Biodegradation of Some Tallow-Based Surface Active Agents in River Water[1]

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Abstract

Thirty-three anionic and six nonionic detergents have been screened for biodegradability using the river water "Die Away" test. Concentrations of nonionic detergents were best determined by the surface tension method. All fat-derived compounds without mid chain substitution were found to degrade in Schuylkill River water at 20C in less than a week.

Introduction

INCREASED ATTENTION to pollution control and the adverse publicity given to the effect of non-biodegradable detergents on our rivers and streams in recent years makes it desirable to know about the biodegradability of each new compound as it is suggested for use as a detergent. Although it can be postulated that all straight chain surface active agents will be easily degraded, the actual tests recorded in the literature are limited. Since this laboratory has synthesized a number of tallow-based compounds, particularly those derived from the α-sulfo fatty acids, we have undertaken to verify the expected susceptibility to biological attack and to study the effects of some unusual structures on ease of degradation. The "River Water Die Aaway" test (5,9) was used because it enabled a large number of detergents to be screened in a relatively short time. Although this test has recognized limitations, it was found to be useful in obtaining preliminary information on ease of biodegradation.

Experimental

Materials. Our previous publications have described the preparations of the α-sulfo fatty acids, their salts, esters, and other derivatives (10), oxyethylated acids and alcohols (15) ether alcohol sulfates (3,11,14), and salts of α-phosphono fatty acid esters (6). The sample of alkylbenzenesulfonate (ABS) used here for comparative purposes was a commercial product, 90% active, with an alkyl chain derived from polypropylene. The degradation curve was very similar to that of a standard ABS supplied by the Soap and Detergent Assoc. after suitable corrections were made for difference in per cent active ingredient. Sodium lauryl sulfoacetate, sodium oleyl N-methyl tauride, sodium dioctyl sulfosuccinate and oxyethylated nonylphenol were also commercial samples 60,72,100 and 100% active, respectively. Correction was made in each case for active ingredient content when stock solutions were prepared.

The River Water Test. Detergent solutions containing 100 ppm active ingredients were prepared in distilled water and then diluted to 5 ppm with fresh river water. With the exception of a few samples used for comparison, all river water used in the tests came from the Schuylkill River at Fairmount Park, Philadelphia.

The solutions containing 5 ppm detergent in river water were stored in the dark at 20C and aliquots were analyzed regularly for detergent content as indicated below.

Analysis for Anionic Detergents. The methylene blue method of Degens (4) was used for all anionic detergents. A 10-ml aliquot of the storage test solution was added to 100 ml distilled water and treated with 5 ml 0.035% methylene blue solution, made acid with 1.2% sulfuric acid. The salt which formed from detergent and methylene blue was removed from the aqueous methylene blue sulfate solution by three 10-ml chloroform extractions. Combined chloroform extracts were made up to 50 ml and absorbance at 650 mμ was measured in a 21-mm diam tube using a Coleman Universal Spectrophotometer.

Analyses of dilutions of 5 ppm sodium dodecanesulfonate solutions showed the straight line relationship between absorbancy and concn predicted by Beer's law. This curve for sodium dodecanesulfonate was used to convert absorbancy values to concn as sodium dodecanesulfonate. Concn as sodium dodecanesulfonate was in turn converted to ppm of each detergent by appropriate mol wt correction, assuming a 1:1 stoichiometric relationship between dye molecule and surface active agent.

Concn determined by the methylene blue method and corrected for molecular weight gave reliable analyses with detergents having one highly ionized hydrophilic group such as the alkyl sulfates, alkanesulfonates or simple alkyl esters of α-sulfo fatty acids. The H_2SO_4 present in the reagent does not permit soaps or carboxylic acids to react and considerably reduces the reaction of the partially ionized phosphonates.

Analysis for Nonionic Detergents. At present there appears to be no satisfactory chemical or physical method for the analysis for nonionic detergent at very low concn (5 ppm or less). Of the chemical methods the colorimetric method of Pitter (8) seemed to be the most promising but considerable difficulty was encountered in separating and washing the precipitated phosphotungstic acid complex. Pitter points out that color development is sensitive to water content, and that the results are poorly reproducible with nonionics that contain a small number of oxyethyl groups.

Two physical methods, the eventual disappearance of foam and the gradual rise in surface tension, were investigated but correlation between the two effects was poor in many cases. Since it was found that high mol wt polyethylene glycols have foaming properties in their own right and some nonionic detergents have no perceptible foam at low concn, the surface tension method was preferred. Accordingly surface tension-dilution curves were obtained for each nonionic, from which surface tension measurements obtained throughout the storage period were converted to concn values.

Results and Discussion

River Water Samples. Sodium dodecanesulfonate was used as a standard to check the biological activity of each river water sample. Figure 1 shows a graph for the disappearance of sodium dodecanesulfonate in samples of water taken from the same spot in the Schuylkill River at various times from August 13, 1962 to March 22 of the following year. With the

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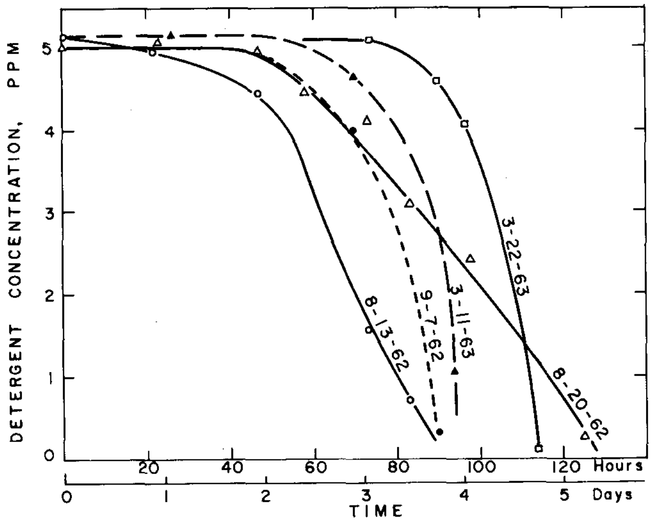


FIG. 1. Seasonal variations of sodium dodecanesulfonate biodegradation in Schuylkill River water: ○ Aug. 13; △ Aug. 20; ● September 7, 1962; ▲ March 11; □ March 22, 1963.

exception of the sample of 8-13-62, taken after a severe summer drought, all of the curves are nearly the same.

Figure 2 shows curves for the disappearance of sodium dodecanesulfonate in samples taken from the Schuylkill River at Fairmount Park, Philadelphia, the Delaware River at Stockton, N. J., and the Susquehanna River near Drumore, Pa. Degradation appears to proceed at about the same rate in all three of these water samples. When the water from small creeks was used, the results were more erratic and blanks varied a great deal more depending on local conditions.

Analysis for water hardness by titration with a standard solution of ethylenediaminetetraacetic acid (2) and pH values for these samples show in Table I. There is not much variation in pH. Water hardness values reflect variations in rainfall.

TABLE I

Analysis of River Water Samples

Water sample	Hardness ppm (2)	pH
Schuylkill River		
August 13, 1962	106	7.93
August 20, 1962	160	7.85
September 7, 1962	129	7.53
September 14, 1962	......	7.44
November 26, 1962	......	7.40
December 10, 1962	100	7.68
March 11, 1963	77	7.58
March 22, 1963	77	7.60
Delaware River		
March 28, 1963	26	7.75
Susquehanna River		
March 30, 1963	35	7.60

Degradation of Anionic Detergents. Figure 3 illustrates the range of materials being considered here: the very soft or very easily biodegraded, sodium alkyl sulfate; the soft sodium alkanesulfonate; an example of a soft α-sulfo fatty acid derivative; and the hard tetrapropyl type ABS, quite resistant to biodegradation.

Figure 4 presents the degradation curves for sevseral α-sulfopalmitates and an α-phosphonopalmitate. Although disodium α-sulfopalmitate has a slightly higher initial value because it is dianionic, the curves for diacid, disodium salt and methyl ester are very much alike. A curve for sodium isopropyl α-phosphonopalmitate shows the effect of the sulfuric acid in the reagent in suppressing the ionization of the weakly acid phosphono group resulting in lower analytical values.

At concn below 1 ppm, the degradation curves sometimes showed erratic behavior, probably because of analytical difficulties at this low concn. More valid comparison could be obtained therefore by comparing the time required for 80% degradation. Table II lists the time required for the disappearance of 80% of each surface active agent. Since the degradation time for sodium dodecanesulfonate showed some variations, the time relative to that for sodium dodecanesulfonate in river water of the same date is recorded in the last column of Table II.

Analysis for the α-phosphono esters by the methylene blue method is not quantitative. Although the analytical values were low for sodium isopropyl α-phosphonopalmitate, they do appear to show 80% degradation in about 75 hr. Sodium amyl α-phosphonopelargonate gave an even lower analysis and greater time was required for degradation. It was not possible to analyze for trisodium α-phosphonopalmitate because a tendency to precipitate in hard

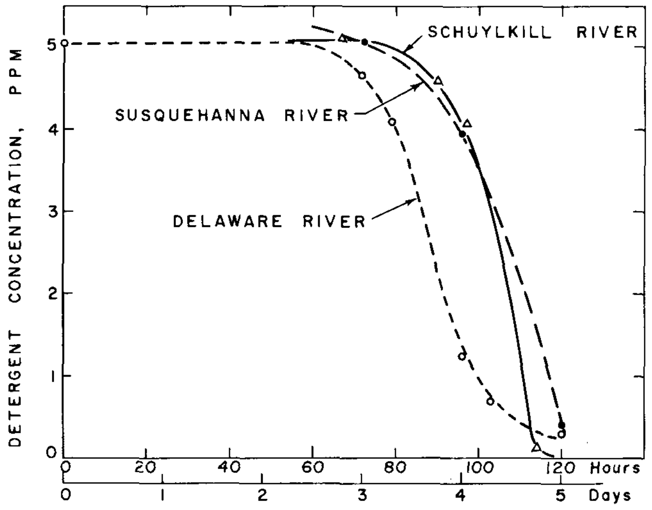


FIG. 2. Biodegradation of sodium dodecanesulfonate in water from different sources: ○ Delaware River, March 28; △ Schuylkill River, March 22, ● Susquehanna River, April 5, 1963.

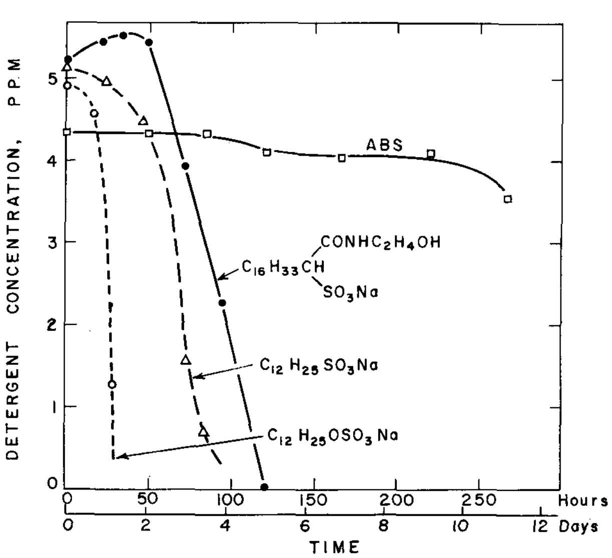


FIG. 3. Biodegradation of anionic surface active agents in Schuylkill River water: ○ sodium dodecyl sulfate; △ sodium dodecanesulfonate; ● sodium N-hydroxyethyl α-sulfostearamide; □ sodium alkylbenzenesulfonate (tetrapropyl type, ABS).

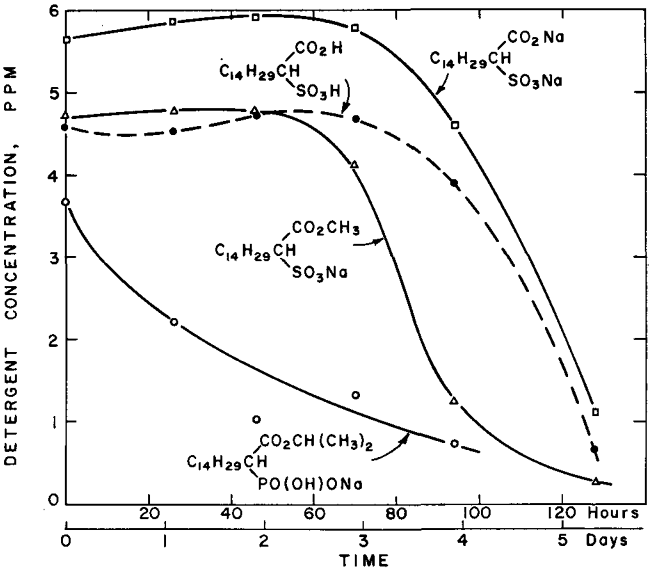


Fig. 4. Biodegradation of α-sulfopalmitates and α-phosphonopalmitate: ○ sodium isopropyl α-phosphonopalmitate; △ sodium methyl α-sulfopalmitate; ● α-sulfopalmitic acid; □ disodium α-sulfopalmitate.

water further complicated the results (6).

From the values listed in Table II, it appears that the surfactants can be divided arbitrarily into four categories: 1) very soft, 2) soft, 3) moderately hard, and 4) hard. Although the first three categories may be considered soft by most workers in this field, we have chosen to make finer distinctions in order to relate structure to biodegradability. It is also possible that these differences may be important where decomposition takes place under the anaerobic conditions of a cesspool or septic tank.

The very soft (0–3 days) group includes the simple alkyl sulfates, carboxylic esters, and amides where the hydrophobic and hydrophilic portions are easily separated by hydrolysis, thus destroying surface activity. Determination of biological oxygen demand does not show that this type of compound uses up oxygen any faster than other straight-chain materials. With the exception of a few unusual chemical structures which will be described shortly, all of the alkanesulfonates, the α-sulfo fatty acids, their salts, esters and amides belong to the soft group (3–7 days).

The moderately hard group (1–2 weeks) includes a number of compounds of unusual structure. They may be suspected of interfering with bacteriological activity by virtue of their exceptional wetting properties or by phenyl or chlorine substitution on the alkyl chain. The curves for disodium α-sulfophenylstearate and disodium dichloro-α-sulfostearate (13), plotted on Figure 5, show some evidence of stepwise breakdown. Disodium dihydroxy-α-sulfostearate on the other hand degrades more rapidly than the unsubstituted α-sulfo fatty acids.

The materials which we have chosen to classify as hard (greater than two weeks) are typified by polypropyl benzenesulfonate (ABS), plotted in Figure 3. This showed only 55–70% degradation after one month under these conditions.

Degradation of Nonionic Detergents. Figure 6 shows biodegradation curves for four nonionic detergents of different structure. Concn for these curves were determined by the surface tension method. Sucrose monopalmitate shows very rapid initial degradation, followed by a slower degradation in the final stages which may indicate that some of the intermediates in the degradation process are surface active. Degradation of the ester type nonionic proceeds immediately and rapidly while the ether type appears to go through a short induction period before rapid degradation proceeds. The curve for hexadecanol with 20 oxyethyl groups and that for hexadecanol with 10 oxyethyl groups were found to be identical by this method. Nonylphenol nonionic shows very little degradation in this time. The apparent initial decrease in concn of nonylphenol nonionic is probably due to ageing effects on surface tension.

Table III compares 80% degradation times obtained from the curves with time required for disappearance of foam. Although hexadecanol with 10 oxyethyl groups showed good agreement between methods, hexadecanol with 20 oxyethyl groups showed

TABLE II
Biodegradation of Anionic Detergents in Schuylkill River Water, 20C

Compound	Time in hr for 80% degradation	Relative ease of biodegradation	
		Class	Relative to $C_{12}H_{25}SO_3Na$, %
Sulfates			
$C_{12}H_{25}OSO_3Na$	32, 42, 42	I	38, 39
$C_{16}H_{33}(OC_2H_4)_3OSO_3Na$	45, 52	I	55, 47
$C_8H_{17}CHClCHCl(CH_2)_6CH_2OSO_3Na$	82	II	110
Esters and Amides			
$C_{12}H_{25}O_2CCH_2SO_3Na$ [a]	40	I	54
$C_{15}H_{31}CO_2C_2H_4SO_3Na$ [a]	19	I	28
$C_{17}H_{33}CON(CH_3)C_2H_4SO_3Na$ [a]	62	I	91
$C_{14}H_{29}NHCO(CHOH)_{3.5}(CHOSO_3Na)_{.5}CH_2OSO_3Na$ [b]	92	II	110
Alkanesulfonates			
$C_{12}H_{25}SO_3Na$	82, 116, 87, 88, 68, 98, 111, 98	II	100
$C_{16}H_{33}SO_3Na$	103	II	89
$C_{17}H_{35}SO_3Na$	117	II	100
α-Sulfo Fatty Acids and Salts			
$C_{14}H_{29}CH(SO_3H)CO_2H$	125	II	128
$C_{14}H_{29}CH(SO_3NH_4)CO_2H$	128	II	130
$C_{14}H_{29}CH[SO_3NH(C_2H_4OH)_3]CO_2H$	126	II	135
$C_{14}H_{29}CH(SO_3Na)CO_2Na$	90, 128	II	82, 130
$C_{16}H_{33}CH(SO_3H)CO_2H$	180	III	220
$C_{16}H_{33}CH[SO_3NH(C_2H_4OH)_3]CO_2H$	91, 115	II	83, 155
$CH_3(CH_2)_xCHPh(CH_2)_yCH(SO_3Na)CO_2Na$ [c]	335	III	300
$C_8H_{17}CHClCHCl(CH_2)_6CH(SO_3Na)CO_2Na$	186	III	250
$C_8H_{17}CHOHCHOH(CH_2)_6CH(SO_3Na)CO_2Na$ [d]	89	II	81
$C_8H_{17}CHOHCHOH(CH_2)_6CH(SO_3Na)CO_2Na$ [e]	84	II	76
Esters of α-Sulfo Fatty Acids			
$C_7H_{15}CH(SO_3Na)CO_2C_6H_{13}$	245, 233	III	265
$C_{14}H_{29}CH(SO_3Na)CO_2CH_3$	103	II	105
$C_{14}H_{29}CH(SO_3Na)CO_2CH(CH_3)_2$	120	II	122
$C_{14}H_{29}CH(SO_3Na)CO_2C_4H_9$	164, 167	II	190
$C_{16}H_{33}CH(SO_3Na)CO_2CH_3$	93	II	80
$C_{16}H_{33}CH(SO_3Na)CO_2CH(CH_3)_2$	120, 115	II	103, 132
$C_{16}H_{33}CH(SO_3Na)CO_2C_2H_4SO_3Na$	130, 116	II	112, 133
$C_8H_{17}O_2CCH_2CH(SO_3Na)CO_2C_8H_{17}$ [a]	113	II	103
α-Sulfo Sulfate and α-Sulfo Amide			
$C_{16}H_{33}CH(SO_3Na)CH_2OSO_3Na$ [g]	92	II	135
$C_{16}H_{33}CH(SO_3Na)CONHC_2H_4OH$	111, 116	II	135, 105
ABS			
$RC_6H_4SO_3Na$ [a, f]	>700	IV	>800

[a] Commercial products.

[b] Sesquisulfate of N-tetradecyl-D-gluconamide (7).

[c] α-Sulfonation of the Friedel-Crafts reaction product from oleic acid and benzene (13).

[d] Sulfonation of *erythro*-9,10-dihydroxystearic acid (13).

[e] Sulfonation of *threo*-9,10-dihydroxystearic acid (13).

[f] 50% biodegradation in 20 days.

[g] Sulfation of 2-sulfooctadecanol (12).

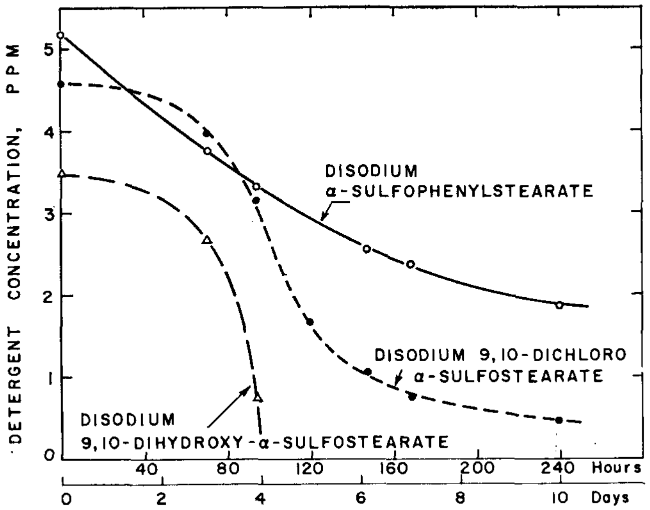
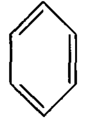


FIG. 5. Biodegradation of some substituted α-sulfostearates:
△ $CH_3(CH_2)_7CHOHCHOH(CH_2)_6CH(SO_3Na)CO_2Na$;
● $CH_3(CH_2)_7CHClCHCl(CH_2)_6CH(SO_3Na)CO_2Na$;
○ $CH_3(CH_2)_xCH(CH_2)_yCH(SO_3Na)CO_2Na$, where $x + y = 14$.



$CH_3(CH_2)_x\underset{C_6H_5}{\underset{|}{CH}}(CH_2)_yCH(SO_3Na)CO_2Na$

no correlation whatsoever; the surface tension method showed 80% degradation in 43 hr but foam persisted for greater than 25 days.

Although it has been possible to make some interesting comparisons of a large number of compounds using the river water die-away test, it must be remembered that this method has serious limitations and the results must be carefully qualified. There is need for a more thorough analysis of degradation products which may be formed in the process either as intermediates or end products. It is also important to know how these materials will perform under actual operating conditions with sewage sludge microorganisms.

Relative Toxicity

Most of this paper concerns the α-sulfo esters, now commercially available under the trade name Bioterge (1). It is pertinent to include here preliminary evaluation of relatively toxicity, and skin tests on α-sulfo and α-phosphono compounds by the Pharmacological Laboratory of the W. Utiliz. Res. and Dev. Div. The data pertain only to *acute oral toxicity*, measured by administration of a single dose, or, when necessary, closely spaced multiple doses, of an aqueous solution to mice via stomach tube. They do not necessarily give any clue to possible effects resulting from repeated ingestion over long periods of time. With this understanding, 22 α-sulfo esters were categorized as follows. LD_{50} dosage range, mg/kg mouse *>1000* and *<2000*: sodium amyl α-sulfopelargonate; *3000 mg/kg*: sodium ethyl and isopropyl α-sulfostearate; *>3000 mg/kg*: sodium 2-ethylhexyl α-sulfopelargonate, sodium propyl α-sulfolaurate, sodium methyl, ethyl and propyl α-sulfomyristate, sodium methyl, ethyl, propyl, isopropyl, butyl, and amyl α-sulfopalmitate, disodium 2-sulfoethyl α-sulfopalmitate, sodium dodecafluoroheptyl α-sulfopalmitate, sodium methyl, propyl, butyl, and amyl α-sulfostearate; *>4000 mg/kg*: disodium 2-sulfoethyl α-sulfostearate, sodium dodecafluoroheptyl α-sulfostearate.

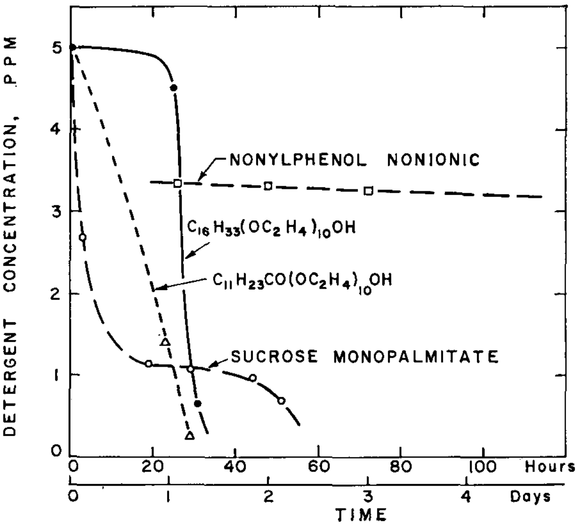


FIG. 6. Biodegradation of nonionic surface active agents.

TABLE III

Degradation of Nonionic Detergents in Schuylkill River Water, 20C

Compound	Surface tension		Time in days for foam disappearance
	% Degradation in 48 hr	Time in hr for 80% degradation	
$C_{11}H_{23}CO(OC_2H_4)_{10}OH$	100	25	3
$C_{18}H_{35}(OC_2H_4)_6OH$	74	64	2
$C_{16}H_{33}(OC_2H_4)_{10}OH$	95	34	1½
$C_{16}H_{33}(OC_2H_4)_{20}OH$	95	43	>25
Sucrose monopalmitate	84	43	10
Branched chain nonylphenol nonionic	....	840	>60

Approxiate oral LD_{50} values for analogous α-phosphono compounds were as follows. *2500 mg/kg* mouse: α-phosphonolauric acid; *3000 mg/kg*: α-phosphonopelargonic acid and sodium isopropyl α-phosphonolaurate; *>3000 mg/kg*: sodium methyl α-phosphonomyristate.

Skin Tests on α-phosphono and α-sulfo compounds were also carried out. A 2% solution of each of the 22 α-sulfo esters listed above was applied to the shaved back of two albino rats. After 15 daily applications and one week after the last application, no signs of irritation were observed for any of the compounds tested. Skin tests were also negative for 2% solutions of α-phosphonopelargonic acid, α-phosphonolauric acid, sodium isopropyl α-phosphonolaurate, and sodium methyl α-phosphonomyristate.

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REFERENCES

1. Anonymous, Chem. Eng. New *41*, No. 11, 102–108, 110–112, 114, 126 (1963).
2. Bersworth Chemical Co., Technical Bulletin No. 2, "The Versenes," Framingham, Mass. (1953).
3. Bistline, R. G., Jr., A. J. Stirton, J. K. Weil, and E. W. Maurer, JAOCS *34*, 516–518 (1957).
4. Degens, P. N., Jr., H. C. Evans, J. D. Kommer, and P. A. Winsor, J. Appl. Chem. *3*, 54–61 (1953).
5. Hammerton, C., *Ibid. 5*, 517–524 (1955).
6. Maurer, E. W., A. J. Stirton, W. C. Ault, and J. K. Weil, JAOCS in press.
7. Mehltretter, C. L., M. S. Furry, R. L. Mellies, and J. C. Rankin, *Ibid. 29*, 202–207 (1952).
8. Pitter, P., Chem. Ind. *1962*, 1832–1833.
9. Sawyer, C. N., R. H. Bogan, and J. R. Simpson, Ind. Eng. Chem. *49*, 236–240 (1956).
10. Stirton, A. J., JAOCS *39*, 490–496 (1962).
11. Weil, J. K., R. G. Bistline, Jr., and A. J. Stirton, J. Phys. Chem. *62*, 1083–1085 (1958).
12. Weil, J. K., F. D. Smith, A. J. Stirton, and R. G. Bistline, Jr., JAOCS *40*, 538–541 (1963).
13. Weil, J. K., A. J. Stirton, R. G. Bistline, Jr., and W. C. Ault, *Ibid. 37*, 679–682 (1960).
14. Weil, J. K., A. J. Stirton, R. G. Bistline, Jr., and E. W. Maurer, *Ibid. 36*, 241–244 (1959).
15. Wrigley, A. N., F. D. Smith, and A. J. Stirton, *Ibid. 34*, 39–43 (1957).

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