SOME FACTORS INFLUENCING DETERGENCY AND GERMICIDAL ACTIVITY OF DETERGENT-SANITIZERS

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THESIS

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SOME FACTORS INFLUENCING DETERGENCY AND GERMICIDAL ACTIVITY OF DETERGENT-SANITIZERS

by

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INTRODUCTION

Public health officials are well aware of the importance of eating utensil sanitization. Lynch and Cumming (1919) have established the fact that saliva-borne disease may be transmitted indirectly through the improperly sanitized utensil. Mallmann and Zaikowski (1947) have shown that, when working with a mechanical dishwasher, it is possible to remove all public health hazard from a properly cleaned dish by rinsing it in 170° F. water. However, since water of this temperature is impractical for the sanitization of beverage glasses, chemical disinfection must be used. Mallmann, in unpublished data, has shown the inefficiency of water at temperatures below 150° F. to materially reduce the number of bacteria remaining on dishes so treated.

In the tavern, the problem of drinking glass sanitization becomes three-fold. First, temperatures usually associated with bacterial destruction are not practical for hand dishwashing. Most establishments prefer to wash and sanitize in water with temperatures ranging from 115° to 120° F. and to rinse in cold water. Second, the time that the glass is being washed must be kept to a minimum if large numbers of stored glasses are impractical from either the standpoints of economy or storage space. Third, during rush periods throughout the working day the tendency to speed up the washing and cooling processes dictates the use of a sanitizing agent which exerts its germicidal action in a few seconds.

The Ingham County Sanitary Code (1948) governing washing and disinfection of eating and drinking utensils states that all utensils shall be washed after each use in a 0.5% alkali cleanser or chemical detergent in water of at least 110° F., rinsed immediately in clean hot water, and "disinfected" immediately after rinsing by complete submersion in a chlorine solution containing at least 200 p.p.m. of available chlorine for at least two minutes, or by any other method approved by the health officer. Inasmuch as any standard method of procedure for utensil sanitization should be based on performance tests, rather than on compounds used, plans are being made to ammend these regulations to emphasize and evaluate the results of a standard bacteriological examination for the control of eating and drinking utensils. At the present time, the United States Public Health Service Bulletin 280 (1943) has set the standard for the maximum permissible number of organisms on drinking glass rims at 100 per swabbed area. The swabbed area is defined as that area covered by a swab in passing three times over the inside rim and three times over the outside rim of the glass under examination.

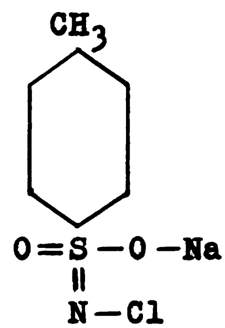
In general, there are two main classes of chemical compounds that have been employed for the sanitization of beverage glasses, i.e., chlorine and quaternary ammonium compounds. The chlorine class may be divided into the hypochlorites and chloramines. The hypochlorites are formed by the action of chlorine gas on sodium or calcium hydroxide. The chloramines used as a disinfectant in this application are known chemically as para-toluene or benzene sodium sulfochloramide and usually referred to as chloramine T and chloramine B, respectively. Quaternary ammonium compounds are built around a nitrogen atom surrounded by a long-chain alkyl group, two methyl or ethyl groups, a benzyl group, and a halogen, usually chlorine or bromine, substituted on the nitrogen atom.

Everyone is in agreement in regard to the fact that in order to have any disinfectant function to the limit of its capability it must be free to exert its action on a clean surface. This effect is usually made possible by first washing the glass in a good detergent to remove any excess organic matter and, incidentally, most of the bacteria, followed by a rinse to remove the wash water, and finally sanitizing by submerging the glass in a solution of disinfectant which should destroy any organisms withstanding mechanical removal by the washing process. If a compound could be developed which would shorten the washing and sanitizing operation to a single, simultaneous process this would facilitate utensil handling and reduce, to a minimum, the number of tanks required to properly sanitize eating and drinking utensils. Several attempts have been made to produce such a detergent-sanitizer. This paper is a presentation of their degree of success in achieving that result.

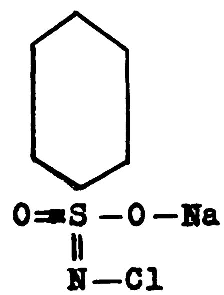
REVIEW OF LITERATURE

The discussion of detergent-sanitizers will be limited to those which have the chloramines and the alkyl dimethyl benzyl ammonium chloride or cetyl dimethyl ethyl ammonium bromide incorporated as the germicidal ingredient.

According to Berliner (1931), the term chloramine includes all amino or imino groups in which one or both hydrogens have been replaced by chlorine. In this paper we are dealing only with chloramine T which is known chemically as para-toluene sodium sulfochloramide and is generally represented by the formula:

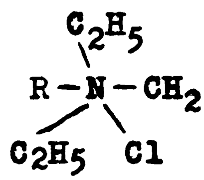


and chloramine B which is benzene sodium sulfochloramide and has the formula

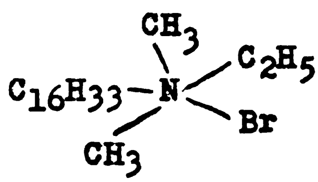


The matter of relative germicidal efficiency of chloramine T compounds has not been established to any degree of satisfaction. The germicidal property of the chloramines was discovered by Dakin and Cohen (1916)and the next year, Dakin et al (1917) selected chloramine T as the best of those tested. Tilley (1920) found that chloramine T, when compared on a weight basis with hypochlorites was less germicidal, but on the basis of available chlorine it was more germicidal than hypochlorites. However, Mallmann (1934) has shown that the methods of measuring available chlorine do not give an indication of germicidal chlorine in the presence of suspended material. Meyers (1930) and others have noticed the slow action of chloramine T compounds. In a comparison of azochloramid, chloramine T and sodium hypochlorite, Schmelkes and Horning (1935) have shown that in the absence of organic matter sodium hypochlorite is superior to chloramine T but that the order is reversed when the activity is measured in the presence of 10% serum. Mallmann and Schalm (1932) noticed the reduced bactericidal activity of chloramine compounds upon the addition of alkali, and later Charlton and Levine (1937) stated that the effect of pH is the most important factor in determining the germicidal efficiency of chlorine compounds, and present data which show that a change in pH from 7.3 to 11.3 increases the killing time of 1000 p.p.m. available chlorine on Bacillus metiens from 20 seconds to 64 minutes. McCulloch (1945) points out the fallacy of combining an alkaline detergent with a chlorine disinfectant.

The quaternary ammonium compounds are a group which is characterized by a long-chain alkyl radical, two methyl or ethyl groups, and generally a benzyl, ethyl, or methyl group substituted on the nitrogen atom. The salts are usually the chloride or the bromide. In this study, the germicidal ingredient of the quaternary detergent-sanitizer is, in one product, alkyl dimethyl benzyl ammonium chloride with the structure,



where R is a long-chain alkyl group found to be a mixture of groups containing anywhere from eight to sixteen carbon atoms in the commercially available products. In the other detergent-sanitizer, the germicidal ingredient is cetyl dimethyl ethyl ammonium bromide with the structure,



The synthetic wetting agents may be separated into three catagories, depending upon their behavior in solution. The anionic compounds ionize with the hydrophobic group in the anion; the cationic compounds which ionize with the hydrophobic group in the cation; and the non-ionic compounds.

Quaternary ammonium salts are of the cationic type and are often referred to as cationic detergents.

These compounds were introduced as disinfectants by Domagk in 1935 who was the first to demonstrate the bactericidal effect of an alkyl dimethyl benzyl ammonium chloride. Prior to 1935, the cationics had reached a high state of development as wetting agents in the dyeing of textiles. They have been reviewed by Rahn and Van Eseltine (1947). Baker, Harrison, and Miller (1941) found that the action of these synthetic detergents can be nullified by lecethin, cephalin, and sphingomyelin. These lipid materials are assumed to protectively coat the surface of a susceptible cell. They also demonstrated neutralization of cationics by anionics. Valko and Dubois (1944) confirmed the neutralization phenomenon and proceeded further to show that organisms apparently dead from the action of cationics could be revived by neutralization with an anionic. Recently, Kivela, Mallmann and Churchill (1948) have shown that the bacteriostatic action of quaternary ammonium compounds may be reversed by dilution and shaking in distilled water or physiological saline solution. In view of the possibility of such a revival of so-called "dead" organisms, it is difficult to evaluate germicidal activity of this class of compounds by the phenol coefficient method of Ruehle and Brewer (1931). In order to stop the action of the bactericide after a stipulated time interval, it becomes necessary to add an inhibitor such as sodium suramine to the subculture tubes as advocated by Lawrence (1947).

EXPERIMENTAL AND DISCUSSION

This study was conducted on six commercial products. A description of each compound is as follows:

Compound "A" - a germicide containing 16% chloramine. T, buffered with sodium carbonate and sodium polyphosphate.

Compound "B" - a detergent-sanitizer containing 14.5% chloramine B (sodium benzene sulfonchloramide), a wetting agent (sodium alkyl benzene sulfonate), and modified soda.

Compound "C" - alkyl dimethyl benzyl ammonium chloride.

Compound "D" - a detergent-sanitizer containing 5% alkyl dimethyl benzyl ammonium chloride, 26% alkyl aryl polyether alcohol, and 69% water.

Compound "E" - cetyl dimethyl ethyl ammonium bromide.

Compound "F" - a detergent-sanitizer containing 10% cetyl dimethyl ethyl ammonium bromide, 25% alkyl aryl polyether alcohol, and 65% borated alkali.

For convenience, these compounds will be designated by the assigned letter in the above description throughout this paper.

The test organisms used were *Salmonella typhosa* (Hopkins strain) and *Micrococcus pyogenes* var. *aureus* #209, as approved by the Food and Drug Administration.

All dilutions of the compounds used in this study are reported in terms of the germicidal ingredient except where otherwise noted.

Determination of Phenol Coefficients

Phenol coefficients of the germicidal ingredients alone and in combination with the detergents were determined in order that the degree of interaction, if any, between the germicide and detergent could be ascertained. The technic and media are in keeping with the standard set by Ruehle and Brewer (1931) for evaluating antiseptics and disinfectants.

The first phase of this study consisted of testing the relative germicidal ability of compounds "A" and "B", representatives of the chloramine type compounds. The results are presented in Table 1.

From these data it is apparent that the phenol coefficient of compound "A" is three times that of compound "B". It must be taken into consideration that compound "B" is buffered to a pH of 9.6 while compound "A" is only slightly buffered to a pH of 8.5 The difference in germicidal activity is apparently due to the difference in the pH of the two compounds, the more acid one being more germicidal. This is in keeping with the findings of Charlton and Levine (1937) who demonstrated that the activity of chloramine T solutions against Bacillus metiens spores is greater in the

Table 1. Germicidal Activity of Compounds "A" and "B" using Salmonella typhosa as the test organism

Test Material	Dilution	Exposure in Minutes* 5	10	15
"A"	1:2,000	-	-	-
	1:3,000	+	-	-
	1:5,000	+	+	-
	1:7,000	+	+	-
	1:10,000	+	+	+
"B"	1:800	-	-	-
	1:1,000	+	-	-
	1:2,000	+	+	-
	1:3,000	+	+	+
Phenol	1:80	-	-	-
	1:90	+	-	-
	1:100	+	+	-
Phenol Coefficient of "A"	3,000 ÷ 90 = 33			
Phenol Coefficient of "B"	1,000 ÷ 90 = 11			

* A plus and minus sign indicates presence or absence respectively of visible turbidity in sub-culture tubes in 48 hours.

lower pH range. Furthermore, they have shown this effect to be more pronounced below pH 7 than above.

The next experiment was designed to show the germicidal activity of the quaternary ammonium compounds when tested alone and in combination with a compatible detergent. The results of this experiment are shown in Table 2.

It can be seen that the germicidal efficiency of the sanitizer, when measured by the phenol coefficient method using S. typhosa as the test organism, is markedly reduced when combined with a wetting agent and/or a borated alkali. Compound "C" has its activity reduced by approximately one-half by the addition of a wetting agent only, whereas compound "E", the sanitizer in the presence of both a wetting agent and borated alkali has its efficiency as a germicide reduced beyond the limits of practicability. When the above tests were repeated, using M. pyogenes (aureus) as the test organism, there was no significant change in the germicidal efficiency of the detergent-sanitizer when compared with the results obtained with the sanitizer only. The results are presented in Table 3.

Phenol coefficients in the presence of organic matter

To demonstrate the effect of organic matter on quaternary ammonium sanitizers, the same technics as described above were employed, except that the dilutions of quaternaries were made up in the presence of serum to give a final concentration of 10% organic matter. Table 4 shows the effect of organic matter on the quaternary ammonium germicides. Referring back

Table 2. Germicidal Activity of Quaternary Ammonium Germicides and Detergent-Sanitizers Using <u>Salmonella typhosa</u> as the test organism

Test Material	Dilution	Exposure in Minutes 5	10	15
"C"	1:28,000	-	-	-
(alkyl dimethyl benzyl ammonium chloride	1:30,000	+	-	-
	1:32,000	+	+	-
	1:34,000	+	+	+
"D"	1:12,000	-	-	-
(A detergent sanitizer with 5% "D"	1:14,000	+	-	-
	1:16,000	+	-	-
	1:18,000	+	+	-
"E"	1:8,000	-	-	-
(cetyl dimethyl ethyl ammonium bromide)	1:10,000	+	-	-
	1:12,000	+	+	-
	1:14,000	+	+	+
"F"	1:100	-	-	-
(a detergent-sanitizer with 10% "E")	1:150	+	+	+
	1:200	+	+	+
	1:250	+	+	+

Phenol Coefficient of "C" 30,000 ÷ 90 = 333
Phenol Coefficient of "D" 14,000 ÷ 90 = 155
Phenol Coefficient of "E" 10,000 ÷ 90 = 111
Phenol Coefficient of "F" 125 ÷ 90 = 1.4

Table 3. Germicidal Activity of Quaternary Ammonium Germicides and Detergent-Sanitizers using *Micrococcus pyogenes* var. *aureus* #209 as the test organism

Test Material	Minimum Killing Dilution
"C"	1:50,000
"D"	1:45,000
"E"	1:28,000
"F"	1:25,000

*Lowest Dilution showing complete kill in 10 minutes but not at 5 minutes.

Table 4. Effect of 10% Organic Matter on Quaternary Ammonium Germicides using <u>Salmonella typhosa</u> as the test organism.

Test Material	Dilution	Exposure in Minutes 5	10	15
"C"	1:4,000	-	-	-
	1:6,000	+	-	-
	1:8,000	+	+	-
	1:10,000	+	+	+
"E"	1:1,000	+	+	+

to Table 2, compound "C" shows kill in a 1:30,000 dilution when the test organism is exposed to the quaternary only, but as shown in Table 4, the germicidal activity is reduced to 1:6,000 by the presence of 10% serum. The same thing is true, but to a much greater degree, when one examines the figures for compound "E". The minimum killing dilution in the absence of organic matter is reduced from 1:10,000 to less than 1:1,000. Thus, the effect of adding 10% serum to these particular compounds reduces the activity five times in the case of compound "C" and greater than ten times in the case of compound "E". Lower dilutions of compound "E" were not made because it was felt that dilutions below 1:1,000 were impractical.

The effect of organic matter has been noted by Quisno and Foter (1946) who have shown that 10% serum reduces the germicidal activity of another quaternary ammonium compound, cetyl pyridinium chloride, from twelve to twenty-five times when tested against Gram negative organisms. This fact is of great importance when such compounds are to be incorporated as part of a detergent-sanitizer since the organic soil from the utensil is removed and placed in intimate contact with the germicide in the wash water.

The Speed of Disinfection

In applying a chemical agent for the purpose of reducing the number of viable organisms remaining on the rim of a drinking glass, the duration of exposure to this agent must be long enough to permit the compound to exert its action. Unfortunately, the personnel responsible for washing the utensils pass the glasses through sanitizing solutions as rapidly as possible without regard to the activity of the disinfectant upon limited exposure.

Chloramine compounds have been shown to be too slow-acting for application as a bactericidal rinse. Meyers (1930) has presented data which indicates that chloramines are too slow in germicidal action to be used as a milk bottle rinse, but states that they are efficient when used as a bactericidal soak for pipe lines and other pieces of dairy equipment. Mallmann, Kivela and Turney (1946) have demonstrated the slow action of chloramine T when used to sanitize beverage glasses.

For comparative purposes and for a preliminary experiment to determine the time required to achieve complete sterilization, the speed of disinfection test was performed. Medication tubes were prepared containing 15 ml. of the indicated dilution of the germicidal ingredient in each detergent-sanitizer. In addition to the tubes containing the germicide, a control tube was prepared which contained 15 ml. of distilled water. All tubes were placed in a water bath maintained at 20° C. during the exposure period. At zero time, 1.5 ml. of a 24 hour broth culture of <u>S. typhosa</u> was delivered to the medication tube and mixed with the disinfectant. At intervals of 15, 30, 45, 60 seconds, 2, 3, 4, 5, 7½, 10 and 20 minutes, a ml. portion of the mixture was removed and discharged into a sub-culture tube containing 9 ml. of F.D.A. broth and an inhibitor. Sodium thiosulfate as advocated by Mallmann and Cary (1933) was used to stop the action of chloramine T and sodium suramine, suggested by Lawrence (1947) was used to stop the action of the quaternary ammonium salts.

The results of this test are shown in Table 5. From these data it is apparent that in the case of chloramine T complete kill is not effected in 10 minutes at the recommended use-dilution of 1:5,000. The quaternary ammonium salts when tested under these conditions show complete kill in 7½ minutes but not at 5 minutes, however, it will be noted that the concentrations used are one-third lower than the recommended use dilution in the case of compound "C" and one-half the recommended concentration in the case of compound "E".

To determine the number of organisms surviving in these low concentrations of quaternary ammonium compounds, the same procedure as outlined above was followed except that appropriate dilutions of the sub-culture tubes were made and plated on tryptone-glucose extract agar, incubated at 37° C. for 48 hours and the number of organisms was recorded. The results of this quantitative survival are shown in Table 6. These data are in keeping with those shown in conjunction with phenol coefficients in that complete kill was obtained at the specified dilutions in 10 minutes but not in 5 minutes. The speed test yields, in addition, more information in regard

Table 5. Speed of Disinfection of a Chloramine ("A") and Quaternary Ammonium ("C" and "E") Type Germicides Using Salmonella typhosa as the test organism

Exposure Period	"A" 1:5,000	"B" 1:15,000	"C" 1:10,000
15 sec.	+	- +	+
30 sec.	+	+	+
45 sec.	+	+	+
1 min.	+	+	+
2 min.	+	+	+
3 min.	+	+	+
4 min.	+	+	+
5 min.	+	+	+
7½ min.	+	-	-
10 min.	+	--	-
15 min.	-	-	-
20 min.	-	-	-
30 min.	-	-	-

Table 6. Survival of *Salmonella typhosa* upon Exposure to Quaternary Ammonium Germicides

Exposure Period	Compound "C" 1:15,000 Number of Survivors	Compound "E" 1:10,000 Number of Survivors
0 sec.	155,000,000	61,000,000
15 sec.	290,000	59,000,000
30 sec.	114,000	39,000,000
45 sec.	33,000	16,000,000
1 min.	13,000	5,000,000
2 min.	6,000	280,000
3 min.	5,100	78,000
4 min.	2,900	13,000
5 min.	300	200
7½ min.	0	0
10 min.	0	0
Control 10 min.	127,000,000	56,000,000

to the true value of these compounds. For instance, in the case of compound "C" the minimum killing dilution of 1:5,000 results in greater than 99% kill in 15 seconds even though complete kill is not attained after 5 minutes exposure. The same is true for compound "E", although to a lesser degree. The phenol coefficient minimum killing dilution of 1:10,000 requires 7½ minutes exposure to effect complete sterilization, but the figures in Table 6 show that 99% kill can be attained in 2 minutes. From the standpoint of utensil sanitization it is not practical nor is it necessary to obtain sterility.

Detergency Evaluation

The available methods for the evaluation of detergents have been summarized by Snell (1932). Briefly, these methods are as follows:

1. Measurement of surface tension against air - by capillary tubes, drop numbers, bubble pressure, or by measuring the amount of froth produced under definite conditions. This measurement cannot be assumed to represent the wetting action of the detergent against a grease soil since the interface measured is between detergent and air rather than between detergent and grease. This method will determine the amount of suds that a given detergent will produce but the relation between sudzing and detergency is of questionable value.

2. Measurement of the interfacial tension between paraffin oil or benzene by drop numbers or measurement of

emulsification. This method should give a closer approximation to the actual value of the detergent, but in the detergent process deflocculation must preceed emulsification if soil particles are to be held in suspension.

3. Measurement of suspending power - by the ability to coat carbon or other powders and measure the rate of sedimentation or protective action in filtration. This measurement is subject to variable results.

4. Protective action as measured by gold numbers. This measurement is affected by particle size, pH, concentration, and degree of dispersion and so results in inconsistant protective action values.

5. Direct washing tests. The greatest limitation to this test is the selection of a suitable soil.

The Direct Washing Test

In the following experiments the direct washing test was selected because it seems to provide a measure of overall detergency of the compounds used. The original procedure devised by Gilcreas and O'Brien (1941) was followed with three modifications as recommended by Mallmann (1942). The first modification incorporated an oil-soluble dye, Sudan III, in the original grease soil. This dye was substituted for carbon dusting to increase contrast between clean and soiled areas. It appears to give more reliable results. The second modification makes use of a milk soil. Inasmuch as many quaternary ammonium compounds are designed for the sanitization of dairy equipment, additional information can be obtained in regard to the efficiency of these compounds when used to remove dried milk films. Possibly, the most noteworthy modification is concerned with the basic design of the photoelectric device for measuring the degree of soil removal. In the original apparatus described by Gilcreas and O'Brien and a later apparatus described by Hughes and Bernstein (1945) there exists the possibility of a grave sampling error. This error is introduced by the small area of the slide that is actually "seen" by the light beam. It is conceivable that a large particle could be embedded in the relatively small sampling area. In the present instrument this sampling area has been increased from approximately 0.012 sq. in to 0.37 sq. in. or an increase in the area "seen" by the light beam of 31 times, thus minimizing error due to inhomgeneity of the soil film.

Removal of Artificial Soil

This experiment is described to show the degree of soil removal attained by compounds "D" and "F" when tested against a grease soil.

Grease Soil

Peanut butter	50 gm.
Butter	25 gm.
Lard	25 gm.
Mineral oil	20 gm.
Xyol	20 gm.
Sudan III	0.25 gm.

The soil was prepared from the ingredients shown above. Clean, scratch-free slides measuring 1 x 3 inches were dipped in this artificial soil. The slides were removed from the soil with a pair of forceps and placed in a vertical position with the lower edge resting on absorbent paper. They were allowed to drain overnight to remove the excess soil and solvent. The slides were then placed in a rack accomodating 12 slides, holding each slide at 45° from the horizontal and approximately 1 inch apart. The rack was attached to a Gilcreas and O'Brien washing apparatus and mechanically agitated horizontally through a distance of 3 inches in a volume of 6 quarts of detergent-sanitizer solution in the specified concentrations for 2 minutes at a temperature of 115° F. The slide rack was removed from the detergent-sanitizer solution and immediately placed in a water rinse at 115° F. and agitated for 1 minute. The rack was then removed from the machine and placed in the air to dry. A rack of control slides were agitated in a similar manner for a period of 3 minutes to ascertain the degree of soil removal by the frictional forces of the water only. The dried slides were measured photoelectrically to determine the amount of soil removal. These readings are recorded in percent transmission. A rack of unwashed slides were examined for soil density to establish the degree and uniformity of soil application to the slides.

The above procedure was repeated using a milk soil. The results of these experiments are shown in Tables 7 and 8. The figures designated as percent removal are calculated to show the removal based on the amount of soil applied to the unwashed control slides.

$$\text{Percent soil removal} = \frac{\text{washed average} - \text{unwashed average}}{100 - \text{unwashed average}}$$

In reference to Table 7, compound "D", which contains 26% alkyl aryl polyether alcohol as the wetting agent, does a fair job of removing the grease soil. Furthermore, it is interesting to note that the amount of soil removed is not changed appreciably by varying the concentration from 6 ml. to 14 ml. per 6 quarts of wash water. On the other hand, when using compound "F", which contains 25% alkyl aryl polyether alcohol and 65% borated alkali, there is a definite decrease in the amount of soil removal when the concentration is reduced from 14 grams to 10 grams per 6 quarts. At this concentration, compound "D" containing only the germicide and a nonionic wetting agent is superior to compound "F" which contains borated alkali. This difference is undoubtedly due to the free draining and rinsing properties of the wetting agent.

Table 8 shows how these compounds act on a milk soil. The most startling thing about these data is that water alone removes the milk soil to a greater extent than either compound "D" or "F". Two explanations may be offered for this unexpected result. Both compounds have been shown to

Table 7. Detergency of the Quaternary Ammonium Detergent-Sanitizers using a Grease Soil

Slide No.	Water Washed Control	Compound "D" 6 ml.	Compound "D" 10 ml.	Compound "D" 14 ml.	Compound "F" 10 gm.	Compound "F" 12 gm.	Compound "F" 14 gm.	Unwashed Control
1	32	92	98	96	87	88	94	32
2	36	93	96	98	90	92	96	29
3	40	89	97	97	87	91	93	31
4	34	96	96	97	87	90	90	26
5	33	94	97	95	91	92	92	30
6	33	96	97	98	90	91	93	32
7	31	93	97	97	84	90	96	29
8	33	96	96	98	89	89	94	32
9	37	94	95	99	87	92	92	27
10	31	95	97	98	88	93	93	31
11	30	98	95	97	88	87	94	30
12	38	97	98	98	90	92	90	32
av. % T.	34	94.4	96.6	97.3	88.2	90.6	93.1	30.1
% Removal	5.5	92	95	96	82	85	90	0

Note - The numbers reported are in terms of percent transmission. Quantities of compounds used are added to siz quarts of tap water.

Table 8. Detergency of the Quaternary Ammonium Detergent-Sanitizers Using a Milk Soil

Slide No.	Water Washed Control	Compound "D"			Compound "F"			Unwashed Control
		6 ml.	10 ml.	14 ml.	10 gm.	12 gm.	14 gm.	
1	97	93	91	90	96	95	92	82
2	94	94	90	90	94	95	91	86
3	94	92	90	89	94	94	92	86
4	95	92	90	87	94	93	89	90
5	96	92	89	88	94	94	89	87
6	96	89	87	89	93	94	90	90
7	95	90	89	88	94	93	87	90
8	96	90	90	88	94	90	90	88
9	95	89	89	89	95	91	90	89
10	93	91	91	89	94	94	88	88
11	98	93	90	89	93	93	90	89
12	93	95	93	91	95	96	88	87
Av. % T.	95	91.6	90	89	94	93.5	90	87.7
% Removal	59	32	19	11	50	47	19	0

Note: The numbers reported are in terms of percent transmission. Quantities of compounds used are added to 6 quarts of tap water.

do a fair job in removing a grease soil. The particles of milk solids in suspension are stabilized by protective layer of butterfat so that when the soil is plated on the slide and dried the film thus prepared will be translucent due to the minute amount of fat present. After the fats have been removed selectively by the wetting agents, leaving the milk solids still attached, the films, in the absence of fat, takes on a chalky appearance and becomes more opaque. The second explanation presupposes the formation of a protein-quaternary ammonium compound complex. In this case, the increased capacity is due to a replacement of the original milk soil film with a complex film formation which is of greater optical density than the soil. Since the degree of soil removal is measured by the amount of light that is transmitted through the soiled slide, either and possibly both of these actions may take place and give rise to less light penetrating the film. The other thing shown in Table 8 is that compound "F" does a slightly better job of milk soil removal than does compound "D". It will be recalled that this detergency is reversed in the case of the grease soil. Furthermore, the results using a milk soil seem to indicate that the higher dilutions of both compounds do a better job of removal than the lower dilutions.

Alkalinity and Degree of Buffering

In the work of Guiteras and Shapiro (1946) they point out that the ideal dishwashing compound would be one which combined the detergent properties of an anionic wetting agent and the bactericidal properties of a cationic agent. They prepared two detergent sanitizers, both containing cetyl dimethyl ethyl ammonium bromide as the sanitizing agent, trisodium phosphate, and an alkylated aryl polyether alcohol as the wetting agent. In one they used 33% sodium carbonate and 33% borax giving a final pH of 11.5 and in the other they used 25% sodium bicarbonate and 25% tetra-sodium pyrophosphate giving a final pH of 10.0. They found that the compound with the pH of 11.5 produced a sharp drop in the number of surviving bacteria up to about 8 minutes exposure to the detergent-sanitizer but then there was an increase in the number of surviving organisms after 10 minutes exposure. This did not happen with the detergent-sanitizer buffered at pH 10.0. They conclude that the detergent must be alkaline enough to be emulsifying but not alkaline enough to saponify fat soil. They postulate that when excess alkalinity is present, the fat is removed, emulsified, saponified, and forms a soap with the alkali which then proceeds to neutralize the cationic sanitizer. Upon further soil removal, more organisms are exposed to a diminishing amount of available quaternary ammonium sanitizer.

A determination of the pH of the various compounds used in this study was performed. In addition, the degree of buffering was determined as the amount of 0.1 N. hydro-chloric acid used to change the initial pH by one unit. The results of this experiment are shown in Table 9.

It will be seen from the figures in Table 9 that in the case of compound "B", a chloramine detergent-sanitizer is rather highly buffered to a pH of 9.6 to 9.9. This renders the compound so stable, as far as available chlorine is concerned, to make it a slow acting compound. Since chlorine compounds are highly susceptible to the pH of the solution and are known to function best in the acid range, their undesirability as a germicidal rinse can be predicted. On the other hand, compound "F", a highly buffered alkaline detergent-sanitizer of the quaternary ammonium type is approaching saponifiable alkalinity and is very well buffered in that range. While compound "E" approaches this alkalinity it has relatively no buffer capacity and would approach the pH of the diluting material. Thus, the efficiency of compound "E" would be predicted to exceed that of compound "F" and both would be expected to exceed the chloramine type detergent-sanitizers.

The Simulated Field Test

An attempt was made to conduct, in the laboratory, a test which would closely approximate the conditions in the field. As pointed out earlier, the most difficult limitation of any laboratory test is in the choice of a suitable soil. Several preliminary tests using a peptone water soil proved

Table 9. Alkalinity and Degree of Buffering

Test Material	Dilution	Initial pH	Degree of Buffering*
A	1:1,000	8.5	3.6
Chloramine T	1-5,000	8.5	0.5
B	1-1,000	9.6	7.9
Chloramine C Det.	1-5,000	9.9	1.4
E QAC and Wetting Agent	1-1,000	8.0	0.25
	1-5,000	9.4	0.10
F QAC and Weting Agent & Borated alk.	1-1,000	9.5	17.4
	1-5,000	9.5	3.7

*Ml. of 0.1 N HCl to change pH one unit.

to be unsuccessful in determining the germicidal efficiency of a quaternary ammonium preparation, due to a steady increase in the number of organisms being introduced on the surface of the glasses. A soil incorporating a slight amount of agar was considered but was later abandoned on the basis that it proved harder to remove than soils usually encountered in a tavern. Cider was finally adopted as the soil of choice because it assumed a viscid consistency when "dry" and yet was removed without too much effort. Pasteurized apple juice was selected and survival numbers, using Escherichia coli as the test organism were prepared.

The soil was prepared by taking 1,000 ml. of pasteurized apple juice and adding 4 grams of dipotassium hydrogen phosphate (K_2HPO_4) and 1.5 grams of potassium dihydrogen phosphate (KH_2PO_4) which gave a final pH of 7. The soil was autoclaved at 15 pounds pressure for 15 minutes. After cooling to room temperature, 30 ml. of a 24 hour broth culture of E. coli were added and mixed.

A control of unbuffered cider was included. Both ciders were maintained at room temperature for 8 hours. At the specified intervals, 1 ml. samples were removed and the number of surviving organisms was determined by standard plating technic. The results are shown in Table 10.

From Table 10, it can be seen that while the organisms are steadily dying off in the acid, unbuffered cider, the number of surviving organisms remains practically constant over a period of 8 hours when the cider soil is buffered to

Table 10. Survival or *Escherichia coli* in buffered and unbuffered cider soil

Sample Removed at - hours	Unbuffered Cider	Buffered Cider pH 7
0	25,500,000	23,000,000
½	24,600,000	20,300,000
1	15,400,000	29,000,000
2	12,100,000	21,900,000
3	9,200,000	21,000,000
5	4,600,000	22,600,000
8	2,160,000	24,700,000

pH 7. Having established a suitable soil, the simulated field test was conducted on compound "F".

Beverage glasses were soiled by dipping them in the buffered cider soil as described above and placed rims up to dry for a period of 30 minutes. Although the soil did not become dry in this time, it became quite viscid. Three tanks were set up to simulate field conditions. The first two tanks designated as the wash and rinse tanks, were filled with 5 gallons of tap water at a temperature of 115° F. at the start. The third tank, designated as the sanitizer tank was filled with 5 gallons of cold water (temp. 68° F.). To the wash tank, one ounce of the detergent-sanitizer "F" was added. The second tank served as a rinse and contained water only. The third tank had a sufficient amount of compound "E", the germicidal ingredient of the detergent-sanitizer, added to give a concentration of 1-5,000 (200 p.p.m.). The soiled glasses were hand-washed in the wash tank, passed through the rinse tank and dipped in the sanitizing solution while remaining in the hands of the worker. The glasses were sampled at intervals in accordance with the Supervised Field Test for Utensil Sanitization as outlined in the U. S. Public Health Bulletin 280 (1943). Briefly, the sampling consists of starting with the tenth glass and sampling every 20th glass which has been sanitized and every 20th glass starting with the twentieth glass which has been rinsed but not sanitized. Each sampled glass is swabbed with a sterile cotton swab, passing it three times over the inner surface of the rim and three times over the outer surface of the rim. The swab is then placed in a bottle containing 10 ml. of a phosphate buffer solution containing 0.002 gm. sodium suramine as an inhibitor. Samples of the wash, rinse, and sanitizer solutions are taken at the start and after every 100 glasses have been processed. The results of a typical experiment are shown in Table 11.

The results of the simulated field test show that under these conditions the compound "F" when used alone permits approximately 25% of the glasses sampled to come through the rinse tank with counts in excess of 100 organisms per rim, the standard of acceptance of the local department of health. When the glasses are passed through a sanitizing tank containing 200 p.p.m. of quaternary ammonium sanitizer, all glasses sampled are within this standard. However, the counts in the wash tank are continuing to rise which indicates that the detergent-sanitizer is not acting on the number of incoming organisms adhering to the soiled glasses. This suspicion was confirmed in the field tests which follow.

Field Studies

The final test to which all of these compounds were subjected was to demonstrate their activity under actual conditions of use in the field. Two local taverns were selected in the vicinity of Lansing. One was chosen because it uses lime-soda softened water with 85 p.p.m. total hardness. The

Table 11. Efficiency of Compound "F" in the Simulated Field Test

Sampling	Bacterial Populations of the tanks: Wash Tank	Rinse Tank	Sanitizing Tank
At Start	17	20	0
After 100 glasses	2,800	42	0
After 200 glasses	3,400	49	0
After 300 glasses	9,000	37	0
After 400 glasses	12,500	54	0

Bacterial Counts of the Glasses

Rinsed Glasses				Sanitized Glasses			
Glass	Count	Glass	Count	Glass	Count	Glass	Count
10	0	230	40	20	0	240	0
30	0	250	60	40	10	260	10
50	120	270	20	60	0	280	20
70	90	290	60	80	0	300	0
90	120	310	20	100	10	320	0
110	110	330	20	120	0	340	20
130	10	350	30	140	0	360	0
150	20	370	80	160	0	380	0
170	20	390	170	180	20	400	0
190	10	410	240	200	C*	420	10
210	30			220	0		

*Accidentally contaminated.

other establishment located outside of the city limits uses hard water with 450 p.p.m. total hardness. Both establishments had the three tank system and a mechanically operated brushing device. Tables 12 and 13 present data obtained when a good anionic detergent is used as a washing compound, lowed by a water rinse to prevent anionic carry-over, and a final rinse in a satisfactory quaternary ammonium type sanitizing agent. These data are presented to show the degree of sanitization possible under proper conditions, and by the use of adequate washing and sanitizing materials. All sampling was conducted in exactly the same manner as described under the simulated field test.

In examination of the results presented in Tables 12 and 13 a marked difference in the degree of sanitization of the finished glasses is noted. The only apparent difference in the conditions of these two surveys is that in one (Table 12) softened water was used while in the other (Table 13) hard water was used. It did not seem logical to the writer that water hardness alone could produce these divergent results. In the case of the establishment using softened water the wash water counts increased approximately three times, the rinse water counts were roughly doubled and the sanitizing solution remained at zero, or more correctly, the sanitizing solutions remained at a population of something less than 1,000 organisms per ml. because a dilution of 1:1,000 was the lowest plating made. One-third

Table 12. Field Test Conducted in a Beverage Establishment Using Lime-Softened Water (85 p.p.m.) Using an anionic detergent and an alkyl dimethyl benzyl ammonium chloride sanitizer

Sampling	Bacterial Counts of Tanks Wash Tank	Rinse Tank	Sanitizer Tank
At Start	84,000	3,300	0
After 100 glasses	98,000	4,100	0
After 200 glasses	111,000	6,800	0
After 300 glasses	243,000	6,000	0

Rinsed Glasses		Sanitized Glasses	
Glass No.	Bacterial Count	Glass No.	Bacterial Count
10	590	20	0
30	1,100	40	0
50	120	60	20
70	120	80	0
90	1,550	100	0
110	1,630	120	30
130	250	140	0
150	400	160	10
170	90	180	0
190	100	200	0
210	190	220	10
230	1,300	240	0
250	200	260	0
270	710	280	20
290	80	300	0

Table 13. Field Test Conducted in a beverage establishment using hard water (450 p.p.m.), an anionic detergent and alkyl dimethyl benzyl ammonium chloride sanitizer before cleaning the wash tanks.

Sampling	Bacterial Counts of Tanks: Wash Tank	Rinse Tank	Sanitizer Tank
At Start	110,000	6,000	0
After 100	15,000,000	190,000	0
After 200	18,100,000	900,000	0
After 300	21,700,000	1,600,000	0

Rinsed Glasses		Sanitized Glasses	
Glass No.	Bacterial Count	Glass No.	Bacterial Count
10	1,930	20	60
30	over 3,000	40	390
50	" 3,000	60	920
70	" 3,000	80	710
90	" 3,000	100	450
110	" 3,000	120	640
130	" 3,000	140	400
150	" 3,000	160	80
170	" 3,000	180	120
190	" 3,000	200	400
210	" 3,000	220	over 3,000
230	" 3,000	240	170
250	" 3,000	260	180
270	" 3,000	280	400
290	" 3,000	300	160
310	" 3,000	320	120

of the glasses sampled prior to immersion in the sanitizer were found to be acceptable as shown by counts of 100 organisms per rim or fewer. After the glasses had been immersed in the sanitizing solution and then sampled, all were acceptable and two-thirds of these were found to be sterile.

In tha analysis of the data obtained in the establishment using the hard water, it can be seen that the wash water counts increased from 110,000 to 15,200,000 organisms per ml. during the washing period of 100 glasses and that after this the counts increased slowly. The rinse water counts increased at a fairly even rate while the sanitizer tank remained at zero. It is of particular significance to note that under these conditions, none of the rinsed glasses and only one-eighth of the glasses which passed through the sanitizing tank showed counts of less than 100 organisms per rim.

Upon investigation of the washing equipment, the writer found that the wash and rinse tanks were covered with an insoluble, porous scale on the bottom and sides. Further investigation revealed the presence of a scum layer embodying decayed food particles embedded between the bristles of the brushing machine. These findings justify the sharp increase in the bacterial count of the wash water as noted at this sampling. When the brush was set in motion the wash water was heavily contaminated by the brushing machine. The sharp increase in the bacterial population of the wash water would, in all probability have been noted shortly after the brush was set in motion. To demonstrate that the insanitary conditions of the brush and tanks, rather than the water hardness, was responsible for these results, the brushing machine was dismantled and thoroughly cleaned. Likewise, the scale deposits on the sides and bottoms of the wash tanks were scoured off until the metal was bright. The test was repeated using the same detergent and sanitizer as in the previous test. The results of this test appears in Table 14. The counts in all wash, rinse and sanitizer tanks remained at zero during the period of sampling with the exception of the sample takan from the wash tank after 200 glasses had been washed. This figure does not represent an actual count of 1000 organisms per ml. any more than do the other samples indicate sterility since the lowest dilution plated was 1:1,000. It is more correct to say that all of these tanks except one, contained somewhat less than 1,000 prganisms per ml. In review of this test, it may be assumed that the bacterial load in the wash tank, rather than water hardness is responsible for the different results obtained in Tables 12 and 13. This demonstrates, rather vividly, the importance of maintaining washing equipment in a sanitary condition if chemical agents are to give satisfactory performance.

The next survey was designed to demonstrate the effectiveness of chloramine type detergent-sanitizers. In the wash tank, a sufficient amount of chloramine B detergent-sanitizer, compound "B" was added to give a concentration of 50 p.p.m. available chlorine. The rinse tank contained 5 gallons of water and the sanitizer solution was made up in 5 gallons of water to a concentration of 200 p.p.m. of available chlorine, using a chloramine T germicide, compound "A". The results of this survey are shown in Table 15. It is apparent that the detergent-sanitizer does not satisfactorily sanitize the glasses in the alloted exposure time of the washing operation, as evidenced by the relatively high numbers of surviving bacteria on the rinsed glasses. When the glasses have been passed through an additional rinse containing chloramine, satisfactory sanitization is effected. All of the sanitized glasses except two have fewer than 100 organisms per rim when exposed twice to the chloramine sanitizer; however, when exposed to the sanitizer only once, excessive number of bacteria remain. The prompt response of the organisms to the second exposure may be due to attenuation brought about by the first exposure may assist in oxidizing the organic matter in the remaining soil so that upon the second exposure, the unprotected organisms succumb to the higher concentration of germicide. In either case, it may be concluded that under the conditions of this test the chloramine type detergent-sanitizer, when used alone, does not do an adequate job of sanitizing beverage glasses. However, when used in combination with a compatible

Table 14. Field Test conducted in a beverage establishment using hard water (450 p.p.m.), an anionic detergent, and alkyl dimethyl benzyl ammonium chloride sanitizer - after cleaning brush and scouring wash tanks

Sampling	Bacterial Counts of Tanks: Wash Tank	Rinse Tank	Sanitizing Tank
At Start	0	0	0
After 100 glasses	0	0	0
After 200 glasses	1,000	0	0
After 300 glasses	0	0	0

Rinsed Glasses: Glass No.	Bacterial Counts	Sanitized Glasses: Glass No.	Bacterial Count
10	120	20	0
30	80	40	0
50	40	60	0
70	0	80	0
90	450	100	0
110	40	120	0
130	20	140	0
150	20	160	0
170	0	180	40
190	20	200	10
210	0	220	0
230	10	240	0
250	20	260	0
270	30	280	10
290	0	300	0

sanitizing rinse solution, all public health hazard is removed as evidenced by the few numbers of organisms surviving this treatment.

Several bacteriological surveys were made in an attempt to evaluate the germicidal efficiency of the quaternary ammonium type of detergent-sanitizer under actual conditions of use in the field. Representative data of these surveys are presented in Tables 16 and 17.

The use dilution of the quaternary ammonium detergent-sanitizer was 1 ounce per 5 gallons of water. This represents a concentration of 0.16%. At this concentration, the sanitizer would be present in a concentration of 78 p.p.m. because the detergent-sanitizer used was composed of 5% germicidal ingredients. These results in Table 16 indicate that satisfactory results can be obtained, as measured by bacterial populations on the sanitized glasses, provided a sanitizer is placed in the final rinse. When so treated, only one glass out of the 25 sampled failed to meet the standard of 100 or fewer organisms. On the other hand, when only the detergent-sanitizer is used, nine glasses or approximately one-third of those sampled failed to meet this requirement. If the concentration of the detergent-sanitizer had been doubled so that a concentration of 150 p.p.m. of sanitizer had been present in the wash tank, counts on the glasses leaving the detergent-sanitizer would have been similar to those obtained from the sanitizing tank. Such results have been obtained in other tests.

Table 16. Field Test Conducted in a beverage establishment using softened water (85 p.p.m.), a detergent-sanitizer compound "D", an alkyl dimethyl benzyl ammonium chloride and wetting agent, and compound "C", alkyl dimethyl benzyl ammonium chloride sanitizer

Sampling	Bacterial Counts of Tanks: Wash tank	Rinse Tank	Sanitizer Tank
At Start	0	250	0
After 100 glasses	0	0	0
After 200 Glasses	0	0	0
After 300 glasses	00	0	0
After 400 glasses	200	0	0
After 500 glasses	500	1,800	0

Rinsed glasses				Sanitized Glasses			
Glass	Count	Glass	Count	Glass	Count	Glass	Count
10	10	270	690	20	50	280	30
30	40	290	370	440	10	300	20
50	70	310	80	60	0	320	0
70	30	330	110	80	0	340	30
90	400	350	40	100	10	360	0
110	60	370	60	120	20	380	0
130	0	390	220	140	0	400	120
150	20	410	130	160	110	420	40
170	170	430	50	180	10	440	20
190	480	450	140	200	20	460	10
210	60	470	30	220	10	480	10
230	20	490	40	240	0	500	20
250	20			260	0		

Table 17. Field test conducted in a beverage establishment using hard water (450 p.p.m.), a detergent-sanitizer, compound "F", a cetyl dimethyl ethyl ammonium bromide and wetting agent and borated alkali and compound "E", cetyl dimethyl ethyl ammonium bromide sanitizer

Sample	Bacterial Counts of Tanks		
	Wash Tank	Rinse Tank	Sanitizer Tank
At Start	144,000	54,000	2,200
After 100 glasses	1,050,000	44,000	800
After 200 glasses	2,710,000	200,000	1,500
After 300 glasses	81,000,000	630,000	1,700
After 400 glasses	102,000,000	1,360,000	2,300

Rinsed Glasses				Sanitized Glasses			
Glass	Count	Glass	Count	Glass	Count	Glass	Count
10	1260	230	over 3000	20	80	240	20
30	2850	250	" 3000	40	200	260	10
50	1970	270	" 3000	60	70	280	260
70	960	290	" 3000	80	2140	300	40
90	Over 3000	310	" 3000	100	550	320	30
110	" 3000	330	" 3000	120	280	340	670
130	" 3000	350	" 3000	140	310	360	30
150	" 3000	370	" 3000	160	60	380	750
170	" 3000	390	" 3000	180	40	400	1600
190	" 3000	410	" 3000	200	10	420	120
210	" 3000			220	320		

In summarizing the results obtained in Table 17, compound "F" failed to do a satisfactory job of sanitizing the soiled beverage glasses by itself and also in combination with 200 p.p.m. of sanitizer in the final rinse. The sanitized glasses show reduced counts but over 50% of these glasses are well above the maximum acceptable number. The use dilution of this detergent-sanitizer is 1 ounce per 5 gallons of water but in this case, 10% of the compound is the germicidal ingredient, which would give a sanitizer concentration of 150 p.p.m. in the wash tank. The rapid increase in the bacterial population of the wash water indicates that this amount is quite insufficient to cope with this load. The phenol coefficient data presented earlier in this study would predict this. The increased populations in the rinse tank are undoubtedly due to carry-over contamination from the wash tank. The counts obtained in the sanitizer tank again exemplify the inefficiency of this sanitizer, inasmuch as the counts increase after a suitable period of self-sanitization of initial contamination is completed. Repeated tests using this detergent-sanitizer result in a film formation which cannot be removed by subsequent washings in this preparation. Again, this can be predicted from the results of detergency evaluation where in compound "F" was shown to have a lower dilution coefficient than compound "D".

SUMMARY

Phenol coefficient studies were performed on one chloramine T compound and two quaternary ammonium compounds used separately and when combined with detergents. The combination detergent-sanitizer reduced the germicidal activity of these compounds to 1/3 in the case of chloramines, 1/2 in the dase of alkyl dimethyl benzyl ammonium chloride, and 1/80 in the case of cetyl dimethyl ethyl ammonium bromide when *Salmonella typhosa* was used as the test organism. This effect was not marked when using *Micrococcus pyogenes* var. *aureus*.

Organic matter in the form of 10% serum reduces the killing dilution of alkyl dimethyl benzyl ammonium chloride from 1:30,000 to 1:6,000. When cetyl dimethyl ethyl ammonium bromide is used, this dilution is reduced from 1:10,000 to less than 1:1,000.

The speed of disinfection is greater for alkyl dimethyl benzyl ammonium chloride than for cetyl dimethyl ethyl ammonium bromide, the former producing 99% kill in 15 seconds at a 1:15,000 dilution while the latter requires 2 minutes to attain 99% kill in a dilution of 1:10,000.

Detergency evaluation of the quaternary ammonium type detergent sanitizers was made. Both compounds do a fair job of grease removal. When a milk soil is used, the efficiency is inversely proportional to the concentration of detergent sanitizer. Water produced the most complete soil removal.

The buffer capacity of the detergent sanitizers was determined.

A technic for performing the simulated field test is described. A suitable soil for this test was found to be cider buffered to pH 7.

Field tests were conducted in two taverns in the vicinity of Lansing, using various combinations of good and poor detergents and sanitizing agents separately and in combination. Data are presented which indicate that satisfactory utensil sanitization can be effected under proper conditions. Of the detergent-sanitizers tested none produce satisfactory sanitization can be effected under proper conditions. Of the detergent-sanitizers tested none produce satisfactory sanitization under the conditions of the test.

When a detergent sanitizer is used as a wash and followed by a germicidal rinse, satisfactory results may be obtained.

A slow acting compound is unsatisfactory when used as a rinse but may function properly when employed as a bactericidal soak.

Factors responsible for these results are:

1. germicidal activity of compound
2. organic matter
3. speed of disinfection
4. nature of soil
5. alkalinity and degree of buffering
6. bacterial density in wash and rinse water.

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