mg/L; in embryo-larval life cycle tests, the NOEC ranged from 0.11 to 8.4 mg/L, depending on chain length.

3. Effects of Environmental Conditions on Toxicity

The toxicity of LAS surfactants varies with environmental conditions. Increasing temperature appears to increase toxicity, as does decreasing dissolved oxygen. Aquatic organisms may also be more sensitive to LAS in hard water than in soft.

Recent work has examined the effect of hardness. Lewis and Perry (1979) showed that the 48 hr LC_{50} to Daphnia decreased from 5.6 to 2.7 mg/L as hardness increased from 35 to 340 mg/L $CaCO_3$. Holman and Macek (1980), however, found water hardness was not a particularly significant factor in determining chronic toxicity to fathead minnows. In looking more closely at this effect, Maki and Bishop (1979) found that toxicity to Daphnia magna was related to the hardness of the culture water as well as the test water. In hard test water

(350 mg/L as $CaCO_3$), toxicity was independent of culture conditions; 48 hr LC_{50} values ranged from 3.2 to 4.0 mg/L. In soft test water (25 mg/L as $CaCO_3$), toxicity was related to the hardness of culture water as follows:

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

This finding may explain previous reports which sometimes showed contrary results.

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

The effects of previous exposure to LAS on toxicity was studied by Maki and Bishop (1979). Daphnia magna were exposed to 0.4 mg/L $C_{11.8}$ LAS for up to seven generations. The 48 hr LC_{50} value for D. magna was 2.6 (95% CL: 1.8 - 3.3) mg/L for cultures acclimated for seven generations, and 3.0 (95% CL: 2.0 - 3.8) mg/L for unacclimated cultures.

In summary, increasing hardness of the test water has been correlated with increasing LAS toxicity. However, the hardness of the culture water may also affect toxicity test results. The addition of suspended solids appeared to decrease LAS toxicity to Daphnia, while acclimation appeared to have no effect

on toxicity to daphnids previously exposed to LAS.

4. Bioaccumulation

The limited information available on uptake and bioaccumulation of LAS by aquatic organisms was summarized in the 1977 Report and was primarily concerned with residues in aquatic organisms. Levels of 0.01 to 2.0 mg/kg MBAS were reported. A single study examined the metabolism and clearance of LAS and found that 5% of the residue in the gall bladder was LAS. Clearance of ^{14}C-activity occurred within 3 days after removal to clean water.

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

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

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

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

Comotto et al. (1979) studied the uptake and depuration of LAS by Daphnia magna and fathead minnow using ^{14}C-labelled LAS surfactants (C_{12} LAS, C_{13} LAS and light and heavy blend C_{14} LAS). In Daphnia, steady-state was reached in 1 day; in fatheads, three days were needed. In both cases, bioconcentration was dependent on chain length and exposure concentration; shorter chain lengths were accumulated less. The bioconcentration factors in Daphnia ranged from 500-4000. In fathead, the dry weight bioconcentration factors were as follows: muscle, 79-372; whole fish, 269-1223; gall bladder, 21,000-70,000. Clearance was rapid in both species upon removal to clean water. These authors also determined the chemical species composition of the accumulated radioactivity. Much of that in Daphnia was in the form of intact LAS. However, in fish, the following percentages of intact LAS were found: gills, 25-75%; carcass, 50-70%; viscera, 15-35%; and gall bladder 2-3%. Thus, it is apparent that fatheads metabolized LAS, while daphnids did not.

These results suggest that LAS are rapidly taken up by fish and concentrated in tissues and organs. Clearance is also rapid, with a half-life of 2-3 days. It should be pointed out that all of these experiments were conducted

using radiolabelled LAS. Thus, the reported accumulation factors assume that the detected radioactivity was associated with intact-LAS. However, one study showed that LAS are at least partially metabolized by fish and the eliminated products may well include some secondary products.

5. Interactions with Other Chemicals

> *LAS have been studied for their effects in combination with some other chemicals, especially metals and pesticides. Additive effects have been observed with zinc, and synergistic effects with copper and mercury. In studies with pesticides, "enhanced" toxicity has been reported with DDT and parathion. Synergism was reported in aquatic organisms with parathion, methyl parathion, ronnel, trithion and trichloronat. No synergism, however, was found with dicapthon, guthion, EPN or dieldrin. LAS have also been shown to increase the toxicity of petroleum products.*

It is unclear whether any of these studies has shown synergistic effects, or merely additive effects due to problems with evaluating data. Recent studies have looked at such interactions somewhat more rigorously. Tsai and McKee (1978) investigated the effects on goldfish (Carassius auratus) of various chemical interactions that might occur in a stream receiving chlorinated sewage effluents. They utilized mixtures of choramines, LAS and copper as representative of possible interactions and found that equal ratios of LAS and chloramines were slightly synergistic at lower concentrations, and additive at higher concentrations; LAS concentrations ranged from 1.8 to 6.5 mg/L. For unequal ratios, the two chemicals were strongly synergistic. When LAS were combined with copper, the toxicity was additive at equal concentrations

and at a ratio of 2:1 (LAS:copper). However, then the ratio was 1:2, the effects were additive at high concentrations and synergistic at low. The ternary mixtures of chloramine, copper, and LAS in various ratios were all additive at high concentrations and synergistic at low concentrations.

Lewis and Perry (1979) examined the effects of surfactant mixtures on Daphnia magna and bluegill. Using $C_{11.8}$ LAS, Neodol™ 45-7 (n-pri-$C_{14-15}AE_7$) and C_{12-14} monomethyl-dihydroxyethyl ammonium chloride, these authors found that a ternary mixture and three binary mixtures were less than additive or additive, depending on the statistical method of analysis used.

The studies discussed in this section indicate that synergistic effects with LAS may be observed, depending on the concentration, with either chloramines or copper. No synergistic effects were observed when LAS were mixed with other surfactants.

B. Effects of LAS on Higher Plants

In the 1977 Report, three studies were cited on the effects of LAS on plants. The growth of pea plants in terms of weight and length was reported to have been inhibited by 50% with a 50 mg/L solution of LAS. Orchid seedling growth was reduced by 60% in 110 mg/L LAS, while 10 mg/L resulted in a fresh weight reduction of 30%. Histological damage in the seedlings occurred after 48 hours of exposure to 1,000 mg/L. However, in five species of hardwoods, 5000 mg/L LAS had no effect on translocation or absorption.

Taniyama and Nomura (1978) reported a significant reduction in paddy rice production for plants watered with 50 mg/L LAS solutions. The reduced grain

yield was due to a reduced number of grains per panicle (7 g vs 30 g for controls) and a significant decrease in the percentage of ripened grains (9.5% compared to 77% in controls) in LAS-treated plants. In separate experiments with potted rice plants, exposure to 50 mg/L LAS solutions produced no effects on plant height, number of tillers (shoots) or dry matter production, but was found to markedly inhibit water absorption by the roots, to inhibit photosynthesis and to result in considerable yellowing of the leaf blade.

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

Seven-day EC_{50} values in duckweed (Lemna minor) were determined by Bishop and Perry (1979). The median effect concentration of $C_{11.8}$ LAS to reduce frond count, dry weight, and root count was 2.7 mg/L.

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

Limited data suggest that the minimum concentration at which LAS exert toxic effects to plants is 3 mg/L. As with other biota, the toxicity of LAS to plants depends largely on alkyl chain length, as well as other factors

such as frequency of toxicant renewal and certain water characteristics (e.g., salinity). Some data also suggest that LAS may stimulate plant growth in some cases.

C. Effects on Birds and Wildlife

The 1977 Report contained no information with respect to the effects of LAS on birds and wildlife. No information has been found since that time.

D. Mode of Action in Aquatic Species

Loss of gill function is the most commonly observed sign of LAS-related effects. However, it is unknown whether gill damage is the cause of death or only a contributing factor.

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

In more recent work, Tomiyama (1978) postulated an initial formation of complexes of surfactants (RSO_3 or RSO_4 groups) with protein components in the gills, resulting in impairment of gill function and death due to oxygen deficiency. This author tested his theory using several different approaches. He exposed fish to LAS and then took an erythrocyte count, which is inversely correlated with oxygen availability. He found an increased erythrocyte count in fish that died upon exposure to LAS. In addition, by

using various proteins, he found that previous formation of surfactant complexes can prevent formation of a complex with the gills, thus reducing toxicity. Complexation appears to be promoted in hard, acidic water.

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

Jackson and Fromm (1977) also found that treatment with LAS affected permeability. They exposed isolated gill arches of rainbow trout to 5 - 100 mg/LAS, and observed an exponential increase in uptake of tritiated water. They concluded that LAS either interacted with the mucous coating of the gill, directly affected the epithelium, or both. Since the mucous serves as a diffusion barrier, they suggested that increased uptake of water would occur. This increased uptake of water might not be lethal in itself, but these authors proposed that it could burden the kidneys.

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

BIBLIOGRAPHY

BISHOP, W. E. and A. W. Maki. 1978 (Oct. 17-18). A critical comparison of two bioconcentration test methods. Presented at the ASTM 3rd Aquatic Toxicology Symposium, New Orleans, La. Proceedings of the symposium (in press).

BISHOP, W.E. and R. L. Perry. 1979 (Oct. 17). The development and evaluation of a flow-through inhibition test with duckweed (Lemna minor L.). Presented to the ASTM 4th Aquatic Toxicology Symposium, Chicago, IL. Proceedings of the symposium (in press).

BROWN, V. M., F. S. H. Abram and L. J. Collins. 1978. The acute lethal toxicity to rainbow trout of an LAS surfactant and of its residues and degradation products. Tenside Detergents 15:57-59.

COMOTTO, R. M., R. A. Kimerle and R. D. Swisher. 1979. Bioconcentration and metabolism of linear alkylbenzene sulfonate by Daphnids and fathead minnows. Aquatic Toxicology, ASTM STP 667, L. L. Marking and R. A. Kimerle, Eds., American Society for Testing and Materials, pp. 232-250.

CONOCO, INC., unpublished data.

DANVILA, A. M. 1977. The toxicity of anionics in the biodegradation process. The behavior of Daphnia magna and Artemia salina in the Guadalquivir River waters and in the Bay of Algeciras. Spanish Petrochemistry (December, 1977), pp. 1-33 (English translation).

DHALIWAL, A. S. A. Campione and S. Smaga. 1977. Effect of linear alkylbenzene sulfonate (11.2 LAS) on the morphology and physiology of Plectonema boryanum and Chlamydomonas reinhardi. J. of Phycology 13 (Suppl):18. (Abstract).

DOLAN, J. M., III. and A. C. Hendricks. 1976. The lethality of an intact and degraded LAS mixture to bluegill sunfish and a snail. J. Water Pollut. Cont. Fed. 48:2570-2577.

DDWDEN, H. G. M., M. J. Lambert and R. Truman. 1979. Salinity damage to Norfolk Island pines caused by surfactants. II. Effects of sea water and surfactant mixutres on the health of whole plants. Aust. J. Plant Physiol. 5:387-395.

E G & G Bionomics, (Aquatic Toxicology Laboratory). 1978. The effects of continuous aqueous exposure to LAS 9-75-1 on hatchability of eggs and growth and survival of fry of fathead minnow (Pimephales promelas). Report submitted to Soap and Detergent Association #BW-78-6-202.

HITCHCOCK, W. S. and D. F. Martin. 1977. Effects and fate of a surfactant in cultures of the red time [sic] organism, Gymnodinium breve. Bull. Environ. Contamin. and Toxicol. 18(3):291-296.

HOLMAN, W. F. and K. J. Macek. 1980. An aquatic safety assessment of linear alkylbenzene sulfonate (LAS): Chronic effects on fathead minnows. Trans. Am. Fish. Soc. 109:122-131.

JACKSON, W. F. and P. O. Fromm. 1977. Effect of a detergent on flux of tritiated water into isolated perfused gills of rainbow trouts. Comp. Biochem. Physiol. 58C:167-171.

KIKUCHI, M., M. Wakabayashi, H. Kohima and T. Yoshida. 1978. Uptake, distribution and elimination of sodium linear alkylbenzene sulfonate and sodium alkyl sulfate in carp. Ecotoxicol. and Environ. Safety 2:115-127.

KIMERLE, R. A., K. J. Macek, B. H. Sleight, III. and M. E. Barrows. 1979. Bioconcentration of linear alkylbenzene sulfonate (LAS) in bluegill. Water Res. (in press).

LEWIS, M. A. and R. L. Perry. Oct., 1979. Acute lethalities of equimolar and equitoxic surfactant mixtures to Daphnia magna and Lepomis machrochirus. Presented to the ASTM 4th Aquatic Toxicology Symposium, Chicago, IL Proceedings of the symposium (in press).

LDPEZ-ZAVALA, A., A. S. de Aluja, B. Elias L., L. Manjarrez, A. Bachmann, L. Mercado and S. Caltenco. 1975. The effects of the ABS, LAS and ADS detergents on fish, domestic animals and plants. Progress in Water Technology 7(2):73-82.

LUNDAHL, P. and R. Cabridenc. 1978. Molecular structure - biological properties relationships in anionic surface-active agents. Water Res. 12:25-30.

MACIOROWSKI, A. F., J. M. Dolan, III and B. C. Gregg. 1977. Histological damage and recovery of Pisidium casertanum exposed to linear tridecyl benzene sulfonate, an anionic surfactant. Comp. Biochem. Physiol. 56C:117-122.

MAKI, A. W. 1979a. Respiratory activity of fish as a predictor of chronic fish toxicity values for surfactants. Special Technical Publ. 667, ASTM, Philadelphia, PA 77-95.

MAKI, A. W. Sept. 24-28, 1979b. An environmental safety evaluation of detergent chemicals in estuarine ecosystems. Proceedings of 14th International Marine Biological Symposium - "Protection of life in the sea." Helgoland, Germany, Sept. 24-28, 1979. Biologische Anstalt Helgoland, Hamburg, Germany (in press).

MAKI, A. W. 1979c. Correlations between Daphnia magna and fathead minnow (Pimephales promelas) chronic toxicity values for several classes of test substances. J. Fish Res. Board Can. 36:411-421.

MAKI, A. W. and W. E. Bishop, 1979. Acute toxicity studies of surfactants to Daphnia magna and Daphnia pulex. Arch. Environ. Contam. Toxicol. 8:599-612.

MANNER, H. W. and C. Muehleman. 1976. LAS inhibition of diffusion and uptake of tritiated uridine during teleost embryogenesis. Env. Biol. Fish. 1(1):81-84.

PROCTER AND GAMBLE COMPANY, unpublished data.

PYBUS, C. 1973. Effects of anionic detergent on the growth of Laminaria. Marine Pollut. Bull. 4(5):73-77.

REIFF, B., R. Lloyd, M. J. How, O. Brown and J. S. Alabaster. 1979. The acute toxicity of eleven detergents to fish: Results of an interlaboratory exercise. Water Res. 13:207-210.

RENZONI, A. 1974. Effect of toxicants on marine invertebrate larvae. Thalassia Jugosl. 10 (1-2):197-211.

SABOUREAU, J. L. and R. Lesel. 1977. Toxicite de substances a des doses sublethales chez le poisson. II-Toxicite de detergents anionique et cationique chez la truite arc-en-ciel (Salmo gairdneri Richardson). [Toxicity of substances to fish in sublethal concentration. II-Toxicity of anionic and cationic detergents toward rainbow trout (Salmo Gairdneri Richardson).] Trib Cebedeau 30(403/404):271-276 (English translation).

SCHÖBERL, P. and E. Kunkel. 1977. Die fischvertraglichkeit der rest-tenside und zwischenprodukte des mikrobiellen abbaus eines linearen alkylbenzolsulfonates. [The compatability of fish to the residual surfactant and intermediate products of the microbial degradation of a linear alkylbenzene sulfonate.] Tenside Detergents 14(6):293-296 (English translation).

SWEDMARK, M., A. Granmo and S. Kollberg. 1976. "Toxicity Testing at Kristinberg Marine Biology Station", in: Pollutants in the Aquatic Environment. FPO/SIDA/TF 108 Suppl. 1 pp. 65-74.

SWISHER, R. D., W. E. Gledhill, R. A. Kimerle and T. A. Taulli. 1978. Carboxylated intermediates in the biodegradation of linear alkylbenzene sulfonates (LAS). VII Internationsl Congress on Surface Active Substances, Proceedings, Moscow, 1976. 4:218-230.

TANIYAMA, T. and T. Nomura. 1978. Effects of various synthetic detergents on photosynthesis, dry matter and grain production in rice plants. Mi Daigaku Kankyo Kagaku Kenkyu Kiyo. 3:93-104.

TATSUKAWA, R. and H. Hidaka. 1978. Avoidance test of chemical substances on fish. Avoidance of detergents by ayu (Plecoslossus altivelis). Nippon Nosei Kasaku Kaishi 52 (7):263-27D.

TOMIYAMA, S. I. 1974. Effects of surfactants on fish. Bull. Jpn. Soc. Sci. Fisheries. 40(12):1291-1296.

TOMIYAMA, S. 1978. On the possible mechanism of the effect on fishes and shellfishes by household detergents. Yukagaku 27(6):347-353 (English translation).

TSAI, C-f, J. A. McKee. 1978. The toxicity to goldfish of mixtures of chloramines, LAS and copper. Water Res. Center, Univ. of Maryland. Technical Report 44:PB 280-554.

VAILATI, G., D. Dalamari, and R. Marchetti. 1975. Effect of alkylbenzene sulfonate (LAS) on the development stages of Salmo gairdneri. Nuovi Ann. Ig. Microbiol. 26(1):69-84. (As cited in Chemical Abstracts 84:700085, O 87).

V. HUMAN SAFETY

The human safety aspects of linear alkylbenzene sulfonates, presented in the 1977 Report, encompassed all data (published and unpublished) available through 1976. We indicated at that time that present day use of LAS did not represent a hazard to human health. Information developed since 1976 and reported below substantiates that assessment.

A. Animal Studies

Studies examining the toxicity of LAS to mammalian species have shown that the acute oral LD_{50} values for rodents range from 650 to 2000 mg/kg. In rats, long-term feeding studies at LAS levels up to 0.5% of the diet (which exceed estimated human consumption by over a thousand-fold) suggest no indications of any deleterious effects. At concentrations of 0.5 and 1.0%, LAS surfactants can induce immediate but reversible ocular congestion and edema in rabbits. Undiluted LAS samples are primary skin irritants in rabbits, but at a concentration of 1% LAS, which is above normal domestic use levels, LAS are non-irritating to laboratory animals.

Acute Toxicity. Ito et al. (1978) compared the acute oral, subcutaneous and intravenous toxicities of the magnesium and sodium salts of C_{10-13} LAS in ICR mice and Sprague-Dawley rats (see Table 1-B). Oral LD_{50} values in rats were comparable to values noted in the 1977 Report. No sex differences were observed for either species, regardless of route of administration, but both formulations were more toxic to rats than mice. Mg-LAS was considerably more toxic than Na-LAS by the intravenous route, particularly in rats. In terms of

TABLE 1-B. ACUTE TOXICITIES OF Mg-C_{10-13} AND Na-C_{10-13} LAS IN MICE AND RATS

Species	Compound	Route	Sex	LD_{50} (mg/kg) ± 95% C.L.	
ICR mice	Mg-LAS	Intravenous	♂	98	(91-106)
			♀	151	(113-200)
	Na-LAS		♂	207	(129-330)
			♀	298	(276-320)
	Mg-LAS	Subcutaneous	♂	1520	(1338-1727)
			♀	1550	(1336-1798)
	Na-LAS		♂	1250	(1078-1450)
			♀	1400	(1239-1582)
Sprague-Dawley rats	Mg-LAS	Intravenous	♂	27.2	(22.4-33.4)
			♀	35.0	(32.3-38.0)
	Na-LAS		♂	119	(106-134)
			♀	126	(97-166)
	Mg-LAS	Subcutaneous	♂	710	(602-838)
			♀	730	(629-847)
	Na-LAS		♂	840	(712-991)
			♀	810	(664-988)
	Mg-LAS	Oral	♂	1900	(1597-2261)
			♀	1840	(1559-2171)
	Na-LAS		♂	1460	(1207-1767)
			♀	1470	(1256-1720)

(Ito et al., 1978)

toxicity, the following rank was observed: intravenous > subcutaneous > oral route.

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

Acute Irritation-Ocular. No new studies were available, but studies cited in the 1977 Report indicated LAS concentrations of 0.5 to 1.0% could induce immediate but reversible ocular congestion and edema in rabbits.

Acute Irritation - Skin. Application of an occluded patch containing 0.1 ml of a 2% aqueous solution of 97.9% pure C_{12} LAS to the shaved backs of guinea pigs for 24 hr resulted in moderate to severe skin irritation; i.e. a skin irritation score of 3 out of a possible 4. A slightly less severe reaction (skin irritation score = 2.75) was seen with a mixed LAS sample (99.8% pure; C_{10} - 7%, C_{11} - 36%, C_{12} - 33.7%, C_{13} - 23.4%) under the same test conditions (Imokawa, 1979).

Skin Sensitization. Guinea pigs injected intradermally with a 1% w/v aqueous solution of LAS (OOBANE JN^{TM}) and topically challenged showed no skin sensitization (Shell Research Limited, unpublished data). This finding agrees with those previously noted in the 1977 Report for humans.

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

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

Subacute Toxicity - Oral/Subcutaneous. Simultaneous oral and subcutaneous administration of C_{10-13} LAS to male and female rhesus monkeys (Macaca mulatta) for 28 days resulted in no adverse effects. Groups of three male and three

female monkeys were simultaneously administered 0, 30, 150 or 300 mg/kg C_{10-13} LAS orally and 0, 0.1, 0.5 or 1.0 mg/kg C_{10-13} LAS, respectively, by subcutaneous injection. At the highest treatment level, the monkeys frequently vomited, usually within 3 hours of administration. An increased frequency of loose or liquid feces was noted in animals in the top two treatment levels. No significant decrease in mean body weight gain was seen, but individual animals in the top treatment group did show a slight depression in body weight gain. Fibrosis of the injection site was seen in all groups, the incidence and severity being dose-related. No other treatment-related responses with respect to histopathology, hematology, urinalyses or opthalmology were reported (Heywood et al., 1978).

This study adds the monkey to the list of species in which subchronic oral administration of LAS results in no permanent adverse effects. This study also contains the first indication of LAS-induced emesis. The fact that emesis occurred up to three hours post-dosing suggests that the emetic action was due to central stimulation of the emetic center rather than local gastrointestinal irritation.

Subacute Toxicity - Subcutaneous. In a related study, Kikuchi (1978) subjected male and female C57BL/TW strain mice to repeated subcutaneous injections of LAS from the day of birth. The injection schedule was a follows:

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

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

0.02 ml of 10% LAS for 5 injections over the next 10 days (days 21-30); and 0.04 ml of 10% LAS every other day for next 30 to 60 days.

Treated mice exhibited epilation around the site of injection with dermatitis frequently noted after several injections. Survival, growth and reproductive parameters were not affected. Necropsy at 60 days revealed an increase in adhesions (60.6% vs 0% in controls) between some organs, particularly between spleen and kidney. Interestingly, Kikuchi noted no adhesions in related experiments if injections were initiated on day 11 of life or later. The relative weights of kidney and liver were increased, and the spleen in females, but not males, was markedly enlarged, especially in mice with dermatitis at the site of injection (See Table 1-C). Histopathological examination of liver, kidney, spleen, adrenal and thyroid revealed no treatment-related changes.

Subacute Toxicity - Inhalation. Data on the inhalation toxicity of LAS were recently reported by Coate et al. (1978). Five male and four female cynomolgus monkeys (Macaca fascicularis) were exposed 6 hr daily 5 days per week for 6 months to an atmosphere containing 100 mg/m^3 of a synthetic detergent dust containing 13%(w/w) C_{12} LAS. The mass median diameter of the dust particles (3 μm) was in the respirable range. Gross signs of respiratory distress, pulmonary histopathological effects and pulmonary function impairment were noted. Histological alterations consisted of chronic bronchiolitis characterized by infiltration of mono-

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

Treatment	No.	Sex	Mean Body Weight (g)	Relative Organ Weight (mg/20g bw)		
				Kidney	Liver	Spleen
LAS	32	M	22.97	315.3	1240	105.9
	12	F	17.48	302.4	1240	144.7
Control	18	M	22.1	278.D	1130	101.8
	3	F	17.3	--	1130	129.3

(Adapted from Kikuchi, 1978)

nuclear macrophages and lymphocytes. The walls of the respiratory bronchioles were moderately to markedly fibrosed and there was a diffuse non-suppurative alveolitis in the proximal alveoli adjacent to respiratory bronchioles. Basal cell hyperplasia and focal squamous metaplasia of the trachea were also noted. The proliferative changes were considered to be a result of irritation. Cumulative tidal volume for nitrogen washout was significantly increased (1.10 to 1.27 L) but returned to normal when animals were held without further exposure. Dust exposure had no apparent effect on flow resistance, or diffusion capacity. Two mortalities were recorded during the study; one animal died during Week 22 of exposure; another was killed in moribund condition during Week 24. No treatment-related effects were noted with respect to hematology, clinical chemistry, urinalysis, skin sensitization tests or chest radiographs. The presence of builders and additives in the dust formulation, however, obscured the relevance of the data with respect to LAS safety.

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

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

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

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

Carcinogenicity and Co-Carcinogenicity.

Both oral and percutaneous exposure of laboratory animals to LAS have been completely negative with respect to carcinogenicity. In rats, LAS was shown to enhance gastric tumor induction by nitroquinoline-N-oxide; however, it is unclear whether this effect is due to enhanced carcinogen absorption or some other physiological mechanisms.

No new studies have been found in this area.

Mutagenicity.

A limited number of studies with LAS have given no indications of mutagenic activity.

Masubuchi and co-workers (1976) examined cytogenetic effects of LAS in mice and rats. Chromosome studies of bone marrow cells after 9 months dietary administration of 0.9% LAS showed no induction of chromosome abnormalities in either ICR mice, Wistar or Sprague-Dawley rats.

In another study, Hope (1977) also reported that the incorporation of C_{10-15} LAS into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosomes of rat bone marrow cells.

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

These data reconfirm previously reported unpublished data, and are important to the assessment of safety of LAS in that they represent the results from genetic experiments done in whole animals.

In vitro studies with both mammalian and bacterial cells exposed to LAS have also proved negative. No chromosomal aberrations were noted in rat liver cells exposed to n-pri-C_{12-13} LAS at concentrations up to 100 µg/ml (Shell Toxicology Laboratory, unpublished data). In another study, Inoue et al. (1980) reported no induction of morphological transformation of hamster embryo cells

exposed in culture to 0.5, 1, 5, 10, 20 or 50 µg C_{10-14} LAS/ml, but did observe cytotoxic effects at the 50 µg/ml level.

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

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

Teratogenesis/Reproduction Studies.

There is no evidence that LAS induces birth malformations or affects reproduction in experimental animals.

Recent studies also show that the adverse effects of LAS on the fetus occur only at levels that are toxic to dams. Three oral studies have been

conducted since the 1977 Report (Tiba et al., 1976; Shiobara and Imahori, 1976; Kuwano et al., 1977); the alkyl chain lengths of the LAS materials tested in these studies were not stated. Tiba et al. (1976) reported that dietary administration of 0.1 or 1.0% LAS to pregnant SD-JCL rats produced no significant differences in body weight, food consumption, reproductive and lactation indices or fetal anomalies when compared to controls.

Shiobara and Imahori (1976) found that daily oral administration of 10, 100 or 300 mg/kg/day of the sodium salt of LAS to pregnant mice from day 6 through 15 of gestation had a retarding effect on the growth of both mothers and litters at the two lower dosages and was lethal at the top treatment level. No teratogenicity was observed.

In a third study, Kuwano et al. (1977), noted that oral administration of 500 ppm LAS on days 6 to 18 of gestation resulted in no teratogenic effects. In a separate experiment, LAS exerted no additive or synergistic effect on the positive teratogenicity of methylmercury chloride given on day 11 of gestation.

Several investigators have also evaluated the effects of dermal application of LAS to pregnant animals in an attempt to disprove reports by the Mikami group (presented in the 1977 Report) that skin application of LAS to pregnant mice and rats was teratogenic. Again, the alkyl chain lengths of the tested materials were not given.

Nishimura (1976) reported that results of a joint study on the effects of

dermal application of LAS to pregnant Wistar rats failed to support earlier reports by Mikami (see 1977 Report) of LAS-induced abnormalities in mice and rats dermally treated with LAS during gestation. Four study groups (including Mikami) conducted parallel studies utilizing identical LAS samples, animal strain and the Mikami protocol. Pregnant rats were treated with 0.5 ml of 0, 1, 5 or 20% LAS, applied daily on days 0-20 of gestation to the backs of unrestrained rats. All rats were killed on day 21. All four study groups, including Mikami, obtained highly analogous results indicating no teratogenic effects. Dams treated with 20% LAS (~600x normal use levels) exhibited reduced feed consumption during the first half of gestation and a primary-type contact dermatitis at the site of application; fetuses in this group had reduced body weights which may have been secondary to decreased feed consumption in the dams, and one of four study groups noted delayed bone ossification at this exposure level. No other noteworthy effects were seen in the other treatment groups. Findings by Mikami's group of bleeding under the skin in fetuses were demonstrated to have been artificially induced during extraction of the fetuses from the uteri.

A consensus report, written by participants in the joint study, regarding earlier published findings of Mikami that dermally applied LAS induced abnormalities in pregnant mice and rats, summarized their conclusions as follows:

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

(2) Reported data were insufficient;

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

(4) No statistical evaluation was done;

(5) Reported abnormalities were most likely artifically

produced during extraction of the fetuses from the uteri; and

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

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

Daly et al. (1980) applied and allowed to remain 0.5 ml of 0.05, 0.1 or 0.5% LAS to the backs of pregnant Wistar rats daily throughout gestation (corresponding to 1,2 or 10 mg/kg/day) or 0.5 ml of 1,5 of 20% LAS for only 30 minutes each day during gestation (corresponding to 20, 100 or 400 mg/kg/day). All dams were killed on day 21. No indications of teratogenicity or embryotoxic effects were seen. Reduced body weight gain was noted in dams in the 20% LAS

group and slight (5% group) to marked (20% group) skin changes observed in dams from the two highest treatment groups. These skin changes consisted of erythema, skin thickening and fissuring.

Application of 20% LAS twice a day to the backs of pregnant ICR/Jcl mice prior to implantation (days 0 to 3) interrupted cleavage of eggs and retarded fetal development. A significantly higher number of embryos were in the oviducts of LAS-treated dams (44.6%) compared to control mice (2.1%) with the majority in the morula stage in contrast to the late blastocyst stage of control embryos. LAS treatment also resulted in an elevated incidence of deformed embryos (21.6%) compared to controls (4.9%), mostly in the one to eight-cell-stage (Nomura et al., 1980).

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

Pharmacology - Absorption and Metabolism. Cresswell et al. (1978) examined the disposition of [^{14}C]-LAS in four adult rhesus monkeys following single and repeated oral or subcutaneous administration. A single 30 mg/kg oral dose of LAS (mean mol. wt. 349) was rapidly excreted principally in urine during the first 24 hours (66.5% in males; 72.1% in females). Mean excretion values at 5 days were 71% in urine, 23% in feces. Similarly, a single subcutaneous injection of [^{14}C]-LAS resulted in the excretion of 64% of the radiolabel in urine and an additional 11% in feces within 5 days, with most excretion occurring within the first 24 hours. No unchanged LAS was detected in urine samples after oral or subcutaneous doses. Five major radioactive components were detected in urine; all were apparently more polar than LAS but were not sulfate or glucuronide conjugates.

Similar peak plasma concentrations of 34, 41 and 36 µg/ml of [^{14}C]-LAS were noted, all at 4 hours, after single oral doses of 30, 150 or 300 mg/kg, respectively. These results could indicate nonlinear absorption processes, or alternatively, the absorption process may be saturated at the 30 mg/kg level. With single subcutaneous doses, peak plasma concentrations increased almost proportionately, representing 0.16, 0.72 and 1.13 µg/ml for the 30, 150 and 300 mg/kg doses, respectively. No accumulation of plasma radioactivity was seen during seven consecutive daily oral (30 mg/kg/day) or subcutaneous (1 mg/kg/day) doses. Mean peak plasma concentrations and biological half-lives were similar after the first and seventh doses.

In another study, Drotman (1977) applied a single cutaneous dose of radiolabelled C_{12} LA^{35}S to the skin of rats, rabbits and guinea pigs. The test material was left in place for 72 hours; the animals were restrained to prevent ingestion. Only traces of radioactivity appeared in urine and feces with

recovery from the site of application being 78% in the guinea pig, 82% in the rabbit and 93% in the rat.

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

B. Human Studies

In man, LAS is rapidly and completely excreted following a single oral dose and does not effectively penetrate skin. Little or no skin irritation is observed in patch tests in humans with concentrations of LAS well above normal use levels (1% or less). Thus, LAS is considered acceptable with respect to human health for consumer and industrial use.

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

No additional information on the human health aspects of LAS surfactants was found.

BIBLIOGRAPHY

CASHNER, F. M. Schuyler, R. Fletcher, H. Ritz and J. Salvaggio. 1980. Immunologic responses of cynomolgus monkeys after repeated inhalation exposures to enzyme and enzyme-detergent mixtures. Toxic. Appl. Pharmacol. 52:62-68.

COATE, W. B., W. M. Busey, W. H. Schoenfisch, N. M. Brown and E. A. Newmann. 1978. Respiratory toxicity of enzyme detergent dust. Toxicol. Appl. Pharmacol. 45:477-496.

CRESSWELL, D. G., G. A. Baldock, L. F. Chasseaud and O. R. Hawkins. 1978. Toxicology studies of linear alkylbenzene sulphonate (LAS) in rhesus monkeys. II. The disposition of [^{14}C] LAS after oral or subcutaneous administration. Toxicology 11:5-17.

OALY, I. W., R. E. Schroeder and J. C. Killeen. 1980. A teratology study of topically applied linear alkylbenzene sulphonate in rats. Fd. Cosmet. Toxicol. 18:55-58.

OROTMAN, R. B. 1977. "Metabolism of cutaneously applied surfactants", In: Cutaneous Toxicity, Academic Press, Inc., V. A. Drill and P. Lazar, ed., pp. 95-109.

HEYWOOD, R., R. W. James and R. J. Sortwell. 1978. Toxicology studies of linear alkylbenzene sulphonate (LAS) in rhesus monkeys I. Simultaneous oral and subcutaneous administration for 28 days. Toxicology 11:245-250.

HOPE, J. 1977. Absence of chromosome damage in the bone marrow rats fed detergent actives for 90 days. Mutation Res. 56:47-50.

IMOKAWA, G. 1979. Study on skin-irritating and biological properties of monoalkyl phosphate anionic surfactants. J. Am. Oil Chem. Soc. 56(5):604-609.

IMOKAWA, G., K. Sumura and M. Katsumi. 1975a. Study on skin roughness by surfactants: II. Correlation between protein denaturation and skin roughness. J. Amer. Oil. Chem. Soc. 52(1):484-489.

INOUE, K. T. Sunakawa and S. Takayama. 1980. Studies of in vitro cell transformation and mutagenicity by surfactants and other compounds. Fd. Cosmet. Toxicol. 18:289-296.

ITO, R., H. Kawamura, H. S. Chang. K. Kudo, S. Kajiwara, S. Toida, Y. Seki, M. Hashimoto and A. Fukushima. 1978. Acute, subacute and chronic toxicity of magnesium linear alkylbenzene sulfonate (LAS-Mg). Toho Igakkai Zasshi 25(5-6): 850875. (J. Med. Soc. Toho, Japan).

KIKUCHI, K. 1978. Observations on some tissues of mice injected with LAS synthetic detergent for varying periods from the day of birth. Kawasaki Med. J. 4(3):193-201.

KUWANO, A., Y. Hamano, K. Inoue, Y. Oda, H. Yamamoto, N. Kunita. 1977. Studies on toxicity of commercial detergents. Part 5. Effects of sodium linear alkylbenzenesulfonate on the teratogenicity of methylmercuric chloride. Osaka-furitsu Koshu Eisei Kenkyusho Kenkyu Hokoku, Shokuhim Eisei Hen. 8:7-10.

MARKHAM, R. J. F. and B. N. Wilkie. 1979. Effect of detergent on protease-induced mortality and pulmonary histopathology. Arch. of Env. Health 34(6): 418-423.

MASUBUCHI, M. A. Takahashi, O. Takahashi and K. Hiraga. 1976. The cytogenetic studies and dominant lethal tests of long-term administration of butylated hydroxytoluene (BHT) and linear alkylbenzene sulfonate (LAS) in mice and rats. Tokyo Toritsu Eisei Kenkyusho Kenkyu Nempo. 27(2):100-104. [Ann. Rep. Tokyo Metr. Res. Lab. P.H. 27(2):100-104].

MASUDA, F. and K. Inoue. 1974. Effects of maternal administration of linear alkylbenzene sulfonate (LAS) upon mouse fetal and neonatal development. Congenital Anomalies 14:309-318.

MASUDA, F., K. Dkamoto and K. Inoue. 1974. Effects of linear alkylbenzene sulfonate applied dermally to pregnant mice on the development of their fetuses. Shokuhin Eiseigaku Zasshi 15(5):349-355.

NISHIMURA, H. 1976. Could skin application of cleansing detergents be teratogenic? Course of the successive joint studies and interpretation of the results. Cong. Anom. 16:175-185. (English translation).

NOMURA, T., S. Kimura, S. Hata, T. Kanzaki and H. Tanaka. 1980. The synthetic surfactants AS and LAS interrupt pregnancy in mice. Life Sci. 26:49-54.

ODA, Y., A. Kuwano, K. Inoue, Y. Hamano, H. Ramamoto, B. Mitsuda and N. Kunita. 1977. Studies on toxicity of commercial detergents. Part 6. Mutagenicity study in bacteria. Osaka-furitsu Koshu Eisei Kenkyusho Kenkyu Hokoku, Shokuhin Eisei Hen. 8:11-16.

SELMECI-ANTAL, M. nad A. Blaskovits. 1977. The effect of sodium dodecylbenzene sulphonate (SDBS) and various diets on rat liver glucose-6-phosphatase (G6P-ase) and Glucose-6-phosphate dehydrogenase (G6PDH) activities Nutr. Metab. 21(Suppl 1): 244-246.

SHELL RESEARCH LIMITED, unpublished data.

SHELL TOXICOLOGY LABORATORIES, unpublished data.

SHIOBARA, S. and A. Imahori. 1976. Effects of linear alkylbenzene sulfonate orally administered to pregnant mice on the mothers and their fetuses. Shokuhin Eiseigaku Zasshi. 17(4):295-301, as cited in Chemical Abstracts 86:12292k.

SWISHER, R. D. W. E. Gledhill, R. A. Kimerle and T. A. Taulli. 1978. Carboxylated intermediates in the biodegradation of linear alkylbenzene sulfonates (LAS). VII. International Congress on Surface Active Substances. Proceedings, Moscow, 1976. 4:218-230.

TIBA, S., S. Shiobara, A. Imahori and T. Kitagawa. 1976. Effects of linear alkylbenzene sulfonate (LAS) on dam, fetus, and newborn rats. Shokuhin Eiseigaku Zasshi 17(1):66-71.

BIBLIOGRAPHY (continued)

WATARI, N., K. Torizawa, M. Kanai and Y. Suzuki. 1977. Ultrastructural observations of the protective effect of glycyrrhizin for mouse liver injury caused by oral administration of detergent ingredient (LAS). J. Clin. Electron Microscopy 10:121-139.

CHAPTER 2

ALKYL SULFATES

(AS)

ALKYL SULFATES

SYNOPSIS

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

There are presently no environmental standards of water quality with respect to alkyl sulfates. Levels of AS, as such, in streams and waterways are not presently being monitored, but MBAS levels would include AS, if present. Biodegradation studies indicate linear primary AS readily undergo primary biodegradation 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.

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

Aquatic algae have exhibited toxicosis in AS concentrations from 10 mg/L

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

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

Ingestion of AS by laboratory animals during gestation produced no terata or deterimental effects on litter parameters except at doses that were severely toxic to the dams. Percutaneous applications of some concentrated AS samples (10-20%) to mice during early gestation were embryotoxic, but skin application of these concentrated samples during later stages of pregnancy were neither embryotoxic nor teratogenic. A decreased number of implantations was noted following application of a lower concentration of AS (2%) during early gestation, but the number of animals examined was too small to allow definitive conclusions to be drawn.

Although concentrated AS samples are primary skin and eye irritants in

laboratory animals, repeated occluded skin exposure to 0.1% AS is non-irritating. Similar results are noted in humans. Little or no ocular irritation is observed in rabbits at concentrations of 1% AS or less.

The minimal amounts of AS in detergent formulations taken with their facile biodegradation and generally low order of toxicity indicates that the use of alkyl sulfates does not pose a significant hazard to human health.

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 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.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

ALKYL SULFATES

I. INTRODUCTION

Up to the mid-1960's, anionic alkyl sulfates (AS) were predominantly used as wool-washing agents or as active ingredients in heavy duty laundry formulations. More recently, AS have been utilized in a wide variety of specialty products such as shampoos, cosmetics, etc. The bulk of AS in these products are linear, primary alkyl sulfates but some linear and branched secondary AS are also utilized. Primary AS are typically prepared by conventional sulfation of the parent alcohol with either sulfur trioxide or chlorosulfonic acid; secondary AS are more readily prepared by reacting the parent alkene with sulfuric acid.

Wordwide consumption of AS in 1976 amounted to 82,000 metric tons, (Matson, 1978). This consumption was distributed as follows:

United States - 36,000 metric tons,
Western Europe - 40,000 metric tons,
Japan - 6,000 metric tons.

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

BIBLIOGRAPHY

MATSON, T. P. 197B. The workhorse surfactants: LAS, alcohol sulfates and ether sulfates. J. Am. Oil Chemists' Soc. 55:66-69.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alkyl sulfates are one of several entities classified as anionic surfactants and thus can be detected with many of the procedures utilized in the detection of LAS (see Chapter 1). The MBAS analytical procedure measures AS along with other anionic surfactants but does not distinguish among them. A simple acid hydrolysis followed by a second MBAS analysis, however, will distinguish between LAS and AS.

B. Water Quality Standards

There are presently no standards in the United States or Europe specifically restricting the concentrations of alkyl sulfates. 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.

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.

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. Laboratory Investigations

AS are readily biodegraded in standard BOD tests and evolved CO_2 procedures. Neither slight branching nor increments in the length of the carbon chain appear to exert a significant effect on the rate of degradation. Die-away tests and simulated treatment processes indicate complete primary biodegradation (as MBAS) within 1 to 3 days, even under anaerobic conditions.

The work of Miura et al. (1979), referred to in Chapter 1, also involved biodegradation of a 100 mg/L sample of sodium dodecylsulfate. With activated sludge inoculum, MBAS disappeared completely in less than 5 days, while removal of TOC and BOD/TOD (total oxygen demand) approached 100% between 10 and 15 days. Similar results were reported by Itoh et al. (1979). The two alkyl sulfates tested, $C_{12\ ave}$ AS and a coconut-alcohol-derived AS, were the most readily biodegraded of all the surfactants tested.

B. Field Studies

Two field studies cited in the 1977 Report indicated 96 to 98% removal of AS (as MBAS) in a trickling filter sewage treatment plant

and complete removal of AS (far infrared analysis) during passage through two Japanese sewage treatment plants.

No additional field studies have been found.

C. Metabolic Pathways of Biodegradation

Linear primary AS readily undergo primary biodegradation via sulfatase enzymes which hydrolyze the sulfate ester group to form inorganic sulfate and the corresponding alcohol which eventually undergoes β-oxidation. This rapid breakdown does not necessarily apply to secondary or branched AS; some, but not all, branched primary and secondary AS are resistant to biodegradation.

The enzymes responsible for removal of the sulfate group from secondary alkyl sulfates (sulfohydrolases) were studied by Matcham et al. (1977). They found differences in specificity for chain-length and position of the sulfate group, among enzymes from two microbial species, Comamonas terrigena and Pseudomonas $C_{12}B$.

In the U.S.S.R., Stavskaya et al. (1976, 1979) have identified several bacteria capable of degrading C_{12} AS, with loss of MBAS and hydrolysis to sulfate and dodecanol. The bacteria involved include Citrobacter freundii, Pseudomonas aeruginosa, Flavobacterium devorans and Achromobacter guttatus. Aerobacter aerogenes, isolated with C. freundii, is apparently able to grow only on the products of the latter organism's attack on C_{12} AS.

BIBLIOGRAPHY

ITOH, S., S. Setsuda, A. Utsunomiya and S. Naito. 1979. Studies on the biodegradation test method of chemical substances. II. Ultimate biodegradabilities of some anionic, nonionic, and cationic surfactants estimated by CO_2 production. Yakagaku 28(3):199-204.

MATCHAM, G. W. J., B. Bartholomew, K. S. Dodgson, J. W. Fitzgerald and W. J. Payne. 1977. Stereospecificity and complexity of microbial sulphohydrolases involved in the biodegradation of secondary alkyl sulphate detergents. FEMS Microbiology Letters 1:197-200.

MIURA, K., K. Yamanaka, T. Sangai, K. Yosyimura and N. Hayashi. 1979. Application of biological oxygen consumption measurement technique to the biodegradation test of surfactants. Yukagaku 28(5):351-355.

STAFSKAYA, S. S., N. A. Yaroshenko, L. A. Taranova and S. G. Yukhenko. 1975. Decomposition of sodium dodecylsulfate by sewage bacteria. Hydrobiol. J. 12(3): 76-77.

STAFSKAYA, S. S., I. A. Krivets, L. S. Samoilenko. 1979. [Study of products of bacterial destruction of sodium dodecyl sulphate]. Prikl. Biokem. Mikro. Biol. 15(5):790-793.

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

The toxicity of AS to aquatic organisms was reviewed in the 1977 Report. Studies conducted since that time, with references to earlier work, are discussed in this section. The methodology used in the present report is described in Chapter 1 of 1977 Report.

1. AS Structure-Activity Relationship

In the aquatic toxicity data surveyed in the 1977 Report, there was no evidence of a correlation between toxicity and alkyl chain length or molecular weight. Moreover, no information on the toxicity of the biodegradation products of AS was found.

The results of a recent study by Lundahl and Cabridenc (1978) indicate a definite trend of increasing toxicity with increasing alkyl chain length. In 24 hr toxicity tests with Daphnia magna, EC_{50} (immobilization) values decreased by approximately one half for every additional carbon in the alkyl chain, resulting in a range of 8200 to 42 mg/L for C_5 to C_{13} AS. Wright (1976) found C_{10} AS approximately ten times as toxic to barnacle larvae (Elminius modestus) as C_8 AS. The most pronounced effects of alkyl chain length were observed in tests by Kikuchi et al. (1976), in which LC_{50} values for the Japanese killifish (Oryzias latipes) decreased by a factor of ten for every two-carbon increase for C_{12} to C_{16} AS (see Table 2-A).

TABLE 2-A. ACUTE TOXICITY OF ALKYL SULFATES TO FISH

Species	Surfactant	LC_{50} Concentration (mg/L)	Experimental Conditions	Reference
Japanese killifish (*Oryzias latipes*)	NaC_{12ave} AS	70	24 hr., distilled water	Kikuchi *et al.* (1976)
	$NaC_{14}AS$	5.9		
	$NaC_{16}AS$	0.78		
Japanese killifish (*Oryzias latipes*)	NaAS	10	48 hr.	Tomiyama (1974)
Bluegill sunfish (*Lepomis macrochirus*)	$NaC_{12}AS$	4.5	96 hr., 125 mg/L hardness pH 7.4, flow-through, nominal	Bishop and Perry (1979)
Rainbow trout (*Salmo gairdneri*)	$NaC_{12}AS$	4.62	24 hr., 350-375 mg/L hardness pH 8.2, flow-through, nominal	Fogels and Sprague (1977)
Zebrafish (*Brachydanio rerio*)	$NaC_{12}AS$	7.79		
Flagfish (*Jordanella floridae*)	$NaC_{12}AS$	8.10		
Phoxinus phoxinus	$C_{12}AS$	30.5	24 hr., pH 7.7, flow-through, 13°C	Lundahl and Cabridenc (1978)
Minnow (*Macrones vittatus*)	C_{12ave} AS	1.39	96 hr., 60-70 mg/L hardness (as $CaCO_3$), pH 7.3, static	Verma *et al.* (1978)
	C_{12ave} AS, triethanolamine salt	1.53		

Increased toxicity with increasing alkyl chain length was also observed to some extent by Tukmache and co-workers (1977) in their work with yeast protoplasts. C_{12} AS had the highest lytic activity for plasma membranes as compared to C_8, C_{10}, C_{11}, C_{12}, C_{13} and C_{14} AS.

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

Lundahl and Cabridenc (1976) conducted experiments on Daphnia to determine the toxicity of the degradation products of C_{12} AS. In this instance, the toxicity of the surfactant, as measured by immobility, increased to a maximum at 30 hours of exposure, then rapidly dropped off to almost negligible toxicity. The authors cited previous work which indicated "that alkyl sulfates degrade by the hydrolysis of the sulfonic ester function followed by the oxidation in acid of the formed alcohol." The authors suggested that this hypothesis would explain the results of the experiment, since dodecanoic acid (from degraded C_{12} AS) is much more toxic than C_{12} AS, but is very short-lived in solution.

2. Acute Toxicity to Fish

The LC_{50} values reported in the 1977 Report on AS toxicity ranged from 2-1,000 mg/L. As

only four species of fish were tested, it was not possible to identify species or groups which were generally more sensitive. Of these four species, however, the bluegill (Lepomis macrochirus) had the lowest LC_{50} (2.13 mg/L) in a toxicity test with branched NH_4C_{15} AS.

More recent data on freshwater fish toxicity are summarized in Table 2-A; most of the toxicity tests were conducted with the sodium salt of C_{12}AS. The range of reported LC_{50} values for alkyl sulfates, both for the Japanese killifish (Oryzias latipes), was 0.78 to 70 mg/L. The variation was attributed to the difference in alkyl chain length used, with NaC_{16}AS being apparently much more toxic than NaC_{12ave}AS. Five other species were also tested for sensitivity to C_{12}AS, for which the range of LC_{50} values was 4.5 to 30.5 mg/L.

Abel (1978) determined mean survival periods (LT_{50}) for two fish species at constant concentrations of NaC_{12ave} AS. The LT_{50} value in these static tests for rainbow trout (Salmo gairdneri) in 42 mg/L was 45 hours; for brown trout (Salmo trutta) in 18 mg/L, the LT_{50} was 24.5 hours. Kikuchi et al. (1976) reported that earlier growth stages in goldfish (Carassius auratus) are the most sensitive.

3. Acute Toxicity to Invertebrates

The 1977 Report surveyed the literature on AS toxicity to aquatic invertebrates, reporting LC_{50} values ranging from 2.8 to more than 200 mg/L. The lowest LC_{50} was for Daphnia magna exposed for 48 hours to NaC_{12-14} AS. 24-hour-old pupae of the mosquito (Culex pipiens q.) exhibited the least sensitivity of all invertebrates tested in Na-2-ethylhexyl sulfate, with an LC_{50} of >200 mg/L (time not given).

Most of the recent studies on invertebrate toxicity have been conducted with marine organisms (see Table 2-B). LC_{50} values from these studies range from 0.35 to 162 mg/L. In a static test, the 48 hr LC_{50} for larvae of the horse clam (Tresus capax) was 0.35 mg/L NaC_{12} AS (Cardwell et al., 1978). In another experiment (Tatem et al., 1976), the grass shrimp (Palaemonetes pugio) was reported to have a maximum 96 hr LC_{50} of 162 mg/L NaC_{12} AS. Tatem et al. (1976) have hypothesized that grass shrimp are less susceptible to C_{12} AS because they are inactive, bottom-dwelling organisms. Since surfactants tend to collect at surfaces, fish and smaller crustaceans are likely to be most sensitive to AS.

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

4. Sublethal Toxicity

No information on the sublethal toxicity of AS was available during the review conducted for the 1977 Report.

Recent studies have reported a variety of sublethal effects, including immo-

TABLE 2-8 ACUTE TOXICITY OF ALKYL SULFATES TO INVERTEBRATES

Species	Surfactant	LC_{50} Concentration (mg/L)	Experimental Conditions	Reference
Pacific oyster larvae (Crassostrea gigas)*	$NaC_{12}AS$, 100%	0.58-1.16 (0.91 avg.)	48 hr., 29°/oo salinity pH 7.8, static, nominal	Cardwell et al. (1977)
Pacific oyster larvae (Crassostrea gigas)*	$NaC_{12}AS$, 100%	1.0	48 hr., 29°/oo salinity pH 7.8, static, nominal	Cardwell et al. (1978)
Horse clam larvae (Tresus capax)*	$NaC_{12}AS$, 100%	0.35		
Grass shrimp, adults (Palaemonetes pugio)*	$NaC_{12}AS$	52.0-162.0	96 hr., 15°/oo salinity 20°C, static, nominal	Tatem et al. (1976)
Daphnia magna	$NaC_{12}AS$	1.8	48 hr., 125 mg/L hardness, pH 7.4, flow-through nominal	Bishop and Perry (1979)
Scud (Gammarus sp.)*	$NaC_{12}AS$	14.4	96 hr.	Bluzat et al. (1976)
Snail (Lymnea sp.)	$NaC_{12}AS$	24.4		
Gnat larvae (Chaoborus sp.)	$NaC_{12}AS$	50		
Daphnia magna	$NaC_{12}AS$	1.8	48 hr., 125 mg/L hardness, pH 7.4, flow-through nominal	Bishop and Perry (1979)

*Marine species

bilization and abnormal development. The lowest concentration at which sublethal effects appeared was 0.1 mg/L C_{12ave} AS, which depressed the olfactory bulbar electric response in whitefish (Coregonus clupeaformis) (Hara and Thompson, 1978). The authors considered this a deleterious effect, because reduced olfactory sensitivity could impair feeding and migrating behavior. In a companion experiment, whitefish were found to be attracted to NaC_{12ave} AS concentrations of 0.1, 0.5, and 1.0 mg/L; the fish exhibited neither attraction to nor avoidance of 0.01 and 10 mg/L concentrations of C_{12ave} AS (Hara and Thompson, 1978).

Other effects were observed at AS concentrations ranging from 0.4 to 8200 mg/L, primarily in marine species. The available data are summarized in Table 2-C.

5. Subchronic and Chronic Toxicity

The 1977 Report reviewed the findings of one chronic toxicity study on clam (Mercenaria mercenaria) and oyster (Crassostrea virginica) larvae. Fertilized egg development was significantly retarded in both species at 1 mg/L AS compared to controls; development was completely inhibited at 2.5 mg/L. Clam mortality was 68% after a 10-day exposure, while oyster mortality was 82% after 12 days in 5 mg/L.

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

TABLE 2-C SUBLETHAL EFFECTS OF ALKYL SULFATES ON AQUATIC ORGANISMS

Species	Surfactant	Concentration (mg/L)	Effects	Experimental Conditions	Reference
Whitefish (Coregonus clupeaformis)	$NaC_{12ave.}AS$	0.1	Depression of olfactory bulbar elec. response	15 min., 78.4 mg/L hardness pH 7.5, 10.5°C, flow-through	Hara and Thomps (1978)
Japanese ayu (Plecoslossus altivelis)	"formulation AS" "pure reagent AS"	4.0 8.4	Est. threshold concentration for avoidance	-	Tatsukawa and Hidaka (1978)
Pacific oyster larvae (Crassostrea gigas)*	$NaC_{12}AS$	0.67-1.04 (0.84 avg)	EC_{5n}, abnormal shell development	48 hr., 29°/oo pH 7.8, static nominal	Cardwell et al. (1977)
Pacific oyster larvae (Crassostrea gigas)*	$NaC_{12}AS$	∿ 0.95	EC_{50}, abnormal development	48 hr., 29°/oo salinity pH 7.8, static, nominal	Cardwell et al. (1978)
Horse clam larvae (Tresus capax)*	$NaC_{12}AS$	0.4			
Barnacle nauplii (Elminius modestus)*	$C_{10}AS$ C_8AS	1.8×10^{-3}M 1.7×10^{-2}M	EC_{50}, immobility	30 min., 15°C	Wright (1976)
Sea urchin embryos (Hemicentrotus pulcherrimus)*	$NaC_{12ave.}AS$	28	Inhib. of micromere formation in eggs	-	Tanaka (1976)
(Temnopleurus toreumaticus)*		20	Cleavage inhibited, cell shape transformation		
(Pseudocentrotus depressus)*					

* Marine species

TABLE 2-C (continued)

Species	Surfactant	Concentration (mg/L)	Effects	Experimental Conditions	Reference
Daphnia magna	C_5AS	8200	EC_{50}, immobilization	24 hr., pH 7.7 13°C, flow-through	Lundahl and Cabridenc (1978)
	C_8AS	4350			
	C_9AS	2300			
	$C_{10}AS$	800			
	$C_{12}AS$	80			
	$C_{13}AS$	42			

2.85 mg/L.

The only information available on chronic toxicity in invertebrates was found in Patzner and Adam (1979). In a 30-day test with the flatworm (Dugesia gonocephala), the authors calculated an LC_0 (the highest concentration at which no lethal effect was observed) of 0.5 mg/LC_{12} AS. However, the regenerative capacity of worms was reduced at concentrations as low as one half the LC_0.

6. Effects of Environmental Variables on AS Toxicity

One study reviewed in the 1977 Report found that NaC_{12ave} 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. Toxicity also increased with the hardness of the acclimatization water. In another toxicity test, goldfish were exposed to 4 mg/L NaC_{12ave} AS for two months, and then tested for susceptibility to DDT. Although some increase in the toxicity of DDT was observed, the differences were not statistically significant.

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

In a toxicity test with carp, Kikuchi et al. (1976) observed increasing

toxicity of linear AS compounds as water hardness increased. No further information was given.

7. Bioaccumulation of AS

In a study reviewed in the 1977 Report, rainbow trout and goldfish were exposed to 70 ml/L NaC_{12} AS for 35 minutes and 112 minutes, respectively. Concentrations in fish tissues ranged from 6.8 µg/g to 85.7 µg/g at the end of these periods. Uptake increased significantly as water hardness increased from 0 to 300 mg/L as $CaCO_3$.

Wakabayashi et al. (1978) exposed carp (Cyprinus carpio) to a 0.5 mg/L solution of ^{35}S-labelled NaC_{12ave}AS for 72 hours in a flow-through aquarium. Maximum levels of ^{35}S were observed after 24 hours; thereafter, residues in the hepatopancreas decreased, while whole body and gall bladder residues were stable. The maximum ^{35}S bioconcentration factors observed were 50 for the hepatopancreas, 700 for the gall bladder, and 4 for the whole body. Upon transfer to fresh water, the ^{35}S body burden was reduced by one half in three days.

Carp were also used by Kikuchi et al. (1978) in a 24-hour uptake test in 1.1 mg/L ^{35}S-NaC_{12ave}AS (water hardness was 25 mg/L as $CaCO_3$). After two hours, the bile bioconcentration factor of 3 was the highest of any measured body part. At the end of 24 hours, bioconcentration factors (measured as ^{35}S) in the gall bladder and hepatopancreas were 5-7x, 1-2x in the skin surface and kidney, and less than 1x in gills, brain, and muscle tissue. After 48 hours in fresh water, the bioconcentration factor in the gall bladder was 80x, while bioconcentration factors in other tissues and organs were less than one.

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

Laumond et al. (1973) reported that the presence of 1 mg/L AS (unspecified) has no significant effect on mercury uptake by phytoplankton (Diogenes sp.) or mussels.

B. Toxicity of AS to Algae and Microorganisms

The 1977 Report reviewed a report of a maximum acceptable concentration for five days (MAC-5day) of 1 - 10 mg/L AS for the marine flagellate (Dunaliella sp.). MgC_{12} AS completely inhibited the growth of 12 species of marine phytoplankton (Chlorophyceae) at concentrations of 100 and 1,000 mg/L. In addition, Nannochloris sp. and Stichococcus sp. were completely inhibited at 10 mg/L MgC_{12} AS.

In addition, a study was reviewed in the 1977 Report on the effects of NaC_{12} AS on ciliates (Cyrtolophosis) at concentrations of 0.02 - 0.2 mg/L for 4 or 15 minutes. In 0.1 and 0.2 mg/L, the cell cytoplasm autolysed, releasing the granular component and nuclear matrix. AS surfactants with varying alkyl chain length inhibited the growth and motility of the bacterium, Proteus mirabilis; longer chain compounds had a stronger effect. Growth inhibition has also been observed in E. coli and various soil and water bacteria at AS concentrations of 200 to >200,000 mg/L; soil bacteria were apparently the most sensitive.

No recent studies on AS toxicity to algae were available for this report. Bernheim (1975) exposed a pigmentless strain of the bacterium, Pseudomonas

aeruginosa, to concentrations of 0.5 x 10^{-4}M to 3.0 x 10^{-4}M NaC_{12} AS. Higher concentrations of AS disrupted both inner and outer membranes which resulted in potassium efflux and lysis. At lower concentrations, only the outer membrane was affected, as shown by loss of Alcian Blue staining, minor potassium efflux, and increased swelling of cells after incubation in LiCl solution. The latter effect was attributed to a loss of cell support normally provided by the outer membrane.

C. Effects of AS on Higher Plants

A single study on the effects of AS on plants was included in the 1977 Report and showed a stimulatory effect. Corn seeds that had been watered with 0.01, 0.1 or 1 g/L C_{12} AS weighed 97%, 130% and 136% respectively, of the control, with similar increases in length and dry weight of corn plants reported.

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

Antonielli and Lupatteli (1977) steeped barley seeds (Hordeum vulgare L.) in NaC_{12ave} AS (100% active) concentrations of 10^{-5}M to 10^{-2}M for 24 hours, and then allowed them to germinate. The lowest concentrations of AS causing significant growth inhibition (11%, as determined by shoot length) was 10^{-3}M.

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

O. Effects of AS on Birds and Wildlife

No studies of AS toxicity to terrestrial biota were found for the 1977 Report.

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

E. Mode of Action

The mode of action of AS in fish has been discussed at length in the 1977 Report; information on other organisms was not available. The usual behavioral response to AS toxicosis is increased swimming activity and respiratory rate, often followed by surfacing, loss of balance, reduced motility, and death. Progressive gill lamellae damage is the most frequently observed pathological effect.

The toxicity of AS to aquatic organisms has been attributed to a variety of factors, such as a decrease in the surface tension of the water, changes in the permeability of certain tissues and interactions between the surfactant and protein components of cell membranes.

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

Abel (1978) has reported a different mode of action of NaC_{12ave} AS on rainbow trout above and below 120 mg/L. At lower concentrations, AS "appears to act at a site in the external membrane of the gill cells. Consequent leakage of metabolites renders the cell non-viable and autolysis occurs, i.e. the cell is destroyed by the action of its own lytic enzymes." This was termed the slow type of toxic action. At concentrations exceeding 120 mg/L, AS appeared to act by chemical denaturation of the cell constituents, causing very rapid cell death. High concentrations were also more conducive to rapid absorption of the surfactant by the fish. The author stated that the two modes of action may occur simultaneously at higher concentrations, but since the macroscopic damage caused by both modes is the same, this hypothesis is not verifiable. Toxicity tests on the brown trout provided no evidence for a dual mode of action in this species.

BIBLIOGRAPHY

ABEL, P. D. 1978. Mortality patterns in trout (Salmo trutta and S. Gairdneri) exposed to an anionic detergent in relation to concentration and mechanisms of toxic action. Freshwater Biol. 8:497-503.

ANTONIELLI, M. and M. Lupattelli. 1977. Un test orzo per misurare l'azione biologica di detergenti e detersivi. [A barley test for measuring the biological action of detergents and detersives]. Agrochimica 21:15-22.

BERNHEIM, F. 1975. A comparison of the effects of sodium dodecyl sulphate and sodium dodecanoic on cell swelling potassium efflux in a strain of Pseudomonas aeruginosa. Cytobios 14:203-210.

BISHOP, W. E. and R. L. Perry. 1979. The development and evaluation of a flow-through growth inhibition test with duckweed (Lemna minor L.). Proc. ASTM 4th Aquatic Toxicol. Xymp., Chicago, Il., October 17, 1979.

BLUZAT, R., F. J. Rodriquez-Ruiz, and J. Seuge. 1976. Toxicite aigue de 5 agents polluants, sur 4 especes d'invertebres habitant les eaux douces. [Acute toxicity of five pollutants to four freshwater invertebrate species]. C. R. Hebd. Acad. Sci. Ser. D 283(9):1089-1092.

BODE, H., R. Ernst and J. Arditti. 1978. Biological effects of surfactants. III. Hydra as a highly sensitive assay animal. Environ. Pollut. 17:175-185.

BROMAGE, N. R. and A. Fuchs, 1976. A histological study of the interrenal cells of the goldfish (Carassius auratus) to treatment with sodium lauryl sulphate. J. Fish Biol. 9:529-535.

CARDWELL, R. D., C. E. Woelke, M. I. Carr, and E. Sanborn. 1978. Variation in toxicity tests of bivalve mollucs larvae as a function of termination technique. Bull. Environ. Contam. Toxicol. 20:128-134.

CARDWELL, R. O., C. E. Woelke, M. I. Carr and E. Sanborn. 1977. Appraisal of a reference toxicant for estimating the quality of oyster larvae. Bull. Environ. Contam. Toxicol. 18(6):719-725.

CHOULES, G. L. W. C. Russell and D. A. Gauthier. 1978. Duck mortality from detergent-polluted water. J. Wildl. Manage. 42(2):410-414.

FOGELS, A. and J. B. Sprague. 1977. Comparative short-term tolerance of zebra-fish, flagfish, and rainbow trout to five poisons including potential reference toxicants. Water Res. 11:811-817.

HARA, T. J. and B. E. Thompson, 1978. The reaction of whitefish, Coregonus clupeaformis, to the anionic detergent sodium lauryl sulphate and its effects on their olfactory responses. Water Res. 12:893-897.

KIKUCHI, M., M. Wakabayashi, H. Kojima and T. Yoshida. 1978. Uptake, distribution and elimination of sodium linear alkylbenzene sulfonate and sodium alkyl sulfate in carp. Ecotoxicology and Environmental Safety 2:115-127.

KIKUCHI, M., M. Wakabayashi, T. Nakamura, W. Inoue, K. Takahashi, T. Kawana, H. Kawahara nad Y. Koido. 1976. A study of detergents. II. Acute toxicity of anionic surfactants on aquatic organisms. Annu. Rep. Tokyo Metrop. Res. Inst. Environ. Prot. 57-69 (English abstract).

LAUMOND, F., M. Neuberger, B. Oonnier, A. Fourey, R. Bittel, and M. Aubert. 1973. Experimental investigations, at laboratory, on the transfer of mercury in marine trophic chains. Res. Intern. Oceanogr. Med. 31/32:47-53.

LUNDAHL, P. and R. Cabridenc. 1978. Molecular structure-biological properties: Relationships in anionic surface-active agents. Water Res. 12:25-30.

LUNDAHL, P. and R. Cabridenc, 1976. [A focus on a method of study of the evolution of the toxicity of pollutants during biodegradation. Application to surface agents]. J. Fran. Hydrol. 7(3):143-149 (English translation).

PATZNER, A. M. and H. Adam. 1979. [Influence of tensids on the regeneration of Dugesia gonocephala (Plathelminthes, Tricladida) and Notoplana humilis (Plathelminthes, Polycladida)]. Zool. Anz. Jena 2D2(3/4):199-208 (English abstract).

TANAKA, Y. 1976. Effects of the surfactants on the cleavage and further development of the sea urchin embryos: 1. The inhibition of micromere formation at the fourth cleavage. Development, Growth, and Differentiation 18(2):112-122.

TANIYAMA, T. and T. Nomura. 1978. Effects of various synthetic detergent on photosynthesis, dry matter, and grain production in rice plants. Mie Daigaku Kankyo Kagaku Kenkyu Kiyo 3:93-104.

TATEM, H. E., J. W. Anderson and J. M. Neff. 1976. Seasonal and laboratory variations in the health of grass shrimp Palaemonetes pugio: Dodecyl sodium sulfate bioassay. Bull. Environ. Contam. Toxicol. 16(3):368-375.

TATSUKAWA, R. and H. Hidaka. 1978. Avoidance test of chemical substances on fish. Avoidance of detergents by ayu (Plecoslossus altivelis). Nippon Nosei Kagaku Kaishi 52(7):263-270. (As cited in Chem. Abstr. 89 158269s).

TOMIYAMA, S. 1978. On the possible mechanism of the effect on fishes and shellfishes by household detergents. Yukagaku 27(6):347-353. (English translation).

TOMIYAMA, S. 1974. Effects of surfactants on fish. Bull. Jpn. Soc. Sci. Fish. 40(12):1291-1296. (English abstract).

TUKMACHEV, V. A., B. IU. Zalavskii and S. V. Rogozhin. 1977. Lysis of yeast protoplasts under sodium alkyl sulfates treatment. Biokhimiia 42:2246-2252.

VERNA, S. R., D. Mohan and R. C. Dalela. 1978. Studies on the relative toxicity of few synthetic detergents to a fish Macrones vittatus. Acta hydrochim. hydrobiol. 6(2):121-128.

WAKABAYASHI, M., M. Kikuchi, H. Kojima and T. Yoshida. 1978. Bioaccumulation profile of sodium linear alkylbenzene sulfonate and sodium alkyl sulfate in carp. Chemosphere 11:917-924.

WAKA8AYASHI, M., M. Kikuchi, W. Inoue, H. Kawahara and Y. Koido. 1975. Toxicity of detergents to some aquatic organisms. I. Acute toxicity of anionic surfactants to Oryza latipes. Suisan Zoshoka 23(3):119-124. (As cited in Chem. Abstr. 85: 117591q).

WRIGHT, A. 1976. The use of recovery as a criterion for toxicity. Bull. Environ. Contam. Toxicol. 15(6):747-749.

ZAHN, R. K., G. Zahn, W. E. G. Muller, I. Muller, R. Beyer, U. Buller-Berger, B. Kurelec, M. Rijavec and S. Britvec. 1977. Consequences of detergent pollution of the sea: Effects on regenerating sponge cubes of Geodia cydonium. Sci. Total Environ. 8:109-151.

V. HUMAN SAFETY

Data presented in the 1977 Report on the human safety aspects of alkyl sulfates indicate that AS as a class are relatively non-toxic. Their low order of acute mammalian toxicity and the absence of chronic effects support the view that present day use of AS poses no threat to human health.

A. Animal Studies

In mammalian species, the acute oral LD_{50} values of AS are generally above 1000 mg/kg; AS in commercial use have oral LD_{50} values between 5000 and 15,000 mg/kg. No deleterious effects were noted in rats fed 1% C_{12ave} AS in the diet for one year. Although AS concentrations of 10% or more are primary eye irritants in rabbits, a concentration of 1% produces little or no ocular irritation. Concentrated AS samples are primary skin irritants in rabbits and guinea pigs but are non-irritating to laboratory animals at a 0.1% concentration.

Acute Toxicity. No additional information was found on the acute toxicity of alkyl sulfates in laboratory animals.

Acute Irritation - Skin. Occluded, 24-hr exposure of the rabbits to 2, 10 or 20% aqueous solutions of the sodium, ammonium or triethanolamine salts of C_{12ave} AS produced moderate to severe skin irritation.

The sodium salt was the most irritating, particularly at the lowest concentration. Primary irritation scores were as follows:

Concentration	Sodium Salt	Ammonium Salt	Triethanolamine Salt
2%	>5 < 5.5	5 < 5.5	3.5
10%	6	5 < 6	5
20%	6	6	5.2

(Ciuchta and Dodd, 1978)

Skin Sensitization.

Several studies cited in the 1977 Report indicated that AS are not sensitizing materials.

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

Instillation of 0.1 ml of 2, 10 or 20% aqueous solutions of the sodium, ammonium or triethanolamine salts of C_{12ave} AS into rabbits' eyes produced mild irritation at the 2% level and moderate to severe irritation which persisted at 7 days at the higher surfactant concentrations (Ciuchta and Dodd, 1978). The

relative irritancy of the three samples could not be determined since they were fairly well grouped together.

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

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

Subchronic Irritation - Skin. Cumulative, open-patch test, application of 0.1 ml of a 2% aqueous solution of C_{12} AS (100% pure) to the shaved backs of guinea pigs twice daily for a total of nine treatments resulted in moderate to severe cumulative skin irritation. A skin irritation score of 3 of a possible 4 points was recorded (Imokawa, 1979).

Subchronic Toxicity - Oral. Administration of 250 mg/kg C_{12} AS by gavage to male New Zealand white rabbits daily for two months was found to slightly inhibit the progression of cholesterol-induced atherosclerosis. Serum cholesterol levels (3000 mg/100 ml) were elevated, however, in rabbits fed the high cholesterol diet plus C_{12} AS by gavage compared to rabbits on the high cholesterol diet alone (2100 mg/100 ml). All rabbits were fed 100 g of a standard commercial rabbit pellet daily. Group 1 received the pelleted diet alone; Group 2 received the diet supplemented with 1% cholesterol and 6% peanut oil by weight; and Group 3 received the supplemented diet plus C_{12} AS by gavage. At two months, the rabbits were killed, aortas removed and serum and tissue cholesterol measured. The percent of aortic surface area involved by atherosclerosis was calculated by planimetry. The extent of cholesterol-induced atherosclerotic lesions in the aorta was reduced by C_{12} AS (32% atherosclerotic lesions compared to 72% in controls fed the high cholesterol diet). Cholesterol levels in aortic tissue for these two groups were 12.4 mg/g and 18mg/g, respectively. Data for the group given the pellets alone were not given (Kwak et al., 1975).

The mode of action of AS in the inhibition of the progression of cholesterol-induced atherosclerotic lesions in not clear. Morin et al. (1974) have reported that NaC_{10} AS, an homolog of C_{12} AS, inhibits cholesterol esterification by aortic microsomes in swine arteries in vitro. These surfactants may, therefore, reduce the accumulation of cholesterol esters in aortic tissue in vivo.

Chronic Toxicity. No new studies were found in this area. Data presented in the 1977 Report indicated no deleterious effects were produced in rats fed 1% C_{12ave} AS in the diet for one year.

Carcinogenicity.

Long-term feeding studies in rats and skin-painting experiments in mice give no indication of carcinogenic activity for alkyl sulfates. Addition of 0.25% C_{12} AS to the drinking water of rats was shown to enhance gastric tumor induction by nitro-N-nitrosoguanidine; however, it is unclear whether this effect is due to enhanced carcinogen absorption or some other physiological mechanisms.

No new studies have been found in this area.

Teratogenesis.

There is no evidence of terata or detrimental effects on litter parameters associated with ingestion of up to 300 mg/kg AS by laboratory animals during gestation. Reduced litter size and fetal loss were seen in mice but not rats or rabbits at doses that were severely toxic to the dams.

Daily application of 0.1 ml of a 20% aqueous solution of AS (alkyl chain length not identified) to the dorsothoracic area of pregnant ICR/Jcl mice on days 1 to 10 of gestation was observed to interfere with embryonic development at the cleavage stage (Nomura et al. , 1980). Implanted embryos were found in only 1/26 (3.9%) of AS-treated dams compared to 18/20 (90%) of water-treated controls. Application of a lower concentration of AS (2%) to the skin of mice on days 1 through 17 of gestation also reduced the number of pregnancies (14/22; 63.6%), but this reduction was not statistically significant, because the number of animals compared was too small. The percentage of early and late deaths, the percentage of living fetuses and the incidence of malformation in AS-treated groups were

comparable to those noted for control mice. Body weights of living fetuses in the 2% AS group were comparable to controls, but were significantly reduced in the 20% AS group (♂:1.21 g; ♀:1.21 g vs. control ♂:1.37 g; control ♀:1.30 g).

Application of 10% AS twice a day to the backs of pregnant mice prior to implantation (days 0 to 3) interrupted cleavage of eggs and retarded fetal development. A significant number of embryos were in the oviducts of AS-treated dams (18.6%) compared to control mice (2.1%) with the majority in the morula stage in contrast to the late blastocyst stage of control embryos. AS treatment also resulted in an elevated incidence of deformed embryos compared to controls, mostly in the one to eight-cell-stage (29.1% vs 4.9% in controls). Application of 2% or 20% AS to mice during late pregnancy (days 12 to 17), however, did not interrupt gestation. The 20% AS treatment reportedly retarded growth of suckling mice, but this effect disappeared after weaning (Nomura et al., 1980).

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

Mutagenicity. Hope (1977) reported that the incorporation of C_{12ave} AS into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosomes of rat bone marrow cells. These negative findings are the first available data on the mutagenicity of AS.

Pharmacology - Metabolism.

Metabolic studies in rats indicate that greater than 80% of the ^{35}S-labelled AS is excreted in the urine within 48 hours, regardless of the route of administration. Butyric acid-4-sulfate is the major metabolite.

Burke et al. (1978) recently examined the effect of substitution at the ω-carbon on the distribution, metabolic fate and mode of excretion of AS. MRC hooded rats received 1 mg/200 g body weight of either 10-undecenyl [^{35}S] sulfate or 10-phenyldecyl [^{35}S] sulfate by intravenous injection.

With 10-undecenyl sulfate, approximately 78% of the injected radioactivity was recovered in urine, largely as the sulfate ester conjugate (68%); an additional 10% was present as inorganic sulfate. Significant amounts of radioactivity were also detected in bile (9.9-11.5%), mainly as the sulfate ester conjugate. Thus, introduction of a terminal unsaturated linkage did not prevent metabolism.

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

Pharmacology-Cellular Effects. In agreement with studies cited in the 1977 Report, Ossipov et al. (1978) found that the hemolytic activity of a homologous

series of AS (C_8 to C_{15}) on dog and human erythrocytes increased with alkyl chain length, reaching maximum activity at twelve carbons. Further elongation of the hydrophobic chain did not produce any marked changes.

B. Human Studies

In two individuals, orally administered C_{16} AS was well absorbed in one individual (80% excreted in urine, 7% in feces by 72 hr), but poorly absorbed in the other test subject (20% in urine, 73% in feces at 118 hr). Skin irritation test results range from little or no response to moderate irritation following exposure to 1% AS under varying conditions.

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

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

Fisher and Maibach (1975) found that application of aqueous 0.5, 1 or 2% solutions of C_{12ave} AS to the backs of healthy male volunteers produced epi-

dermal hyperplasia. Treatment with a 1% concentration produced an approximately 30-fold increase in mitotic activity which peaked 48 hr post-treatment. Treatment with either 0.5% or 2% C_{12ave} AS produced similar changes but to a smaller degree.

Imokawa et al. (1975a) evaluated the relative intensity of skin roughness produced on the surface of the forearm of human volunteers from contact with 1% aqueous solutions of AS with varying alkyl chain length (C_8, C_{10}, C_{12}, C_{14}). Skin roughening potential increased with increasing alkyl chain length, reaching maximum intensity at twelve carbons. In later studies (Imokawa et al., 1975b; Imokawa and Mishima, 1979), these investigators noted that the relative degree of skin roughening in vivo correlated with the extent of protein denaturation, but did not coincide with the irritancy potential as evaluated by the closed patch test.

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

BIBLIOGRAPHY

BURKE, B., A. H. Olavesen, C. G. Curtis and G. M. Powell. 1978. The distribution and excretion of non-biodegradable anionic surfactants. Xenobiotica 8(3):145-155.

CIUCHTA, H. P. and K. T. Dodd. 1978. The determination of the irritancy potential of surfactants using various methods of assessment. Drug and Chemical Toxicol. 1(3):305-324.

DAHL, M.V. and R. J. Trancik. 1977. Sodium lauryl sulfate irritant patch tests: Degree of inflammation at various times. Contact Dermatitis 3: 263-266.

DAVIES, R. E., S. R. Kynoch and M. P. Liggett. 1976. Eye irritation tests - an assessment of the maximum delay time for remedial irrigation. J. Soc. Cosmet. Chem. 27:301-306.

DOMINGUEZ, J. G., J. L. Parra, M. R. Infante, C. M. Pelejero, F. Balaguer and T. Sastre. 1977. A new approach to the theory of adsorption and permeability of surfactants on keratinic proteins: the specific behaviour of certain hydrophobic chains. J. Soc. Cosmet. Chem. 28:165-182.

HOLST, R. and H. Moller. 1975. One hundred twin pairs patch tested with primary irritants. Brit. J. Dermatol. 93:145-149.

HOPE, J. 1977. Absence of chromosome damage in the bone marrow of rats fed detergent actives for 90 days. Mutation Res. 56:47-50.

IMOKAWA, G. 1979. Study on skin-irritating and biological properties of monoalkyl phosphate anionic surfactants. J. Am. Oil Chem. Soc. 56(5):604-609.

IMOKAWA, G. and Y. Mishima. 1979. Cumulative effect of surfactants on cutaneous horny layers: Adsorption onto human keratin layers in vivo. Contact Dermatitis 5:357-366.

IMOKAWA, G., K. Sumura and M. Katsumi. 1975a. Study on skin roughness caused by surfactants: I. A new method in vivo for evaluation of skin roughness. J. Am. Oil Chem. Soc. 52(12):479-483.

IMOKAWA, G., K. Sumura and M. Katsumi. 1975b. Study on skin roughness caused by surfactants: II. Correlation between protein denaturation and skin roughness. J. Am. Chem. Soc. 52(12):484-489.

KWAK, Y. S., K. T. Lee and D. N. Kim. 1975. Study of drugs affecting cholesterol-induced atherosclerosis in rabbits. In: Advances in Experimental Medicine and Biology Vol. 67:149-167. (Atherosclerosis Drug Discovery, Charles E. Day, ed. Plenum Press, New York.

BIBLIOGRAPHY

MORIN, R. J., G. G. Edralin and J. M. Woo. 1974. Esterification of cholesterol by subcellular fractions from swinearteries, and inhibition by amphipathic and polyanionic compounds. Atherosclerosis 20: 27-39.

NOMURA, T., S. Kimura, S. Hata, T. Kanzaki and H. Tanaka. 1980. The synthetic surfactants AS and LAS interrupt pregnancy in mice. Life Sci. 26:49-54.

OSSIPOV, N.N., B. Yu. Zaslavsky and S. V. Rogozhin. 1978. Action of surface-active substances on biological membranes. I. Effect of chemical modification of membranes on hemolysis of erythrocytes by sodium alkyl sulfates. Colloid and Polymer Sci. 256:1105-1109.

CHAPTER 3

ALCOHOL ETHOXYLATES

(AE)

ALCOHOL ETHOXYLATES

SYNOPSIS

Alcohol ethoxylates (AE) comprise a major portion of the growing nonionic surfactant market. Their superior cleaning of man-made fibers, their tolerance to water hardness and their ability to perform well in cold water have increased their use in a large number of detergent formulations and specialty products.

Since it is often difficult to distinguish between different classes of nonionic surfactant or their degradation products analytically, it is not possible to ascertain which class of surfactant may contribute to levels of nonionics in waterways with current analytical methodology. There are presently no national criteria for levels of nonionic surfactants in waters of the United States, nor is there any indication of a need for criteria.

As a class, AE undergo extensive, rapid primary and ultimate biodegradation under both laboratory and field conditions. Neither variations in alkyl chain length nor increments in the length of the ethoxylate portion of the molecule (within the range generally utilized in detergent formulations were found to affect the rate or extent of AE degradation. The major degradative pathway of AE appears to be hydrolysis of the ether linkage and subsequent oxidation of the alkyl chain and EO portion of the molecule. Secondary and slightly branched primary AE appear to be degraded at slightly slower rates than linear primary AE.

AE are toxic to aquatic fauna, with LC_{50} values generally between 0.4 to 180 mg/L; sublethal effects have been observed at concentrations as low

as 0.26 mg/L. The toxicity of AE generally increases as the ethoxylate chain decreases in length from 20 to 2 EO units. The relationship of toxicity to alkyl chain length is not totally clear; toxicity appears to decrease for AE with alkyl chain lengths less than 10 or greater than 12 carbons. Toxicity also decreases as the surfactant is biodegraded. The effect of water hardness on AE toxicity is uncertain; some studies have found that AE are less toxic in hard water, while others report no significant effect with variations in water hardness. AE are taken up by bluegill with whole body bioconcentration factors of 445-700; the greatest accumulation occurred in the gall bladder. Bioconcentration, however, was measured using ^{14}C, and thus the effects of metabolism are unknown. The toxic effects of AE are attributed to gill damage and disruption of cell membranes.

Acute toxic concentrations of AE for aquatic microflora range between 0.05 mg/L and 1000 mg/L, depending on the species tested. Soil microorganisms have been adversely affected by AE concentrations of 1000 mg/L. The growth and development of higher plants have been inhibited by watering with AE solutions of 4-1000 mg/L, while the aquatic duckweed was affected at 1.9 mg/L.

AE exhibit a low order of acute toxicity in laboratory animals. Oral LD_{50} values range from 870 to >25,000 mg/kg, with toxicity increasing rapidly as the length of the ethoxylate chain increases up to a maximum toxic level at about 10 EO units/molecule. Two separate chronic studies with rats indicated no significant treatment-related effects resulted from ingestion of up to 1% AE in the diet for two years. Tumor incidence in treated animals were comparable to controls. There is no evidence of mutagenic or teratogenic effects resulting from AE exposure. Use of certain AE as analgesics and anesthetics

in human therapy have produced no untoward reactions. These data suggest that use of AE as a component of detergent formulations poses no threat to environmental quality or to human safety.

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).

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

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

ALCOHOL ETHOXYLATES

I. INTRODUCTION

Alcohol ethoxylates (AE) comprise a major portion of the growing nonionic surfactant market. Their superior cleaning of man-made fibers, their tolerance of water hardness, facile biodegradability and low-foaming properties have enhanced their use in newer detergent formulations. AE are also widely used in specialty products and in processes where their emulsifying and wetting properties are needed. Prepared commercially by reaction of an alcohol and ethylene oxide, AE and other nonbenzenoid ethers accounted for 501 million pounds of surface-active agents sold in the United States during 1973, of which 377 million pounds were mixed linear AE.

Total United States production of alcohol ethoxylates and other non-benzenoid ethers in 1978 amounted to 655 million pounds, of which 476 million pounds were mixed linear alcohol ethoxylates (U.S. International Trade Commission, 1979). These figures reflect the increased use over a five year period of an additional 100 million pounds of mixed linear AE.

BIBLIOGRAPHY

U. S. INTERNATIONAL TRADE COMMISSION. 1979. Synthetic Organic Chemicals. United States Production and Sales of Surface-Active Agents, 1979.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

A number of analytical methods employed in the determination of the presumptive levels of nonionic surfactants in the environment and/or in biodegradation studies were reviewed in the 1977 Report. These included: BIAS (bismuth iodide active substances), CTAS (cobalt thiocyanate active substances), picrate analysis, several less developed methods, and various chromatographic methods (thin-layer, paper and gas). These methods vary considerably in their specificity for AE. Indeed, due to the similarity of the responses of intact surfactant and degradation products, it is often difficult to distinguish between them analytically. A prepurification step (e.g. sublation) is frequently incorporated into the analytical procedure, particularly for analysis of environmental samples, in an attempt to separate intact surfactant from interferring substances. Polyethylene glycols, the major degradation intermediates of AE can also be separated from the parent compound by thin-layer chromatography.

The use of Amberlite XAD-4 resin as an extractant for low levels of AE and PEG (polyethylene glycols) was evaluated and used in studies of their fate in various water systems (Jones and Nickless, 1978 a,b). Analyses were performed by (1) separation using the barium chloride-phosphomolybdic acid method followed by measurement of absorbance at 310 nm, and (2) thin-layer chromatography and use of the Dragendorff reagent for detection of AE and PEG. Concentrations of a secondary AE as low as 10 ppb could be determined with large volumes (liters) of water being analyzed, and interfering substances were removed by subsequent purification procedures. Application of this method to a sewage plant influent and effluent (1978b) showed a great removal of AE (by ~90%). PEG was not signifi-

cantly removed. A further decrease in the concentration of AE (~100 times lower) was observed in the stream below the plant, due either to dilution or further biodegradation.

The potassium picrate method (PPAS) was used to analyze nonionic surfactants after extraction of standard and seawater samples with 1,2-dichloroethane (Favretto et al., 1978). The use of $C_{12}AE_6$ as a standard was recommended. Interference from anionic and cationic surfactants was eliminated primarily by the extraction procedure. Seawater samples from the Adriatic (Trieste Harbor) showed PPAS values of 0.039-0.187 ppm.

Recent work by Sones et al. (1979), using gas chromatography for determination of alcohol and ether sulfates, as well as alkyl carbon distribution, was directed toward characterizing detergent formulations.

B. Water Quality Standards

There are presently no national criteria for allowable nonionic surfactant concentrations in waters of the United States.

C. Nonionic Surfactants in Natural Water Bodies

Concentrations of nonionic surfactants, including AE, in the environment were discussed in the 1977 Report. Since in most instances, current analytical methods do not distinguish between specific classes of nonionics and/or their degradation products, it is not yet possible to ascertain which class of surfactant contributed to levels of nonionics in waterways. Concentrations reported are almost al-

ways lower than anionics at the same sampling point. With respect to polyethylene glycol concentrations, a single study noted increases in several English rivers over an eight-year interval.

Alcohol ethoxylates are not presently being monitored, as such, in the United States.

BIBLIOGRAPHY

FAVRETTO, L., B. Stancher and F. Tunis. 1978. Extraction and determination of polyoxyethylene alkyl ether nonionic surfactants in water at trace levels. Analyst 103:955-962.

JONES, P. and G. Nickless. 1978a. Characterization of nonionic detergents of the polyethoxylated type from water systems. I. Evaluation of amberlite XAD-4 resin as an extractant for polyethoxylated material. J. Chromatog. 156:87-97.

JONES, P. and G. Nickless. 1978b. Characterization of nonionic detergents of the polyethoxylated type from water systems. II. Isolation and examination of polyethoxylated material before and after passage through a sewage plant. J. Chromatog. 156:99-110.

SONES, E.L., J. L. Hoyt and A. J. Sooter. 1979. The determination of alcohol and ether sulfates and their respective alkyl carbon distributions in detergent formulations by gas chromatography. J. Am. Oil. Chem. Soc. 56(7):689-700.

III. BIODEGRADATION

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

A. Laboratory Investigations

Primary biodegradation of AE in seawater and fresh water at two temperatures (20-23°C and 3-4°C) was studied by Schöberl and Mann (1976). They compared three AE's containing a primary branched-chain C_{11-14} alcohol with 7, 9 or 11 EO units. Analyses were done with Wickbold's BIAS method. Degradation of the AE's was extensive in fresh water at the higher temperature but was slower at the lower temperature as expected. There was less difference between rates in seawater at the two temperatures. Under most conditions, the rates in seawater were higher than in freshwater. Increased length of the EO chain resulted in decreased rates of degradation, particularly at low temperature in fresh water.

In a similar study, the extent of primary biodegradation (measured as CTAS response) of n,pri-$C_{14-15}AE_7$ in estuarine and ocean waters as compared to river water was studied. Greater than 85% primary biodegradation occurred in both estuarine and river waters in 11 days or less. Degradation was slower in ocean water, requiring 21-35 days to achieve 85% primary biodegradation (Procter and Gamble Company, unpublished data).

There has been an extensive laboratory investigation of the degradation of n-pri-$C_{14-15}AE_7$ (Dobanol 45-7^{TM}) in an activated-sludge/mineral salts system

(Cook, 1979). From an initial concentration of 500 mg/L, with a TOC of ∿325 mg/L, TOC was reduced 69% in three days and to the background (sludge) level in 15 days. Intact surfactant was no longer detectable by three days (TLC analyses) and a polyglycol-like compound appeared; it disappeared in 6-10 days. There was also a transient appearance of acid-extractable materials in the early stages of degradation. These results were obtained by several methods and showed similar patterns or were consistent with each other.

A brief report on degradation of high concentrations of sec-$C_{12-14}AE_9$ in a continuous flow-activated sludge system showed it to be rapidly degraded (Kurata and Koshida, 1976). The criteria applied included CTAS, TOC and COD. After a few months, the low values of all three measures indicated nearly complete removal of the secondary AE.

A series of papers on the ultimate biodegradability of AE has emanated from the Shell laboratories (Kravetz et al., 1978; Kravetz et al., 1979; and Scharer et al., 1979). These were primarily shake-flask studies, using either raw sewage or secondary wastewater effluent as inocula, with determination of degradation after a period of acclimation. Analyses were run for CTAS, TOC, DOC, CO_2 production and H8r cleavage followed by GLC for analysis of alkyl and polyoxyethylene (POE) residues. BOD analyses were also run with unacclimated inocula. One radioactive-tracer labelled surfactant was synthesized with ^{14}C in the EO chain and ^{3}H in the alkyl chain. One major difference between this study and previous ones (which showed only partial mineralization of the EO chains) was the use of an atmosphere containing 70% oxygen "to provide sufficient oxygen for complete surfactant oxidation to CO_2". Thus, the results are most pertinent to demonstrating "biodegradability," rather than as a demonstration that biodegradation (in actual situations) would occur at the rates observed or to the extent observed in the time-frame "of 29 days or longer where required."

AE made from different alcohols with differing degrees of branching, combined with EO chains of different lengths were tested. The alkyl chain was degraded more rapidly than the EO chain, with little dependence on degree of branching, but the primary branched chain ethoxylates were degraded more rapidly than a "100% linear" secondary alcohol ethoxylate. The EO chains of the former were extensively mineralized (80-95+%) as determined from H8r cleavage, DOC reduction and CO_2 release (chemical and radioactive).

In the second series with a similar test system and experimental conditions (Kravetz et al., 1979), EO chain lengths of 7, 18, 30 and 100 units were used on a common alcohol (n-pri-C_{12-15} alcohol). Results showed only slight decreases in ultimate biodegradation up to 30 units (all up to 90% or more in 21 days). The AE containing 100 EO units per mole showed only 20% ultimate biodegradability, and that may be attributed to the relatively few short POE chains in this material. The tracer results are best interpreted as indicating the primary attack was "near" the α-carbon of the alkyl group. It was suggested that results of other studies indicating an initial attack on the ω-end were due to the presence of other microbes in the inocula used. The initial α-carbon oxidation might produce an ester, which would be split by esterases, releasing the POE chain as PEG (polyethylene glycol-type intermediates).

In developing a kinetic model for estimating biodegradation potential of xenobiotics (Larson, 1979), some data were developed on ultimate AE-biodegradation. Test conditions involved an acclimated inoculum developed in a semi-continuous activated sludge system (in which 100% removal by soluble organic carbon analysis of $C_{14-15}AE_7$ in 5 days has been determined), and the use of the test substance as sole carbon source in a BOD medium. The rate and extent of CO_2 evolution for two AE's ($C_{13-14}AE_7$ and $C_{11-12}AE_{6.5}$) were high, 95.0-99.8, and 98.7-86.5%, respectively, at 10 and 20 mg/L. This indicates a high degree of

mineralization. A detailed study of the kinetics of AE biodegradation in Ohio River water (Larson and Perry, 1980) showed rapid biodegradation of AE including the EO chain. Methods included the use of the electrolytic respirometer to measure oxygen consumption, and ^{14}C-labelling in the EO chain. The commercial, unlabelled linear AE was $C_{12.5}AE_{6.5}$ and the labelled AE, $C_{12}AE_9$. The rate of linear AE degradation, measured by oxygen consumption, showed evidence of saturation at AE concentrations of 5 mg/L or greater. However, degradation of the ethoxylate chain of $C_{12}AE_9$ was rapid and directly proportional to concentration at levels normally found in surface waters (10 ppb). In experiments where CO_2 evolution from labelled (in the EO portion) and unlabelled AE were measured, the extent of degradation averaged 88-97% of theoretical across a range of test conditions and concentrations. This again indicates a rapid and high degree of degradation and mineralization of the EO chain, as well as the alkyl moiety. In addition, the rate of degradation was five-fold higher at 10 ppb with labelled AE than that found in screening studies with 20 ppm, suggesting that screening results may underestimate the true environmental rate.

The microbial degradation of PEG from surfactants has been studied separately by several groups. A detailed review (Cox, 1978), presented results and problems to date, including the diversity of microbial types which have various degrees of activity against compounds of differing chain lengths.

Three species of bacteria illustrating this latter point were studied by Jenkins et al. (1980). Cook (1978) presented a method for the isolation of such bacteria, and Haines and Alexander (1975) performed detailed biochemical studies with Pseudomonas aeruginosa.

B. Field Studies

The available data on the performances of AE in the field were quite limited for the 1977 Report, but indicated extensive degradation of AE occurred in the field.

A municipal sewage plant (activated sludge) was used to study the treatability of n-pri-$C_{14-15}AE_7$ (Sykes et al., 1979). Surfactant was dosed into the plant inflow at 5 or 10 mg/L, and reduction of AE concentration in the primary and secondary effluents was measured by CTAS. Removal in the secondary process averaged 80% before and after dosing (recovery) and 90% during the dosing periods. Total plant reductions were only slightly higher. These results paralleled the removal of total BOD and MBAS, indicating little, if any, effect on the performance of the plant. Results were remarkably consistent through summer and winter periods.

Similar results were observed in a German activated sludge plant (Wagner, 1978) in which influent and effluent concentrations of nonionic surfactants of the AE type were followed by BIAS for a year. No dosing was done, but a special heavy duty detergent, apparently containing AE, was used in the area during the test period. The mean BOD_5 reduction was 96% with 91% for BIAS (nonionics) and 94% for MBAS (anionics).

A similar study, conducted in England during the winter, also showed high removal of AE in a trickling filter plant (Abram et al., 1977). Measurements by the Wickbold method (CTAS), showed 96-98% loss of Dobanol 45-7^{TM} ($C_{14-15}AE_7$) and Dobanol 45-11^{TM} ($C_{14-15}AE_{11}$), at influent concentrations of 10 and 25 mg/L. The effluents were non-toxic to rainbow trout.

The results of Jones and Nickless (1978b) have been discussed in connection with their methodological development.

C. Effect of Chemical Structures

Neither variations in the alkyl chain length nor increments in the length of the ethoxylate portion of the molecule (within the range generally utilized in detergent formulations) was found to affect the rate and extent of AE degradation. However, increments beyond 20-30 EO units definitely retard the degradation of the molecule.

D. Metabolic Pathways of Biodegradation

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 glycol-like materials and ultimately, CO_2 and H_2O.

BIBLIOGRAPHY

ABRAM, F. S. H., V. M. Brown, H. A. Painter and A. H. Turner. 1977. The biodegradability of two primary alcohol ethoxylate nonionic surfactants under practical conditions, and the toxicity of the biodegradation products to rainbow trout. Paper presented at the IVth Yugoslav Symposium on Surface Active Substances, Dubrovnik, October 17-21, 1977.

COOK, K. A. 1978. A rapid method for the detection of nonionic surfactant-degrading micro-organisms. J. of Appl. Bacteriol. 44:299-303.

COOK, K. A. 1979. Degradation of the nonionic surfactant Dobanol 45-7 by activated sludge. Water Res. 13:259-266.

COX, D. P. 1978. "The biodegradation of polyethylene glycols" in: Advances in Applied Microbiology. 23:173-193.

HAINES, J. R. and M. Alexander. 1975. Microbial degradation of polyethylene glycols. Appl. Microbiol. 29(5):621-625.

JENKINS, L. D. L., K. A. Cook and R. B. Cain. 1980. Microbial degradation of polyethylene glycols. J. Appl. Bacteriol. 48 (In Press).

JONES, P. and G. Nickless. 1978b. Characterization of non-ionic detergents of the polyethoxylated type from water systems. II. Isolation and examination of polyethoxylated material before and after passage through a sewage plant. J. Chromatog. 156:99-110.

KRAVETZ, L., H. Chung, J. D. Rapean, K. F. Guin and W. T. Shebs. 1978. Ultimate biodegradability of detergent range alcohol ethoxylates. Water Res. 13 (In Press).

KRAVETZ, L., H. Chung, K. F. Guin and W. T. Shebs. 1979. Ultimate biodegradation of alcohol ethoxylates. 2. Surfactant concentration and polyoxyethelene chain effects. Water Res. 14 (In Press).

KURATA, N. and K. Koshida. 1976. Biodegradability of sec-alcohol ethoxylate-experimental results in continuous-flow activated sludge tests. Yukagaku 25(8): 499-500.

LARSON, R. J. 1979. Estimation of biodegradation potential of xenobiotic organic chemicals. Appl. Environ. Microbiol. 38(6):1153-1161.

LARSON, R. J. and R. L. Perry. 1980. Use of the electrolytic respirometer to measure biodegradation in natural waters. Water Res. (in press).

Procter and Gamble Company, unpublished data.

SCHARER, D. H., L. Kravetz and J. B. Carr. 1979. Biodegradation of nonionic surfactants. Tappi 62(10):75-78.

SCHÖBERL, P. and H. Mann. 1976. Temperatur-Eisfluß auf den biologischen Abbau nicht-ionischer Tenside in See-und Süßwasser. [The influence of temperature on the degradation of nonionic tensides in sea- and freshwater]. Arch. Fisch. Wiss. 27(2):149-158.

SYKES, R. M., A. J. Rubin, S. A. Rath and M. C. Chang. 1979. Treatability of a nonionic surfactant by activated sludge. J. Water Pollut. Contr. Fed. 51(1): 71-77.

WAGNER, R. 1978. Über das verhalten von MBAS and BIAS auf einer kommunalen klaranlage. [Behavior of MBAS and BIAS in a municipal purification plant]. Gas-Wasserfach, Wasser-Abwasser 119(5):235-242 (English translation).

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

A general consideration of the environmental safety aspects of AE was presented in the 1977 Report. Studies conducted since that time are discussed in this section, with reference to earlier work. The methodology used in the present report is described in Chapter 1 of the 1977 Report.

1. AE Structure-Activity Relationship

An increase in aquatic toxicity of AE is observed with decreasing ethoxylate chain length, ranging from 2 to 20EO units. Also noted is an association between decreasing alkyl chain length and decreasing toxicity as ethoxylate chain length remains constant. One study, however, found that the most toxic of the AE_3 compounds tested had an alkyl chain of ten carbons, and that toxicity decreased when the alkyl chain was shorter than C_{10} or longer than C_{12}.

The degradation of AE reduces their toxicity to fish. Most of the mortalities caused by AE in static toxicity tests occur within 24 hours; the reduced toxicity with time is attributed to degradation of the surfactants. One exception to this was reported for Plurafac RA 43, a modified AE; toxicity to an annelid decreased only slightly during 28 days of exposure. Brine shrimp were found to be relatively tolerant of ethylene glycols, intermediate degradation products of AE.

These findings have been confirmed by more recent information on AE toxicity. The trend of increasing toxicity with decreasing ethoxylate chain length is evident in the data of Shell Chemical Co., (unpublished

reports) and Kurata et al. (1977), as presented in Table 3-A. Maki and Bishop (1979) explicitly demonstrated this relationship in a comparison of the toxicities of $C_{14}AE$ samples with ethoxylate chains ranging from one to nine EO units in length. In a consistent pattern, LC_{50} values increased by a factor of 12, from 0.83 mg/L for AE_1 to 10.1 mg/L for AE_9.

The effect of biodegradation on toxicity of AE has been examined by several investigators (Maki et al, 1979; Abram et al., 1977; Reiff, 1976; Kurata et al., 1977). Maki and coworkers (1979) examined the effects of sewage treatment on the toxicity of $C_{14.5}AE_7$ to fathead minnow. In a series of tests, these investigators determined the acute toxicity of this surfactant in various dilution waters, in sewage effluent and in the effluent subsequent to spiking of the influent with the surfactant. Degradation tests were also conducted to determine the decreased toxicity in both effluent and river water with biodegradation. In addition, a stream survey was conducted using various indices of the community structure to determine the impacts of the sewage treatment plant as well as surfactant spiking. The results of these in-depth investigations were extensive, but can be summarized as follows:

- Toxicity varies with the dilution media. A medium high in suspended solids and organic matter, and thus bacterial populations, results in reduced acute toxicity. For example, the 96 hr LC_{50} values for fathead minnow increased from 1.2 to 1.38 to 2.48 mg/L for $C_{14.5}AE_7$ in tap water (virtually no SS or COD measurable), stream water (3-20 mg/L SS; 5 mg/L COD and secondary effluent (50 mg/L SS; 33-124 mg/L CDD), respectively.

- An overall 90% removal of surfactant in wastewater treatment was found. No differences in toxicity to fathead minnows were observed between effluent collected during the background period and during the spiking of the influent with 10 mg/L $C_{14.5}AE_7$. The authors therefore concluded that the acute toxicity of 10 mg/L of the surfactant was eliminated during passage through the sewage treatment plant.

- In biodegradation tests, the surfactant was reduced to non-toxic levels in both stream water and effluent within 24 hours when the initial surfactant concentration was 3 mg/L or less. At a concentration of 10 mg/L, toxicity associated with the surfactant was observed for 5 days in stream water and for 2-3 days in secondary effluent.

- The biodegradation products of the surfactant are apparently non-toxic to fathead minnow. In a die-away test conducted with gently aerated, 100% secondary effluent spiked with 30 mg/L $C_{14,5}AE_7$, the CTAS level was 0.57 mg/L at 8 days. None of the fish added at that time died or exhibited abnormal behavior.

- Significant differences were found in community structure among upstream, downstream, and recovery zone locations of the receiving stream, but no effects attributable to the surfactant dosing were observed within each location.

Abram et al. (1977) conducted a similar study, adding $C_{14.5}AE_7$ and $C_{14.5}AE_{11}$ to domestic sewage influent at both 10 and 25 mg/L. Removal by trickling filter

treatment was 96-98% as measured by CTAS response, and the effluent showed no toxicity to rainbow trout.

The data of Reiff (1976) and Kurata et al. (1977) also indicate that the biodegradation products of AE surfactants are much less toxic than the parent compounds. The 48 hr LC_{50} values for goldfish for $C_{12-14}AE_9$ and $C_{12-15}AE_9$ increased by factors of 18 and 65, respectively, after four days in solution (Kurata et al. 1977). Reiff (1976) reported that at a high initial loading of 20 mg/L, an unspecified AE compound lost its toxicity to rainbow trout in 10-14 days.

2. Acute Toxicity to Fish

The 1977 Report cited LC_{50} values ranging from 0.7 mg/L $C_{16-18}AE_{14}$ for the rainbow trout to 150 mg/L $C_{12}AE_{20}$ for the golden orfe.

Table 3-A summarizes recently published data on the acute toxicity of AE to finfish. The LC_{50} values range from 0.4 mg/L TAE_3 for the brown trout (Salmo trutta), to 12 mg/L $C_{12-14}AE_{12}$ for the goldfish (Carassius auratus).

3. Acute Toxicity to Aquatic Invertebrates

Invertebrates show a much broader range of sensitivity to various AE surfactants, with LC_{50} values varying between 1.1 mg/L $C_{12-18}AE_{14}$ for Daphnia, to more than 180 mg/L $C_{8-10}AE_{15}$ for mosquito larvae (Culex pipiens). One study found that surfactant hydrophilicity was inversely related to toxicity in planaria.

TABLE 3-A. ACUTE TOXICITY OF ALCOHOL ETHOXYLATES TO FISH

Species	Surfactant (Trade Name)	LC_{50} centration (mg/L±95%CL)	Test Duration	Reference
Rainbow trout, fingerling (*Salmo gairdneri*)	$C_{9-10}AE_{2.5}$ (Dobanol 91-2.5™)	5.7	48 and 96 hr	Shell Chemical Co., unpublished data
Rainbow trout, fingerling (*Salmo gairdneri*)	$C_{9-11}AE_5$ (Dobanol 91-5™)	8.9	48 and 96 hr	Shell Chemical Co., unpublished data
Rainbow trout, fingerling (*Salmo gairdneri*)	$C_{12-15}AE_3$ (Dobanol 25-3™)	1.3-1.7	48 and 96 hr	Shell Chemical Co., unpublished data
Rainbow trout, fingerling (*Salmo gairdneri*)	$C_{12-15}AE_3$ (Dobanol 235-3™)	1.0	48 and 96 hr	Shell Chemical Co., unpublished data
Rainbow trout, fingerling (*Salmo gairdneri*)	$C_{14-15}AE_{18}$ (Dobanol 45-18™)	5.0-6.3	48 and 96 hr	Shell Chemical Co., unpublished data
Bluegill sunfish, juvenile (*Lepomis macrochirus*)	$C_{14.5}AE_7$	0.7 (0.5-0.9)	96 hr	Bishop and Perry (1979)
Bluegill sunfish, juvenile (*Lepomis macrochirus*)	$C_{14.5}AE_7$ (Neodol 45-7™)	0.66 (0.51-0.86)	96 hr	Lewis and Perry (1979)
Fathead minnow (*Pimephales promelas*)	$C_{14.5}AE_7$ (Neodol 45-7™)	2.48 (2.09-2.92) 1.38 (1.29-1.50) 1.20 (1.13-1.26)	96 hr, 350mg/L hardness 96 hr, 250mg/L hardness 96 hr, 100mg/L hardness	Maki *et al.* (1979)
Brown trout (*Salmo trutta*)	TAE_3 $C_{12-14}AE_{10-11}$ $C_{12-14}AE_8$	0.4 0.18-1.8 0.8	96 hr 96 hr 96 hr	Reiff *et al.* (1979)
Harlequin fish (*Rasbora heteromorpha*)	TAE_3 $C_{12-14}AE_{10-11}$ $C_{12-14}AE_8$	0.7 1.6-2.8 1.2	96 hr 96 hr 48 hr	Reiff *et al.* (1979).

TABLE 3-A. ACUTE TOXICITY OF ALCOHOL ETHOXYLATES TO FISH (continued)

Species	Surfactant (Trade Name)	LC_{50} Concentration (mg/L±95%CL)	Test Duration	Reference
Golden orfe (Idus idus melanotus)	TAE_3	2.3-2.5	96 hr	Reiff et al. (1979)
	$C_{12-14}AE_{10-11}$	4.1-4.5	96 hr	
	$C_{12-14}AE_8$	1.8-2.7	96 hr	
Golden orfe (Leuciscus idus melanotus)	AE_{10}	8.3	48 hr	Fischer and Gode (1978)
Goldfish, juvenile (Carassius auratus)	AE_{10}	7.1	48 hr	Fischer and Gode (1978)
Goldfish, juvenile (Carassius auratus)	$C_{12-14}AE_7$ (SEC-7™)	3.3	48 hr	Kurata et al. (1977)
Goldfish, juvenile (Carassius auratus)	$C_{12-14}AE_9$ (SEC-9™)	5.1	48 hr	
Goldfish, juvenile (Carassius auratus)	$C_{12-14}AE_{12}$ (SEC-12™)	12.0	48 hr	
Goldfish, juvenile (Carassius auratus)	$C_{12-15}AE_9$ (LA-9™)	1.9	48 hr	
Goldfish, juvenile (Carassius auratus)	$C_{12-15}AE_9$ (OXO-9™)	1.4	48 hr	
Minnow (Macrones vittatus)	Swanic 6L™	0.5 (0.42-0.56)	96 hr	Verma et al. (1978)
Mummichog[1] (Fundulus heteroclitus)	$C_{14.5}AE$	1.45	96 hr	Maki (1979b)

[1]Marine species.

The range of LC_{50}'s found in recent studies is from 0.29 mg/L $C_{14.5}AE_7$ for Daphnia magna, to 30.9 mg/L of the same compound for the blue crab (Callinectes sapidus). The LC_{50} for D. magna (see Table 3-B) is the lowest toxic level reported to date, suggesting that the daphnid is the most sensitive of the species tested. Bode et al. (1978) reported "lethality" in 24 hr toxicity tests with Hydra attenuata exposed to 2×10^{-2} mM concentrations of a series of AE with varying alkyl (10-18 carbons) and ethoxy (5-18EO) chains. Two surfactants, $C_{10}AE_{6.7}$ and $C_{18}AE_7$, were somewhat less toxic, causing mortality at 2×10^{-1} mM.

4. Acute Toxicity to Algae

In the 1977 Report, AE surfactants were reported to exert adverse effects on algae at concentrations ranging from 5 mg/L to greater than 1000 mg/L. The most common effect was inhibition of growth and cell division.

Kutt and Martin (1974) exposed cultures of a red tide dinoflagellate (Gymnodinium breve) to various concentrations of coconut alcohol-derived AE for periods of up to 10 days. A concentration of 0.05 mg/L produced 33 % mortality on the second day after inoculation; recovery was not described.

In a recent study, Payne and Hall (1979) argue that mortality values for a percentage of the test organisms (e.g. LC_{50}) are not useful because of the rapid growth rate of algal cultures upon removal to clean water.

TABLE 3-B. ACUTE TOXICITY OF ALCOHOL ETHOXYLATES TO AQUATIC INVERTEBRATES

Species	Surfactant (Trade Name)	LC_{50} Concentration (mg/L±95%CL)	Test Duration	Reference
Daphnia magna	$C_{14}AE_1$	0.83 (0.73-0.91)	48 hr	Maki and Bishop (1979)
	$C_{14}AE_9$	10.1 (9.46-10.66)	48 hr	
Daphnia magna	$C_{12.5}AE_{6.5}$	1.14 (0.96-1.37)	96 hr	Maki (1979c)
	$C_{14.5}AE_7$	0.43 (0.37-0.51)	96 hr	
Daphnia magna	$C_{14.5}AE$	0.7 (0.5-0.9)	48 hr	Bishop and Perry (1979)
Daphnia magna	$C_{14.5}AE_7$ (Neodol 45-7™)	0.34 (0.26-0.39)	48 hr, 35mg/L hardness	Lewis and Perry (1979)
		0.29 (0.22-0.31)	48 hr,181mg/L hardness	
		0.40 (0.3-0.5)	48 hr,340mg/L hardness	
Daphnia magna	$C_{14.5}AE_7$ (Neodol 45-7™)	0.36 (0.17-0.45)	48 hr, 25mg/L hardness	Procter and Gamble Company, unpublished data
		0.34 (0.26-0.43)	48 hr,125mg/L hardness	
		0.40 (0.35-0.46)	48 hr,225mg/L hardness	
		0.38 (0.29-0.47)	48 hr,325mg/L hardness	
Mussel[1] (Mytilus edulis)	TAE_{10}	50	96 hr	Swedmark et al. (1976)
Pink shrimp, juvenile[1] (Penaeus duroarum)	$C_{14.5}AE_7$	0.78 (0.34-1.8)	96 hr	Maki (1979b)
Blue crab[1] (Callinectes sapidus)	$C_{14.5}AE_7$	30.9 (26.2-36.5)	96 hr	Maki (1979b)

[1]Marine species.

In their own toxicity studies, the authors confirmed the report of Hall (1973) that cultures of the blue-green alga (Microcystis aeruginosa) survive $C_{12-14}AE_6$ concentrations of 1000 mg/L with no adverse effects. $C_{12-14}AE_6$ was algistatic (causing no change in cell number during exposure, but permitting regrowth in fresh media) to populations of the diatom, Navicula seminulum, and the green alga, Selenastrum capricornutum, at concentrations of 5-10 mg/L and 50 mg/L, respectively; $C_{12-14}AE_6$ was algicidal at 100 mg/L and 1000 mg/L, respectively. The authors concluded that a 50% reduction in standing crop for algal species does not represent a good measure of toxicity, especially in the logarithmic phase of growth.

5. Sublethal Toxicity

A variety of sublethal effects was discussed in the 1977 Report, most of which occurred at AE concentrations of 0.5-2 mg/L. In rainbow trout and cod, progressive sublethal effects included erratic and exaggerated swimming, loss of balance, and complete passivity. In invertebrates, responses to sublethal concentrations of AE were characterized mainly by reductions in growth rate and loss of swimming ability in larvae.

The only recent information on sublethal effects of AE in finfish is from a study by Maki (1979a) on bluegill sunfish (Lepomis macrochirus). In 48 hr tests, concentrations of 0.26-1.2 mg/L, $C_{12.5}AE$ suppressed ventilation rates by 30-50% compared to the controls as concentrations were increased from 0 mg/L to 1.2 mg/L. $C_{14.5}AE$ also decreased respiratory

activity but less strongly than $C_{12.5}AE$. The authors concluded that the no effect concentrations for ventilation were 0.26-0.54 mg/L for $C_{14.5}AE$ and greater than 1.56 mg/L for $C_{12.5}AE$. The NOEC for $C_{12.5}AE$ agreed with the chronic MATC for fathead minnow, while the NOEC (>1.56 mg/L) did not agree with the chronic MATC (<0.21>0.28 mg/L) for $C_{14.5}AE$, suggesting that this method may not be a good predictive tool for some nonionic surfactants.

In a test with barnacle nauplii (Elinius modestus), Wright (1976) determined a 30-minute EC_{50} value for immobilization of 580 mg/L $C_{10}AE_{20}$. Recovery of 50% of the affected specimens occurred within 20 minutes after removal to clean water; all organisms recovered within 48 hours. Maki (1979b) exposed larval Eastern oysters (Crassostrea virginica) to various concentrations of $C_{14.5}AE$; the resultant 48 hr EC_{50} value for inhibition of larval development was 0.11 mg/L.

In summary, sublethal effects in aquatic fauna have been observed at concentrations of 0.11 mg/L to more than 2 mg/L with various AE surfactants. The lowest value was reported for the larvae of the Eastern oyster. The lowest AE concentration producing a distinct sublethal effect in fish was 0.26 mg/L in the bluegill.

6. Chronic Toxicity

No chronic toxicity studies with AE were included in the 1977 Report.

Granmo and Jorgensen (1975) performed chronic toxicity tests on the mussel (Mytilus edulis) to determine the effects of TAE_{10} on spawning and fertilization. Spawning ability was not affected by long-term (5 months) exposure to TAE_{10} concentrations as high as 1.5 mg/L. However, fertilization of the gametes of the exposed mussels decreased with increasing TAE_{10} concentration, and was almost completely inhibited at 2 mg/L. A no-effect concentration was not established because development was inhibited at the lowest concentration tested (0.1 mg/L).

No observed effect concentrations for fathead minnows (Pimephales promelas) were reported by Maki (1979c). From a chronic toxicity test with special attention to egg production and spawning rate, the author derived a NOEC of 0.32 mg/L for $C_{12.5}AE$, the highest concentration tested. An embryo-larval test (30-day continuous exposure to embryos and newly hatched fry) was conducted with $C_{14.5}AE$. A NOEC of 0.18 mg/L was indicated for fathead minnow. From 21-day exposure studies, a NOEC of 0.24 mg/L was established for both surfactants for Daphnia; the 96 hr LC_{50} values were 1.44 and 0.43 mg/L, respectively.

Limiting concentrations of 1 mg/L and 0.5 mg/L $C_{13-15}AE_{10}$, i.e., the highest concentrations at which no mortality occurred, were determined for the flatworms Dugesia gonocephala and Notoplana humilis, respectively, by Patzner and Adam (1979) in 30-day tests. Regeneration of the worms, however, was inhibited at concentrations of half these values.

7. Effects of Environmental Variables on AE Toxicity

The 1977 Report discussed the influence of water characteristics on the aquatic toxicity of AE. Rainbow trout and goldfish were found less susceptible to $C_{12}AE_{3.25}$ in hard water than in soft water, even when acclimated in soft water.

Recent studies have examined the effects of several environmental variables on the toxicity of AE surfactants. Two reports (Maki and Bishop, 1979; Maki et al., 1979) suggest a slight decrease in AE toxicity as water hardness increases. Maki and Bishop (1979) found the 48-hour LC_{50} for $C_{14.5}AE_7$ for Daphnia increased from 0.36 mg/L at 50 mg/L $CaCO_3$ hardness, to 0.90 mg/L at 350 mg/L hardness. However, no such trend was observed in other tests for the same AE surfactant and species over a range of 25-325 mg/L hardness for the test waters when the Daphnia were cultured in hard water (350 mg/L) (Procter and Gamble Company, unpublished data). Maki and Bishop (1979) found that the hardness of the culture water in which D. magna were raised had no effect on sensitivity to $C_{14.5}AE_7$ in tests with soft water and only minimal effects in hard test waters. Overall, water hardness appears to exert no marked effect on the toxicity of AE to D. magna.

Lewis and Perry (1979) also tested the toxicity of $C_{14.5}AE_7$ to daphnids for synergism or antagonism with other (anionic or cationic) surfactants. Their results indicate that a mixture of AE and a cationic surfactant (C_{12-14} monomethyldihydroxyethyl ammonium chloride) exerted a more-than-additive toxic effect. However, additive and less than additive results

were indicated in other cases studied. Mixtures of AE with LAS or all three types of surfactants together exerted no more than an additive toxicity to Daphnia. Binary and ternary mixtures were also less than additive or additive to bluegill.

Maki and Bishop (1979) conducted parallel tests with Daphnia magna to determine the effect of suspended solids on AE toxicity. In 48-hour tests, the presence of 50 mg/L kaolinite clay did not significantly alter the toxicity of $C_{10}AE_3$, $C_{14}AE_3$, or $C_{18}AE_3$.

8. Bioaccumulation of AE

No information was available for the 1977 Report on uptake and bioaccumulation of AE by aquatic organisms.

Two studies have since examined the bioconcentration of AE by bluegill sunfish (Lepomis macrochirus). In 28-day tests, Bishop and Maki (1979) exposed juvenile bluegill to ^{14}C-labelled $C_{14}AE_7$ in concentrations of 0.02 mg/L or 0.20 mg/L. Uptake of AE was rapid, and whole body bioconcentration factors reached a plateau of 700 at both concentrations within 120 hours. Approximately 85% of the accumulated ^{14}C activity was eliminated after 432 hours of exposure in clean water.

For ^{14}C-labelled $C_{14}AE_7$ (0.016 mg/L), the whole body bioconcentration factor was somewhat greater for small (0.6 g) fish at 613, than for the

larger (4 g) fish, at 445, but the authors concluded that they were not significantly different. The gall bladder accumulated the most AE of any organ with a BCF of 35,056, and muscle tissues the least with a BCF of 156 (Procter and Gamble Company, unpublished data). It should be noted that these BCF are based on ^{14}C-activity and assume that all AE are intact. Thus, these values represent maximum BCF.

9. Comparison of Toxicity Data with Environmental Concentrations of AE

The toxicity of AE surfactants is difficult to assess in terms of observed environmental concentration in that monitoring data are limited. Maki (1979b), however, has attempted to assess the hazard of $C_{14.5}AE$ to estuarine aquatic organisms through the use of modeling techniques. Surfactant concentrations were estimated using assumed input from sewage treatment plants and appropriate dilution for twenty different estuarine locations; no degradation was allowed for. In that no monitoring data on nonionics were available, the resultant concentrations were compared to the limited monitoring data available on anionic surfactants, measured as MBAS. In the two locations where comparisons could be made for anionic surfactants, the agreement between Maki's model and monitoring data was good. Maki, therefore assumed that agreement between his model and monitoring data for nonionic surfactants would also be good. Using Maki's model, the estimated maximum AE concentrations for the estuaries ranged from 0.2 µg/L in Penobscot Bay, Maine to 19.8 µg/L in the Hudson River.

The geometric mean of AE concentrations for all estuaries was 3.2 μg/L. Results of acute toxicity tests and Maki's calculated safety factors are shown in Table 3-C. The results indicate that oyster larvae are the most sensitive, with a chronic safety factor of 9. It should be pointed out, however, that the calculated AE concentrations were based on a conservative or high estimate of inflow from sewage treatment plants, and that no degradation was allowed for. Thus, the calculated safety factors are also probably conservative.

B. Effects of AE on Soil Microorganisms

The 1977 Report contained results of several experiments on the effects of alcohol ethoxylates on soil microorganisms. The nitrification process in two soil types was slightly inhibited by a 0.1% soil concentration of $C_{12}AE$. In soil watered with a 1000 mg/L solution of $C_{11-15}AE_9$, microfungi biomass was reduced by 16% compared to controls. No reduction in number of species was observed. At 10 mg/L of this compound, the growth of several species was accelerated, indicating a possible use of the AE as a carbon source. Other effects, such as reduced sporulation, pigment diffusion, and reduced exudate production were observed at concentrations less than 1000 mg/L.

No further information on this aspect of AE toxicity has been found.

TABLE 3-C. TOXICITY OF C_{14-15}AE TO AQUATIC ORGANISMS AND CALCULATED SAFETY FACTORS

Species	Effect	EC_{50} (mg/L)	NOEC (mg/L)	Mean Acute Safety Factor*	Chronic Safety Factor**
Oyster larvae	48 hr larval devel.	0.11	0.06	44.7	9
Pink Shrimp	96 hr LC_{50}	0.78	0.56	418	84
Blue Crab	96 hr LC_{50}	30.9	10.0	7461	1492
Mummichog	96 hr LC_{50}	1.45	1.0	746	149

(Maki, 1979b)

* Calculated from the acute NOEC divided by the estimated maximum estuarine concentration.

** The product of the acute safety factor and an application factor (i.e., chronic NOEC/96 hr LC_{50}) of 0.2.

C. Effects of AE on Higher Plants

AE surfactants were found to have adverse effects on plant growth and development in solutions of 4-1000 mg/L. It was hypothesized that the site of surfactant action is the cytomembrane.

Bishop and Perry (1979) exposed the aquatic duckweed (Lemna minor) to flow-through exposures of $C_{14.5}AE$. The calculated 7-day EC_{50} was 21 mg/L on the basis of frond count (a measure of growth performance), and 1.9 mg/L on the basis of root length.

An extensive study on the effects of ten AE surfactants on two species of grass was conducted by Valoras and Letey (1978). Barley and rye grasses were exposed to 50 mg/L or 100 mg/L solutions of each surfactant. Growth greater than or equal to control was seen with all surfactants at 50 mg/L concentrations. At 100 mg/L, n-pri-$C_{12-13}AE_3$ and n-pri-$C_{12-15}AE_3$ were the most toxic for both grasses; barley growth was reduced by 25% and 20%, respectively. Rye grass was generally more sensitive than barley with growth reductions of approximately 50% and 80%, respectively, for these two surfactants, although growth in both species was inhibited by 100 mg/L concentrations of all the surfactants tested (i.e., n-pri-$c_{12-15}AE_7$, -AE_9, -AE_{12}, -AE_{20}, -AE_{30}; n-pri-$C_{9-11}AE_{2.5}$, -AE_6, -AE_8). The least phytotoxic compounds for both barley and rye grasses were n-pri-$C_{12-15}AE_{20}$, n-pri-$C_{9-11}AE_6$ and n-pri-$C_{9-11}AE_8$.

The phytotoxicity of AE in the field was tested in a study which investigated the utility of utilizing AE to reduce soil hydrophobicity in burned areas to allow reseeding. Neodol 91-8™ (80%) was applied to 3 plots at 16 or 32 lb/acre following seeding with ryegrass. The site was rapidly revegetated with perennials and annuals, and no phytotoxicicity to the ryegrass was observed (Shell Chemical Co., unpublished data).

D. Effects of AE on Birds and Wildlife

A single study tested $C_{11-15}AE_9$ (PA-14) for use as an avian stressing agent for control of starlings and blackbirds. This surfactant, applied as an aerial mist, breaks down oil in the feathers of birds, thus removing their natural waterproofing. If feathers become water-soaked, lethal hypothermia results. The oral toxicity of PA-14 to sparrow hawks was found to be 6,300 mg/kg.

No other information on avian or terrestrial toxicity was available.

E. Mode of Action

All theorized modes of action by AE were summarized in the 1977 Report. One author attributed the toxic effects to changes in cell membranes, which result from a reduction in the surface tension of the water caused by AE dissolution. Toxicosis is usually evidenced by gill injury, the first sign of which may be an accumulation of mucus.

Critical micelle concentrations of AE in fish tissues may also be a factor. However, no single hypothesis has yet been able to explain the mode of action of AE.

No additional information was available in this area.

BIBLIOGRAPHY

ABRAM, F.S.H., V.M. Brown, H.A. Painter and A.H. Turner. 1977. The biodegradability of two primary alcohol ethoxylate nonionic surfactants under practical conditions, and the toxicity of the biodegradation products to rainbow trout. Paper presented at the IVth Yugoslav Symposium on Surface Active Substances, Dubrovnik, October 17-21, 1977.

BISHOP, W.E. and R.L. Perry. 1979. The development and evaluation of a flow-through growth inhibition test with duckweed (Lemna minor L.). Proc. ASTM 4th Aquatic Toxicology Symposium, Chicago, IL, October 17, 1979.

BISHOP, W.E. and A.W. Maki. 1978. A critical comparison of two bioconcentration test methods. Proc. ASTM 3rd Aquatic Toxicology Symposium, October 17-18, New Orleans, LA.

BODE, H., R. Ernst, and J. Arditti. 1978. Biological effects of surfactants. III. Hydra as a highly sensitive assay animal. Environ. Pollut. 17(3):175-185.

FISCHER, W.K. and P. Gode. 1978. [Comparative investigation of various methods of examining fish toxicity, with special consideration of the German golden orfe test and the iso zebra fish test.] Z.f. Wasser Abwasser Forsch 11(3/4):99-105 (English translation).

GRANMO, A. and G. Jorgensen. 1975. Effects on fertilization and development of the common mussel, Mytilis edulis after long-term exposure to a nonionic surfactant. Mar. Biol. 33:17-20.

HALL, R.H. 1973. An algal toxicity test used in the safety assessment of detergent components. Presented at 36th Annual Meeting, American Society of Limnology and Oceanography, Inc. Salt Lake City, Utah. June 12, 1973.

KURATA, N., K. Koshida, and T. Fujii. 1977. Biodegradation of surfactants in river water and their toxicity to fish. Yukagaku 26(2):115-118.

LEWIS, M.A. and R.L. Perry. 1979. Acute lethalities of equimolar and equitoxic surfactant mixtures to Daphnia magna and Lepomis macrochirus. Proc. ASTM 4th Aquatic Toxicol. Symp.

MAKI, A.W. 1979a. Respiratory activity of fish as a predictor of chronic fish toxicity values for surfactants. Spc. Tech. Publ. 667, Am. Soc. Test Materials.

MAKI, A.W. 1979b. An environmental safety evaluation of detergent chemicals in estuarine ecosystems. Proc. 14th Int. Mar. Biol. Symp. - "Protection of Life in the Sea". Helgoland, Germany. Sept. 24-28, 1979. Biologische Anstalt Helgoland, Hamburg, Germany. In press.

MAKI, A.W. 1979c. Correlations between Daphnia magna and fathead minnow (Pimephales promelas) chronic toxicity values for several classes of test substances. J. Fish. Res. Bd. Can. 36:411-421.

MAKI, A.W. and W.E. Bishop. 1979. Acute toxicity studies of surfactants to Daphnia magna and Daphnia pulex. Arch. Environ. Contam. Toxicol. 8:599-612.

MAKI, A.W., A.J. Rubin, R.M. Sykes and R.L. Shank. 1979. Reduction of nonionic surfactant toxicity following secondary treatment. J. Water Poll. Control Fed. 51(9):2301-2313.

PATZNER, V.A.M. and H. Adam. 1979. Influence of tensids on the regeneration of Dugesia gonocephala (Plathelminthes, Tricladida) and Notoplana humilis (Plathelminthes, Polycladida). Zool. Anz., Jena 202(3/4):199-208.

PAYNE, A.G. and R.H. Hall. 1979. A method for measuring algal toxicity and its application to the safety assessment of new chemicals. Spec. Tech. Pub. 667, Am. Soc. Test Mat.

PROCTER AND GAMBLE COMPANY, unpublished data.

REIFF, B. 1976. 7th International Congress on Surface Active Substances, Moscow, September 1976, as cited in Scharer et al. (1979).

REIFF, B., R. Lloyd, M.J. How, D. Brown and J.S. Alabaster. 1979. The acute toxicity of eleven detergents to fish: results of an interlaboratory exercise. Water Research 13:207-210.

SCHARER, D.H., J.B. Carr, and L. Kravetz. 1979. Biodegradation of nonionic surfactants. Tappi 62(10):75-78.

SHELL CHEMICAL COMPANY, unpublished data.

SWEDMARK, M., A. Granmo and S. Kollberg. 1976. Toxicity testing at Kristineberg Marine Biology Station, in: Pollutants in the Aquatic Environment, FAO/SIDA/TF, 108 Suppl. 1, 1976 Report.

VALORAS, N. and J. Letey. 1978. Screening of Neodol chemicals for potential use in erosion control. Report of the University of California, Riverside, to Shell Chemical Co., Houston, Texas. Manuscript.

VERMA, S.R., D. Mohan, and R.C. Dalela. 1978. Studies on the relative toxicity of a few synthetic detergents to a fish Macrones vittatus. Acta hydrochem. hydrobio. 6(2):121-128.

WRIGHT, A. 1976. The use of recovery as a criterion for toxicity. Bull. Environ. Contam. Toxicol. 15(6):747-749.

V. HUMAN SAFETY

The human safety data on alcohol ethoxylates presented in the 1977 Report indicated that although adequate chronic animal tests were not available, prolonged use of certain AE as human analgesics had revealed no untoward reactions to AE at doses many times those expected from environmental sources.

A. Animal Studies

AE exhibit a low order of acute toxicity in experimental animals. Oral LD_{50} values range from 1600 to >25,000 mg/kg with toxicity increasing rapidly as the length of the ethoxylate chain increases up to a maximum at about 10EO units/ surfactant molecule. The length of the alkyl chain exerts a negligible effect on toxicity. No chronic studies were available for AE but ingestion of 1% AE in the diet for one month was without effect in rats. Acute skin irritation studies with undiluted AE samples produced slight to extreme irritation reactions in rabbits but these exposures are considerably greater than exposure under normal use conditions. Eye irritation studies indicate that higher concentrations (10-100%) of AE produce varying degrees of reversible irritation but only transient irritation at a 1% concentration.

Acute Toxicity - Oral. Oral LD_{50} values in rats for a series of undiluted n-pri-AE with varying EO chain length, ranged from 87D

mg/kg for $C_{14-15}AE_{11}$ to 10,000 mg/kg for $C_{12-15}AE_3$ (Shell Chemical Co., unpublished data). These values are consistent with data cited in the 1977 Report, and reflect the trend of increasing oral toxicity with increments in the length of the ethoxylate chain.

Acute Toxicity - Dermal. The dermal LO_{50} values in rabbits for a series of undiluted n-pri-AE ($C_{9-11}AE_{2.5}$, $C_{9-11}AE_6$, $C_{12-15}AE_7$, $C_{14-15}AE_7$, $C_{14-15}AE_{11}$, $C_{14-15}AE_{13}$) ranged from 2300 to greater than 5000 mg/kg (Shell Chemical Co., unpublished data). Similarly, application of undiluted n-pri-$C_{12-15}AE_3$ to rat skin resulted in a dermal LO_{50} value of greater than 2000 mg/kg (Shell Chemical Co., unpublished data).

Acute Irritation - Ocular. Eye irritation studies in rabbits with several undiluted n-pri-AE samples ($C_{9-11}AE_{2.5}$, $C_{12-15}AE_7$, $C_{14-15}AE_7$, $C_{14-15}AE_{11}$, $C_{14-15}AE_{13}$) produced maximum average scores ranging from 18 (moderate) to 43.5 (severe) of a possible 110 points. An identical experiment with the same surfactants in which the eye was washed with 300 ml of tap water 30 seconds post-treatment produced maximum average scores of 12 to 31 points, generally within one hour of treatment (Shell Chemical Co., unpublished data). Several undiluted linear pri-AE ($C_{9-11}AE_6$, $C_{12-15}AE_3$, $C_{14-15}AE_{13}$) were mild to severe eye irritants in rabbits according to the Draize criteria. These samples were non-irritating at 1% concentrations (Shell Chemical Co., unpublished data).

Acute Irritation - Skin. Acute skin irritation studies in rabbits with undiluted n-pri-AE, occluded for 24 hr, resulted in primary irritation scores ranging from 3.59 to 7.75 of a possible 8 points. The materials examined included: $C_{9-11}AE_{2.5}$ (extreme irritation, necrosis), $C_{12-15}AE_7$ (severe), $C_{14-15}AE_7$ (severe), $C_{14-15}AE_{11}$ (moderate) and $C_{14-15}AE_{13}$ (moderate) (Shell Chemical Co., unpublished data). These reactions, however, occurred at concentrations considerably greater than exposure under actual use conditions.

Subacute Skin Irritation and Sensitization. Slight or no irritation was noted following three 6 hour applications of 10% aqueous solutions of n-pri-$C_{14-15}AE_{13}$ or n-pri-$C_{9-11}AE_6$ to occluded, intact rabbit skin. Identical treatment with undiluted samples of these two surfactants resulted in mild skin irritancy and severe necrosis, respectively (Shell Chemical Co., unpublished data).

In another experiment, application of 10% aqueous solutions of n-pri-$C_{9-11}AE_6$ or n-pri-$C_{14-15}AE_{13}$ daily, five days per week, for 4 1/2 weeks to the skin of guinea pigs and rabbits (unoccluded) produced minimal to mild irritancy; no irritation was noted with 1% concentrations (Shell Chemical Co., unpublished data).

Applications of patches containing 0.1% n-pri-$C_{12-15}AE_7$, n-pri-$C_{14-15}AE_7$, n-pri-$C_{14-15}AE_{11}$ or n-pri-$C_{14-15}AE_{13}$ to guinea pig skin on days 9, (initial treatment), 16, 23 and 37 of a 42 day study were

non-sensitizing. Similar results were also noted with 1% n-pri-$C_{9-11}AE_{2.5}$ and n-pri-$C_{9-11}AE_6$ (Shell Chemical Co., unpublished data).

Topical induction with 50% n-pri-$C_{12-15}AE_3$ and subsequent challenge by topical application with a 25% concentration of the surfactant resulted in a very weak skin sensitizing reaction in guinea pigs; i.e., 1 out of 20 test animals showed a trace response which persisted for 48 hours after patch removal (Shell Chemical Co., unpublished data). Similar tests in guinea pigs with n-pri-$C_{14-15}AE_{13}$ or n-pri-$C_{9-11}AE_6$ following topical (5%) or intradermal (0.05%) induction and subsequent topical challenge with 2.5% concentrations produced negative sensitization responses (Shell Chemical Co., unpublished data).

Chronic Toxicity - Oral. Dietary studies with two AE surfactants have been completed since the 1977 Report. Sprague-Dawley rats were fed 0, 0.1, 0.5 or 1% $C_{12-13}AE_{6.5}$ in the diet for 104 weeks (Procter and Gamble Company, unpublished data). Dose-related body weight depression was observed for females in the upper two treatment levels and for males at the 1% level. Food consumption, however, was also reduced in these groups and was attributed to the poor palatability of the diet. At termination of the study, elevated organ-to-body weight ratios were noted for the following organs: liver, kidney and brain - 0.5 and 1% females; liver - 1% males; heart - 1% females.

The only pathology finding of note was focal myocarditis, which in males, increased in frequency as the dose increased. Incidence data at

24 months is presented below:

Dietary Level	Percent Incidence of Focal Myocarditis	
	♂	♀
0%	24	19
0.1%	20	0
0.5%	44	22
1.0%	58	27

Focal myocarditis is a common spontaneous type of lesion found in relatively high frequency in aging populations of rats, and the incidence noted in the 0.5% and 1% male treated groups are comparable to reported background incidences of focal myocarditis in rats of this age while the control animals exhibited an unusually low incidence. (Altman and Katz, 1979; Simms, 1967). No other significant treatment-related histopathology was found and no significant increase in tumor incidence was observed (Procter and Gamble Company, unpublished data).

A second feeding study was conducted with Charles River CD-rats fed 0, 0.1, 0.5 or 1.0% $C_{14-15}AE_7$ in the diet for two years (Procter and Gamble Company, unpublished data). As in the above study, the significant finding was a reduced body weight gain at doses of 0.5% (females) and 1% (both sexes) which was attributed to diet palatibility. No effects were noted in behavior, appearance, survival, or in hematological and biochemical parameters. Decreased absolute organ weights for liver, kidney, heart and thyroid/parathyroid glands were recorded for the 1% female

group and for brain and adrenals for the 1% treated males. Focal myocarditis was also prevalent in all groups of rats in this study. The incidence rates at 12 and 24 months are presented below:

Dietary Level	Incidence of Focal Myocarditis (Ave. Severity Score)							
	12 mo.				24 mo.			
	♂		♀		♂		♀	
0.0%	10/15	67%(2.7)	4/14	29%(2.2)	9/10	90%(3.4)	3/10	30%(3.0)
0.1%	12/15	80%(2.0)	10/14	71%(2.1)	8/9	89%(3.1)	8/10	80%(2.8)
0.5%	15/15	100%(2.8)	15/15	100%(2.8)	10/10	100%(2.9)	5/10	50%(2.4)
1.0%	14/14	100%(3.0)	10/15	67%(2.0)	8/10	80%(3.3)	3/10	30%(2.3)

Although the gross incidences of focal myocarditis of all levels of severity increase with increased dose of AE at 12 months, when the severity of the lesions are taken into account, no treatment related effect is evident. The large incidence (90%) noted in male controls at 24 months may be attributable to the small number of rats remaining in this group at termination. No additional significant histopathology was noted except for the usual types of lesions occurring in aging rat populations.

Chronic Toxicity - Dermal. Repeated dermal application of 0.1 ml of an aqueous solution of 0, 0.2, 1.0 or 5.0% $C_{12-13}AE_{6.5}$ to the backs of ICR Swiss mice three times per week for 18 months produced no remarkable findings. Although one skin papilloma and one squamous cell carcinoma were observed, each in one of 150 low dose (0.2%) females, the lack of any such lesions in the mid-dose and high-dose groups, along with an examination of the background

incidence of spontaneous skin lesions in ICR Swiss mice, indicates that the effect was not related to the application of $C_{12-13}AE_{6.5}$. A second skin papilloma (not at treatment site) was also noted in this group but was not considered treatment related (Procter and Gamble Company, unpublished data).

Carcinogenicity. The chronic oral toxicity studies with rats described above give no indication of any carcinogenicity which could be ascribed to ingestion of up to one percent AE for two years. In addition, the percutaneous application of an aqueous solution containing up to five percent AE to Swiss ICR mice three times weekly for eighteen months did not result in any compound-related carcinogenic response either on the skin or systemically (Procter and Gamble Company, unpublished data).

Mutagenicity.

> *In vitro and host-mediated mutagenicity tests give no evidence that AE are mutagenic.*

No chromosomal aberrations were induced in rat liver cells exposed in culture to n-pri-$C_{12-13}AE_3$ at concentrations up to 10 µg/ml. Negative results were also observed in Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1538 strains; 2000 µg/plate), Escherichia coli (WP2, WP2uvrA strains; 2000 µg/plate) and Saccharomyces cerevisiae (JD1; 5 mg/ml) with or without microsomal activation (Shell Toxicology Laboratory, unpublished data).

Teratogenicity/Reproduction Studies

No indications of teratogenesis or adverse reproductive effects were attributable to the oral administration of up to 0.5% AE in the diet of either rats or rabbits.

Pharmacology-Absorption and Metabolism

Metabolic studies with rats indicate rapid absorption and excretion of orally or topically administered AE. Following oral administration, 54% of administered radioactivity was excreted in urine, 26% in feces and 3% in expired air within 72 hr. Dermal application resulted in excretion of 29, 8 and 11%, respectively, over the same time period.

In a series of studies with various AEs labelled either at the hydroxyl-bearing carbon or the α-carbon of the alkyl group, Drotman (1980) noted that rats dosed orally with either $C_{12}AE_6$, $C_{13}AE_6$ or $C_{14}AE_7$ (labelled in either moiety) readily absorbed >75% of the dose. The major portion of the radioactivity appeared in urine (52-55%) with smaller amounts in feces (23-27%) and expired air (2-3%) by 72 hours. Variations in the length of the alkyl chain or the ethoxylate chain produced the same pattern of disposition when the ^{14}C-label was at the hydroxyl-bearing carbon; however, when the label was in the α-position of the alkyl chain, increasing the alkyl chain length from C_{12} to C_{15} gave rise to more CO_2 (up to ∿50%) and less urinary and fecal elimination than with shorter alkyl chains. Similar results were obtained with cutaneously dosed materials, although absorption was somewhat slower by this route (∿50% at 72 hours).

B. Human Studies

The use of certain AE as analgesics and anesthetics in human therapy has revealed no untoward reactions, even following exposures of several months duration at doses many times those expected from environmental sources. Slight skin irritation is noted with repeated application of highly concentrated AE but no indications of sensitization have been reported.

Drotman (1980) reports that absorption, distribution and excretion of AE by humans closely patterns the disposition of AE in rats. Male volunteers (60-90Kg) ingested 50 mg ^{14}C-labelled AE of varying alkyl chain length. The samples were labelled with ^{14}C in either the hydroxyl-bearing carbon ($C_{12}A^*E_6$; $C_{13}A^*E_6$) or the α-carbon of the alkyl group ($^*C_{12}AE_6$; $^*C_{13}AE_6$). For all these compounds, most of the radioactivity generally appeared in the urine (63-80%), with smaller amounts in feces (3.8-6.9%) and expired CO_2 (3-13%) by 144 hours. The addition of a single carbon to the alkyl chain radiolabelled on the α-carbon was observed to produce an increased excretion of CO_2 (13 vs 3%) and a drop in urinary excretion (63 vs 75%). Thus, the distribution and excretion of the ethoxylate groups of the AEs were similar, but the metabolism of their alkyl chains was a function of chain length, with the longer chained compounds giving rise to more CO_2 and less urinary elimination products than the shorter chained compounds.

Following dermal application of 1 ml of 50:50 ethanol:water solution containing 100 mg $C_{12}{}^*E_6$ to the outer part of the forearm of two male subjects

(protected by a nonocclusive metal shield for 8 hr), most of the radioactivity was recovered at 144 hours by swabbing the skin with alcohol-soaked gauze (74 and 88%). Only 1-2% appeared in the urine and none was found in feces or expired air by 144 hours (Drotman, 1980).

BIBLIOGRAPHY

ALTMAN, P.L. and D.D. Katz. 1979. Inbred and Genetically Defined Strains of Laboratory Animals, Part 1 Mouse and Rat Biological Handbooks, Volume III. Federation of American Society of Experimental Biology, Maryland.

DROTMAN, R.B. 1980. The absorption, distribution, and excretion of alkylpolyethoxylates by rats and humans. Toxicol. Appl. Pharmacol. 52:38-44.

PROCTER AND GAMBLE COMPANY, unpublished data.

SHELL CHEMICAL COMPANY, unpublished data.

SHELL TOXICOLOGY LABORATORY, unpublished data.

SIMMS, H.S. 1967. Pathology of Laboratory Rats and Mice, pp. 733-747, Oxford Publications.

CHAPTER 4

ALCOHOL ETHOXY SULFATES

(AES)

ALCOHOL ETHOXY SULFATES

Synopsis

Alcohol ethoxy sulfates (AES) are primarily used as components of light duty liquid dishwashing products, shampoos and other household specialty products. A total of 128 million pounds were produced in the United States in 1978.

There are presently no national criteria for concentrations of AES in waters of the United States; concentrations of AES, as such, are not presently monitored. The MBAS analytical procedure would measure AES if present, along with other anionic surfactants, but does not distinguish among them. The limited data available on the biodegradability of AES indicate that they are readily biodegraded under both aerobic and anaerobic conditions in the field as well as in laboratory tests. Neither the length of the alkyl chain nor the length of the ethoxylate portion of the molecule (in the range normally used in detergent formulations) appears to influence the rate of biodegradation.

AES are acutely toxic to aquatic fauna, with LC_{50} values in the 1 to 450 mg/L range; sublethal effects have been noted at a concentration of 0.22 mg/L. Toxicity appears to increase with increasing alkyl chain length to a maximum at C_{16} beyond which toxicity decreases. Toxicity also appears to decrease with increases in EO chain length for AES with alkyl chains of less than C_{16}.

A low order of acute and chronic toxicity is noted in experimental animals exposed to AES. Oral LD_{50} values in the rat range from 1700 to >5000 mg/kg. Ocular and skin irritation studies indicate exposures to concentrations of 1% AES or less produce minimal to slight irritation; more intense reactions are noted with higher concentrations. Ingestion of 0.5% AES in the diet for 2 years produced no deleterious effects in rats, and there is no evidence of carcinogenic, teratogenic or mutagenic effects associated with AES exposure. Recently, trace levels of 1,4-dioxane, an animal carcinogen, have been detected in AES concentrates. Since there are no data which indicate that commercial AES are mutagenic, teratogenic or carcinogenic, and in that substantial dilutions of these concentrates are made during formulation of commercial products, and once again at the time of use, consumers do not appear to be at risk from use of these products.

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.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

ALCOHOL ETHOXY SULFATES

I. INTRODUCTION

Alcohol ethoxy sulfates (AES) are principally used as components in light duty liquid dishwashing products, shampoos and other household specialty products. Commercially, AES are produced by ethoxylation of a fatty alcohol, followed by sulfation with sulfur trioxide or chlorosulfonic acid and finally, neutralization to form either the sodium or ammonium salt. Some formulated AES also contain ethanol. A total of 220 million pounds of anionic sulfated ethers, 106 million pounds of which were AES, were produced in 1973.

The most recent U.S. International Trade Commission figures (1979) indicate a total of 285 million pounds of anionic sulfated ethers were produced in 1978, of which approximately 128 million pounds were AES, an increase of 22 million pounds over a five year period.

BIBLIOGRAPHY

U.S. INTERNATIONAL TRADE COMMISSION. 1979. Synthetic Organic Chemicals. United States Production and Sales of Surface-Active Agents, 1979.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alcohol ethoxy sulfates are one of several entities classified as anionic surfactants, and as such can be detected with many of the procedures utilized in the detection of LAS (See Chapter 1). The MBAS analytical procedure measures AES along with other anionic surfactants but does not distinguish among them.

Wickbold (1976) has developed a chromotographic method utilizing a macroporous anion exchange which separates an aqueous AES solution into various components (ether sulfate, alcohol ethoxylate, polyglycol sulfate, sodium sulfate, sodium chloride). The procedure involves passing an ethanolic surfactant solution through a strong acid cation exchanger and a macroporous weakly basic anion exchanger. The nonionic components are contained in the eluent. Fractional elution of the anion exchanger first with aqueous ammonium hydrogen carbonate solution, then with an isopropanol-aqueous ammonium hydrogen carbonate mixture selectively releases (1) inorganic chloride, inorganic sulfate and polyglycol sulfate, and (2) AES, respectively. This eluate is evaporated to dryness, converted into sodium salts and weighed. The procedure is suitable for the investigation of AES with EO chain lengths up to 4 units/mole (i.e., AES in the commercial range). Above this length, the hydrophilic nature of the surfactant is such that it is eluted completely, or in part, along with polyglycol sulfate and the inorganic components.

BIBLIOGRAPHY

U.S. INTERNATIONAL TRADE COMMISSION. 1979. Synthetic Organic Chemicals. United States Production and Sales of Surface-Active Agents, 1979.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alcohol ethoxy sulfates are one of several entities classified as anionic surfactants, and as such can be detected with many of the procedures utilized in the detection of LAS (See Chapter 1). The MBAS analytical procedure measures AES along with other anionic surfactants but does not distinguish among them.

Wickbold (1976) has developed a chromotographic method utilizing a macroporous anion exchange which separates an aqueous AES solution into various components (ether sulfate, alcohol ethoxylate, polyglycol sulfate, sodium sulfate, sodium chloride). The procedure involves passing an ethanolic surfactant solution through a strong acid cation exchanger and a macroporous weakly basic anion exchanger. The nonionic components are contained in the eluent. Fractional elution of the anion exchanger first with aqueous ammonium hydrogen carbonate solution, then with an isopropanol-aqueous ammonium hydrogen carbonate mixture selectively releases (1) inorganic chloride, inorganic sulfate and polyglycol sulfate, and (2) AES, respectively. This eluate is evaporated to dryness, converted into sodium salts and weighed. The procedure is suitable for the investigation of AES with EO chain lengths up to 4 units/mole (i.e., AES in the commercial range). Above this length, the hydrophilic nature of the surfactant is such that it is eluted completely, or in part, along with polyglycol sulfate and the inorganic components.

B. Water Quality Standards

There are presently no standards in the United States or Europe specifically re-restricting concentrations of alcohol ethoxy sulfates. These anionic surfactants are included among those measured in the environment using the MBAS method. The criteria applying to MBAS levels were discussed in Chapter 1.

C. AES in Natural Water Bodies

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

BIBLIOGRAPHY

WICKBOLD, R. 1976. Die untersuchung sulfatierter alkoholoxäthylate [Investigation of sulfated alcohol ethoxylates.] Tenside Detergents 13(4):181-184.

III. BIODEGRADATION

The limited information available on the biodegradability of AES indicates that they are readily biodegraded under both aerobic and anaerobic conditions in the field as well as in laboratory tests.

A. Laboratory Investigations

AES are substantially biodegraded as determined by BOD (35-68% at 5 days) and evolved CO_2 data (71-100%). Neither the length of the alkyl chain nor the length of the ethoxylate portion of the molecule, at least in the range normally used in detergent formulations (i.e. 2 to 4 EO units/mole), appears to significantly influence the rate of biodegradation.

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

In another study, Itoh et al. (1979) examined the biodegradability (evolved CO_2 production) of 20 mg/L of coconut alcohol-derived AE_3S, oxo-alcohol (C_{12-15}) AE_3S and sec-alcohol (C_{12-15}) AE_3S. All 3 surfactants evolved approximately 40-50% of the theoretical CO_2 by 5-10 days. Primary biodegradation was complete (100% MBAS) by 10 days.

B. Field Studies

Fourteen homes with either septic tank or aerobic cavitette sewage treatment systems which exclusively utilized household detergent products containing 10-13% AE_3S for one year averaged 46-66% surfactant removal (as MBAS). Another field study cited in the 1977 Report indicated complete removal of AES during passage through Japanese sewage treatment plants.

BIBLIOGRAPHY

ITOH, S., S. Setsuda, A. Utsunomiya and S. Naito. 1979. Studies on the biodegradation test method of chemical substances. II. Ultimate biodegradabilities of some anionic, nonionic, and cationic surfactants estimated by CO_2 production. Yakagaku 28(3):199-204.

MIURA, K., K. Yamanaka, T. Sangai, K. Yoshimura and N. Hayashi. 1979. Application of biological oxygen consumption measurement technique to the biodegradation test of surfactants. Yukagaku 28(5):351-355. (English translation).

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. Fish

The LC_{50} values listed in the 1977 Report ranged from 0.3 mg/L $C_{15.9}AE_{2.1}S$ to 375 mg/L $C_{10}AE_{2.1}S$, both for the bluegill sunfish (Lepomis macrochirus). The majority of LC_{50} values, however, were between 1 and 10 mg/L. Toxicity appeared to increase with increasing alkyl chain length to a maximum at C_{16}, beyond which toxicity decreased. Conversely, AES compounds with alkyl chains of less than 16 carbons had decreasing toxicity with each increment in the ethoxylate chain from 2 to 6 EO units. Longer-chain AES compounds ($>C_{16}$) increased in toxicity as the ethoxylate chain was lengthened from 2 to 6.

More recent data on the toxicity of AES to fish are summarized in Table 4-A; included are two studies which reported sublethal effects in the fathead minnow and the bluegill. The lowest level at which sublethal toxicosis was observed was 0.22 mg/L $C_{13.7}AE_{2.25}S$, which caused growth inhibition in the fathead minnow during a one-year chronic exposure test. A NOEC of 0.1 mg/L was established for this species (Maki, 1979a). In 0.39 mg/L $C_{16}AE_{3}S$, the ventilation rate of bluegills increased significantly during 48 hours of exposure. The NOEC for ventilation rate was between 0.24 and 0.39 mg/L of exposure (Maki, 1979b). In a comparison of sensitivities of fry and juvenile life stages of fathead minnow, no significant differences in measured LC_{50} values were observed after 4, 7, 14, 28 or 45 days' continu-

TABLE 4-A. TOXICITY OF ALKYL ETHOXY SULFATES TO FISH

Species	Surfactant (Trade Name)	Concentration (mg/L±95% CL)	Effects	Experimental Conditions	Reference
Fathead minnow (Pimephales promelas)	$C_{13.7}AE_{2.25}S$	0.22 0.1	Growth inhib. NOEC	1 yr., flow-thru, 21°, 120 mg/L hard. pH 7.4	Maki (1979a)
Fathead minnow fry (Pimephales promelas)	$C_{14-16}AE_{2.25}S$	0.63 (0.45-0.88)	LC_{50}	45 day, flow-thru	Procter and Gamble Co. (Unpublished data)
Fathead minnow juvenile (Pimephales promelas)	$C_{14-16}AE_{2.25}S$	0.94 (0.61-1.98)	LC_{50}	45 day, flow-thru	
Sheepshead minnow * (Cyprinodon variegatus)	$C_{14-16}AE_{2.25}S$	0.39 (0.3-0.53)	LC_{50}	96 hr, static, 21°, pH 7.9, salinity 30°/°°	
Bluegill sunfish (Lepomis macrochirus)	$C_{16}AE_3S$	0.39	Incr. ventilation rate	48 hr, flow-thru, 13°, 120 mg/L hard., pH 7.4	Maki (1979b)
Brown trout (Salmo trutta)	$C_{12-15}AE_3S$	1.0-2.5	LC_{50}	96 hr, flow-thru, 26-30 mg/L hardness	Reiff et al. (1979)
Brown trout (Salmo trutta)	$C_{12-15}AE_3S$	1.5	LC_{50}	(250 mg/L hard.)	
Golden orfe (Idus idus melanotus)	$C_{12-15}AE_3S$	3.3-6.2	LC_{50}	(268 mg/L hard.)	
Golden orfe (Idus idus melanotus)	$C_{12-15}AE_3S$	5.7	LC_{50}	(48 hr, 150 mg/L hard.)	
Harlequin fish (Rasbora heteromorpha)	$C_{12-15}AE_3S$	3.9	LC_{50}	(96 hr, 20 mg/L hard.)	
Rainbow trout (Salmo gairdneri)	$C_{12-15}AE_3S$ (Dobanol 25-3S/27)	8.9 (7.3-10.3)	LC_{50}	96 hr, static, 15° 260 mg/L hard., pH 8.2-8.6	Shell Chemical Co. (unpublished data)
	$C_{12-13}AE_2S$ (Dobanol 23-2S/28)	28 (23-35)	LC_{50}		
	$C_{9-10}AE_{2.5}S$ (Dobanol 91-2.5S)	400-450	LC_{50}		
Japanese killifish (Oryzias latipes)	AES	10	LC_{50}	48 hr	Tomiyama (1974)

* Marine species

ous flow exposure to $C_{14-16}AE_{2.25}S$. The 45 day LC_{50} values for fry and juveniles were 0.63 (95%CL: 0.45-0.88) mg/L and 0.94 (95%CL:0.61-1.98) mg/L, respectively. In addition, no significant effects on length and weight of either life stage were noted (Procter and Gamble Company, unpublished data).

Acute toxicity levels as measured by LC_{50} values, ranged between 1 and 450 mg/L in the data surveyed. The lowest reported LC_{50} value (0.63 mg/L) was for fathead minnow fry exposed to $C_{14-16}AE_{2.25}S$ in a 45-day continuous flow toxicity test (Procter and Gamble Co., unpublished data). The data of Shell Chemical Company (unpublished) support the finding of the 1977 Report that toxicity increases with increasing alkyl chain length. In toxicity tests with rainbow trout, the 96-hour LC_{50} value decreased from 400-450 mg/L for $C_{9-10}AE_{2.5}S$ to 8.9 mg/L for $C_{12-15}AE_3S$.

2. Invertebrates

> *The 1977 Report surveyed the literature on AES toxicity to aquatic invertebrates, reporting 24-hour LC_{50} values ranging from 5 to 37 mg/L, for Daphnia magna. The sodium salt of $C_{12-14}AE_3S$ was found to be slightly less toxic than the ammonium salt of the same compound. The only other species for which an acute toxicity value was found was mosquito larvae (Aedes aegypti), with a 24-hour LC_{50} of 11 mg/L.*

Further information on aquatic invertebrate toxicity was provided in a chronic toxicity test with Daphnia magna (Maki, 1979a). The 21-day EC_{50} for inhibition of reproduction (with respect to total young produced) was

0.37 mg/L $C_{13.67}AE_{2.25}S$ in continuous-flow conditions and 120 mg/L $CaCO_3$ water hardness. The 96-hr and 21-day LC_{50} values for the same surfactant were 1.17 mg/L and 0.74 mg/L, respectively. A chronic NOEC of 0.27 mg/L was established for this species.

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

Pink shrimp (Penaeus duorarum) have also been tested for their susceptibility to AES. The 96 hr LC_{50} for $C_{14-16}AE_{2.25}S$ was 350 (95%CL: 220-590) mg/L; the no observed effect level for this acute exposure was less than 120 mg/L $C_{14-16}AE_{2.25}S$ (Procter and Gamble Co., unpublished data).

B. Toxicity of AES to Algae and Microorganisms

Three AES compounds were found to have inhibitory effects on the growth of E. coli in culture plates. The lowest concentrations of $C_{12}AE_3S$, $C_{12}AE_3S$ (Ziegler-derived), and $C_{12-14}AE_{2.2}S$ (natural alcohol-derived) which prohibited the development of more than 5 colonies per plate (over 5 days at 37°) were 18, 4, and 2 g/L, respectively. MAC (minimum algistatic concentrations for a 5-day exposure) values were reported for 3 species of algae, and ranged between >10 mg/L and <1000 mg/L AES.

No information on AES toxicity to bacteria was found in this survey, although two studies on aquatic flora were reviewed. Kutt and Martin (1974)

exposed a red tide dinoflagellate (Gymnodinium breve) to various concentrations of a coconut-alcohol-derived ethoxy sulfate for 48 hours. A concentration of 2.5 µg/L caused 87% mortality, 12.5 µg/L resulted in 63% mortality, and only 44% perished in 50 µg/L. No explanation was offered for the abnormal inverse relationship between toxicity and surfactant concentration.

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

BIBLIOGRAPHY

KUTT, E.C. and D.F. Martin. 1974. Effect of selected surfactants on the growth characteristics of Gymnodinium breve. Marine Biology 28:253-259.

LUNDAHL, P. and R. Cabridenc. 1976. A focus on a method of study of the toxicity of pollutants during biodegradation. J. Francais d'Hydrologie 7(3) #21:143-150. (English translation).

MAKI, A.W. 1979a. Correlations between Daphnia magna and fathead minnow (Pimephales promelas) chronic toxicity values for several classes of test substances. J. Fish. Res. Bd. Can. 36:411-421.

MAKI, A.W. 1979b. Respiratory activity of fish as a predictor of chronic fish toxicity values for surfactants. Spec. Tech. Publ. 667, Am. Soc. Testing and Materials.

PROCTER AND GAMBLE CO., unpublished data.

PYBUS, C. 1973. Effects of anionic detergent on the growth of Laminaria. Marine Pollution Bulletin 4(5):73-77.

REIFF, B., R. Lloyd, M.J. How, D. Brown, and J.S. Alabaster. 1979. The acute toxicity of eleven detergents to fish: Results of an interlaboratory exercise. Water Res. 13:207-210.

SHELL CHEMICAL CO., unpublished data.

TOMIYAMA, S. 1974. Effects of surfactants on fish. Bull. Jpn. Soc. Fish. 40(12):1291-1296 (English abstract).

V. HUMAN SAFETY

AES exhibit a low order of acute and chronic toxicity in experimental animals. Oral LD_{50} values range from 1700 to >5000 mg/kg in the rat. Dermal LD_{50} values reported in rabbits range from 4700 to 12,900 mg/kg. Ingestion of 0.5% AES in the diet for 2 years produced no deleterious effects in rats. Ocular and skin irritation studies in rabbits indicate care should be exercised against direct eye contact or excessive dermal exposure to concentrations of AES greater than 1-2%.

A. Animal Studies

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

Undiluted n-pri-$C_{12-13}AE_2S$ produced severe eye irritation in rabbits; moderate eye irritancy was noted with a 10% aqueous solution of this surfactant, while a 1% concentration was practically non-irritating. No irritation was seen with a 0.1% solution (Shell Research Limited, unpublished data).

Acute Irritation - Gastrointestinal. Ciuchta and Dodd (1978) also examined the gastrointestinal irritation induced by the above surfactant in a mouse writhing test. Mice were injected intraperitoneally with 0.2 ml of various concentrations of an aqueous solution of $C_{12ave}AES$ and observed until a positive response was elicited. A concentration of 0.23% (0.15-0.36%) was calculated to produce writhing in 50% of the animals.

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

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

Morikawa et al. (1978) tested the skin sensitizing potential of a series of AES surfactants in guinea pigs. All animals were induced by intradermal injection (8% aqueous solution) and topical application (20% aqueous solution, 48 hr, occluded) and challenged two weeks later by

topical application (8% aqueous solution). The surfactants tested included: $C_{11-15}AE_1S$, $C_{12}AES$, $C_{12}AE_1S$, $C_{12}AE_5S$, $C_{12-16}AES$, $C_{12-16}AE_1S$, $C_{12-16}AE_3S$, $C_{12-16}AE_5S$, $C_{14}AE_1S$, $C_{14}AE_3S$ $C_{16}AE_3S$. None induced skin sensitization in guinea pigs. In addition, Morikawa tested two samples of $C_{12}AE_3S$ sulfated either with sulfur trioxide or chlorosulfonic acid. The chlorosulfonic acid preparation was not a skin sensitizer (0/10) in guinea pigs while 15/15 animals gave positive reactions with the sulfur trioxide preparation. Allergenic activity was found by chromatographic separation techniques to be concentrated in one fraction of the sulfur trioxide preparation. This allergenic material was isolated and identified as 1-dodecene-1, 3-sultone, formed as a by-product during industrial synthesis.

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

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

In another experiment, application of a 10% aqueous solution of n-pri-$C_{12-13}AE_2S$ daily, five days per week, for 4 1/2 weeks to the skin of guinea pigs and rabbits (unoccluded) produced severe skin irritation; no irritation was noted with either 1% or 0.1% concentrations (Shell Research Limited, unpublished data).

Intradermal and topical induction of guinea pigs with hypochlorite bleached and unbleached samples of n-pri-$NH_4C_{12-15}AE_3S$, n-pri-$NaC_{12-15}AE_3S$, or n-pri-$C_{12-13}AE_2S$ followed by topical challenge produced no skin sensitization (Shell Research Limited, unpublished data). Similar skin sensitization studies with 12 separate batches of n-pri-$C_{12-15}AE_3S$ also resulted in no skin sensitization in guinea pigs (Shell Toxicology Laboratory, unpublished data).

Carcinogenicity.

No indications of increased tumor incidence were noted in two feeding studies with rats given 0.5% AES in the diet or 0.1% AES in drinking water for 2 years. Skin-painting studies in mice with 5% $C_{12}AE_3S$ or with a 10% TES/LAS mixture for 2 years did not induce a carcinogenic response.

No additional studies have examined the carcinogenic potential of AES surfactants. Recently, trace levels of 1,4-dioxane, an animal carcinogen, have been reported to be present in formulated AES products (Shell Oil Co., 1979; Sherex Chemical Co., 1980). This material is a by-product formed during conversion of alcohol ethoxylates to alcohol ethoxy sulfates.

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

Concentrated sodium and ammonium salts of AES have been found to generally contain between 250 and 600 ppm 1,4-dioxane (Shell Oil Co., 1980). The actual concentration of 1,4-dioxane in formulated household dishwashing products would appear to be so low as to pose no substantial risk to users; <1 to 45 ppb assuming an $\sim$2000-fold consumer dilution of a commercial formulation containing 15% AES concentrate. Shampoos present a different picture since the product is at least momentarily handled undiluted just prior to lathering the hair. 1,4-Dioxane values of 100 ppm or less can be calculated for such exposures. However, this kind of exposure is very short, and any 1,4-dioxane will be greatly diluted and removed with water after a few minutes. The negative results reported for AES surfactants in both oral and dermal carcinogenicity tests, in addition to negative mutagenicity and teratogenicity findings, indicate that use of commercial AES products should not present a hazard to consumers.

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

Mutagenicity.

No indications of mutagenicity were noted in a dominant lethal study or in vivo or in vitro cytogenetic studies with a 55% AES:45% LAS mixture.

Recent in vivo and in vitro cytogenetic studies indicate that AES alone are not mutagenic. Hope (1977) reported that the incorporation of C_{12-15}AES into the diet of rats at a maximum tolerated dose (1.13% active ingredient) for 90 days had no effect on the chromosomes of rat bone marrow cells.

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

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

Teratogenesis/Reproduction Studies.

No data are available on the teratogenic effects of AES administered alone. Oral administration of a commercial mixture of AES and LAS, however, gave no indications of any embryotoxic or teratogenic effects in mice, rats or rabbits. In addition, no adverse effects on fertility, lactation, litter size, survival or growth of off-spring were seen in rats fed diets containing 0.1% $C_{12}AE_3S$ for two generations.

Pharmacology - Absorption and Excretion.

Orally administered [^{14}C]-$C_{16}AE_3S$ was readily absorbed by rats and men and excreted principally in urine with lesser amounts found in feces and expired air. The major metabolite in urine was identified as 2-(triethoxy sulfate) acetic acid.

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

B. Human Studies

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

BIBLIOGRAPHY

CIUCHTA, H.P. and K.T. Dodd. 1978, The determination of the irritancy potential of surfactants using various methods of assessment. Drug and Chemical Toxicol. 1(3):305-324.

CONOCO CHEMICALS. 1980. Unpublished data.

HOPE, J. 1977. Absence of chromosome damage in the bone marrow of rats fed detergent actives for 90 days. Mutation Res. 56:47-50.

IMOKAWA, G. 1979. Study on skin-irritating and biological properties of monoalkyl phosphate anionic surfactants. J. Am. Oil Chem. Soc. 56(5): 604-609.

INOUE, K., T. Sunakawa and S. Takayama. 1980. Studies of *in vitro* cell transformation and mutagenicity by surfactants and other compounds. Fd. Cosmet. Toxicol. 18:289-296.

KOCIBA, R.J., S.G. McCollister, C. Park, T.R. Torkelson and P.J. Gehring. 1974. 1,4-Dioxane. I. Results of a 2-year ingestion study in rats. Tox. Appl. Pharmacol. 30:275-286.

MORIKAWA, F., M. Tejima, S. Fujii and S. Hyakutake. 1978. [Contact hypersensitivity and the sensitizing mechanism of solutions contaminated during alkyl ethoxy sulfate production.] Arerugi 27(7):648-661.

NCI (National Cancer Institute). 1978. Bioassay of 1,4-Dioxane for possible carcinogenicity. National Cancer Institute Carcinogenesis Technical Report Series No. 80, U.S. Dept. Health, Education and Welfare, Publication No. (NIH) 78-1330.

SHELL OIL COMPANY. 1979. "1,4-Dioxane in Ethoxysulfates," submitted to EPA under Section 8 (e) of the Toxic Substances Control Act, September 25, 1979.

SHELL OIL COMPANY. 1980. Addendum submitted to EPA in follow-up to the September 25, 1979 submission under Section 8 (e) of the Toxic Substances Control Act, January 18, 1980.

SHELL RESEARCH LIMITED, unpublished data.

SHELL TOXICOLOGY LABORATORY, unpublished data.

SHEREX CHEMICAL COMPANY, INC. 1980. Sherex reports dioxane found in detergents, shampoos. Toxic Materials News, p. 14, January 9, 1980.

TAYLOR, A.J., G.M. Powell, D. Howes, J. Gordon Black and A.H. Olavesen. 1978. Metabolism of the surfactants sodium undecyltriethoxy sulphate and sodium dodecyltriethoxy sulphate in the rat. Biochem. J. 174:405-412.

YOUNG, J.D., W.H. Braun, L.W. Rampy, M.B. Chenoweth and G.E. Blau. 1977. Pharmacokinetics of 1,4-dioxane in humans. J. Toxicol. Environ. Health 3:507-520.

CHAPTER 5

Alkylphenol Ethoxylates

(APE)

ALKYLPHENOL ETHOXYLATES

Synopsis

Alkylphenol ethoxylates (APE) are one of several groups of chemical entities classified as nonionic surfactants. The practice of utilizing APE as components of domestic household products has been largely replaced by the use of more readily biodegraded nonionics, but APE 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.

There are presently no national criteria for levels of APE in waters of the United States. Since in most instances, analytical methods do not distinguish between specific classes of nonionics and/or their degradation products, it is not possible to ascertain which type of surfactant contributes to levels of total nonionics in waterways.

It is generally accepted that APE undergo primary biodegradation in a variety of test systems and field studies provided sufficient acclimation time is allowed. Laboratory studies indicate that significant primary biodegradation occurs within 3 to 10 days with straight-chain-derived APE and somewhat more slowly with branched compounds. The rate of ultimate biodegradability of APE, however, is slow in comparison to other classes of nonionic surfactants.

With respect to the toxicity of APE to aquatic fauna, laboratory studies indicate fish are more sensitive to the toxic effects of APE than mollusks

or crustaceans. Early growth stages of both vertebrate and invertebrate aquatic species appear to be more sensitive than adult members of the same species; higher temperatures also increase toxicity. Acute toxicity data indicate LC_{50} values are generally in the 4 to 12 mg/L range for fish and invertebrates. Sublethal effects such as erratic swimming, impaired locomotion, reduced burrowing, byssus and syphon activities have been reported in aquatic organisms at APE concentrations between 2 and 10 mg/L.

In relation to human safety, animal studies show a low order of acute and chronic oral toxicity with most APE with the exception of APE in a narrow molecular weight range (APE_{15}-APE_{25}); this latter group of compounds has been linked to an increased incidence of cardiotoxicity in dogs and guinea pigs, but not in rats following dietary exposures of relatively short intervals (5-14 days). Acute irritation studies indicate APE present no problem from accidental cutaneous or ocular exposure. No indications of carcinogenic activity were noted in chronic feeding studies with APE; no information is available on the effects of APE with respect to mutagenicity, teratogenicity or reproductive performance.

From currently available information, it appears that the use of APE does not pose a hazard to the environment or to human health. Some areas of uncertainly, however, such as findings of cardiotoxicity with certain APE, and the absence of mutagenicity and teratogenicity information, could be addressed by further investigation.

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).

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

ALKYLPHENOL ETHOXYLATES

I. INTRODUCTION

Alkylphenol ethoxylates (APE) are one of several groups of chemical entities classified as nonionic surfactants. The practice of utilizing APE as components of domestic household products has been largely replaced by the use of more readily degraded nonionics but APE still find considerable use in industrial and agricultural applications. Commercially, alkylphenols are manufactured by the addition of phenol to the double bond of an olefin in the presence of a catalyst such as boron trifluoride; the alkylphenol is purified by distillation, then reacted with several moles of ethylene oxide to produce APE. APE surfactants comprised less than 4% of all nonionics used in household cleaning applications during 1965.

Total production of all types of nonionics amounted to 1324 million pounds in 1978, an increase of 200 million pounds over the last five years (U.S. International Trade Commission, 1979). The percentage of nonionic surfactants actually used in household cleaning products cannot be determined from these Trade Commission figures, but as noted in our 1977 Report, APE surfactants comprise a very small portion of this volume.

BIBLIOGRAPHY

U.S. INTERNATIONAL TRADE COMMISSION, 1979. Synthetic Organic Chemicals. United States Production and Sales of Surface-Active Agents.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alkylphenol ethoxylates are one of several entities classified as nonionic surfactants and thus can be detected with many of the procedures utilized in the detection of AE (See Chapter 3).

B. Water Quality Standards

There are presently no national criteria for limiting nonionic surfactants in waters of the United States.

C. Nonionic Surfactants in Natural Water Bodies

Levels of nonionic surfactants, including APE, in the environment were discussed in the 1977 Report. Since in most instances, analytical methods do not distinguish between specific classes of nonionics and/or their degradation products, it is not possible to ascertain which type of surfactant contributes to levels of nonionics in waterways. Levels reported are almost always lower than anionics at the same sampling point. With respect to polyethylene glycol levels, a single study noted increases in several English rivers over an eight-year interval (1967-1974)

Alkylphenol ethoxylates are not presently being monitored, as such, in the United States.

III. BIODEGRADATION

It is generally accepted that APE undergo primary biodegradation in a variety of test systems and field studies provided sufficient acclimation time is allowed. Laboratory studies indicate significant primary biodegradation occurs within 3 to 10 days with straight-chain-derived APE and somewhat more slowly with branched compounds. The rate of ultimate biodegradability of APE, however, is slow.

A. Laboratory Investigations

Itoh et al. (1979) examined the biodegradability of a test solution of br-$C_9APE_{9.5}$ (20 mg/L) incubated under aerobic conditions at 27°C. Approximately 10% of the CTAS response was lost between days 3 and 10, but no evidence of ultimate degradation, as measured by evolved CO_2, was evident at 10 days.

Utilizing an activated sludge inoculum (30 mg/L), Miura et al. (1979) found that 100 mg/L of either $C_{12}APE_{8.5}$ or $C_9APE_{9.5}$ underwent complete primary biodegradation (100% loss of CTAS) by 5 days while TOC removal and BOD/TOD approached 50% between 5-10 days, when tested according to the specifications of Japanese regulations.

Schöberl and Mann (1976) investigated the biodegradation of C_9APE_9 (10 mg/L) at two temperatures (20-23°C, and 3-4°C) in both fresh and seawater. After 50 days at 20-23°C, the surfactant had biodegraded (loss of BIAS response) 33-36% in freshwater and 95% in seawater. At the lower ambient temperature, degradation in freshwater was the same (37%) but decreased in seawater (15%).

In laboratory shake-flask tests, highly branched C_8APE_{10} underwent essentially complete primary biodegradation (CTAS) in a period somewhat longer than 45 days. Studies of the ultimate biodegradability of this surfactant indicated less than 20% of theoretical CO_2 was evolved after 28 days (Scharer et al., 1979). In another shake-flask study with linear C_9APE_{10}, Davis et al. (1979) reported 27% biodegradation (as measured by a phosphomolybdic acid colorimetric method) after one week in sewage wastewater and 40% degradation in flasks inoculated with a mixed bacterial culture developed from soil and sewage organisms.

In a study of the degradation of APE by anaerobic organisms, Citernesi and co-workers (1976) reported between 50 and 75% adsorption of added C_{12-15} APE_9 (Nonidet P40™) or $C_{12-15}APE$ (Nonidet SH30™) to soil/water system after 15 days, but virtually no further change by 30 days. Analysis of the supernatant showed 90-100% removal (as measured by TLC) between 15-30 days; the combined disappearance of the surfactant from soil and the supernatant material was 83-84%.

B. Field Studies

Two field studies cited in the 1977 Report indicate APE undergo primary biodegradation under field conditions; i.e., greater than 90% removal (CTAS) of C_8APE_{10} was noted in an extended aeration-activated sludge sewage treatment plant after 20-44 days while 80% degradation (TLC method) of C_8APE_{8-9} occurred in a trickling filter sewage treatment plant.

No additional field studies have been found.

C. Effect of Chemical Structure

The rate of degradation of APE is influenced by: (1) the degree of branching -- less branching results in faster rate of degradation; (2) the number of ethylene oxide units/mole -- an increase in number slows the rate of degradation; and (3) the position of attachment of the benzene ring to the alkyl chain -- attachment to a primary carbon results in a faster rate of biodegradation than attachment to a secondary carbon.

D. Metabolic Pathways of Biodegradation

The major degradative pathway of alkylphenol ethoxylates appears to be shortening of the ethoxylate chain and some carboxylation of the alkyl chain, perhaps by ω-oxidation. The extent of further degradation of either the alkyl chain or the benzene ring is unknown.

BIBLIOGRAPHY

CITERNESI, U., R. Materassi and F. Favilli. 1976. [Interaction of synthetic detergents with anaerobic microorganisms in the soil]. Nuovi Annali d'Igeime e Microbiologia 27(1):39-49.

DAVIS, L., J. Blair and C. Randall. 1979. Mixed bacterial cultures break "non-biodegradable" detergent. Industrial Wastes 25(3):pp26, 27, 34, May/June.

ITOH, S., S. Setsuda, A. Utsunomiya and S. Naito. 1979. Studies on the biodegradation test method of chemical substances. II. Ultimate biodegradabilities of some anionic, nonionic, and cationic surfactants estimated by CO_2 production. Yakagaku 28(3):199-204.

MIURA, K., K. Yamanaka, T. Sangai, K. Yoshimura and N. Hayashi. 1979. Application of biological oxygen consumption measurement technique to the biodegradation test of surfactants. Yukagaku 28(5):351-355 (English translation).

SCHARER, D.H., L. Kravetz and J. B. Carr. 1979. Biodegradation of nonionic surfactants. Tappi 62(10):75-78.

SCHÖBERL, P. and H. Mann. 1976. Temperatur-EinfluB auf den biologischen Abbau nicht-ionischer Tenside in See-und SüBwasser.* [The influence of temperature on the degradation of non-ionic tensides in sea-and freshwater]. Arch. Fisch Wiss. 27(2):149-158.

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. Acute Toxicity to Fish

In the literature reviewed in the 1977 Report, short-term LC_{50} values for fish ranged from 1.3 mg/L C_8APE_{30} to 1080 mg/L C_9APE_4, both for the bluegill. However, most LC_{50} values were between 4 and 12 mg/L. Most of the APE surfactants tested had similar configurations (i.e., alkyl chain length of 8 or 9, ethoxylate chain of 8-10 units), although there was evidence that surfactants with shorter ethoxylate chains were more toxic. Other studies indicated greater sensitivity in fish in early life stages and at higher temperatures.

Acute toxicity data from more recent studies, as presented in Table 5-A, show a narrow range of LC_{50} values. The brown trout (Salmo trutta) was the most sensitive of the species tested, with a 96-hr LC_{50} of 1 mg/L C_9APE_{9-10} (Reiff et al., 1979), while the minnow (Phoxinus laevis) was least sensitive, with a 48-hr LC_{50} of 65 mg/L APE (Hamburger et al., 1977). All other species cited had acute values below 18 mg/L. The data were not extensive enough to permit comparisons of LC_{50} values under varying environmental conditions for the same species. However, Fischer and Gode (1978) reported that the "critical concentration range" was higher (12-20 mg/L) at 15°C than at 20°C (6-11 mg/L) for the golden orfe (Leuciscus idus melanotus), suggesting increased toxicity (or increased fish sensitivity) with increasing temperature.

TABLE 5-A ACUTE TOXICITY OF ALKYLPHENOL ETHOXYLATES TO FISH

Species	Surfactant	LC_{50} Concentration (mg/L ± 95% CL)**	Experimental Conditions	Reference
Harlequin fish (Rasbora heteromorpha)	C_9APE_{9-10} -99.9% active	8.6	Dynamic* 96 hr 20 mg/L hard., 20°	Reiff et al. (1979)
Brown trout (Salmo trutta)	C_9APE_{9-10}	1.0	26-30 mg/L hard., 15°, dynamic, 96 hr	
Golden orfe (Idus idus melanotus)	C_9APE_{9-10}	7.0 11.2	268 mg/L hard., 20° dynamic, Static, both 96 hr	
Goldfish (Carassius auratus)	C_9APE_9	18	48 hr	Tomiyama (1974)
Goldfish (Carassius auratus)	C_9APE_{10}	5.4	48 hr	Kurata et al. (1977)
Minnow (Phoxinus laevis)	APE	65	Static, 48 hr	Hamburger et al (1977)
Rainbow trout (Salmo gairdneri)	APE	4-6.3 2	Static, 48 hr Flow-through, 48 hr	
Golden orfe (Leuciscus idus)	APE	3.7->10	Static, 48 hr	
Cod (Gadus morrhua)	C_9APE_{10}	6	Flow-through, 96 hr 32-34 0/00 salinity, 4-16°	Swedmark et al. (1976)

*Continuous or intermittent replacement of test solution.

**95% Confidence Limits were not reported for any of these studies.

The toxicity of APE surfactants diminishes with time as a result of biodegradation, although it apparently occurs less rapidly than for other commonly used surfactants. Kurata et al. (1977) exposed goldfish (Carassius auratus) to C_9APE_{10} which had been maintained in river water for 4 days, and determined a 48-hr LC_{50} of 3.7 mg/L. The LC_{50} for the intact surfactant was 5.4 mg/L, indicating no biodegradation had occurred during the 4-day period. Reiff (1977) reported that an initial concentration of 20 mg/L APE became nontoxic to rainbow trout (Salmo gairdneri) after 70 days. The same concentration of AE, on the other hand, produced no toxicity after 10-14 days.

2. Acute Toxicity to Invertebrates

In the 1977 Report, LC_{50} values varied widely between species. Barnacle larvae (Balanus balanoides) were the least tolerant of the species tested, with a 96-hr LC_{50} of 1.5 mg/L C_9APE_{10}. Mosquitoes in the pupal and larval stages were extremely resistant to APE, with LC_{50}'s exceeding 400 mg/L. APE compounds with shorter ethoxylate chains and branched alkyl chains were the most toxic forms in tests with mosquito pupae. Spider crabs and barnacles in the larval stage were more susceptible to APE than during the adult phase, while the decapod Leander adspersus was more sensitive in the post-molt stage than during intermolt. The same study reported that higher temperatures increased the toxicity of C_9APE_{10} to mussels and clams. A 10 mg/L APE solution was found to decrease in toxicity to marine annelids over a period of 28 days.

Only one additional study was found that detailed the acute toxicity of APE to aquatic invertebrates. Swedmark, et al. (1976) reported a 96-hr LC_{50} of 12 mg/L C_9APE_{10} for the adult common mussel (Mytilus edulis) in a flow-through toxicity test.

3. Toxicity to Microflora

Various algae species exposed to APE exhibited toxic effects over a wide range of concentrations. Growth inhibition occurred at APE concentrations of 10-1000 mg/L in 12 species of marine phytoplankton; APE compounds with longer ethoxylate chains were less toxic. The growth of freshwater algae was inhibited at 20-50 mg/L APE. In one case, 50 mg/L APE stimulated the cell activity of blue-green algae.

No other information on APE toxicity to algae was found.

4. Sublethal and Chronic Toxicity

In the studies surveyed by the 1977 Report, a variety of sublethal effects in aquatic organisms was reported. At $C_9APE_{9.5}$ concentrations in excess of 5-6 mg/L, rainbow trout swam erratically, lost balance and eventually became immobilized. By comparison, the 96-hr LC_{50} value for this species was 10-12.5 mg/L. Invertebrates exhibited impaired locomotion and reduced burrowing activity, heart rate and syphon activity, at 0.5-100 mg/L C_9APE_{10}. During chronic exposures of 3 weeks duration, impaired swimming in crustaceans persisted at all concentrations greater than 10 mg/L.

Swedmark et al. (1976) reported sublethal effects of C_9APE_{10} to cod (Gadus marrhua) and common mussels (Mytilus edulis) exposed for 96 hours. Cod exhibited increased swimming activity, and avoidance reactions, as a result of exposure to 2 mg/L, and decreased swimming activity (impaired reactions) in 4 mg/L. The authors observed decreased byssal activity (formation of filament used for attachment) and shell closure ability in the mussel at 5 and 10 mg/L, respectively.

Patzner and Adam (1979) exposed two species of Plathelminthes worms, Dugesia gonocephala and Notoplana humilis, to APE (Hostapur CX^{TM}) in long-term tests. The threshold for lethal effects over 30 days was 3 and 1 mg/L for D. gonocephala and N. humilis, respectively.

5. Interactions with Dther Chemicals

The 1977 Report cited a study in which APE_{8-11} and LAS (DOBANE JNX^{TM}) acted synergistically in their foaming behavior. One APE compound, ORTHO HDD^{TM}, increased the rate of initial penetration of an insecticide into citrus leaves. Other studies reported that C_9APE_9 doubled the uptake of picloram by eucalyptus leaf disks, and that C_8APE_{10} increased phosphorus absorption by apple leaves by a factor of 7.

No other information on the chemical interactions of APE was found.

B. Effects of APE on Microorganisms

One study noted in the 1977 Report found that a 1000 mg/L solution of C_8APE slightly

inhibited the nitrification process in two soils tested. The bacterium, Aerobacter aerogenes was exposed to Macrocyclon™, a polyethylene glycol ether of p-tertoctylphenol -formaldehyde cyclic tetramer with 12 ethylene oxide residues per molecule; concentrations of 200 to 100,000 mg/L were toxic to 25% of the population.

Lamikanra and Allwood (1976) tested C_8APE_5 (Triton X-45™) and C_8APE_{10} (Triton X-100™) for their anti-bacterial action on Staphylococcus aureus. C_8APE_5 was the more toxic of the two surfactants, producing growth inhibition in the bacteria at concentrations as low as 0.0585 µM. The threshold for growth inhibition using C_8APE_{10} was 0.159 µM.

Higher concentrations of both surfactants brought about a complete inhibition of growth over the 6 hour observation period. The authors concluded that C_8APE_5 was more toxic because it was taken up more rapidly by the bacteria. Uptake rates did not increase when the concentration exceeded 0.468 µM C_8APE_5 or 0.795 µM C_8APE_{10}.

Hislop et al. (1977) conducted toxicity tests on a variety of microorganisms using different Triton™ surfactants. The most extensive testing was for the purpose of controlling apple scab (Venturia inaequalis). The following list shows the degree to which ascospore release was inhibited by dipping infected apple leaves into a 50,000 mg/L solution of various surfactants:

Surfactant	Ascospores Released (% of Control)
C_8APE_5 (Triton X-45™)	2.7
C_8APE_{7-8} (Triton X-114™)	16.7
C_8APE_{10} (Triton X-100™)	29.5

Surfactant	Ascopores Released (% of Control)
C_9APE (Triton N-101™)	7.4
C_8APE_{12-13} (Triton X-102™)	32.6
C_8APE_{70} (Triton X-705™)	3.3

The finding that toxicity is inversely related to ethoxylate chain length is consistent with data from other types of toxicity studies. Spotts and Feree (1979) found that neither C_8APE_{10} (Triton X-100™) nor C_9APE_6 (Triton N-57™) sprayed on dormant Cortland apple trees (Malus domestica) has any effect on powdery mildew, apple scab, or fruit set.

Low concentrations (5 mg/L) of C_8APE_5 (Triton X-45™) had no effect on the growth of various soil microorganisms, including gram-positive bacteria, yeasts, and various fungi cultured in agar. At concentrations of 50 mg/L C_8APE, growth was inhibited in all microorganisms except the yeasts (Hislop et al., 1977).

As part of the same study, Hislop et al. (1977) dipped earthworms (Lumbricus terrestris) into various 5,000 and 50,000 mg/L solutions of Triton X-45™, Triton X-100™ or Triton X-705™. Effects were observed only in worms exposed to 50,000 mg/L Triton X-705™ (C_8APE_{70}), with a 30% mortality rate over 20 days.

C. Effects of APE on Higher Plants

Various effects of surfactants on plants were noted in studies reviewed by the 1977 Report.

Leaf damage was noted in six crop plants treated with various APE surfactants, particularly with 10,000 mg/L solutions. Other effects included suppression of root elongation in cucumber seedlings, reduced pollen germination in the bitter-gourd, and cell damage in the beet at APE concentrations between 25 and 100 mg/L.

In experiments with sorghum (Sorghum bicolor), Horowitz and Givelberg (1979) exposed seedling roots and leaves to various concentrations of Agral 90™, a 92% active alkylphenol-ethylene oxide condensate. Significant growth reduction occurred in the shoots and roots of seedlings after 8 days of root exposure to a 10 mg/L solution of Agral 90™. After 24 hours in 10,000 mg/L Agral 90™, the roots leaked amino acids and inorganic ions such as Na, K, Ca, and Mg. An exposure period of 2-3 days in 10,000 mg/L produced wilting in the test plants.

Spotts and Ferree (1979) gave single spray treatments of C_9APE (Triton N-57™) or C_8APE_{10} (Triton X-100™) in 10,000-50,000 mg/L concentrations to various crop plants. In apple trees (Malus domestica), spray treatments of 30,000 and 50,000 mg/L of either surfactant produced a 2-5 day delay in bud break (compared to controls), and bud kills of 30-50%. The same concentrations were lethal to 100% of the buds on Concord grape vines; only C_9APE produced a bud delay in Aurore grapes (Vitus spp.). Both APE compounds caused a 67-100% bud kill in peach trees (Prunus persica) at concentrations of 10,000-50,000 mg/L. Among surviving peach buds, only C_9APE_6 (30,000 and 50,000 mg/L treatments) produced a delay in bud break of 2 days. Neither of the APE surfactants had any effect on pear buds (Pyrus spp.) at any of the concentrations tested.

Stolzenberg and Olson (1977) exposed the cut ends of excised barley plants (Hordeum volgare) to 150 mg/L C_8APE_{10} (Triton X-100™). Transpiration and photosynthetic oxygen evolution continued at 75% of the rate of controls when surfactant residues in the tissues reached $\leq$ 150 mg/g fresh weight.

The growth rate of cress seedlings grown on filter paper in 5,000 or 50,000 mg/L solutions was reduced by six APE surfactants tested by Hislop et al. (1977) (see IV-B). In addition, the authors sprayed 50,000 mg/L C_8APE_5 (Triton X-45™) on potting soil used to grow barley and peas. No effects on the seedlings were observed.

O. Mode of Action

According to the studies reviewed in the 1977 Report, the mode of action of APE varies depending on the organism. In fish, the gill lamellae are affected by edema and mucosis; in addition, there is a possible narcotic effect on motor control. In bivalves, ion balance, osmoregulation, and respiration have been disrupted by exposure to APE. Mosquito pupae may drown as a result of decreased surface tension; survival among larvae is higher because of their ability to respire through the cuticle.

The only additional information on APE mode of action was found in a study by Ottoson and Rydqvist (1978). Those authors examined the effects of C_8APE_5 (Triton X-45™) and C_8APE_{10} (Triton X-100™) on the stretch receptor neuron of the crayfish (Astacus fluviatilis). Concentrations of 38-50 mg/L of either surfactant inhibited the receptor potential of the neuron after 60-90

minutes; responsiveness returned to normal after replacement in normal saline water. This effect was attributed to a selective action on the sodium channels in the membrane of the receptor neuron.

BIBLIOGRAPHY

Fischer, W. K., and P. Gode. 1978. Comparative investigation of various methods of examining fish toxicity, with special consideration of the German golden orfe test and the iso zebra fish test. A. F. Wasser - und Abwasser Forschung 11 (3/4): 99-105 (English translation).

Hamburger, B., H. Haberling, and H. R. Hitz. 1977. Comparative tests on toxicity to fish using minnows, trout, and golden orfe. Arch. Fischerewiss. 28(1): 45-55 (English abstract).

Hislop, E. C., V. M. Barnaby and R. T. Burchill. 1977. Aspects of the biological activity of surfactants that are potential eradicants of apple mildew. Ann. Appl. Biol. 87:29-39.

Horowitz, M. and A. Givelberg. 1979. Toxic effects of surfactants applied to plant roots. Pestic. Sci. 10:547-557.

Kurata, N., K. Koshida and T. Fujii. 1977. Biodegradation of surfactants in river water and their toxicity to fish. Yakagaku 26(2):115-118.

Lamikanra, A. and M. C. Allwood. 1976. The antibacterial activity of non-ionic surface-active agents. Microbios Letters 1:97-101.

Ottoson, D. and B. Rydqvist. 1978. The effects of Triton-detergents on the stretch receptor of the crayfish. Acta physiol. scand. 103:9-18.

Patzner, V. M. and H. Adam. 1979. Influence of tensids on the regeneration of Dugesia gonocephala (Plathelminthes, Tricladida) and Notoplana humilis (Plathelminthes, Polycladia). Zool. Anz., Jena 202(3/4, 5):199-208.

Reiff, B., R. Lloyd, M. J. How, D. Brown and J. S. Alabaster. 1979. The acute toxicity of eleven detergents to fish: Results of an interlaboratory exercise. Water Res. 13:207-210.

Reiff, B. 1979. VIIth International Congress on Surface Active Substances. Moscow. September 1979 (1977). (As cited in Scharer et al., 1979).

Scharer, D. H., L. Kravetz and J. B. Carr. 1979. Biodegradation of nonionic surfactants. Tappi 62(10):75-76.

Spotts, R. A. and D. C. Ferree. 1979. Effect of a dormant application of surfactants on bud development and disease control in selected deciduous fruit plants. Hort. Science 14(1):38-39.

Stolzenberg, G. E. and P. A. Olson. 1977. Behavior and fate of ethoxylated alkyl-phenol detergents in barley plants, in Abstract of Papers, 173rd ACS Meeting, New Orleans, March 20-25.

Swedmark, M., A. Granmo and S. Kollberg. 1976. Toxicity testing at Kristinberg Marine Biology Station, in: Pollutants in the Aquatic Environment. FAO/SIDA/TF 108 Suppl. 1.

Tomiyama, S. 1974. Effects of surfactants on fish. Bull. Jpn. Soc. Sci. Fish. 40(12):1291-1296.

V. HUMAN SAFETY

The data available for the 1977 Report indicated that the use of alkylphenol ethoxylates did not appear to pose a significant risk to human health. However, some areas of uncertainty (e.g., cardiotoxicity of a narrow molecular weight range of APE, carcinogenicity, mutagenicity and teratogenicity) required further investigation.

A. Animal Studies

A low order of acute and chronic oral toxicity is noted in experimental animals with APE, with the exception of APE in a narrow molecular weight range (APE_{15}-APE_{25}); this latter group of compounds has been linked to an increased incidence of cardiotoxicity in dogs and guinea pigs following dietary exposures of relatively short intervals (5-14 days). Oral LD_{50} values in rats range from about 1000 to 30,000 mg/kg, depending on the length of the ethylene oxide adduct. Ocular effects also exhibit a structure dependence for irritant potency with highest toxicity noted with surfactants containing 8-10 ethylene oxide units. Acute irritation studies, however, suggest that APE present no substantial problem from accidental cutaneous or ocular exposure. Chronic feeding studies with rats and dogs fed 1000 mg/kg/day for two years indicated no significant toxicity.

Acute Toxicity.

Acute Irritation - Skin, - Ocular

Subchronic/Chronic Toxicity

No new acute, subchronic or chronic studies have been conducted with APE since the 1977 Report. Similarly, no data were found on APE surfactants in the areas of acute skin and eye irritation or skin sensitization.

Carcinogenicity.

No indications of carcinogenic activity were noted in chronic feeding studies with APE. A single study reported a cocarcinogenic effect for C_9APE (2000 mg/L) added to the drinking water of rats in the induction of gastric tumors by N-methyl-N'-nitro-N-nitrosoguanidine; however, it is unclear whether this effect is due to enhanced carcinogen absorption or some other physiological mechanisms.

Mutagenicity.

No information is available on the mutagenicity of APE.

Teratogenicity/Reproduction Studies.

No data are available on the effects of APE exposure either to the conceptus or on the reproductive performance of experimental animals.

No data on the carcinogenic, mutagenic or teratogenic activity of APE surfactants have been published since the 1977 Report.

Pharmacology - Absorption and Metabolism.

Absorption and metabolism studies indicate that both rats and dogs excrete about 90% of ingested APE within 72 hours. Reduced urinary excretion with a reciprocal increase in fecal excretion is observed with increasing ethylene oxide adduct length.

The principal urinary metabolites are mono- and dicarboxylic acids of polyethylene glycol and the glucuronic acid conjugate of the alkyl-phenol.

Pharmacology - Cardiotoxicity.

APE in a narrow molecular weight range (APE_{15}-APE_{25}) have been linked to an increased incidence of cardiotoxicity in dogs and guinea pigs. Focal cardiac necrosis was reported in dogs fed 40 mg/kg/day C_9APE_{20} for 90 days with lesions evident within 5 days at higher doses. Rats, however, fed 5000 mg/kg/day under the same treatment regimen exhibited no cardiac pathology.

B. Human Studies

Skin irritation studies with APE materials containing 1 to 13 ethylene oxide units indicated neither primary irritation following 48-hour patch tests nor sensitization upon subsequent challenge two weeks later.

Vontver et al. (1979) recently examined the effects of topical treatment of culture-proved genital herpes simplex virus in 37 men and 32 women with a cream containing Nonoxynol-9™ (C_9APE_9). The cream was applied five times a day until the lesions healed or for a maximum of seven days. Female patients also inserted the medication into the vagina each evening. No adverse effects were noted nor did the treatment result in any inhibitory effect on the course of genital herpes or on new lesion formation.

BIBLIOGRAPHY

VONTVER, L.A., W. C. Reeves, M. Rattray, L. Corey, M. A. Remington, E. Rolentino, A. Schweid, K.K. Holmes. 1979. Clinical course and diagnosis of genital herpes simplex virus infection and evaluation of topical surfactant therapy. Am. J. Obstet. Gynecol. 133(5):548-554.

CHAPTER 6

Alpha Olefin Sulfonates

(AOS)

ALPHA OLEFIN SULFONATES

Synopsis

Alpha olefin sulfonates (AOS) are one of several groups of chemical entities classified as anionic surfactants. AOS surfactants possess good detergency and foaming characteristics in hard water. Some 10,000 metric tons of AOS were consumed in 1976.

At present, there are no water quality criteria for limiting AOS in surface waters of the United States and levels of AOS, as such, are not presently being monitored in the United States or Europe. MBAS measurements, however, would include AOS surfactants, if present. Biodegradation studies with AOS are somewhat limited, but the data available indicate AOS are readily biodegraded under both laboratory and environmental conditions.

Recent studies support earlier findings that the acute aquatic toxicity of AOS increases with increasing alkyl chain length. LC_{50} values range from 0.3 mg/L to 21 mg/L. Insufficient data exist to establish the extent of AOS toxicity to juvenile life stages of aquatic organisms, higher plants or microorganisms. Toxicity to fish appears to be directly related to changes in the interfacial tension between gills and water.

AOS surfactants exhibit a moderately low order of toxicity in rodents. No adverse effects were observed in rats fed 1000 mg/kg/day for 90 days or 5000 mg/kg of diet for two years. AOS concentrations up to 1% are not ocular

irritants in rabbits, but concentrations greater than 5% are capable of producing reversible mild to severe ocular irritation. Acute skin irritation studies in rabbits range from slight to severe skin irritation; AOS concentrations of 1-2% produce negligible to mild irritancy in humans.

Long-term studies of the possible carcinogenicity of AOS by oral and percutaneous routes have been negative. In addition, a number of in vitro and host-mediated mutagenicity tests have produced negative results. A single set of experiments with one commercial sample showed a positive response in a host-mediated assay with rats; however, chemical fractionation studies suggest that this response may be due to materials having no direct relationship to the surfactant.

An increase in cleft palates was seen in offspring of mice given 300 mg/kg/day AOS by gavage 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 in which the dams exhibited toxic responses.

Although data on the reproductive effects of AOS and by-products are limited, all of the human safety information presently available suggests that normal use levels of AOS do not pose either an environmental hazard or a significant risk to human health.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

I. INTRODUCTION

Alpha olefin sulfonates (AOS) are one of several groups of chemical entities classified as anionic surfactants. They possess good detergency and foaming characteristics in hard water and thus, are useful surfactants for heavy duty powder detergents of low phosphate formulation. AOS are commercially prepared by direct sulfonation of n, α-olefins with a dilute stream of vaporized sulfur trioxide 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. AOS have thus proved difficult to characterize chemically, and commercial AOS products contain a mixture of alkene sulfonates, hydroxyalkane sulfonates and disulfonates. 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. 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.

Matson (1978) reports that 10,000 metric tons of AOS, evenly divided between the United States and Western Europe, were consumed during 1976.

BIBLIOGRAPHY

MATSON, T.P. 1978. The workhorse surfactants: LAS, alcohol sulfates and ether sulfates. J. Am. Oil Chem. Soc. 55:66-69.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

As anionic surfactants, alpha olefin sulfonates can be detected with many of the procedures utilized in the detection of LAS (See Chapter 1). As with LAS, the mainstay for the determination of presumptive levels of AOS in the environment has been the MBAS method and, therefore, is subject to the inherent limitations of this procedure. In addition, the 1977 Report discussed several commonly used physicochemical analyses which, with slight modifications in experimental procedures, have been adapted to AOS determinations.

A simple, rapid method for the quantitative determination of alkene-1-sulfonates, the principal components of commercial AOS products, without interference from hydroxyalkane-1-sulfonates also present in the commercial mixture has recently been developed (McClure, 1978). The analysis consists of titrating the sample in 98% acetic acid-2% water solution with a stream of ozone to a photometric endpoint using Rouge Organol BS indicator. The presence of both unreacted olefins and sodium alkene-1-sulfonates is detected with this method. An extraction-GC process is utilized to distinguish the typically small amounts of unreacted olefins present, and the alkene-1-sulfonate content is determined by difference. Duplicate results were reported not to differ from the mean by more than 1% for samples containing 0.50 to

1.50 meq/g of unsaturation. Comparison of analyses of a known sample by ozone titration and NMR gave values which were within 2% of each other.

Three papers have also reported the use of physicochemical techniques for the analysis of residual amounts of sultones which may be present in AOS (Slagt et al., 1976; Wolf and McPherson, 1976; Callahan et al., 1976). Slagt and co-workers (1976) described a high pressure liquid chromatographic method for the quantitative determination of both 1,3- and 1,4-sultones with a lower limit of detection of 10 mg/kg. Duplicate determinations of a known sample gave standard deviations of 8-9 mg/kg at a level of 300 mg 1,4-sultone/kg. The reliability of the method was tested by the addition of known amounts of 1,3- and 1,4-sultones. All of the added 1,4-sultone was recovered quantitatively, even when extracted 11 days after spiking. The 1,3-sultone, which is far less stable, was not recovered quantitatively; 85% was recovered one hour after spiking and only 45% of the added amount was detected after 11 days, the rest probably being decomposed.

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

Callahan et al. (1976) have developed a gas chromatographic method specifically for the analysis of C_{14} delta, unsaturated gamma and 2-chloro gamma sultones but the method has been used for other sultones. The method is reportedly capable of detecting less than 1 ppm of these sultones in a 38% AOS solution. In recovery studies with C_{14} delta sultone, a standard deviation of ± 2 ppm was observed at 10-30 ppm level of sultone.

B. Water Quality Standards

There are presently no water quality criteria for concentrations of alpha olefin sulfonates in surface waters of the United States.

C. AOS in Natural Water Bodies

AOS are not presently being monitored as such in the United States or Europe. If present, AOS surfactants, as well as other anionics, would be included in MBAS measurements.

BIBLIOGRAPHY

CALLAHAN, J., L. Gildenberg and N. Omelczenko. 1976. Gas chromatographic analysis for long chain sultones in olefin sulfonates. J. Amer. Oil Soc. 54:343-346.

McCLURE, J.D. (1978). The determination of alkene sulfonates in olefin sulfonates by ozone titration. J. Amer. Oil Chem. Soc. 55:905-908.

SLAGT, C., W.G.B. Huysmans and A.W.J. Raaijmakers. 1976. The determination of sultones in α-olefin sulphonates by gas and high pressure liquid chromatography. Tenside Detergents 13(4):185-187.

WOLF, T. and B.P. McPherson. 1976. Analysis of long chain sultones by thin layer chromatography. J. Amer. Oil Chem. Soc. 54:347-350.

III. BIODEGRADATION

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

A. Laboratory Investigations

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

With an activated sludge inoculum, Miura et al. (1979) found that the MBAS response to 100 mg/L AOS virtually disappeared by 15 days while removal of TOC approached 90% by 8 days.

In another study, Itoh et al. (1979) reported complete biodegradation (100% loss of MBAS response) of C_{12}AOS and approximately 30% of theoretical CO_2 evolved by 10 days.

Utilizing a modified shake-flask test with secondary wastewater effluent as inocula, Kravetz and coworkers (1977) determined the primary and ultimate biodegradability of a series of single carbon cut ADS (C_{12},C_{14},C_{16},C_{18}). The

tested AOS samples were found to undergo 98-99% primary biodegradation (as measured by M8AS) within 3 days. Alkyl chain length was noted to affect the rate of ultimate biodegradation of the samples, however. Although C_{12}AOS and C_{14}AOS degraded similarly (~65% evolved CO_2 by 30 days), increasing the alkyl chain from C_{14} to C_{18} decreased the rate and amount of evolved CO_2. All AOS test samples, however, reached greater than 50% mineralization within two weeks; 75 to 80% of a glucose sample was mineralized in the same time period.

B. Field Studies

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

No additional field studies have been found.

BIBLIOGRAPHY

ITOH, S., S. Setsuda, A. Utsunomiya and S. Naito. 1979. Studies on the biodegradation test method of chemical substances. II. Ultimate biodegradabilities of some anionic, nonionic, and cationic surfactants estimated by CO_2 production. Yakagaku 28(3):199-204.

KRAVETZ, L., H. Chung and J.C. Rapean. 1977. Ultimate biodegradation studies of alpha olefin sulfonates. Paper presented at the AOCS 68th Annual Spring Meeting, New York, New York, May 9-12.

MIURA, K., K. Yamanaka, T. Sangai, K. Yoshimura and N. Hayashi. 1979. Application of biological oxygen consumption measurement technique to the biodegradation test of surfactants. Yukagaku 28(5):351-355. (English translation).

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. Fish

In the 1977 Report, reported LC_{50} values for AOS ranged from less than 0.3 mg/L to 21 mg/L. In toxicity tests with bluegill, 24-hour LC_{50} values for dimer olefin sulfonate and vinylidine AOS were 97 mg/L and 58 mg/L, respectively. Mortality after the first 24 hours was higher under continuous-flow than static conditions, indicating possible surfactant biodegradation or absorption in static toxicity tests. Longer-chain AOS were consistently more toxic than those with shorter alkyl chains.

Acute toxicity data from more recent studies are summarized in Table 6-A. The range of LC_{50} values from these reports is 0.5 mg/L to 5.7 mg/L; of the species tested, harlequin fish were the most sensitive. The data of Reiff et al. (1979) support the earlier finding that AOS surfactants with longer alkyl chains are more toxic; i.e., for C_{16-18}AOS, the LC_{50}'s were generally 4-10 times lower than for C_{14-16}AOS.

2. Invertebrates

Daphnia magna was the only invertebrate for which toxicity data were found in the literature surveyed for the 1977 Report. LC_{50}

TABLE 6-A. ACUTE TOXICITY OF AOS TO FISH

Species	Surfactant	Concentration (mg/L)‡	Effects	Experimental Conditions	Reference
Harlequin fish (Rasbora heteromorpha)	C_{14-16} AOS 90% active *	3.3	LC_{50}	20 mg/L hardness 20°C, dynamic,** 96 hr	Reiff et al. (1979)
	C_{16-18} AOS 90% active	0.5	LC_{50}	"	
Brown trout (Salmo trutta)	C_{14-16} AOS 90% active	2.5-5.0	LC_{50}	26-30 mg/L hardness 15°C, dynamic, 96 hr	
	C_{16-18} AOS 90% active	0.5	LC_{50}	"	
Golden orfe (Idus idus melanotus)	C_{14-16} AOS 90% active	4.9	LC_{50}	268 mg/L hardness, 20°, dynamic, 96 hr	
		3.4		268 mg/L hardness, 20°C, static, 96 hr	
		5.7		150 mg/L hardness, 20°C, static, 48 hr	
Golden orfe (Idus idus melanotus)	C_{16-18} AOS	1.0	LC_{50}	268 mg/L hardness, 20°C, dynamic, 48 hr	
		0.9		268 mg/L hardness, 20°C static, 96 hr	
		1.9		150 mg/L hardness, 20°C, static, 48 hr	

*Percent by weight of the surfactant component of the formulation.
**Dynamic refers either to continuous or intermittent replacement of the test solution.
‡Expressed as active material. No 95% confidence limits were reported for any of the cited studies.

TABLE 6-A (Continued)

Species	Surfactant	Concentration (mg/L)	Effects	Experimental Conditions	Reference
Japanese himedaka	AOS	3	LC_{50}	48 hr 0 mg/L $CaCO_3$ hardness	Tomiyama (1978)
		1		10 "	
		1		20 "	
		1		50 "	
		1		100 "	
		0.5		500 "	
Golden orfe (Leuciscus idus melanotus)	50-50 mixture of olefin sulfonate and succinic acid mono-sulpho-ester	5	LC_{20}	48 hr	Mann and Stach (1974)
		6	LC_{100}	48 hr	
Rainbow trout (Salmo gairdneri)		7.5	LC_{50}	24 hr	"

values ranged from 7.0 mg/L for C_{16-18}AOS to 18.0 mg/L C_{14-16}AOS. Vinylidene AOS was the most toxic, however, and caused 50% mortality in 24 hours at a concentration of 2.47 mg/L.

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

3. Sublethal and Chronic Toxicity

The hatching of fathead minnow eggs was reduced by 60-70% (compared to controls) as a result of exposure to 7.5 mg/L C_{14-16}AOS, while a 3.2 mg/L concentration caused 100% mortality in hatchlings. No mortality was observed at 1.8 mg/L of the surfactant. Midges were exposed continuously to several concentrations of C_{14-16}AOS through two life cycles. Survival was decreased by 24-52%, compared to controls, in two tests at 9.0 mg/L. Second generation survival was also reduced at 9.0 mg/L, while a 4.5 mg/L concentration had no effect on either generation.

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

4. Interactions with Other Chemicals

One study reviewed in the 1977 Report found no conclusive evidence of synergism between linear tridecyl benzene sulfonate and C_{14-16} AOS or C_{16-18}AOS when tested on bluegill.

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

The data of Tomiyama (1978) indicate that the 48-hour LC_{50} value for Japanese himedaka decreases slightly as water hardness (as $CaCO_3$) increases (See Table 6-A). The author attributed this trend to increased uptake rates and complex formation in harder water; it is unclear, however, if the reduction in toxicity is significant.

B. Effects of AOS on Higher Plants

The 1977 Report reviewed one study dealing with the effects of AOS on seed germination in tomato, barley, and bean plants. Watering solutions of 10, 25, or 40 mg/L AOS had no significant effect on germination and growth when compared to control plants.

No additional studies on AOS toxicity to plants were found.

C. Toxicity of AOS to Microorganisms

The only information available on AOS toxicity to microorganisms is a study with Escherichia coli incubated on gelatin media. An AOS concentration of 150 mg/L was the lowest level which limited E. coli growth to 5 colonies per plate.

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

D. Mode of Action

The studies reviewed in the 1977 Report identify the gills as the primary site of toxic action by AOS. Toxicity is directly related to changes in interfacial (between gill and water) tension; it was postulated that oxygen absorption is severely hindered when the tension decreases beyond a certain critical point. Another investigation cited protein complexing between dissolved surfactants and gill surface tissues as the primary mode of action.

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

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

BIBLIOGRAPHY

LUNDAHL, P., and R. Cabridenc. 1976. A focus on a method of study of the evolution of the toxicity of pollutants during biodegradation as applied to surfactants. J. Fr. Hydrol. 7, 3, #21:143-149. (English translation).

MANN, V.H., and H. Stache. 1974. Effect of a mixture of succinic acid mono-sulpho-ester and olefin sulfonate on fish. Arch. Fisch. Wiss. 25(1/2): 53-56. (English abstract).

REIFF, B., R. Lloyd, M.J. How, D. Brown, and J.S. Alabaster. 1979. The acute toxicity of eleven detergents to fish: Results of an interlaboratory exercise. Water Res. 13:207-210.

TOMIYAMA, S. 1974. Effects of surfactants on fish. Bull. Jpn. Soc. Sci. Fish. 40(12):1291-1296. (English abstract).

TOMIYAMA, S. 1978. On the possible mechanism of the effect on fishes and shell-fishes by household detergents. Yukagaku 27(b):347-353. (English translation).

V. HUMAN SAFETY

Data available for evaluation for the 1977 Report suggested that normal use of AOS surfactants did not pose a significant risk to human health. However, the lack of reported data on the teratogenic/reproductive effects of AOS and skin irritancy of AOS and by-products of AOS manufacture suggested a need for further studies.

A. Animal Studies

AOS exhibit a moderately low order of toxicity in both rats and mice. Acute oral LD_{50} values in mice are higher (2500 to > 4000 mg/kg) than intravenous, intraperitoneal or subcutaneous values ($\leq$ 1600 mg/kg), indicating either a low rate or incomplete absorption, or rapid metabolism and elimination. No adverse effects were noted either in rats fed 1000 mg/kg/day AOS in the diet for 90 days or rats given 5000 ppm AOS in the diet for two years (mean daily intake: 195 mg/kg males; 259 mg/kg females). Rats also appeared to tolerate well both repeated dermal and inhalation exposures to AOS.

AOS are slightly to severely irritating to rabbit skin but data are inconsistent, probably due to such factors as purity, method of production, etc. Skin sensitization studies in guinea pigs indicate AOS are generally non-sensitizers; a few positive responses were either unexplainable, attributed to incomplete hydrolysis of the product or irreproducible. AOS concentrations of 1% are not ocular irritants in rabbits but concentrations greater than 5% are capable of producing reversible, mild to severe ocular irritation.

Acute Toxicity.

Acute Irritation - Ocular, Skin.

Skin Sensitization.

No additional information has been published in these areas since the 1977 Report.

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

Subchronic/Chronic Toxicity. No new long-term studies have become available since our 1977 Report.

Carcinogenicity.

> *Two studies cited in the 1977 Report indicated negative carcinogenic responses in rodents from AOS exposure. No increased incidence of tumors was recorded for CFX rats fed up to 5000 ppm C_{14-16}AOS in the diet for 2 years and no adverse effects were noted in Swiss Webster mice after 2 years of twice-weekly percutaneous applications of 5% aqueous solutions of either C_{15-18}AOS, hexadecane-1,4-sultone or a sultone concentrate (64% active) extracted from the process stream during the sulfonation process.*

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

(1) deionized water (vehicle controls)
(2) essentially hydrolyzed, composite sample of C_{14-16}AOS and C_{16-18} AOS (30.0% active)
(3) partially hydrolyzed sample of AOS (30.9%) identical to (2) but containing a residual level of sultone
(4) commercial C_{14-16}AOS (38.9% active)

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

A dermal carcinogenicity study has also been conducted with Swiss Webster mice. Groups of mice (40/sex/group) were treated three times a week for 92 weeks with 0.02 ml of test materials applied to the shaved

interscapular surface. The study contained six treatment groups: 20% C_{14-18}AOS, 25% C_{14-18}AOS, 20% C_{14-16}AOS, 25% C_{14-16}AOS, 6.7% C_{16}-1,4(delta) sultone, 8.3% C_{16}-1,4-sultone. There were also untreated, water and acetone control groups. A brief summary of the study (no data reviewed) stated that no significant toxicity or histopathology attributable to treatment was found (International Alpha Olefin Sulfonate Group, unpublished data).

Mutagenicity.

A number of in vitro and host-mediated mutagenicity tests have produced negative results. A single set of experiments showed a positive response in a host-mediated assay with rats; however, the response may be due to materials having no direct relationship to the surfactant.

Teratogenicity/Reproduction Studies

There are no data available on the effects of AOS on reproductive performance in laboratory animals. With respect to teratogenic effects, an increase in cleft palates was found in offspring of mice given 300 mg/kg of AOS by gavage during gestation as well as an increased incidence of minor skeletal anomalies in both mice and rabbits at this dosage level. These responses generally occurred in groups in which the dams exhibited toxic responses. No adverse effects were noted, however, in pregnant rats or their offspring following administration of 600 mg/kg AOS during gestation.

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

B. Human Studies

Negligible to mild skin irritation was observed in human volunteers in 24-hour patch tests with 1-2% active AOS samples. In another study, increased irritation was noted as the study progressed in a 10 day occlusive patch test with a 0.8% active concentration of AOS. The evidence indicates that AOS surfactants are not skin sensitizers in humans.

BIBLIOGRAPHY

COLGATE PALMOLIVE COMPANY, unpublished data

IMOKAWA, G. 1979. Study on skin-irritating and biological properties of monoalkyl phosphate anionic surfactants. J. Am. Oil Chem. Soc. 56(5): 604-609.

INTERNATIONAL ALPHA OLEFIN SULFONATE GROUP, unpublished data.

MINEGISHI, K.I., M. Osawa and T. Yamaha. 1977. Percutaneous absorption of α-olefin sulfonate (AOS) in rats. Chem. Pharm. Bull. (Tokyo) 25(4): 821-825.

SOAP AND DETERGENT ASSOCIATION, unpublished data.

Chapter 7

Secondary Alkane Sulfonates (SAS)

SECONDARY ALKANE SULFONATES

Synopsis

The production and use of the anionic secondary alkane sulfonates (SAS) are largely limited to Western Europe where they are principally used as components of liquid surfactant formulations; some 50,000 metric tons of SAS were consumed in 1976.

There are presently no water criteria for SAS in either the United States or Europe. Levels of SAS, as such, in surface waters are not presently being monitored, but MBAS measurements would include SAS, if present. The contribution of SAS to MBAS levels in the United States is negligible since these surfactants are not extensively used in this country.

The limited information available on the biodegradability of SAS surfactants suggests that they are readily biodegraded under both field and laboratory conditions. Alkyl chain length does not appear to influence the rate of biodegradation but reduced ambient temperatures do retard the degradation rate, as with all materials.

Human safety and aquatic toxicity data are scant and do not allow any general conclusions to be drawn in terms of environmental acceptability or human safety of SAS surfactants. The limited data available, however, suggest no significant hazards to either man or the environment.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

I. INTRODUCTION

The production and use of anionic secondary alkane sulfonate (SAS) surfactants are largely limited to the European continent. Due to their high water solubility characteristics, SAS are principally utilized as components of liquid surfactant formulations. Predominantly linear with the sulfonate group attached to a secondary carbon and randomly positioned along the carbon chain, commercial SAS are produced via a sulfoxidation reaction with n-paraffins in the C_{14} - C_{18} range. The final product generally contains 85-87% alkane monosulfonate, 7-9% alkane disulfonate, <5% sodium sulfate and 1% unreacted paraffins.

Matson (1978) reports that large quantities of SAS are available only in Western Europe which consumed 50,000 metric tons of SAS in 1976.

BIBLIOGRAPHY

MATSON, T.P. 1978. The workhorse surfactants: LAS, alcohol sulfates and ether sulfates. J. Am. Oil Chem. Soc. 55:66-69.

II. ENVIRONMENTAL LEVELS

A. Analytical Methods

Alkane sulfonates 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).

Takeshita et al. (1976) developed a thin-layer chromatographic method for separating C_4 - C_{18} SAS and C_1 - C_{14} ABS. Basically, the process involves separation of the surfactants on polyamide TLC plates with aqueous ammonia-pyridine (SAS with carbon chains $< C_{14}$; ABS $< C_{10}$) or aqueous ammonia-pyridine-methanol systems (SAS $> C_{14}$; ABS $> C_{10}$). The surfactants are detected as yellow fluorescent spots by spraying with pynacryptol yellow reagent followed by observation under UV light. Limits of detection ranged from 0.05 to 2 µg for SAS and 0.1 to 2 µg for ABS depending on the carbon chain length of the surfactant and the solvent system used.

B. Water Quality Standards

There are presently no water quality criteria in the United States or Europe specifically for secondary alkane sulfonates (SAS). These anionic surfactants are included among those measured in the environment using the MBAS method.

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 I. 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.

BIBLIOGRAPHY

TAKESHITA, R., N. Jinnai and H. Yoshida. 1976. Detection of sodium alkanesulphonates and alkylbenzenesulphonates by polyamide thin-layer chromatography. Journal of Chromatography 123:301-307.

III. BIODEGRADATION

The limited information available on SAS surfactants indicates that they are readily biodegraded under both field and laboratory conditions.

A. Laboratory Investigations

Available data indicate SAS are readily biodegraded; BOD values ranged from 20 to 56% of theoretical at 5 days for SAS with 13 to 18 carbon atoms and exceeded 70% of theoretical oxygen demand by 20 days. Complete primary biodegradation (loss of MBAS response) is generally seen within 2-3 days in various die-away tests. Alkyl chain length does not appear to influence the rate of biodegadation, but reduced ambient temperatures do retard the degradation rate, as occurs with all materials. Metabolically, alkane sulfonates appear to be desulfonated and subsequently degraded via β-oxidation.

The mineralization of 5-10 mg of uniformly labelled ^{14}C-sec-n-heptadecanesulfonate at 20° C was studied by Lötzsch and co-workers (1979) in a manometric BOD apparatus (Hach apparatus) inoculated with 30,000 colony-forming bacteria (presumably from a sewage treatment plant but not explicitly stated). Formation of $^{14}CO_2$ began within 24 hours (17% of radioactivity) and reached a value of 60.8% by 12 days. Approximately 30% of the total radioactivity added was associated with the bacteria and 10% remained in the nutrient solution at 12 days.

In a modified BOD "one-pot" process using sewage water from a sewage treatment plant as the inoculum n-C_{13-17} SAS (1% < C_{13}; 58% C_{13-15}; 39% C_{16-17}; 2% > C_{17}), incubated in the dark at 20° C, lost 75% of the MBAS response after

5 days; removal of DOC was 60% and BOD was 13% of theoretical. The quantity of surfactant used corresponded to a carbon concentration of 5 mg/L. By 10-15 days, complete primary biodegradation (100% loss of MBAS response) had occurred and BOD was 85% of the oxygen needed for complete oxidation of SAS. The elimination of DOC, a measure of the progressive mineralization of SAS by the microorganisms, reached a value of 70% by 10 days, 85% after 15 days and 90% at 30 days (Lötsch et al., 1979).

B. Field Studies

Two field studies conducted in trickling filter sewage treatment plants indicate extensive biodegradation of SAS occurs, even during the winter months.

No additional field studies have been reported since the 1977 Report.

BIBLIOGRAPHY

LÖTZSCH, K., A. Neufahrt and G. Täuber. 1979. Comparative studies on biodegradation of secondary alkanesulfonates using ^{14}C-labelled preparations. Tenside Detergents 16(3):150-155 (English translation).

IV. ENVIRONMENTAL SAFETY

A. Aquatic Toxicity

1. Fish

The literature surveyed in the 1977 Report included toxicity tests for SAS on four species of freshwater fish. The LC_{50} values for the bluegill sunfish, Lepomis macrochirus, ranged from 1.3 mg/L for C_{18} SAS to 144 mg/L for C_{13} SAS. Other species of fish had LC_{50}'s from 3.1 mg/L to 23.6 mg/L. Two studies reported a distinct positive correlation between alkyl chain length and toxicity.

From 24-hour acute toxicity tests with the freshwater fish, Phoxinus phoxinus, Lundahl and Cabridenc (1978) found the highest LC_{50} values corresponded with the SAS surfactant with the shortest alkyl chain length; i.e., toxicity decreased with decreasing alkyl chain length (See Table 7-A).

2. Invertebrates

One study reviewed in the 1977 Report observed 24-hour LC_{50} values for Daphnia magna from 9.1 mg/L for C_{13-17} SAS to 282 mg/L for C_{12} SAS. Again, toxicity increased substantially with increasing alkyl chain length.

Table 7-A. Toxicity of Various SAS Surfactants to Aquatic Organisms

SAS Chain Length	Phoxinus phoxinus 24-hr LC_{50} (mg/L)	Daphnia magna 24-hr EC_{50} (immob.) (mg/L)	Chlamydomonas variabilis 24-hr EC_{50} (immob.) (mg/L)
$C_{10.3}$	-	319	125
$C_{11.2}$	-	133	74.9
C_{14}	34.5	111	32.4
C_{15}	8.5	34.2	15.8
C_{16}	3.1	30.1	9.4
C_{17}	-	12.3	3.9
$C_{18.9}$	-	3.3	3.7
$C_{20.7}$	-	6.3	8.4

Lundahl and Cabridenc (1978)

Lundahl and Cabridenc (1978) exposed Daphnia magna to eight SAS compounds with varying alkyl chain lengths. The resultant 24-hour EC_{50} values for immobilization ranged from 3.3 mg/L for $C_{18.9}$ SAS to 3.9 mg/L for $C_{10.3}$ SAS (see Table 7-A). As noted with several other surfactant classes, increasing SAS toxicity is associated with increasing chain length up to a point. However, at $C_{20.7}$, the toxicity apparently begins to decrease.

In tests with Daphnia magna and brine shrimp (Artemia salina), Danvila (1977) observed decreasing toxicity in Hostapur SAS-60™ solutions over time which was attributed to biodegradation of SAS.

3. Microflora

Lundahl and Cabridenc (1978) tested the green alga, Chlamydomonas variabilis, for sensitivity to SAS compounds of varying alkyl chain length. The EC_{50} values for immobilization ranged between 3.7 mg/L and 125 mg/L with increasing toxicity as alkyl chain length increased (see Table 7-A). $C_{20.7}$ SAS was less toxic than the two next longest chains tested ($C_{18.9}$, C_{17}), suggesting that SAS surfactants attain maximum toxicity at a chain length approximately 17 to 19 carbons.

B. Toxicity of SAS to Microorganisms

Growth inhibition was observed in the bacterium, Escherichia coli, when exposed to SAS in culture plates. The following concentrations of SAS prevented the development of more than 5 colonies per plate: 20 g/L C_{14-15} SAS; 200 g/L C_{14} SAS; and > 200 g/L C_{13-17} SAS.

BIBLIOGRAPHY

OANVILA, A. M. 1977. The toxicity of anionics in the biodegradation process. The behavior of Daphnia magna and Artemia salina in the Guadalquivar River waters and in the Bay of Algeciras. Spanish Petrochemistry (Dec. 1977) pp. 1-33 (English translation).

LUNDAHL, P. and R. Cabridenc. 1978. Molecular structure-biological properties: Relationships in anionic surface-active agents. Water Res. 12:25-30.

V. HUMAN SAFETY

The information available for the 1977 Report dealing with the human safety aspects of SAS was scant and did not allow any definitive conclusions to be drawn regarding the safety of SAS to man.

A. Animal Studies

Acute oral toxicity values for SAS range from about 1000 to 3000 mg/kg in the rat and between 500 and 1000 mg/kg for dogs. Skin and ocular irritation studies with concentrated SAS materials (20-60% active) suggest SAS are positive skin and eye irritants in experimental animals; tests with more typical use concentrations are unavailable. Application of 10% SAS and subsequent challenge gave no indication of sensitization in guinea pigs. A single report noted no toxic effects in rats fed 300 mg/kg/day SAS for 45 days and no indications of adverse effects were reported for rats given 100 ppm SAS in their drinking water for one year. No data are available to assess the chronic oral effect of SAS or their potential for induction of carcinogenic lesions in experimental animals.

Acute Toxicity

Acute Irritation - Ocular, Skin

Skin Sensitization

Subchronic/Chronic Toxicity

Carcinogenicity

Mutagenicity

Teratogenicity/Reproduction Studies

No new information has been published with respect to acute toxicity, acute skin and eye irritation, chronic toxicity, carcinogenicity, mutagenicity, or teratogenicity of SAS since the 1977 Report.

B. Human Studies

> *The 1977 Report contained two skin irritation studies conducted with human volunteers. In a repeated patch test with 0.25% SAS, skin irritation occurred in 16% of the test subjects; two 30-minute applications of 0.1% SAS daily for one week resulted in mild to distinct irritation in 58% and 23% of the test individuals, respectively.*

No studies with humans have become available since the 1977 Report.