The Status of Biodegradability Testing of Nonionic Surfactants[1]

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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 biodegradability 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 detergency 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 below, 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-

Some Typical Nonionic Surfactants

1. $RO(C_2H_4O)_nH$ ..... Primary alcohol ethoxylate
2. $HCO(C_2H_4O)_nH$ (R on C, CH_3 on C) ..... Secondary alcohol ethoxylate
3. $R-C_6H_4-O(C_2H_4O)_nH$ ..... Alkylphenol ethoxylate
4. $R-C(=O)-NHC_2H_4OH$ ..... Alkyl monoethanolamide
5. $R-C(=O)-N(C_2H_4OH)_2$ ..... Alkyl diethanolamide
6. $R-N(CH_3)_2 \rightarrow O$ ..... Alkyl dimethyl amine oxide

Note: R = C_8 — C_{18} alkyl chain

[1] Prepared under the auspices of The Subcommittee on Biodegradation Test Methods of The Soap and Detergent Association.

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 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, they are often modified chemically so that their original identity is lost, or they 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 non-members 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 Sub-Committee on Biodegradation Test Methods has, since 1967, concentrated its research efforts on the four 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:

(a) Primary biodegradation: Biodegradation to the minimum extent necessary to change the identity of the compound. (b) Environmentally acceptable biodegradation: Biodegradation to the minimum extent necessary to remove undesirable properties of the compound such as foaminess or toxicity. (c) 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:

1. 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.

2. 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.

3. 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.

4. 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.

5. 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.

6. 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 of 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.

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.

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 the activated-sludge type of sewage treatment plants. The equipment, operating procedures and criteria of adequate biodegradability to be applied in this test are all specified by German law.

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.

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.

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.

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.

Publications by Patterson et al. (9,10) discuss the applicability of this procedure to nonionics using both chromatographic and foam measurement techniques.

The Bunch-Chambers Method (11)

Determination of biodegradability by this method involves a series of four consecutive die-away tests,

TABLE I
Nutrient Media Used in Semi-Continuous Activated Sludge Tests

Nutrients	Standard procedure for anionics mg/liter	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[a]	130[a]
Ammonium sulfate	25	.....	25	.....	.....

[a] This concentration could be raised if acidic conditions were encountered.

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. Neither the method of analysis nor criteria of biodegradability are specified since they are dependent upon the specific material under test.

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. 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 material 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 considerably 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 15 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 No. 2 (see Table I). However, better pH stability was achieved with the medium used in test series No. 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, 6, 17 and 23 hr were evaluated at initial surfactant concentrations of 10 mg/liter and 20 mg/liter.

One of the burdensome features of any method requiring daily care is the necessity of weekend feeding. Some cooperating laboratories serviced their Semi-Continuous Activated Sludge units daily from Mondays through Fridays but omitted weekend 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 weekend 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 included in a forthcoming publication of the SDA (13). The three procedures used in the biodegradability testing program are discussed 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 this 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

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/cm²		Foam loss, %		CTAS-MBAS loss, %		Surface tension, dynes/cm²	
			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	100	100	100	100	100	70	71	98	100	100	100	70	71
Linear secondary alcohol ethoxylate	1.8	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	95	43	80	81	100	48	52	0	0	15	44	40	44
Branched tridecyl alcohol ethoxylate	4.0	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 hr 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/liter) and shake-flask (30 mg/liter) initial levels.
[b] Initial and weekly samples of the die-away systems were analyzed by the colorimetric, foam and surface-tension techniques. The surface tension value reported compares to an initial median value of 43 dynes/cm². Test concentration was 10 mg/liter.
[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/liter.

several limitations, it was believed 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 1. 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, 11 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 described more fully in Appendix A 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 11 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 and 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 2. 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 pro-

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-Octylphenoxy-nonaethoxyethanol	<60	4	0	0

[a] Bunch-Chambers (FWPCA) die-away test system consisted of 20 mg/liter surfactant and 50 mg/liter 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/liter or reducing the surfactant level to 10 mg/liter.

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-Octylphenoxy-nonaethoxyethanol	<60	9	2	0
Methyl branched linear primary alcohol ethoxylate	99	0	1	7

[a] Laboratory activated-sludge units were operated on a 23 hr aeration cycle with degradation monitored by reduction in foaming character of the clarified unit effluent after 5 and 17 hr. The 17 hr 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 hr detention tests; several laboratories reported values below 80%.

posed 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 hr. 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/liter of surfactant gave a foam volume equivalent to a control effluent spiked with 1 mg/liter 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 2, 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 hr. The source of the sludge inoculum was recognized as a critical factor. It was stipulated that sludge should be obtained from a plant 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/liter and 20 mg/liter 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/liter and 1.0 mg/liter. 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

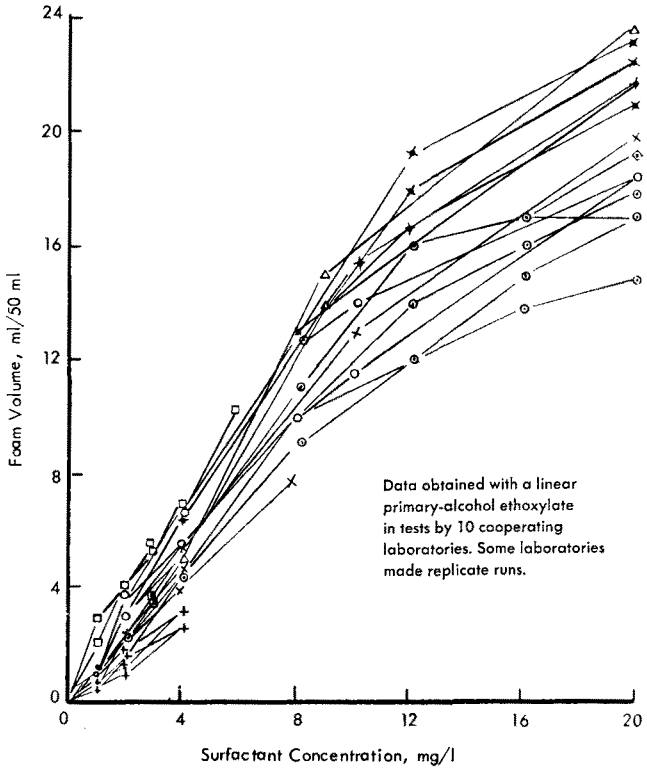

FIG. 1. Typical foam calibration curves.

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 A).

When the Semi-Continuous Activated Sludge method was operated at a 5 hr 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 hr 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

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-Octylphenoxy-nonaethoxyethanol	<60	10	0	0

[a] Laboratory activated-sludge units were operated on a delayed surfactant-feeding basis, yielding per cent degradation values for 6 and 17 hr detention periods. The 17 hr data with 10 mg/liter surfactant dosage are shown in this Table; the 6 hr 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] Per cent degradation was obtained by comparing unit-effluent foam values with foam measurements on surfactant spikes (1 and 2 mg/liter) 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.

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 (14), since the specific field environment is often most resourceful in bringing about the degradation of synthetic chemicals (4). Laboratory data obtained during this phase of the cooperative study are summarized in Table V.

Phase 3. 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 2 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 A. 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 hr 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 11 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 per cent 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 measurements made during

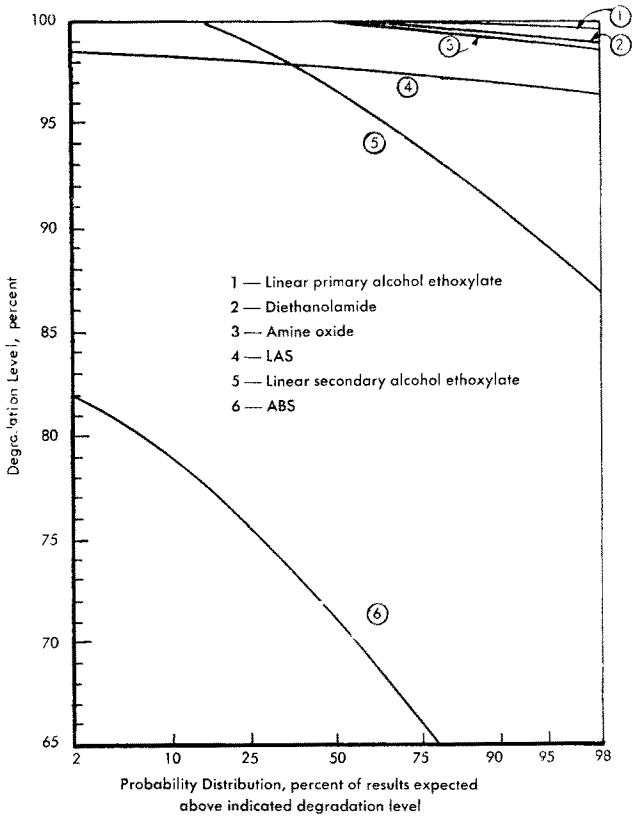

FIG. 2. Statistical analysis of biodegradation results (Phase 3). LAS, linear alkylate sulfonate; ABS, tetrapropylene-derived.

the test could be converted directly into per cent degradation. These measurements were generally the responsibility of one individual in each laboratory so that a high degree of reproducibility could be assured. Figure 1 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 was developed from such data (Table VI). These data have also been plotted in terms of statistical probability as shown in Figure 2.

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.

Applicability of Procedure

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

TABLE VI
Biodegradation Test Results With Modified Semi-Continuous Activated Sludge Test, Fourth Cooperative Study[a]

Surfactant	Degradation, %
Linear primary alcohol ethoxylate	99.7
Linear secondary alcohol ethoxylate	96.2
Diethanolamide	99.8
Amine oxide	99.6
LAS	97.5
ABS	70.3

[a] Laboratory activated-sludge units were operated on a 23 hr-aeration/1 hr-settling cycle at 2500 (±500) mg/liter 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/liter) and synthetic food (300 mg/liter glucose, 200 mg/liter nutrient broth, and 130 mg/liter 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 limited to 32 days.

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 sponsorship; however, individual companies have conducted such research on several of the materials evaluated during Phases 2 and 3. 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.

Although these field studies have been described in the literature (14,15,16) 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 (15). Overall removal was in the order of 94% with an initial surfactant concentration of 22 mg/liter. 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 (14). An extended aeration type sewage treatment plant was also used in this study. Initial surfactant concentrations of 5.3 mg/liter, 9.5 mg/liter and 11.0 mg/liter 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 (16). 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.

Discussion

Based on the results of this three year program, 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. The exhaustive review by the Subcommittee of the available chemical analytical methods indicates that, at present, neither a single method nor group of methods is available to measure accurately both the original molecule and the intermediate degradation products of the many, multistructured compounds which are broadly classified as nonionic surfactants. For this reason, physical techniques such as the measurement of surface tension changes and foam loss seem to offer some promise. Of the two, greater consistency was achieved through the use of a foam loss procedure. However, great variability was noted between laboratories and even within the same laboratory (when the test was run by different operators). This suggests that 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 considered in the overall 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 3 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.

This report is only 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

Thanks to the members of the Subcommittee on Biodegradation Test Methods of The Soap and Detergent Association and to their member companies for advice and assistance, to M. A. Goldberg of Lever Bros. who served as Chairman of the Subcommittee, and to

Leslie Bacon of Wyandotte Chemical Corp. who developed, tested and promulgated analytical methods. The Federal Water Pollution Control Administration and the Surfactant Analysis Subcommittee of The Soap and Detergent Association also contributed valuable assistance.

REFERENCES

1. Subcommittee on Biodegradation Test Methods, JAOCS *42*, 986 (1965).
2. Schick, M. J. "Nonionic Surfactants," Vol. 1, First Edition, Marcel Dekker, Inc., New York, 1967, p. 1.
3. U.S. Tariff Commission, U.S. Government Printing Office, "Synthetic Organic Chemicals," TC Publication 248, Washington, D.C., *1968*, p. 157.
4. Standard Methods Committee, Subcommittee on Biodegradability, J. Water Pollution Control Federation *39*, 1232 (1967).
5. Federal Council of the German Federal Republic, "Regulation Concerning the Degradation of Detergents in Washing and Cleansing Agents," Publication 253/62, German federal law (1962).
6. Standing Technical Committee on Synthetic Detergents, "Supplement to the Eighth Progress Report," Her Majesty's Stationery Office, London, 1966.
7. Glass, A. C., and K. H. Jenkins, "Statistical Summary of 1962 Inventory Municipal Waste Facilities in the United States," Public Health Service Publication No. 1165, U.S. Government Printing Office, Washington, D.C., 1964.
8. Hammerton, C., J. Appl. Chem. *5*, 517 (1955).
9. Patterson, S. J., C. C. Scott and K. B. E. Tucker, JAOCS *44*, 407 (1967).
10. Patterson, S. J., C. C. Scott and K. B. E. Tucker, Ibid., 528 (1968).
11. Bunch, R. L., and C. W. Chambers, J. Water Pollution Control Federation *39*, 181 (1967).
12. Bogan, R. H., and C. N. Sawyer, Sewage Ind. Wastes *26*, 1069, (1954).
13. The Soap and Detergent Association, "The Status of Biodegradability Testing of Nonionic Surfactants," Scientific and Technical Report No. 6, in press.
14. Lashen, E. S., and J. C. Lamb III, Water Wastes Eng. *4*, 56 (1967).
15. Conway, R. A., C. A. Vath and C. E. Renn, Water Works Wastes Eng. *2*, 28 (1965).
16. Brenner, T. E., JAOCS *45*, 433 (1968).
17. Satterthwaite, F. E., Biometrics Bulletin *2*, 110 (1964).

Appendix A

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

Linear Primary Alcohol Ethoxylate. An average 10 mole 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 mole 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 mole 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 mole 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 moles 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 is 85% para and 15% ortho.

Branched Tridecyl Alcohol Ethoxylate. An average 9 mole 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 mole 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 mole 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 B

Statistical Methodology Employed

The data, as received from the participating laboratories, were expressed in milliliters of foam. Foam calibration data (foam height vs. mg/liter 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 per cent of surfactant removed in each instance.

Examination of the data expressed as per cent of surfactant removal revealed that the variance within runs or between runs within laboratories was a function of per cent removed. Small variances were associated with large per cent removals 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 per cent removal 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 per cent 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 with 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 (17) 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 per cent of surfactant removed.

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