The Status of the Biodegradability Testing of Nonionic Surfactants

THE SOAP AND DETERGENT ASSOCIATION
SCIENTIFIC AND TECHNICAL REPORT No. 6
October 1969

A shorter version of this report appeared in the Journal of the American Oil Chemists' Society; JAOCS, *46* 432-440 (Sept., 1969). The authors express their appreciation to the Society for having granted permission to publish this material.

A publication of
THE SOAP AND DETERGENT ASSOCIATION
485 Madison Avenue, New York, New York 10022

The Subcommittee on Biodegradation Test Methods of
The Soap and Detergent Association

A Report on

The Status of Biodegradability Testing of Nonionic Surfactants

by

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ABSTRACT

A wide range of nonionic surfactants was studied in an extensive inter-laboratory biodegradability testing program carried out by member companies of The Soap and Detergent Association over a three-year period. The objectives were: to determine the biodegradability of a variety of nonionic surfactants; and to develop a reliable laboratory scale test method which could be used to evaluate the biodegradability of new candidate materials.

The results of this research and testing confirm that the primary and secondary alcohol ethoxylates, the alkyl alkanolamides, and the alkyl amine oxides are all highly biodegradable. These materials represent the important classes of nonionics used in household and institutional synthetic detergents. The removal of these materials under conditions of normal secondary wastewater treatment can be anticipated.

The diversity of structures represented in the complete nonionic surfactant spectrum, and the problems of residue analysis imposed serious obstacles in the development of a single standard laboratory procedure which will correlate well with the limited field data presently available. The objective of establishing a standard test for all nonionics was not achieved.

Residues of nonionic surfactants from household and institutional synthetic detergents do not appear to contribute to esthetic water pollution or to interfere with waste treatment processes. A variety of biodegradable assessment procedures, applicable to specific nonionics or nonionic groups are currently available and should assure that their residues will not adversely affect the quality of receiving waters. The Subcommittee plans to maintain a program for continued research in nonionic biodegradability testing.

Introduction

The interest of the detergent industry in biodegradability testing developed in the late 1940's and early 1950's when a possible relationship was first established linking detergent surfactant residues with some foaming incidents in sewage treatment plants and, occasionally, in natural waters. With the commitment of the industry to develop, manufacture and market more biodegradable detergents which would largely eliminate these problems, it appeared desirable to have effective test procedures and standards against which the many candidate materials under consideration could be evaluated. In 1961, The Soap and Detergent Association (SDA), through its Technical Advisory Committee, established a Subcommittee on Biodegradation Test Methods composed of representatives from most of the principal detergent manufacturers and raw material suppliers. This group was charged with the responsibility of evaluating methods then being used to determine the biodegradability of surface active agents, and if necessary, developing as soon as possible, methods and standards, which would meet the particular needs of the detergent industry in this country.

Since the use of alkyl benzene sulfonate (ABS) far exceeded that of all other surfactants in household detergent formulations, initial emphasis was placed on the development of test methods for anionic materials of that type. As the research progressed, data were obtained for both branched chain ABS and the new, highly biodegradable linear alkylate sulfonate (LAS), which replaced ABS in household detergents. This phase of the work was completed with the publication, in late 1965, of The Soap and Detergent Association's two-step procedure for the determination of ABS/LAS biodegradability.[1] Publication of the method closely coincided with the completion of the detergent industry's voluntary conversion to LAS and other biodegradable surfactants in mid-1965. The procedure has since been adopted by governmental and private groups such as the Department of Defense and The American Society for Testing and Materials.

Although not nearly as important from a volume standpoint, nonionic surfactants are also used in the formulation of household and institutional detergents. Upon completion of the studies on ABS and LAS, the Subcommittee was charged with the development of biodegradability methods and standards applicable to a broad range of nonionics.

This paper describes the activities of the Subcommittee in this specific area, its progress and achievements to date, inherent problems that have been encountered, and the current state of the art in nonionic biodegradability testing.

Definition and Current Use of Nonionic Surfactants

In general, surfactants are divided into four major categories: anionics, nonionics, cationics, and amphoterics. Schick[2] describes nonionic surfactants as being chiefly polyoxyethylene and polyoxypropylene derivatives but also including such other materials as anhydrohexitol derivatives, sugar and glycol esters, alkyl alkanolamides and alkyl amine oxides. Some typical nonionics are shown diagrammatically in Fig. 1 demonstrating some of the variable structures which can exist.

In recent years, nonionics have played an important role in the formulation of low-sudsing heavy-duty detergents and sulfated derivatives of nonionics are extensively used in light-duty dishwashing compounds. Nonionics also have a variety of non-detergent applications including cosmetics, agricultural chemicals, paints, textiles, paper making, and in other products and processes where their dispersing, emulsifying, wetting, and foaming properties are needed.

Data on surfactant production and

FIGURE 1

Some Typical Nonionic Surfactants

1. $RO\,(C_2H_4O)n\,H$ ——— Primary Alcohol Ethoxylate

2. $\begin{matrix} & R & \\ & | & \\ HCO & (C_2H_4O)n\,H & \\ | & & \\ CH_3 & & \end{matrix}$ ——— Secondary Alcohol Ethoxylate

3. $R\quad O\,(C_2H_4O)n\,H$ ——— Alkylphenol Ethoxylate

4. $\begin{matrix} & O & H \\ & \| & | \\ R\text{-}C\text{-}N & C_2H_4OH & \end{matrix}$ ——— Alkyl Monoethanolamide

5. $\begin{matrix} & O & \\ & \| & \\ R\text{-}C\text{-}N & (C_2H_4OH)_2 & \end{matrix}$ ——— Alkyl Diethanolamide

6. $\begin{matrix} & CH_3 & \\ & | & \\ R\text{-}N & \rightarrow O & \\ & | & \\ & CH_3 & \end{matrix}$ ——— Alkyl Dimethyl Amine Oxide

NOTE: $R = C_8 - C_{18}$ Alkyl Chain

sales are published annually by the U.S. Tariff Commission. The Tariff Commission report for 1966 indicated that 686 million pounds of nonionics of all types were produced in the United States.[3] Based on this information, nonionics accounted for about 20% of the total surfactant production in 1966, second only to that of the anionics. For comparison, it is estimated that about 75% of the U.S. surfactant production was of anionic materials.

However, it is not possible to determine the actual use of nonionic surfactants in U.S. household and institutional synthetic detergents from the Tariff Commission reports, since nonionics find application in a broad range of commercial and domestic products, are often modified chemically so their original identity is lost, or may be exported. Data were needed so that the efforts of the group working on biodegradability test procedures could be concentrated on those nonionic surfactants which were extensively used by the detergent industry. Therefore, a special committee was given the responsibility of resolving this question and concluded that a national survey of all detergent manufacturers was needed to supplement published data. A survey form was sent to over 350 companies, both members and nonmembers of The Soap and Detergent Association. Information was requested on the use of six classes of nonionics in

1965 and 1966. All data were handled on a confidential basis. It was estimated that nonionic use by the companies responding to the survey constituted between 80% and 90% of the total nonionic use by the detergent industry in household and institutional cleaning products. The survey indicated that in 1965, over 80% of the nonionics used fell into four categories: primary alcohol ethoxylates, secondary alcohol ethoxylates, alkyl ethanolamides and alkyl amine oxides.

The remaining types of nonionics make up less than 4% of the total surfactants used in household cleaning applications. Because this fraction is small and represented by so many diverse structures, The Soap and Detergent Association's Subcommittee on Biodegradation Test Methods has, since 1967, concentrated its research efforts on the 4 major classes of material.

Biodegradability Testing Background, Theoretical, and Practical Considerations

Since a great deal of experience had been gained through the development of the ABS/LAS procedure, the Subcommittee program was initially concerned with the application of these methods, where possible, to the testing of nonionic surfactants. This previous work had been limited to a specific class of materials. Therefore, broad concepts which relate to the biodegradability of all organic compounds had not been developed. Until recently, no single, accepted definition of biodegradability existed. Even today the definition proposed by a committee of the Water Pollution Control Federation[4] is in fact a three part definition, with specific limitations as to the applicability of each portion. This group has suggested three criteria of biodegradability:

Primary Biodegradation: Biodegradation to the minimum extent necessary to change the identity of the compound.

Environmentally Acceptable Biodegradation: Biodegradation to the minimum extent necessary to remove undesirable properties of the compound such as foaminess and/or toxicity.

Ultimate Biodegradation: Biodegradation to inorganic end products.

To be meaningful, biodegradability of compounds and end products must be assessed in terms of their effect on the environment. To insure that a specific compound will not adversely affect the environment in which it is used normally requires that its biodegradability exceed the primary level but does not require ultimate biodegradability. Thus, the concept of Environmentally Acceptable Biodegradation is suggested. This is further defined as "susceptability to biodegradation yielding end products which are totally acceptable to the receiving environment which includes air, soil and water although principal interest may lie in treatability in waste disposal facilities."

This basic concept has guided the industry in the development of procedures and standards for surfactant biodegradability. Product residues after use and proper waste treatment should not contribute to foam nor should their presence have any other adverse effect on waste treatment processes or on the quality of receiving waters.

Most household and institutional detergent residues find their way into sewers and ultimately into natural waters. Production and use data would indicate that nonionic surfactant residues from such products would not create a significant water quality problem simply because their use is not that extensive. So while no problem may exist insofar as nonionic residues from household and institutional detergents are concerned, it was necessary to develop methods which relate to the degradation of these compounds under actual conditions of treatment and to demonstrate their rapid biodegradability to innocuous end products.

In developing such procedures, the industry Subcommittee postulated general principles which were applicable to all phases of their activities. The more significant of these include the factors discussed below:

A) While bench-scale simulation of actual sewage treatment plants is not essential, it is imperative that results obtained in laboratory tests correlate with those experienced in such plants. To accomplish this, the test procedure must be sensitive enough so that differences in biodegradability resulting from chemical structure can be observed. This, in turn, requires the availability of accurate and sensitive analytical methods.

B) Besides the basic chemical structure of the compound under test, some other factors affecting biodegradability in nature are: opportunity for bacterial acclimation, bacterial concentration, concentration of the test compound, temperature, and contact time, All of these factors were considered by the Subcommittee in their studies. Where possible, other conditions such as dissolved oxygen levels, pH, nutrients, mixing, etc. should be kept at near optimum conditions to keep the number of variables within recognized and controllable limits.

C) Natural waters, soil, sewage and even air can serve as the source of the microbiological cultures used in biodegradability testing. These cultures can be sustained on both degradable organic compounds and on inorganic nutrients.

D) Generally speaking, test surfactants are fed to cultures at gradually increasing levels to allow adaptation to occur prior to the start of the test itself. Once the degradability of a specific material has been established, these acclimated cultures can be used to measure both the extent and rate of degradation of a homologous series over a wide range of conditions. When testing materials of unknown degradability, the use of cultures developed from domestic activated sludge seems more desirable. In any event, the test period should be limited to preclude the development of a predominant, atypical culture and to limit the cost and inconvenience of lengthy testing. At the present state of the art, the choice of test period is necessarily arbitrary.

E) Materials which are known to be degradable to acceptable levels, such as LAS, should be evaluated along with the actual test material. Also, it is often useful to include a difficult-to-degrade material so that a range of values is obtained. In all cases, a control system, operated identically to the actual test unit, except for the absence of the test surfactant, should be maintained to provide a base line of physical, biological and chemical properties for comparison purposes.

F) As with all biological testing, constant attention to detail is required if meaningful results are to be obtained. As an example, particular care must be taken to assure the maintenance of test materials in a dissolved state at all times. Other factors which deserve consideration include the degree of adsorption of the test material on biological growths, the need for replicate testing to assure statistically valid results, and the determination of differences which can develop between operators and laboratories.

Biodegradability Test Methodology

Over the last decade a relatively wide range of biological systems has been proposed and evaluated, both here and abroad, for the measurement of surfactant biodegradability. In some cases, methods have been officially adopted by law[5] and in others, while not official in the legal sense,[6] they have the weight of government approval.

The tests which have been developed differ significantly in approach, in operational complexity and in extent to which they simulate actual waste treatment conditions. Six of these procedures which were examined in depth by the Subcommittee are briefly described below to give some indication of the variations involved.

Since the activated sludge process and its variants appeared to be the waste treatment system of choice by most pollution control authorities, a laboratory test method which gave results similar to those observed in this type plant seemed most desirable. Also, from a volume standpoint, the majority of wastes receiving secondary treatment are treated by this process making a test of this type even more meaningful.[7] Although procedures which could be correlated with other field conditions were considered (e.g. Bench-Scale Trickling Filters, River Die-Away Tests, etc.), the bulk of the research conducted by the Subcommittee concerned itself with activated sludge performance.

1) *SDA Procedure for ABS and LAS*[1]

The SDA procedure for determining the biodegradability of ABS and LAS is a two-stage method combining two independent microbiological tests. The component tests are complementary. Due to their different biological features, they provide both rapid laboratory screening and reliable evaluation of the performance to be expected in activated-sludge type sewage plants. The procedure is versatile and comprehensive for ABS and LAS types of surfactants. It has reportedly also been successfully applied to other anionic surfactants.

In the first stage of the SDA biodegradability test procedure, the Shake Flask method serves as a simple, rapid, and relatively inexpensive presumptive test. Every surfactant to be tested is evaluated by the procedure. The microorganisms used in the Shake Flask Test are obtained either from natural sources (sewage, activated sludge, soil, water, etc.) or from a specifically acclimated culture, and degradation is measured by the standardized methylene blue active substance (MBAS) analysis. Thirty mg/l of test surfactant is added to a flask containing a specified basal medium and the mixture is inoculated with a biological culture. The test flask (along with control flasks) are continuously shaken on a reciprocating or gyratory shaker. Total duration of the test is 14 days, which includes 2, 72-hour adaptive transfers. If a surfactant's biodegradability exceeds 90% no further testing is required. Conversely, if it falls below 80%, it is not considered biodegradable.

A modification of the Semi-Continuous Activated Sludge Test comprises the second or Confirming Test stage of the SDA procedure. Surfactants whose biodegradability falls between 80% and 90% by the Shake Flask Test are evaluated by this procedure.

Activated sludge from a sewage treatment plant processing mainly domestic wastes, the surfactant to be tested, and a specific synthetic sewage are combined in a specially designed vessel. The mixture is aerated for 23 hours, allowed to settle for one hour, supernatant liquid is withdrawn, and an equal amount of synthetic sewage containing 20 mg/l of the test surfactant is added to the settled sludge to restore the working volume. This cycle is repeated daily.

The minimum duration of a test is 15 days which includes 5 days for incremental surfactant feeding to encourage acclimation of the organisms to the test material, 3 days of equilibration at 20 mg/l of added surfactant before analyses are begun, and at least seven days of level operation. Biodegradation is measured, as in the Shake Flask Test, by the decrease in MBAS concentration. Biodegradability must exceed 90% in this

procedure for a material to be considered acceptable.

2) *The Official German Test for Anionic Surfactant Biodegradability*[5]

This procedure is of the Continuous Activated Sludge type. It typifies several published methods which simulate the operating schemes of activated-sludge type sewage treatment plants. The equipment, operating procedures, and criteria of adequate biodegradability to be applied in this test are all specified by German law.

Sewage containing 20 mg/1 of test substance is fed into a 3-liter aeration chamber from which it overflows into a sedimentation chamber (2-liter capacity). A residence time of 3 hours in the aeration chamber is specified to conform to German sewage plant practice. Sludge is recirculated from the settler to the aerator by means of an airlift. The clarified overflow from the sedimentation chamber, corresponding to treated sewage, is composited and analyzed daily for residual surfactant content by a standardized methylene blue analytical method, and the percent reduction of MBAS input is calculated.

A special feature of the German procedure is the development of the culture (activated sludge) in each test by spontaneous inoculation from the air. This lengthens the duration of the tests, not only for the development of an adequate sludge, but also for its acclimation. The acclimation period varies from test to test and is a characteristic of each test product. Following acclimation, each test is continued with daily determinations of percentage degradation for 21 days of steady-state operation. A degradability of at least 80% by this procedure is demanded by law for detergents containing anionic surfactants.

Testing of finished detergent products is prescribed by German law. However, extraction procedures have been developed for isolating the surfactants from formulated products which contain strong bacteriostats or bactericides.

The principal disadvantages of the method are the relatively long and uncertain duration of tests, the bulkiness of the equipment when it is necessary to test a large number of samples, the large volumes of surfactant and sewage which must be fed, and the high cost, primarily for labor.

3) *The River Die-Away Method*[8]

This is one of the earliest methods used to measure the biodegradability of synthetic surfactants. It involves simply the incubation of the test substance in actual river water under aerobic conditions, and the periodic analysis of the system for the material being tested. It has been discussed in many papers, and its advantages of convenience, economy of materials and effort, and modest equipment requirements are well known. Also widely recognized are its serious shortcomings as a standard test: the variations from time to time in bacterial count between different rivers, between different points in the same river, and even at the same point in a given river. These variations in inocula and the equally serious fluctuations in nutrients and toxins can cause variable results between laboratories studying the biodegradability of the same material.

4) *The Standard Method for Anionic Surfactants in the United Kingdom*[6]

This method, adopted in 1966 by the British Standing Technical Committee on Synthetic Detergents, is a modification of the River Die-Away Test. By using a standard seed and medium, some of the disadvantages of the River Die-Away Test are overcome. Surfactant is added once, at the beginning of each test, and its disappearance is followed for a specified period of time. Biodegradability is expressed as the percent reduction of the initial surfactant concentration during the test. The

test is continued until the percentage of surfactant remaining falls by less than two units in five days; but in any case, the maximum duration is 21 days.

The test is performed as follows: a solution containing 10 mg of surfactant per liter of standard BOD dilution water is inoculated with 30 mg of air-dried activated sludge per liter and is gently stirred at 20°C ±1°C in the dark for a period of up to three weeks. Samples are removed at appropriate intervals and the residual concentration of surfactant is determined by the Longwell and Maniece methylene blue method.

Publications by Patterson, Scott and Tucker[9, 10] discuss the applicability of this procedure to nonionics using both chromatographic and foam measurement techniques. A more detailed discussion on the use of thin layer chromatography for nonionic analysis is included in Appendix A.

5) *The Bunch-Chambers Method*[11]

Determination of biodegradability by this method involves a series of four consecutive die-away tests, each of one week duration. This feature is intended to provide opportunity for acclimation to occur. The method is designed to be suitable for determining the biodegradability of any organic compound.

Ninety ml of BOD dilution water containing 5.0 mg of yeast extract and 2.0 mg or other suitable amount of test compound is inoculated with 10 ml of settled sewage. For each test material, three such systems are prepared in three separate 250-ml Erlenmeyer flasks. The flasks are stored loosely capped, without agitation, at room temperature for 7 days. Weekly transfers of 10 ml are made from each flask to another containing the 90 ml of BOD dilution water, yeast extract, and surfactant for three consecutive weeks. After incubation for seven days, each sub-culture is analyzed to determine the extent of degradation of the test material. Neither the method of analysis nor criteria of biodegradability are specified since they are dependent upon the specific material under test.

6) *The Warburg Method of Determining Biodegradability*[12]

The Warburg manometric technique was frequently used in early studies of the biodegradability of surfactants, and it has been fully described in the literature. In this method, the diluted test material and bacterial inoculum are agitated in a closed oxygen or air atmosphere at constant temperature. The sample is agitated and the oxygen utilized is measured with respect to time by noting the decrease in gas pressure at constant volume; the CO_2 evolved is eliminated by absorption in potassium hydroxide in a small center well of the test flask. With the development of more specific biodegradability methods which are more readily correlatable with practical biodegradability, Warburg use has sharply declined. It is generally not applied today if another biodegradation procedure is suitable for the materials under study.

Methods and Modifications Used in SDA Biodegradation Studies of Nonionic Surfactants

A total of 17 company laboratories and one laboratory of the Federal Water Pollution Control Administration participated in these studies during the three years of cooperative research by the Subcommittee. Biodegradation tests were performed upon several different types of nonionic surfactants using four of the test procedures described above. The four approaches employed were the Shake Flask, the Semi-Continuous Activated Sludge, the Bunch-Chambers, and the River Die-Away tests. These methods span the full range of current biodegradability methodology, with the exception that no continuous test was used. This exception was not considered to be an important limitation since based on an

evaluation of field data, it has been shown that the Semi-Continuous Activated Sludge Test can be operated to achieve the same results (and at considerable less effort) as those obtained by continuous test procedures.

No modifications were made in the microbiological aspects of either the Bunch-Chambers or River Die-Away procedures. The Shake Flask method for anionic surfactants was modified in that the duration of the test was extended to fifteen days and the extent of biodegradation was measured on days 11 and 15 as well as days 0, 7, and 8.

In the earliest series of Subcommittee cooperative tests of nonionics, the Semi-Continuous Activated Sludge procedure was judged to offer the greatest promise as the basis for developing a suitable method for this class of surfactants. This procedure gave more consistent results in inter-laboratory comparisons and the biodegradability data which it yielded were more compatible with other laboratory results and the limited field data available. Therefore, greater effort was made to modify it so as to achieve a satisfactory laboratory test procedure in as short a time as possible.

Some of the cooperating laboratories reported poor cell growth and pH decline in the latter stages of some of their Semi-Continuous Activated Sludge tests. In extreme cases of pH drift, values as low as 6.3 were noted. Various changes were made in the nutrient medium in an effort to provide adequate cell growth and to avoid pH decline in any laboratory. These are summarized in Table I. The pH control was improved by increasing the amount of buffer in the formula, as in test series #2 (see Table I). However, better pH stability was achieved with the medium used in test series #1, 3, and 4.

A study was made of the effect of residence time in the Semi-Continuous Activated Sludge Test upon the biodegradation of the nonionic surfactants. Aeration times of 5 hours, 6 hours, 17 hours, and 23 hours were evaluated at initial surfactant concentrations of 10 mg/1 and 20 mg/1.

One of the burdensome features of any method requiring daily care is the necessity of week-end feeding. Some co-

TABLE I

Nutrient Media Used in Semi-Continuous Activated Sludge Tests

	Milligrams Per Liter				
	Standard Procedure For Anionics	Cooperative Study			
		#1	#2	#3	#4
Glucose	130	300	130	300	300
Nutrient Broth	130	200	130	200	200
Beef Extract	130	—	130	—	—
Dipotassium Hydrogen Phosphate	130	130	500	130*	130*
Ammonium Sulfate	25	—	25	—	—

*This concentration could be raised if acidic conditions were encountered.

operating laboratories serviced their Semi-Continuous Activated Sludge units daily from Mondays through Fridays but omitted week-end care. This, of course, led to abnormal conditions in the tests on Mondays and to a lesser extent on Tuesday. However, by collecting data on Wednesdays, Thursdays, and Fridays only, those laboratories omitting week-end care obtained data comparable to those providing daily care. Consequently, in later tests, all laboratories took data only on Wednesdays, Thursdays, and Fridays of the second, third, and fourth weeks of the tests regardless of the feeding schedule.

Analytical Measurements – Methods Used in Cooperative Studies

Concurrent with studies on biodegradation, The Surfactant Analytical Subcommittee of SDA studied and evaluated all known available methods which might be useful in estimating the degree of surfactant removal. The wide range of nonionic surfactant types, their limited reactivity, plus the need for sensitive and accurate measurements pose a severe if not insurmountable barrier to a single universal test. A review of analytical procedures considered to date is presented in Appendix A. In addition, three procedures used in the biodegradability testing program are discussed briefly below:

The colorimetric extractive procedure, in which the intensity of blue color formed by complexing ethoxylate surfactants with ammonium cobaltothiocyanate is measured, was used in the early phase of testing. Although it was found that this procedure gave reproducible results, its inherent shortcoming of poor correlation with loss of foaming potential and surface tension depression precluded its use as a standard analytical procedure, and other methods were sought.

The general ability of surfactants to reduce the surface tension of water was evaluated as a means of following the biodegradability of nonionics utilizing a standardized procedure and a du Noüy tensiometer. Interferences prevented reproducible results and the method was also considered unsatisfactory.

Failure of extensive efforts to produce a suitable chemical analytical method and the inherent shortcomings of surface tension measurements for routine testing led to the direct measurement of loss of foaming ability. Although this method also has several limitations, it was felt that, overall, it produced the most meaningful results and was therefore used in later phases of the cooperative biodegradability testing. In the course of the cooperative studies a closely standardized procedure was evolved.

Results Obtained From Cooperative Studies

During the cooperative test program, three distinct phases evolved. These could be categorized as an initial screening phase; a phase in which efforts were concentrated on optimizing the Semi-Continuous Activated Sludge method for nonionic testing; and a final phase in which specific nonionics used extensively in detergents were studied. In all, four cooperative, round robin tests were conducted.

Phase I – Preliminary Assessment of Alternative Methods of Biodegradation Testing and Analysis

Initial plans for the cooperative test phase of the study were prepared in May 1965. The test methods selected for evaluation were the Shake Flask test, the Semi-Continuous Activated Sludge Test and the River Die-Away Test. Degradation was followed by the three analytical procedures indicated above. In all, eleven materials of different structure were tested: eight were nonionics, and three anionics (ABS, LAS and a dodecene-1 derived LAS) were used for control purposes. The eight nonionics, which are de-

TABLE II
First Cooperative Screening Study

Surfactants Tested	Semi-Continuous Activated-Sludge Degradation(a)		River Die-Away Median Degradations (b)						Shake Culture Median Degradations Using Initially Unacclimated Seed (c)					
	Residual Effluent Foam, ml/50 ml	CTAS-MBAS Loss, %	Foam Loss, %		CTAS-MBAS Loss, %		Surface Tension, dynes/cm^2		Foam Loss, %		CTAS-MBAS Loss, %		Surface Tension, dynes/cm^2	
			Wk 2	Wk 4	Wk 2	Wk 4	Wk 2	Wk 4	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Linear Alkylate Sulfonate Commercial composite	0.0 (Initially 20 ml)	97	99	100	91	93	70	72	88	91	97	95	60	66
Dodecene-1-derived	0.0 (Initially 20 ml)	100	100	100	99	99	72	71	90	100	96	99	58	70
Tetrapropylene-derived ABS	3.5 (Initially 20 ml)	66	80	93	68	68	59	69	10	7	17	19	42	41
Linear Primary Alcohol Ethoxylate	0.0 (Initially 15 ml)	100	100	100	100	100	70	71	98	100	100	100	70	71
Linear Secondary Alcohol Ethoxylate	1.8 (Initially 15 ml)	94	81	98	93	100	63	71	50	62	88	93	60	61
Random Linear Nonylphenol Ethoxylate	5.0 (Initially 15 ml)	94	58	92	77	100	47	59	0	0	57	68	42	42
Nonrandom Linear Decylphenol Ethoxylate	2.4 (Initially 15 ml)	99	85	99	96	100	63	70	6	6	74	87	50	55
p,t-Octylphenoxynonaethoxyethanol	15.0 (Initially 15 ml)	95	43	80	81	100	48	52	0	0	15	44	40	44
Branched Tridecyl Alcohol Ethoxylate	4.0 (Initially 15 ml)	63	10	91	87	100	56	65	0	0	18	31	41	45
Tripropylene-derived Nonylphenol Ethoxylate,	4.8 (Initially 9 ml)	95	92	92	93	98	45	49	0	0	3	19	39	40
Tetrapropylene-derived Dodecylphenol Ethoxylate	2.0 (Initially 9 ml)	96	55	87	16	98	39	47	8	15	3	0	30	33

(a) Laboratory activated-sludge units were operated on a 23-hour aeration cycle with degradation measured by cobaltothiocyanate (CTAS) and methylene blue (MBAS) colorimetric procedures and reduction in foaming character of clarified unit effluent. The surfactant and synthetic food were added at the same time in this study. Median data during the fourth operating week are reported. Initial foams were estimated from average of die-away (10 mg/1) and shake-flask (30 mg/1) initial levels.

(b) Initial and weekly samples of the die-away system were analyzed by the colorimetric, foam and surface-tension techniques. The surface tension value reported compares to an initial median value of 43 dynes/cm.^{2} Test concentration was 10 mg/1.

(c) Seed was obtained from domestic activated-sludge treatment plants and given two adaptive transfers prior to the test period. Surfactant test concentration was 30 mg/1.

scribed more fully in Appendix C, were as follows:

A) Linear Primary Alcohol Ethoxylate
B) Linear Secondary Alcohol Ethoxylate
C) Random Linear C_9 Phenol Ethoxylate
D) Nonrandom Linear C_{10} Phenol Ethoxylate
E) p, t-Octylphenoxynonaethoxyethanol (Alkylphenol Ethoxylate)
F) Tripropylene-derived Alkylphenol Ethoxylate
G) Tetrapropylene-derived Alkylphenol Ethoxylate
H) Branched Tridecyl Alcohol Ethoxylate

Results from this initial round robin study in which eleven laboratories took part indicated that the primary alcohol ethoxylate was highly biodegradable under all test and analytical conditions (Table II). Slightly lower degradation was observed for the secondary alcohol ethoxylate and for the non-random decylphenol ethoxylate. Under the specific test conditions established, the branched-chain alkylphenol ethoxylates (E, F, & G), the random linear nonylphenol ethoxylate and the tridecyl alcohol ethoxylate showed the lowest rates of biodegradation. With the exception of the primary alcohol ethoxylates, foam loss for nonionics in the Shake Flask test was fair, at best.

Throughout this test series, poor correlation of surface activity loss with cobaltothiocyanate response was found. This was particularly true in the case of the Semi-Continuous Activated Sludge method. In some cases, no foam loss could be observed even though cobaltothiocyanate analysis indicated 95% degradation. Surface tension data were erratic in all cases and, while useful in a qualitative sense, did not appear to warrant further effort to develop this technique as a primary analytical method.

In each of the test methods, the anionic control materials performed about as expected based on previous experience with these compounds.

Phase II — Study of Test Variables in the Semi-Continuous Activated Sludge Test

After reviewing results obtained in the first round-robin study, revisions in test methodology were proposed to improve discrimination among various test compounds. In the case of the Semi-Continuous Activated Sludge method, this was attempted by lowering the total aeration time from the previously used 23 hours. In addition, it was agreed to devote more effort to the application of foam loss as a measure of biodegradation. To put foam measurement on a quantitative basis, the Subcommittee accepted the concept of estimating biodegradability by comparing foam volume of the effluent with foam value of a reference effluent containing known quantities of added surfactant. As a material underwent degradation additional foam measurements were made and compared to a blank reference effluent to which known amounts of surfactant had been added. For example, if a test effluent which had started with 10 mg/l of surfactant gave a foam volume equivalent to a control effluent spiked with 1 mg/l of that surfactant, then degradation of test sample would be said to have proceeded to 90% as measured by foam loss.

Although other biodegradability test procedures were evaluated during Phase II, e.g. the Bunch-Chambers method (Table III), primary emphasis was placed on the Semi-Continuous Activated Sludge method since it seemed to offer the most promise from the standpoint of reproducibility and discrimination. During the course of this work, the method was evaluated at aeration detention times of 5, 6 and 17 hours. The source of the sludge inoculum was recognized as a critical factor. It was stipulated that sludge should be obtained from a plant

TABLE III

Summary of Bunch-Chambers Die-away Test Data From Third Cooperative Study (a)

Surfactant Tested	Median 4th Week Foam (or MBAS) Loss, %	Number of Laboratories Reporting Specified Foam (or MBAS) Loss Level		
		<80%	80%-90%	>90%
Linear Alkylate Sulfonate (LAS)	94 (91)	0 (0)	0 (2)	4 (3)
Tetrapropylene-derived ABS	<60 (35)	2 (5)	0 (0)	2 (0)
Linear Secondary Alcohol Ethoxylate	85 (b)	1	3	1
Nonrandom Linear C_{10} Phenol Ethoxylate	75	3	1	0
p, t-Octylphenoxynonaethoxyethanol	<60	4	0	0

(a) Bunch-Chambers (FWPCA) die-away test system consisted of 20 mg/l surfactant and 50 mg/l yeast extract diluted in a 90/10 mixture of BOD dilution water and settled sewage. After incubating for seven days, the mixture was analyzed and sub-cultures were set up in fresh media and recharged surfactants. The above data were collected at the completion of the fourth die-away period.

(b) Data reported by one laboratory show that higher removals can be obtained by reducing the yeast concentration to 25 mg/l or reducing the surfactant level to 10 mg/l.

treating predominantly domestic sewage since the test was being developed to measure the degradability of cleaning products. The initial surfactant concentration was also recognized as a parameter requiring careful consideration. Evaluations were carried out at starting levels of 10 mg/1 and 20 mg/1 during this phase of the study. These nonionic levels are many times higher than those encountered in domestic sewage except under highly unusual conditions. Nevertheless, it was found necessary to work with these concentrations in order to assure meaningful and reproducible analytical measurements since many nonionics have foam thresholds between 0.5 mg/l and 1.0 mg/l. The high initial concentrations coupled with the shock-feed conditions, tend to compensate for the relatively long detention times used.

In a separate set of experiments, the Subcommittee confirmed that acceptable results could be obtained by comparing foam levels from a surfactant sample undergoing degradation with a sample containing 1/10 of the initial surfactant concentration. The surfactants investigated during this phase of the study included two anionics (ABS and LAS) which again served as references. Three nonionics were deleted because they lacked broad commercial application and one new nonionic surfactant was added. The three materials deleted were the tripropylene and tetrapropylene alkylphenol ethoxylates and the branched tridecyl alcohol ethoxylate. The new material was a primary alcohol ethoxylate in which the alcohol contained approximately 25% methyl groups in the beta position (Appendix C).

When the Semi-Continuous Activated Sludge method was operated at a 5-hour aeration detention time, findings indicated that this test condition was too restrictive to yield generally useful biodegradability data. Results obtained with the units operating at a 17-hour detention time are shown in Table IV. In this procedure, the diluted synthetic sewage was added to the settled sludge and the mixture was then aerated. After a specified period of time, the test surfactant was added to the aerating mixture. It was hoped, that in so doing, the organisms would be in a semi-starved condition at the time of surfactant addition and that results, more consistent with field experience, would be obtained. However, the desired effect was not observed.

Operational difficulties were also encountered. Several participating laboratories reported a significant decline in pH throughout the test. Others reported problems with maintaining a uniform sludge solids level, and with drifting pH. Still other difficulties developed with respect to feeding and in the measurement of foam. In this latter situation, wide variations were reported for the same, known surfactant concentration. All of these factors pointed to the fact that a single, reproducible and generally applicable test method was still not in hand. The results obtained did not entirely correlate with available field test data. Field studies had been carried out on the removal of ABS, LAS, secondary alcohol ethoxylates, and p, t-octylphenoxynonaethoxyethanol in sewage treatment plants. In the case of the last material, considerably higher degradation was observed in the sewage treatment plant studied in the field test than was found in the laboratory conditions discussed above[13] since the specific field environment is often most resourceful in bringing about the de-

Table IV

Second Cooperative Semi-Continuous Activated Sludge Study (a)

Surfactants Tested	Median Foam Loss Reported by Cooperating Laboratories, %	Number of Laboratories Reporting Specified Foam-Loss Levels		
		<80%	80%-90%	>90%
Linear Alkylate Sulfonate (LAS)	96 (b)	0	4	10
Tetrapropylene-derived ABS	<60	10	1	1
Linear Primary Alcohol Ethoxylate	99	0	0	13
Linear Secondary Alcohol Ethoxylate	93 (b)	1	4	8
Nonrandom Linear C_{10} Phenol Ethoxylate	83 (b)	4	7	0
p, t-Octylphenoxynonaethoxyethanol	<60	9	2	0
Methyl Branched Linear Primary Alcohol Ethoxylate	99	0	1	7

(a) Laboratory activated-sludge units were operated on a 23-hour aeration cycle with degradation monitored by reduction in foaming character of the clarified unit effluent after 5 and 17 hours. The 17-hour data are reported in this table. The surfactant and synthetic food were added at the same time in this study.

(b) Foam-loss values for these materials were lower in the 5-hour detention tests; several laboratories reported values below 80 percent.

gradation of synthetic chemicals.[4] Laboratory data obtained during this phase of the cooperative study are summarized in Table V.

TABLE V

Third Cooperative Semi-Continuous Activated Sludge Study (a)

Surfactants Tested	Median Foam (or MBAS) Loss Reported by Cooperating Laboratories, % (b)	Number of Laboratories Reporting Specified Foam (or MBAS) Loss		
		<80%	80%-90%	>90%
Linear Alkylate Sulfonate (LAS)	99 (96)	0 (0)	0 (0)	9 (9)
Tetrapropylene-derived ABS	85 (76)	3 (4)	4 (2)	2 (0)
Linear Secondary Alcohol Ethoxylate	96	0	2	8
Nonrandom Linear C_{10} Phenol Ethoxylate	90	1	4	4
p, t-Octylphenoxynonaethoxyethanol	<60	10	0	0

(a) Laboratory activated-sludge units were operated on a delayed surfactant-feeding basis, yielding percent degradation values for 6- and 17-hour detention periods. The 17-hour data with 10 mg/l surfactant dosage are shown in this table; the 6-hour degradation levels were considerably lower. The diluted synthetic waste (yeast, glucose, buffer) was added to the settled biomass, and the mixture was aerated for an appropriate period before the surfactant solution was added. The delayed surfactant addition allowed the food supply to be reduced by the organisms before they were exposed to the surfactants.

(b) Percent degradation was obtained by comparing unit-effluent foam values with foam measurements on suractant spikes (1 and 2 mg/l) added to the blank unit effluent. Methylene-blue-active substances (MBAS) were calculated from determinations of feed and effluent values. Data used were collected 16 to 32 days after startup.

Phase III — Application to Nonionics Extensively Used in Detergents

In order to bring within controllable proportions the problem of developing a test procedure, the Subcommittee, upon completion of Phase II of the cooperative study, elected to concentrate its efforts on those surfactants which were most widely used in household and institutional detergents. As described previously these were found to be: primary alcohol ethoxylates, secondary alcohol ethoxylates, alkanolamides and amine oxides. The representative samples tested are described more fully in Appendix C. In addition, ABS and LAS were retained as controls. Operational conditions established for the Semi-Continuous Activated Sludge Test, which now appeared to be the method of choice, included the following: 23 hour aeration detention time, maximum duration of the test, 32 days, and weekend feeding preferred. The first four or five days of the test were set aside for incremental build-up of the surfactant concentration and a maximum of two weeks was allowed for sludge to reach the desired concentration and pH.

Each of the eleven cooperating company laboratories ran the test until level operation was achieved. Level operation was defined as six days of data collection in which the difference in average percent removal for the first three days and for the last three days was no more than 3%.

Foam calibration curves were prepared in each laboratory so that foam measure-

ments made during the test could be converted directly into percent degradation. These measurements were generally the responsibility of one individual in each laboratory so that a high degree of reproducibility could be assured. Fig. 2 demonstrates some of the inherent variability in foam measurements between laboratories. The individual curves are based on data submitted by individual laboratories during the evaluation of primary alcohol ethoxylate biodegradability, which was one of the more uniform correlations. Even though variations in absolute foam volume measurements can be fairly large from person to person or laboratory to laboratory, reproducible and reliable biodegradability information

FIGURE 2

Typical Foam Calibration Curves

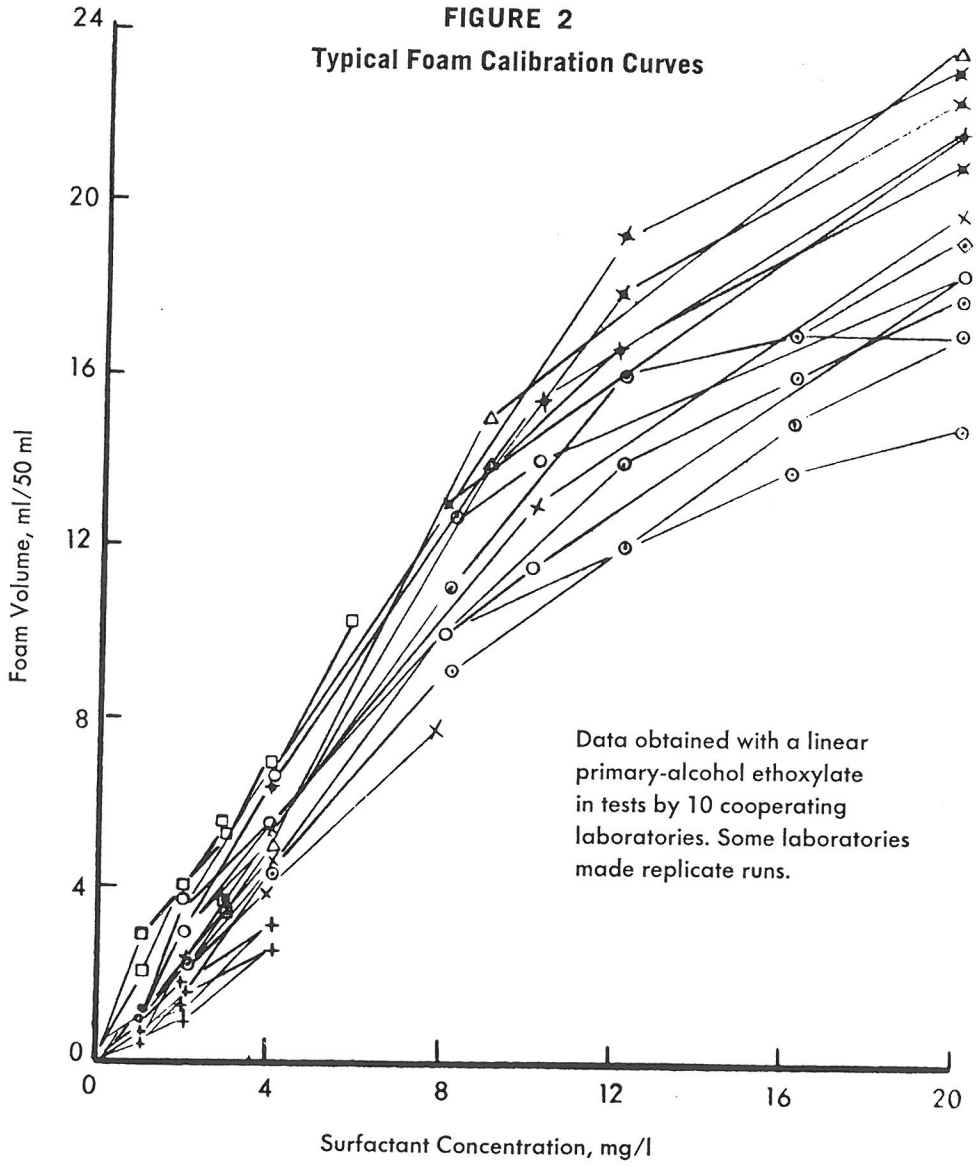


TABLE VI

Biodegradation Test Results With Modified Semi-Continuous Activated Sludge Test

Fourth Cooperative Study

Surfactant	Degradation, %
Linear primary alcohol ethoxylate	99.7
Linear secondary alcohol ethoxylate	96.2
Diethanolamide	99.8
Amine oxide	99.6
Linear alkylate sulfonate (LAS)	97.5
Tetrapropylene-derived ABS	70.3

NOTE:

Laboratory activated-sludge units were operated on a 23-hour-aeration/1-hour-settling cycle at 2500 (±500) mg/l solids concentration. Degradation of the nonionics was monitored by reduction in foaming character of the clarified unit effluent. The degradation of LAS and ABS is presented in terms of MBAS reduction. The surfactant (20 mg/l) and synthetic food (300 mg/l glucose, 200 mg/l nutrient broth, and 130 mg/l K_2HPO_4) were added to the settled sludge at the same time in this study. Units were started with biological solids from domestic activated-sludge plants; the test duration was limted to 32 days.

was developed from such data (Table VI). These data have also been plotted in terms of statistical probability as shown in Fig. 3.

A description of the statistical methodology used in evaluating collected data is included as Appendix B of this report. Confidence in the statistical analysis is high even though one of the cooperating laboratories had difficulty in meeting the requirement of six days of level operation.

Discussion

Throughout these studies, it became increasingly evident that biodegradability testing of this type is extremely complex because of the sensitive biological systems involved and the inherent variability of foam measurement techniques.

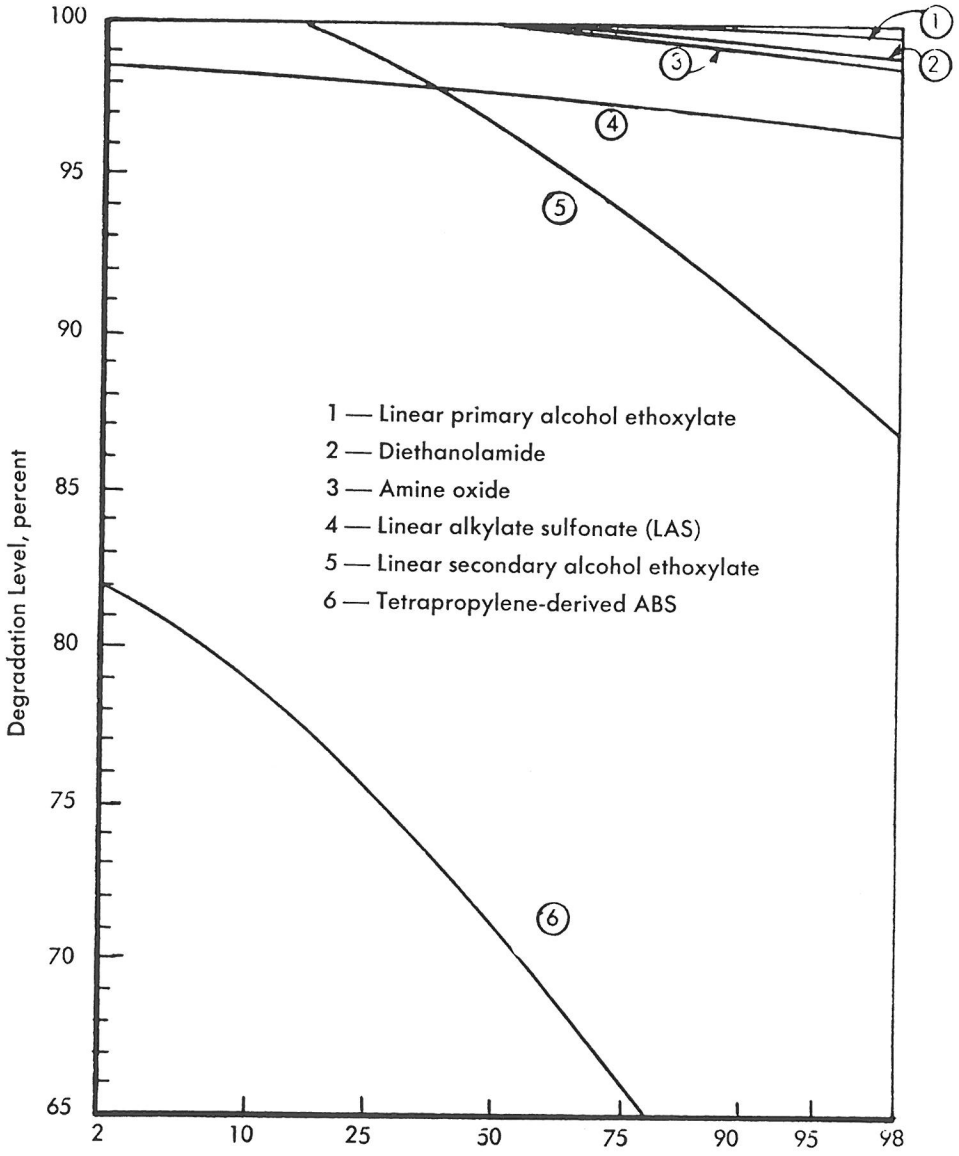
Thus, while a valuable procedure was developed, it has application only in the hands of skilled and experienced investigators and should not be viewed as a universal method for the determination of nonionic biodegradability. However, the procedure is considered to be of the "Fail-Safe" type, that is, if a nonionic material is found to be highly degradable in the test, it can be expected to be so in nature as well. If field and laboratory data lead to conflicting conclusions, experience indicates that greater reliance should be placed on field test results under the intended use conditions. Even when data from sewage treatment plants are available, care must be taken in their interpretation due to wide variations which may exist in field conditions, e.g. aeration detention time, activated sludge concentrations, acclimation of the microorganisms.

No field studies on nonionics were carried out under Subcommittee spon-

sorship; however, individual companies have conducted such research on several of the materials evaluated during Phases II and III. Good correlation with laboratory test results was observed for linear alcohol ethoxylate and for the control anionics. In the case of p, t-octylphenoxynonaethoxyethanol, the Subcommittee's test data did not show comparable correlation.

FIGURE 3

Statistical Analysis of Biodegradation Results (Phase III)



Although these field studies have been described in the literature[13, 14, 15] a brief discussion of the principal findings is warranted. The biodegradability of secondary alcohol ethoxylate under field conditions was evaluated in an extended aeration activated sludge plant.[14] Overall removal was in the order of 94% with an initial surfactant concentration of 22 mg/1. This level of biodegradability compares favorably with that found in the laboratory during the cooperative studies.

Conversely, in a field trial in which the biodegradability of p-t-octylphenoxynonaethoxyethanol was studied, significantly higher biodegradability was observed than was noted in cooperative laboratory testing.[13] An extended aeration type sewage treatment plant was also used in this study. Initial surfactant concentrations of 5.3 mg/1, 9.5 mg/1 and 11.0 mg/1 were used in various phases of the test. Overall degradation exceeded 90% under the three test conditions as determined by various chemical and physical analytical methods. BOD removal approximated 95% in all cases. This paper also describes laboratory conditions under which the test material's biodegradability correlates with the cited field experience.

Numerous field studies, involving a variety of waste treatment processes, have been conducted on the anionic control materials.[15] All of these results correlated well with the laboratory findings and indicated a high level of biodegradability for LAS, while significantly lower levels of biodegradability were noted in the case of ABS.

Summary and Conclusions

After an extensive three-year program, which involved literally thousands of laboratory tests, it would appear that no single, simple, standard method exists at this time to determine the biodegradability of all types of nonionic surfactants. However, the cooperative study carried out by the SDA's Subcommittee on Biodegradation Test Methods, has shown that those nonionics which are used extensively in household and institutional detergents are highly biodegradable and can be readily removed under conditions of normal secondary sewage treatment.

One of the principal problems in developing a universally applicable nonionic biodegradability test procedure exists in the analytical rather than the biological phase of the methodology. An exhaustive review by the Subcommittee of the available chemical analytical methods indicates that, at present, neither a single method or group of methods is available to measure accurately both the original molecule and the intermediate degradation products of the many, multi-structured compounds which are broadly classified as nonionic surfactants. For this reason, physical techniques, such as the measurement of surface tension changes and foam loss, were examined to determine their suitability for use. Of the two, greater consistency was achieved through the use of a foam loss procedure. Nevertheless, great variability was noted between laboratories and even within the same laboratory (when the test was run by different operators). Thus, unless the procedure is carried out by experienced technicians, misleading results may be encountered.

Of the many biological procedures evaluated, the Semi-Continuous Activated Sludge type, because of its high reproducibility and correlation with field experience, seems to be the best approach to the determination of the biodegradability of the major nonionics used in household and institutional detergent products.

Before conclusions can be reached regarding the biodegradability of other nonionics by using the methodology outlined in this report, supporting data, preferably that obtained in the field under intended use conditions, must be con-

sidered in the over-all evaluation. Even when field test data are available, care must be taken in interpreting such findings due to the variations which may exist in waste treatment plants and in nature. However, the Subcommittee is satisfied that the procedures used in Phase III are inherently of the "Fail-Safe" type. That is, if a nonionic material is found to be highly degradable in the test, it will undoubtedly be so in nature. If, however, its biodegradability under laboratory conditions is doubtful, other supporting information is needed to determine its actual biodegradability.

As suggested in the title, this report is a summation of the status of nonionic biodegradability testing to the present time and should not be considered as a final, definitive resolution of the problem. Even though residues of nonionic surfactants emanating from synthetic detergents do not appear to contribute to esthetic water pollution or to interferences with waste treatment processes, continued research is planned by the Subcommittee. This is planned as a three-phase program which will concern itself with the refinement of existing biological and analytical methods, the evaluation of new methods as they become available, and the examination of nonionic surfactants which may be used in household and institutional detergents in the future.

Acknowledgments

The authors express their appreciation to the members of the Subcommittee on Biodegradation Test Methods of The Soap and Detergent Association and to their companies for their many contributions both during the cooperative test program and in the preparation of this report. The current membership and company affiliation of the Subcommittee are as follows:

M. Mausner, Chairman, Witco Chemical Co.
R. A. Wiles, Allied Chem. Corp.
R. Taylor, ARCO Chemical Co.
E. Jungermann, Armour-Dial Inc.
R. House, Chevron Chem. Co.
F. Hudson, Colgate-Palmolive Co.
R. C. Allred, Continental Oil Co.
F. J. Lowes, Dow Chemical Co.
S. B. Creselius, Economics Laboratory, Inc.
J. E. Shewmaker, Esso Res. & Eng. Co.
R. L. Johnson, Ethyl Corporation
F. Krupin, GAF Corporation
L. J. Garrison, Jefferson Chem. Co.
M. A. Goldberg, Lever Brothers Co.
R. D. Swisher, Monsanto Co.
J. R. Duthie, The Procter & Gamble Co.
K. A. Booman, Rohm and Haas Co.
R. J. Pikaar, Shell Chemical Co.
R. A. Conway, Union Carbide Corp.
J. W. Compton, Wyandotte Chemicals Corp.
R. L. Bunch, Federal Water Pollution Control Administration (Ex Officio)

Particular appreciation is extended to Dr. M. A. Goldberg of Lever Brothers Company, Dr. Leslie Bacon of the Wyandotte Chemical Corporation, and to the Federal Water Pollution Control Administration.

Dr. Goldberg served as Chairman of the Subcommittee during the early phases of cooperative testing and formulated many of the concepts that led to the successful initiation of the program and its continued support.

Dr. Bacon developed, tested and promulgated analytical methods, which were badly needed to evaluate effectively the biodegradability of the many materials tested throughout the program.

Over the years, the Federal Water Pollution Control Administration, represented by several highly-respected scientists, has contributed advice and counsel, and has actively participated in the activities of the Subcommittee. These efforts are appreciated by the individual Subcommittee members and the companies they represent.

The continued assistance of the Surfactant Analysis Subcommittee of The Soap & Detergent Association also deserves special mention. Their efforts in evaluating new and existing analytical test methods contributed significantly to the effectiveness of the Biodegradability Testing Program.

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APPENDIX A

Review of Available Analytical Techniques

A. Requirements for an Analytical Method

The objective of providing a general method to measure all nonionics in surface waters, sewage, and laboratory samples which is rapid, easy to use, inexpensive, and analyzes a large number of samples at one time does not seem readily attainable at this time. In general, methods which have been prepared for nonionics are suitable for specific materials under particular conditions and while research continues, the prospects for the development of a universal method do not appear good.

The nonionic surfactants comprise a wide variety of materials, including alkyl amides, amine oxides, alkanolamides, fatty acid esters of glycols and glycerol, numerous polyoxyalkylene compounds as well as ethylene oxide and propylene oxide block polymers. These compounds are all similar in that they lack a strongly reactive functional group.

The fatty acid esters of glycols and glycerol are very similar to those compounds present in intermediary fat metabolism. Thus, in surface waters, sewage, and other related systems, it is not possible to distinguish between surfactant residues and those from other sources. Since fatty materials undergo enzymatic action quite readily, degradation should proceed very rapidly.

The fatty amides are split by the enzymes called amidases to form fatty acids and nitrogen compounds. The materials can also be metabolized like degradation products from fats and proteins.

The polyoxyalkylene compounds include the oxyethylene and oxypropylene derivatives of alkylphenols, alcohols and mercaptans, fatty acids and alkanolamides. In addition, there are polyoxyalkylene glycols and block polymers. This review is restricted to these polyoxyalkylene compounds.

These nonionic surfactants present a much more diverse and complex spectrum for analysis than do the anionic surfactants, because each member of each group is a mixture, consisting of a broad distribution of oxyethylene species for any one product. The diversity of materials and their limited reactivity make an ideal method that is equally applicable to all of these materials highly improbable.

Ideally, the analytical procedure should detect and measure all of the nonionic components in surface waters, effluents, sewage, and in laboratory samples.

During biodegradation, lower homologs of the components are produced as well as other derivatives, such as carboxylates. Some of these derivatives are surface active and can produce foam; and should also be measured by the analytical test.

The concentration of nonionic surfactants that must be detected is very low. The levels of nonionics in rivers or other surface water are generally 0.05 mg/1 or lower and only occasionally reach as high as 0.2 mg/1. In sewage nonionic levels range between 0.1 mg/1 and 2.0 mg/1. As noted previously, laboratory biodegradability testing is usually carried out in the 0.5 mg/1 to 20.0 mg/1 range. The lower limit of detection for any widely used method should, therefore, be no more than 0.05 mg/1.

The systems to be analyzed are generally complex and may contain other natural nonionics. The analysis must be highly specific and the preparation of the sample becomes an important part of the method. Preparative techniques usually are different for each situation but in general involve extraction, often with an added salt, precipitation, centrifugation, or ion exchange cleanup.

B. Chemical and Instrumental Procedures

In recent years, a variety of methods has been suggested for the estimation of nonionic surfactants in laboratory test systems, surface waters, and sewage effluents. An excellent review of the colorimetric methods has recently been published by Heinerth.[1] The following discussion summarizes the current status of the various available methods.

1. *Cobaltothiocyanate Colorimetric Method*

Several modifications of the cobaltothiocyanate procedure have been proposed. Crabb and Persinger[2] extracted a large sample from neutral solution using diethyl ether as the solvent. The ether residues were complexed with ammonium cobaltothiocyanate followed by chloroform extraction of the complex. The absorbance was determined at 620 mμ and the concentration of nonionic surfactants was determined by comparison to a standard curve. Greff, Setzkorn, and Leslie[3] saturated a suitable aliquot with sodium chloride, followed by complexation with ammonium cobaltothiocyanate and extraction of the complex with benzene. The absorbance of the benzene solution containing the complex was measured at 319 mμ.

The Surfactant Analysis Subcommittee of The Soap and Detergent Association also used a preliminary diethyl ether extraction from a suitable aliquot containing the sample. The ether residue was reacted with cold ammonium cobaltothiocyanate, extracted with cold chloroform and the absorbance determined at 319 mμ. Reference was made to a standard curve to determine the concentration of the test sample.

The application of the ammonium cobaltothiocyanate reaction to biodegradation test systems suffers from several serious shortcomings. Among these are the following:

a. At least 3.5 units (average) of ethylene oxide per molecule are required for complex formation with the ammonium cobaltothiocyanate reagent; thus, compounds containing less ethylene oxide than this amount would not be detected by the method. If a trace of water is present at least 5 to 6 units of ethylene oxide are required.[4]

b. The method also loses sensitivity for high-molecular-weight nonionic compounds. For example, the sensitivity of the method is very low for a typical C_{12} hydrophobe containing more than 15 moles of ethylene oxide, and the response of the method is practically zero for compounds of the polyoxyethylene glycol type.

c. An even more serious objection to the method is the large difference in molar absorptivity of compounds containing the same hydrophobe but differing in ethylene oxide content. For example, the absorbance of 20 mg/l solutions of n-dodecanol ethoxylates containing 3.5, 5.4, and 9.1 units of ethylene oxide was found to be about 0.02 for the 3.5 mole adduct, 0.230 for 5.4 mole adduct and 0.450 for the 9.1 mole adduct. While this variation in molar absorptivity might not be a serious drawback in the analysis of known compounds in laboratory test systems, this behavior would cause a tremendous variation in reporting the concentration of nonionics in surface waters and sewage effluents containing nonionic surfactants of unknown structure.

d. Probably the most serious problem in the cobaltothiocyanate test for measuring nonionic detergent biodegradation is the lack of correlation between the values so obtained

and the corresponding values obtained by foam and surface tension measurements. Nonionic compounds based on a straight chain alcohol hydrophobe ordinarily show good agreement in the various biodegradation test systems using the cobaltothiocyanate test and comparing the results to foam and surface tension measurements. However, the phenol based nonionic detergents, even those with linear side chains, ordinarily do not show good agreement between the colorimetric values and the foam and surface tension measurements.

Since most proposed colormetric methods are based upon similar chemistry, namely reaction with the ether chain of the nonionic molecule, it is doubtful that any colorimetric method would yield basically different results than those shown by the ammonium cobaltothiocyanate method.

2. *Ultraviolet and Infra-red Methods*

Measurement of ultraviolet (UV) absorption by aromatic nuclei has been the primary application of this technique. Griffith[5] found that for direct UV examination of aqueous solutions the lower limit of detection was 50 mg/1 for alkyl phenol ethoxylates. Since this does not provide sufficient sensitivity for practical applications, any procedure employing UV measurements will require a concentration step. Weibull and Thorsell[6] extracted a large sample with chloroform. The absorbance of the chloroform solution at 278 mμ was corrected for background absorption at 250 and 300 mμ and compared with a standard curve. Since the identity of the surfactant must be known for calibration purposes, this limits the method to the study of known systems, such as laboratory degradation tests.

The UV method is also subject to many interferences from various impurities, especially LAS in sewage and waste waters. Cleanup procedures, including extractions, ion exchange treatments, and foaming have been suggested to minimize this problem. The Surfactant Analysis Subcommittee of The SDA evaluated the treatment of a large sample with a mixed-bed ion exchange resin, followed by adsorption on activated charcoal, and desorption with methanol and chloroform. The absorbance of a chloroform extract at 275 mμ, corrected for background, is a measure of the alkylphenol ethoxylate content. The procedure was cumbersome, and was not convenient for handling large numbers of samples.

Calibration of the UV method requires a known or arbitrary standard. As degradation proceeds, therefore, the UV procedure will give erroneously high results proportional to the molecular weights of the standard and the species in the sample. To overcome this effect, Osburn and Benedict[7] combined the UV measurement with infra-red (IR) estimation of average molecular weight.[8]

Although this method is relatively easy to perform and moderately rapid, it is applicable to only the alkylphenol ethoxylates. Since the lower limit of detection is approximately 0.5 mg/1, the method is not sufficiently sensitive to survey various surface waters. Thus, the primary application of the UV and IR methods is in laboratory biodegradation tests.

3. *Chromatographic Methods*

Thin layer chromatography (TLC) is one of the newer analytical techniques and has recently been applied to the analysis of river waters and sewage. As is true for other analytical methods, TLC procedures require a preliminary cleanup.

Patterson, Hunt, and Tucker[9] extract the nonionic material with chloroform in three stages. The residue from the extract is spotted on a thin layer plate coated with silica gel and developed in a mixed solvent. Modified Dragendorff spray is

used to detect the separated materials. The concentration of the nonionic suffactant can then be estimated by bracketing the sample with known concentrations of standard nonionic surfactant on the same chromatoplate.

A brief collaborative test of the TLC method by the Analytical Group of the Standing Technical Committee on Synthetic Detergents (Great Britain) indicated satisfactory reproducibility at 0.4 mg/1 of alkylphenol ethoxylate. The recovery for several different commercial surfactants is claimed to be quantitative.

Komorniczyk[10] applied the TLC method to the higher alcohol ethoxylates. Those components with six units or more of ethylene oxide per molecule produce identical spots. Emulsion difficulties encountered during extraction were overcome by boiling the original sample for one hour with concentrated HC1 to hydrolyze naturally occuring emulsion stabilizers.

Although the same response is given for several different ethoxylates, the compounds containing less than 6 moles of ethylene oxide will show a decreasing color response, and therefore, during degradation these materials would yield low results. Most of the reported work pertains to ethoxylates and the response of the propylene oxide derivatives is unknown but would probably be less than the ethoxylate analogs. It is possible also that wide variations in the type of hydrophobe or the length of the ethoxylate chain could affect either completeness of extraction or response on the TLC plate. The carboxylated intermediates formed during degradation would be expected to migrate separately which would contribute to variable comparisons with foam and surface tension measurements.

4. *Phosphotungstic Acid Method*

The ether oxygen of the polyoxyethylene chain present in nonionic surfactants will often form precipitates with heteropoly acids such as phosphomolybdic acid or phosphotungstic acid. The well-known compexation reaction with phosphotungstic acid was converted by Burttschell into a sensitive analytical procedure for various ethoxylates in sewage.[11]

The cleanup procedures include centrifuging to get rid of solids, ion exchanging to remove ammonia, amines, and LAS, and finally extraction with butanone. Separation is achieved by complexation of the ethoxomer with phosphotungstic acid and the amount of tungsten determined colorimetrically by reacting it with dithiol. Blank determinations on the reagents are necessary and should be taken into account. The lower limit of detection is approximately 0.1 mg/1.

Only a limited number of different surfactants are reported in the evaluation. The maximum variation in response for the different nonionics was 1.0 to 1.4, with many being within 1.0 to 1.1. Therefore, an arbitrary standard must be used for calibration purposes.

Biodegradability tests would require further calibration with other known nonionics and the degradation products of these materials. No indication is given as to how well the disappearance of surfactant correlates with the loss in foaming tendency.

The types of interferences which are listed, especially the high variability given for the reagents alone, limit the reproducibility in routine applications, particularly at low levels of surfactant.

Pitter[12] also used phosphotungstic acid precipitation but determined the amount of tungsten by colorimetric measurement with hydroquinone. Since no cleanup procedure is employed, proteins, monoglycerides, and high levels of sulfate can be expected to interfere.

5. *Phosphomolybdic Acid Method*

The other well-known precipitation reaction with heteropoly acids involves

phosphomolybdic acid. Stevenson[13] precipitated alkylphenol ethoxylates directly from solution with $BaCl_2$ and phosphomolybdic acid. The precipitate was isolated by centrifuging and then was dissolved in concentrated sulfuric acid. The color was measured at 520 mμ. No cleanup procedure was used. The lower limit of detection was 1 mg/1, but the procedure had limited applicability. Although LAS did not interfere, alkyl sulfates did cause low results. In addition, carboxymethyl cellulose was an interference.

The Joint Committee of the Association of British Chemical Manufacturers and The Society for Analytical Chemistry[14] issued a tentative method based upon the same complex formation, but from an aqueous ethanolic solution of an ether extract of the sample. The precipitated complex is measured either by: 1) reading the UV absorbance at 310 mμ, or 2) determining the phosphate colorimetrically. A known alkylphenol ethoxylate must be used for calibration, with a lower limit of detection of 10 mg/1.

Proteins interfere in this procedure and a deproteinization step is included in the method for those samples that require it. However, recovery of the non-ionics is incomplete and an arbitrary correction must be applied to the results. This interference seriously limits the applicability of this particular test procedure.

6. *Iodobismuthate Method*

The ether oxygens of nonionic surfactants form precipitates with complex halides such as $HBiI_4$ and often barium ion is added to the mixture because it aids in the precipitation. Either the amount of precipitate or the amount of bismuth in it may be measured as an estimate of the ethoxylate present.

Burger[15] described a method for the range of 0.5 to 10 mg/1 ethoxylate in a variety of sources. The sample is extracted with butanone and the residue from the extraction is transferred to a special centrifuge tube with a small-bore base, and precipitated with a modified Dragendorff reagent (iodobismuthate). The precipitate is centrifuged into the small bore of the tube and the sediment volume measured. By comparison to known standards the amount of nonionic is determined.

The procedure was modified by Wickbold[16] and colorimetric estimation added in place of the sediment measurement. The color is developed with pyrrolidine dithiocarbamate and read at 390 mμ. A separate calibration is required for each nonionic. The lower limit of detection is 0.3 mg/1.

A similar procedure was proposed by the Wyandotte Chemical Corporation.[17] An addition to this procedure is the use of two different extractions to isolate both the nonionic surfactant and the degradation products plus polyoxyethylene materials. The sample with added salt is first extracted with ethyl acetate to recover the surfactants and then with chloroform to isolate the degradation products. Each extract is then processed separately by evaporating to dryness, dissolving in water, and precipating with Dragendorff reagent. After centrifugation the excess bismuth in the supernatant liquid is measured by reaction with KI-ascorbic acid reagent. The absorbance is read at 337 mμ. The difference between this value and the absorbance due to the total bismuth content is proportional to the nonionic surfactant.

The major limitation to these methods is the need for separate calibration curves for each individual nonionic surfactant. Reactions usually only take place if at least 5-6 oxyethylene units are present. This means that samples of unknown composition yield results of unknown accuracy. In addition, little data are available comparing the analytical results by these methods with foam height and sur-

face tension measurements in laboratory degradation studies.

The initial extraction techniques of Burger and of Wickbold appear to be more widely applicable than other proposed procedures. However, the completeness of extraction for degradation products has not been established.

7. *Conversion of Nonionic to Anionic Surfactants*

Instead of analyzing the materials as nonionics they may be converted to anionic or cationic surfactants and the appropriate methods applied. Such a procedure was devised by Han[18] and involves sulfation of the nonionic followed by the methylene blue determination. The sample is saturated with salt and extracted with chloroform. A portion of the chloroform extract is sulfated and then analyzed by the well-known methylene blue procedure.[19]

The method covers the range of 0.2 to 20.0 mg/1 nonionic. However, the response of methylene blue to sulfated ethoxylates, especially those with varying chain lengths, has not been studied widely and therefore, the correlation with die-away tests is unknown. In addition, the methylene blue method is subject to many interferences in unknown samples. These include phenols, alcohols, alkanol amides, amines, and probably hydrocarbons.

Although this method may have applicability in laboratory degradation tests with known materials, it has little advantage over the cobalt thiocyanate procedure for most unknown samples.

C. Physical Methods

In considering the variations in chemical structures among materials classified as nonionic surfactants, it became apparent that development of analytical methods based on chemical functional groups represented a major stumbling block in the assessment of degradation.

It was logical, therefore, that some effort should be directed toward use of physical measurement which might be appropriate and more generally applicable. Both surface tension and foaming measurements were actively studied in cooperative testing programs. A brief discussion of these methods and their applicability are outlined below:

Surface Tension — The Soap and Detergent Association Biodegradation Subcommittee adopted a standardized method of surface tension determination. In this procedure, which employs a du Noüy Tensiometer with a platinum ring, measurements are taken on a 50 ml aliquot in a suitable pre-cleaned container (such as a four-ounce, wide-mouth jar). A rough measurement is taken by quickly increasing the tension of the wire while lowering the sample table until the film at the interface breaks. The ring is then reimmersed, and the tension increased to three dynes/cm.[2] less than the initial breaking point. After a one-minute equilibration period, the tension is then slowly increased at the rate of one or two scale units per minute until the film breaks. The scale reading when the film breaks is the apparent surface tension which is corrected according to the standard table supplied by the tensiometer manufacturer to yield the true surface tension expressed in dynes/cm. The platinum ring should be thoroughly rinsed and flamed between measurements of different solutions. If the same solution were retested after flaming the ring, some of the trace surfactant would have been removed and a higher surface tension would result. The fact that most nonionics are very efficient in reducing surface tension means that residual concentrations show a substantial surface tension depression when present at 1-2 mg/1 and with some materials even at 0.5 mg/1. Below a critical concentration for each nonionic surfactant, surface tension values approach those of plain water. A sample, which

under a given test condition, will give degradation in the range of 90% (from an initial concentration of 10-20 mg/1), may then with slight variations in testing give a wide range of surface tension values and make interpretation difficult.

Surface tension of nonionic compounds does not always give good correlation with foam measurements when dealing with different compounds. Its main utility is to indicate the presence of partially degraded intermediates. Since the foam criterion is more directly related to potential problems in the environment, it has to be the preferred method of approach for general purpose testing where surface tension measurements fail to predict foam. Additionally, the special equipment required for surface tension measurements coupled with the careful maintenance needed, led many laboratories to favor alternate procedures.

Foam Measurements — The failure of the SDA Committee to find a chemical analytical method for nonionic surfactants which could be correlated with actual loss of foaming ability and the similar experience of other investigators led to the direct measurement of the loss of foaming power.

A method of residual foam measurement was developed, modified through contributions of individual laboratories, and finally standardized for committee use.

The standard procedure for determining foaming capacity of surfactant solutions was as follows: a 50-ml aliquot of the sample whose foaming ability was to be measured was placed in a scrupulously cleaned, 100-ml, glass-stoppered, graduated cylinder. The cylinder was shaken by hand for 15 seconds at a rate of 2 to 3 vigorous strokes in a vertical direction per second. The volume of foam was visually read from the calibrations on the cylinder after the vessel had set for 15 seconds and 60 seconds. The foam volume was read in milliliters and reported as "ml of foam per 50 ml of solution." A foam reading of zero was reserved for the condition of absolutely no foam. When the surface of the test solution was only partially covered with foam, such as by a ring of small bubbles around the periphery of the graduate, a special measuring technique was used. The volume of foam was estimated to the nearest 0.1 ml by viewing from above and comparing to a standard set of diagrams. The cylinders used were thoroughly cleaned and rinsed prior to and between foam measurements. The precision of foam measurements was improved significantly by having all determinations in a series of tests performed by the same person.

Prior to foam measurements during a biodegradability test, calibration curves were prepared by shaking appropriate standard concentrations of the agent in test effluent (from the test unit fed synthetic sewage containing no added surfactant, that is, the "blank"), measuring the milliliters of foam generated, and plotting the data. By reference to such a curve, the foam readings taken during a biodegradability test were convertible to mg of surfactant per liter. As a further check, known concentrations of a test substance (usually at 10 and 20% but sometimes also at 5 and 15% of the initial feed concentration of the substance) were prepared in effluent from the "blank" test unit, and the foaming capacity of these standard solutions was measured at the same time as the effluent from the biodegradability tests.

The testing of this procedure did reveal, however, that foam as a measurement method has a number of basic flaws:

1. It can only be applied to surfactant materials that make a stable measurable foam and that exhibit an increase in foam with concentration over the range of interest.

2. It represents, at best, a reliable measure of degradation only when the degradation products make no foam or substantially less foam than the starting materials.
3. It does not reliably provide, of itself, any information about the final fate of the total molecule – i.e., it does not indicate how complete the degradation is or even whether some other reaction may have taken place that inhibits sudsing.
4. It does not apply equitably to a broad class of nonionics – i.e., a material with quite low foaming and with relatively low change in foam volume with concentration would face a severe qualification test.
5. It requires testing at surfactant levels generally well above those likely to be found in the environment.
6. It has no possibility (because it lacks specificity) of application outside the laboratory since other foaming materials, of man-made or natural origin, may be present.

In practice, the above fundamental limitations have been found to be real. Specifically, some nonionics do not produce a satisfactory concentration vs. foam volume curve; some partially degraded materials foam more than the original material; and some nonionics cease to foam when degradation has proceeded only to a limited degree. Nevertheless, the measurement of ability to foam after exposure to biodegradation testing conditions does contribute useful information. An important criterion for "environmentally acceptable residues" is ability to cause foam. For the nonionics of interest, certainly for those most widely used in household and industrial detergents, this may be the most important criterion.

D. Biological Methods

Where chemical and physical analytical approaches have limitations for broad applicability, it is proper to consider the possible application of biological methods. Biological methods of assessing the extent of biodegradation normally involve one of three approaches:

1. The measurement of oxygen consumed by microorganisms (i.e., BOD)
2. The measurement of by-products of microbial metabolism (i.e., CO_2 evolved)
3. The measurement of microorganisms produced using the test material as the basic energy source.

Only cursory consideration was given to such approaches in the Subcommittee's work on nonionics because earlier work on anionic surfactants had suggested limitations that made them unsatisfactory. Some of the limiting factors in the use of the biological approach are outlined below:

BOD/COD Assessment–Although the Biochemical Oxygen Demand (BOD) test by itself or combined with Chemical Oxygen Demand (COD) does not require knowledge of specific structure, it does give, on many occasions, results that have been misinterpreted. While BOD will provide confirmation on certain easily oxidized structures and may properly delineate certain others that are extremely bioresistant, there are problems of assessing many intermediate compounds of interest. The relatively low bacterial seed and the limited time for adaptation in the BOD procedure can give results that suggest bioresistance, a resistance that has often been disproven when the compound is subjected to conditions more closely paralleling waste water treatment.

CO_2 Evolution – For basically carbonaceous surfactant molecules, the measurement of CO_2 evolved from a seeded sample can provide an indication of the progress of bacterial metabolism of the test material. Yields must be calculated

by determination of the theoretical production of the test structure or by comparison with compounds of known and similar structure. This test is of unquestionable value in early screening, but it does have limitations when comparing materials of substantially different structure.

Biological Growth — the availability of test material as a food source can be measured by the production of cells. The source and previous history of the culture used are so important that standardization between laboratories or between tests is difficult. This makes this indirect approach of limited use in a standard qualification-type test.

References

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14) Joint Committee of the Association of British Chemical Manufacturers and The Society for Analytical Chemistry, "Recommended Methods for the Analysis of Trade Effluents," p. 96, W. Heffer and Sons, Ltd., Cambridge, England, (1958).
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16) R. Wickbold, Paper presented during the conference, "Sewage Analysis" at the House of Technology, Essen, Germany, February 18, 1966 (being printed).
17) Wyandotte Chemical Corp., Industrial Chemicals Group, trade literature.
18) K. W. Han, Tenside, *4*, p. 443, (1967).
19) APHA, "Standard Methods for the Examination of Water and Wastewater, 12th ed., p. 296, American Public Health Association, New York, (1965).

APPENDIX B

Statistical Methodology Employed

The data, as received from the participating laboratories, were expressed in ml. of foam. Foam calibration data (foam height vs. mg/1 surfactant) were also available for each product. These calibration curves differed significantly from laboratory-to-laboratory. The proper product-laboratory curve was applied in translating the raw foam data to "percent surfactant removed" in each instance.

Examination of the data expressed as "percent surfactant removed" revealed that the variance within runs and/or between runs within laboratories was a function of "percent removed." Small variances were associated with large "percent removed." This is typical of data bounded by some limit, such as "100% removed." The classical analysis of variance techniques does not apply in this instance. Therefore, the "percent removed" data were transformed to a new scale X, where

$$X = \sqrt{100.1 - \%\ \text{Degraded}}$$

This transformation removed the bulk of the cited defect. All subsequent analyses were in terms of X, with a final transformation of tolerance limits back to "percent degraded."

All of the available data were treated in an unbalanced nested analysis of variance. The levels considered were:

1. Laboratory-to-laboratory (between laboratories within products)
2. Run-to-run within laboratories (between runs within laboratories)
3. Day-to-day within runs (between days within runs)

A "run" is defined here as the continuing degradation of a product with a single laboratory system.

Generally, the variation between runs within a laboratory was of the same magnitude (at the 0.10 level of significance) as the variation within runs. Such data were then pooled into two levels, with the appropriate degrees of freedom estimated by Satterthwaite's[1] approximation.

Statistical tolerance limits (95%) were

computed in the usual fashion, using appropriate values of k from published tables. These limits were transformed from the X-scale to "percent surfactant removed."

Translation of the raw foam data to "percent surfactant removed" was accomplished with graph paper. Transformation to the X-scale and all subsequent analyses were performed with the aid of digital computers.

1) F. E. Satterthwaite, Biometrics Bulletin, 2, p. 110, (1964).

APPENDIX C

Description of the Various Nonionic Surfactants Used in the Three-Year Cooperative Biodegradability Study

Linear Primary Alcohol Ethoxylate
An average 10-mol ethylene oxide derivative of a C_{12} - C_{14} primary alcohol of natural origin derived from coconut oil. The 1% aqueous cloud point is approximately 62.3°C and the test material has a hydroxyl number of 107.

Linear Secondary Alcohol Ethoxylate
An average 9-mol ethoxylate C_{11} - C_{15} random secondary alcohol. The surfactant was derived by ethoxylation of isomeric secondary alcohols. With the exception of the terminal carbons, the attachment was random along the alkyl chain. The 0.5% cloud point is 62°C and the Specific Gravity at 20/20°C is 1.013. The average hydroxyl molecular weight is 594.

Random Linear C_9 Phenol Ethoxylate
An average 9-mol ethoxylate which was made by alkylating phenol with a close cut mixture of alpha olefins averaging C_9. This resulted in a side chain attached to the ring at a (2, 3, 4 position) secondary carbon. It was ethoxylated to a cloud point of 54°C.

Nonrandom Linear C_{10} Phenol Ethoxylate
An ethoxylated C_9 - C_{10} linear alkylphenol containing an average of 10.4 ethylene oxide units. This material has an alkyl chain length distribution of 70% decyl and 30% nonyl. The purity of the starting alkylphenol was greater than 95%. The position of the phenol ring (mainly p-substituted) along the alkyl chain is as follows:

1	11%
2	85%
3	2%
4, 5	2%

Alkyl branching was less than 5%.

p, t-Octylphenoxynonaethoxyethanol
An average 10-mol ethoxylated branched chain alkylphenol. This material has a molecular weight of 647 of which the ethylene oxide constitutes 68%. The aqueous cloud point was 65°C.

Tripropylene-derived Alkylphenol Ethoxylate
A branched chain nonyl (90-95% para) phenol which was made by using a mixture of isomeric branched chain trimers of propylene. It was ethoxylated to a cloud point of 55°C and the molecular weight is 620. The material contains an average of 9.1 mols of ethylene oxide.

Tetrapropylene-derived Alkylphenol Ethoxylate
A tetrapropylene derived dodecylphenol containing an average of 10 ethylene oxide units. The material was made from distilled alkylphenol, thus being predominantly the monododecyl derivative. Typically, this compound of 85% para and 15% ortho.

Branched Tridecyl Alcohol Ethoxylate
An average 9-mol ethylene oxide derivative of a C_{13} oxo alcohol derived from tetrapropylene. This compound finds application in industrial products and its 1% aqueous cloud point is approximately 60°C.

Methyl Branched Linear Primary Alcohol Ethoxylate
An average 9-mol ethoxylate of C_{12} - C_{15} primary alcohol. The primary alcohol contained approximately 25% branching, predominantly the 2-methyl isomer. The 1% aqueous cloud point is 75°C and the material has a hydroxyl number of 92. The 9-mol ethoxylate has an average molecular weight of 603.

Alkyl Diethanolamide
A commercial lauric diethanolamide based upon a 90% lauric acid chain and containing 5% excess diethanolamine.

Alkyl Amine Oxide
Coco dimethylamine oxide derived from distilled coco amine and having a molecular weight of 244. The compound is a 30% active aqueous solution.

APPENDIX D

Supplementary Bibliography on Nonionic Surfactant Biodegradability

The following references and abstracts have appeared since the publication of Schick's *Nonionic Surfactants,* Marcel Dekker, Inc., New York (1967). The final chapter of this book contains an excellent bibliography on biodegradability. Also included are a number of earlier references that were not reviewed by

Schick as well as some that are specific to the present publication. In some cases, identical citations have appeared previously either in the main text or in preceding appendices. They are included again to provide both ready reference and to permit the inclusion of short abstracts.

K. A. Booman, J. Dupre, and E. S. Lashen, *Biodegradable Surfactants for the Textile Industry,* Am. Dyestuff Rept. 56, p. 82, (1967).

Laboratory tests indicate that secondary alcohol ethoxylates and alkylphenol ethoxylates are biodegradable. A field study on secondary alcohol ethoxylates showed that the acclimation required for optimum degradability in laboratory tests actually occurs in the field. The use of acclimated microorganisms is very import- and in order to obtain meaningful laboratory biodegradation results. The most reliable test methods are those which are most closely related to actual waste treatment conditions, such as the river water die-away test or the semi-continuous activated sludge test. Where sewage treatment facilities are adequate, neither LAS, secondary alcohol ethoxylates or alkylphenol ethoxylates should cause foaming in the waterways.

C. Borstlap, and C. Kortland, *The Biological Degradability of Nonionic Detergents Under Aerobic Conditions,* FSA *69,* p. 736, (1967).

The relation between the structure and the biological degradability of nonionic detergents was studied by determining the non-volatile organic matter after a fixed degradation time. It was found that benzene rings, branched alkyl chains and long ethylene oxide chains influence the degradation adversely.

R. L. Bunch and L. W. Chambers, *A Biodegradation Test for Organic Compounds,* J. Water Pollution Control Federation, *39,* p. 181, (1967).

The method is designed to be suitable for determining the biodegradability of any organic compound. A series of 4 consecutive die-away tests each of one week duration, are employed.

R. A. Conway, C. A. Vath, and C. E. Renn, *Biodegradable Detergents,* Water Works Wastes Eng. *2* (1), p. 28, (1965).

Nonionic and anionic ethoxylated adducts of linear secondary alcohols (11-15 C atoms) were removed by biooxidation in a conventional activated sludge process. Foam generation capacity of the plant effluent was reduced by 95%. Specific surfactants used were: an adduct of a linear secondary alcohol and 9 moles ethylene oxide, ammonium and sodium salts of sulfated three-mole adduct of the same linear secondary alcohol.

R. A. Conway and G. T. Waggy, *Biodegradability Testing of Typical Surfactants in Industrial Usage* Am. Dyestuff Reporter *55,* p. 607, (1966).

The susceptibility of surfactants to bacterial oxidation in industrial wastewater treatment plants and surface waters can be established through many fundamental approaches, including oxygen utilization and definitive residue tracing. The quality of various samples of materials of established biodegradability can be monitored by simple screening tests, eg, shake-culture, activated sludge, and river die-away. Biodegradability data were obtained using these techniques to test several commercial surfactants of interest to wet-processing industries. Detailed comparisons of linear secondary-alcohol ethoxamers with alkylphenol-based surfactants were made by several test procedures. The linear secondary-alcohol ethoxylates were indicated to be efficiently biodegraded under a range of environmental conditions, while the alkylphenol materials have not as yet been demonstrated to meet these degradation criteria.

J. Cuta, et. al., *Hygienic Problems of Detergents. IV. The Biochemical Oxidation of Anionic and Nonionic Saponates,* Cesk Hyg. *9,* p. 507, (1964).

Nonionics: Easiest degradation in low ethylene oxide adducts of natural substances. Medium degradation in high ethylene oxide adducts of natural substances or synthetic substances of low toxicity. Worst degradation in high adducts of toxic substances.

L. J. Garrison and R. D. Matson, *A Comparison by Warburg Respirometry and Die-Away Studies of the Degradability of Select Nonionic Surface Active Agents,* J. Amer. Oil Chemists' Soc. *41,* p. 799, (1964).

The relative biodegradability of several classes of nonionic surfactants has been determined by Shake Flask, Die-Away and Warburg Respirometer tests. Analytical techniques used to follow the degradation processes involved the measurement of loss of surfactant properties (surface tension of foamability), colorimetric determinations and oxygen uptake studies. Nonionic products prepared from naturally occurring or synthesized straight chain hydrophobes were shown to exhibit a higher degree of biodegradability than products based upon branched chain materials. A good correlation of data by the various analytical techniques was obtained on the straight chain based products.

H. H. Goldthorpe and J. Nixon, *Further Experiments with Synthetic Detergents at Huddersfield, Particularly with respect to their action on Percolating Beds,* J. Roy

Sanit. Inst. *70*, p. 116, discussion p. 127, (1950).

The treatment of domestic sewage with 200 ppm of detergent lessened acid precipitation necessitating extended aeration periods. An alkylphenol ethylene oxide adduct was oxidized by permanganate but was inert to biological purification.

K. W. Han, *Determination of the Biodegradability of Nonionic Detergents with Sulfation and Methylene Blue Extraction,* Tenside *3*, p. 109. (1966).

Followed biodegradation of primary alcohol, secondary alcohol, alkylphenol, and alkanolamide ethoxylates in a laboratory semi-continuous activated sludge system. Polyethoxylates are extracted from aqueous solution with chloroform, sulfated, and neutralized. Ether sulfates formed are determined by a modified Longwell-Maniece method.

H. J. Heinz, and W. K. Fischer, *The International State of the Methods for the Determination of the Biodegradability of Detergents and the Possibilities of an International Standardization,* Fette Seifen, Anstrichmittel, *68*, p. 955, (1966) and *69*, p. 188, (1967).

Review of existing methods proposing a standard combination of three integrated test methods.

R. Howell, Jr., *New LAS Surfactants Have a Wide Use in Chemical Specialty Formulations* Detergent Age *3*, (7), p. 26, (1966).

Laboratory batch activated sludge tests and field tests were applied to branched and linear alkyl phenol and secondary alcohol based nonionic surfactants. Alcohol derived products were found equivalent to linear alkylate sulfonate.

R. L. Huddleston and R. C. Allred, *Determination of Nonionic Surfactant Biodegradability: Physical Properties vs. Colorimetry,* J. Amer. Oil Chemists' Soc. *42*, p. 983, (1965).

Biodegradation studies with three alkylphenol nonionic products show that loss of cobaltothiocyanate colormetric sensitivity may not necessarily correlate with loss of surface tension lowering and foaming properties. Where this type of disagreement between analytical methods is present, only the surface tension and foam data are valid measurements of biodegradation.

R. L. Huddleston, *Biodegradable Detergents for the Textile Industry,* Am. Dyestuff Rept. *55*, (2), p. 52, (1966).

Demonstrated biodegradability of primary straight chain alcohol nonionic structures and the bioresistance of straight and branched chain alkylphenol nonionic structures. Three analytical methods were used including foam and surface tension measurements.

R. L. Huddleston, *Biodegradability Benefits for the Textile Industry,* Detergent Age *2* (9), p. 16, (1966).

Biodegradability of branched and straight-chain alkylphenol and straight-chain alcohol based nonionic surfactants was compared in laboratory Continuous Activated Sludge and River Die-Away tests. Alcohol based products were found equivalent to LAS. They were, therefore, judged adequately degradable at textile mills having simple effluent treatment systems.

J. V. Hunter and H. Heukelekian, *Determination of Biodegradability Using Warburg Respirometer Techniques,* Proceedings of the 19th Purdue Industrial Waste Conference, p. 616, Purdue University.

Advantages and disadvantages of "oxygen uptake" as a measure of biodegradability. Significant variable, and interpretation of results are discussed.

E. S. Lashen and K. A. Booman, *Biodegradability and Treatability of Alkylphenol Ethoxylates — A Class of Nonionic Surfactants,* Water and Sewage Works, Reference Number, p. R-155, (1967). See also Lashen, E. S.; Trebbi, G. F.; Booman, K. A.; and Dupre, J.; Soap and Chem. Specialties, *43*, (1), p. 55-58, p. 122, (1967).

90% degradation of an octylphenol ethoxylate in an extended aeration package waste treatment plant. Acclimation of river microflora was studied in parallel laboratory tests.

J. H. McFarland and P. R. Kinkel, *Performance and Properties of Nonionic Surfactants from Linear Secondary Alcohols,* J. Amer. Oil Chemists' Soc. *41*, p. 42, (1964).

Performance properties in detergent formulations are defined by the results of detergency and foam stability tests. Properties have been compared to the properties of the less degradable nonylphenol nonionics and to the nonionic surfactants from the linear alkylphenol, oxo alcohol and Ziegler alcohol hydrophobes.

L. W. Oldham, *Effects of a Nonionic Synthetic Detergent on Biological Percolating Filters,* Inst. Sewage Purif., J. Proc., Pt. 2, p. 136, (1958).

An alkylphenol ethylene oxide adduct (13 and 26.5 ppm) was added to settled sewage just prior to its feed to percolating filters. No evidence of biochemical oxidation of the detergent was detected.

Q. W. Osburn and J. H. Benedict, *Polyethoxylates Alkyl Phenols — Relation of Structure to Biodegradation Mechanism,* J. Amer. Oil Chemists' Soc., *43*, p. 141, (1966).

Using various isolation procedures and measurement by infra-red spectroscopy, the mechanism of biodegradation of the polyethoxylated alkyl phenols in the river water die-away test is described. Degradation is shown to proceed by carboxylation of the alkyl chain and, in cer-

tain cases, by degradation of the ethylene oxide chain. Degradation of the ether chain reportedly takes place only when the chain contains ten or less units of ethylene oxide.

S. J. Patterson, et. al., *Nonionic Dets. and Related Substances in British Waters,* 3rd International Conf. on Water Poll. Res., Munich, Germany, Sept. 5-9, 1966.

The thin layer chromatographic method was applied in a study of the occurrence and degradation of the nonionic detergents and polyglycols in the effluents and rivers in the Yorkshire wool manufacturing district of the United Kingdom.

S. T. Patterson, E. C. Hunt, and K. B. E. Tucker, *Determination of Commonly Used Nonionic Detergents in Sewage Effluents by a Thin Layer Chromatographic Method,* J. & Proc. of Inst. Sew. Purif., Part 2, p. 190, (1966).

The TLC method for determination of polyethoxylated nonionic detergents is described in detail. Results are given for the undegraded nonionic detergent content of a number of treated sewage effluents and river waters in the United Kingdom.

S. T. Patterson, C. C. Scott, and K. B. E. Tucker, *Nonionic Detergent Degradation, I, Thin-Layer Chromatography and Foaming Properties of Alcohol Polyethoxylates,* J. Amer. Oil Chemists' Soc. *44,* p. 407, (1967).

Method used for the chemical assessment of alcohol polyethoxylates and material derived from them during degradation under simple laboratory conditions. Foaming capacity also measured. With one exception (a material with a highly branched alkyl chain) the disappearance of all the alcohol-ethoxylates tested was rapid; a small increase in time required for complete disappearance was observed with the more highly ethoxylated materials, and with the materials which had some slight branching in the alkyl chain. Foam formation could be closely correlated with the results obtained by using the thin-layer chromatographic procedure.

S. T. Patterson, C. C. Scott, and K. B. E. Tucker, *Nonionic Detergent Degradation, II, Thin-Layer Chromatography and Foaming Properties of Alkyl Phenol Polyethoxylates,* J. Amer. Oil Chemists' Soc. *45,* p. 528, (1968).

Continuation of preceding paper which describes the application of the same procedures to the less degradable class of alkylphenol ethoxylates. It is noted that with these materials, the rate and extent of degradation is dependent upon the environment in which the degradation occurs.

The authors note that, as in the previously published work, the foaming capacity during degradation could be closely correlated with the results obtained using the thin-layer chromatographic procedure.

P. Pitter, *Colorimetric Determination of Nonionic Surfactants with Hydroquinone and Contribution to Determination of Anionic and Cationic Surfactants,* Techn. of Water *6,* 1, p. 547, (1962).

Nonionic: the isolated surfactant—barium—tungstophosphoric acid complex gives a red color with hydroquinone in concentrated sulfuric acid medium. The method is sensitive and it may be possible to determine tenths of ppm of isolated surfactant. Reproducibility of the determination at 3.0 ppm. Slovasol O (condensation product of ethylene oxide with an oleyl-cetyl alcohol) is $\pm$ 0.1 ppm.

P. Pitter and J. Trauc, *Synthetic Surface-Active Agents in Waste Waters IV, Biological Degradation of Nonionic Agents in Laboratory Models of Aeration Tanks,* Sb. Vysoke Skoly Chem. Technol. Praze, Technol. Vody 7 (1), p. 201-16, (1964).

Condensation product of oleyl and cetyl alcohol with 20 mols. of ethylene oxide is not assimilated by bacteria, while lauryl alcohol and 4 mols. of ethylene oxide is readily attacked. Up to 20 mg/l of both substances does not affect purification and nitrification in the activated sludge process, but at the maximum concentration considerable foaming occurs.

P. Pitter, *An Improved Technique for the Determination of Polyoxyethylene Surfactants with Hydroquinone,* Chem. & Ind., p. 1217, (1966).

This technique is sensitive to compounds containing three or more moles of ethylene oxide. It covers the concentration range 0-5 ppm. Sulfates do not interfere because barium was replaced by calcium.

E. Ruschenburg, *Structure Elements of Detergents and Their Influence on Biochemical Degradation,* Vom Wasser, 30, p. 232, (1963).

Addition of ethylene oxide to a C_{16} primary alcohol reduced susceptibility of biochem. degradation in proportion to the increasing length of the oxyethylene chain.

W. Schonborn, Paper presented during conference at House of Technology, Essen, Germany, Feb. 18, 1966.

Modification of iodobismuthate method for ethoxylate type nonionics. Substitutes colorimetric technique in place of sediment measurement.

L. H. Smithson, *Properties of Ethoxylate Derivatives of Nonrandom Alkylphenols,* J. Amer. Oil Chemists' Soc. *43,* p. 568, (1966).

Optimum biodegradability of C_9 - C_{18} nonrandom linear alkylphenol ethoxylates is obtained when the phenol is attached near the end of the alkyl side chain. Performance of ethoxylates and ethoxysulfates evaluated in typical heavy and light duty formulations.