

# A BACTERICIDAL DETERGENT FOR EATING UTENSILS<sup>1</sup>

ALBERT F. GUITERAS AND REBECCA L. SHAPIRO

*Foster D. Snell, Inc., Brooklyn, New York*

Received for publication August 26, 1946

A good detergent should depress the interfacial tension at a water-oil interface below 5 dynes per cm (Snell, 1932), and in addition it should have good deflocculating and dispersing power (Snell, 1933). As detergents, anion-active agents are generally superior to cation-active and nonionic agents, but as bactericides, cationics are superior. An ideal dishwashing compound would be one that combines the detergent properties of the anion-active agents with the bactericidal properties of the cation-active. Since these two types of surface-active agents are incompatible, it is impossible to combine them, because they would neutralize each other with the formation of an insoluble precipitate, resulting in the loss of detergent as well as bactericidal properties. The purpose of this investigation was to develop a detergent suitable for washing and sanitizing eating utensils.

## EXPERIMENTAL

Two detergent compositions were prepared, one with and one without a cation-active bactericide.

	No. 1	No. 2
Trisodium phosphate.....	33	33
Sodium carbonate.....	33	33.5
Borax.....	33	33.5
Ethyl cetab <sup>2</sup> .....	3	
Nonionic wetting agent <sup>3</sup> .....	1.5	3.0
	<hr/> 103.5	<hr/> 103.0

An artificial soil was prepared according to Gilcreas and O'Brien (1941), consisting of:

100 g raw eggs (white and yolks mixed)  
20 g butter  
20 g lard  
20 g peanut butter  
20 g milk  
10 ml *Staphylococcus aureus* culture (24-hour)  

---

190 g

<sup>1</sup> This investigation was supported by the Rhodes Chemical Corporation, Philadelphia, Pennsylvania.

<sup>2</sup> Cetyl dimethyl ethyl ammonium bromide (Rhodes Chemical Corporation).

<sup>3</sup> An alkylated aryl polyether alcohol.

Twenty-four clean microscope slides were immersed in the artificial soil at 37 C. They were then removed with forceps, and 12 slides each were placed in two monel metal staining racks (Fisher Scientific Company, catalogue no. 8-820) and allowed to drain and dry in the air at room temperature for one-half hour.

Two detergent solutions were prepared with formula no. 1 and formula no. 2 by dissolving 3 g of each respectively in 400 ml distilled water (1 oz per gallon). The solutions were warmed to 37 C and maintained at that temperature throughout the test. The pH of both solutions was 11.5.

After the contaminated slides had dried for one-half hour, one rack was placed in detergent no. 1 and the other in detergent no. 2. The slides were completely covered by the detergent solutions. Each rack was then repeatedly raised out of and then reimmersed in the solution about once a second for a period of 10 minutes. At intervals of 1, 2, 3, 4, 5, and 10 minutes two slides were removed from each rack and placed in separate petri dishes containing 20 ml of sterile FDA broth. At the end of the 10-minute test period all petri dishes were gently agitated to insure mixing, and 1-ml portions were withdrawn from each under sterile conditions. Pour plates were then made with these portions in FDA nutrient agar, using 1 ml each of three dilutions, 1:10, 1:100, and 1:1,000. All petri dishes containing the original microscope slides as well as all pour plates were incubated at 37 C for 24 hours. They were then examined for bacterial growth, and the number of colonies on each pour plate was counted.

It was found that formula no. 2 (without ethyl cetab) is not bactericidal, although there was a gradual drop in the bacterial count, probably due to the alkalinity of the solution. Formula no. 1 produced a very marked sharp drop in the bacterial count up to the 5-minute washing period, at which point the pour plates and the broth were sterile; but at the 10-minute washing period there was a heavy growth of *Staphylococcus aureus* in the broth as well as on the agar plates.

We believe that the explanation for this apparent anomaly is as follows: The bacteria were thoroughly distributed throughout the artificial soil, which consisted of over 30 per cent fat. The slides were then immersed in this soil, which was then allowed to dry on the slides. When the slides were placed in the detergent solution, the detergent quickly removed the soil from the slides and emulsified the fat. The ethyl cetab killed the bacteria in the aqueous phase of the detergent solution and on the clean slides, but did not kill the bacteria which were entrapped within the minute fat particles of the emulsion. After a time, the alkaline detergent began to act on the fat particles in the emulsion and saponified some of the fat, forming a soap, which then inactivated the bactericidal properties of the detergent by precipitating the ethyl cetab out of solution. As more fat particles were saponified, they released more entrapped bacteria to the solution, which was then no longer able to kill them.

To test this theory we made up a second set of detergent compositions that were designed to be emulsifying but nonsaponifying.

	No. 3	No. 4
Trisodium phosphate.....	50	50
Sodium bicarbonate.....	25	25
Tetrasodium pyrophosphate.....	25	25
Rodicide A <sup>4</sup> .....	6	
Nonionic wetting agent <sup>5</sup> .....		3
	106	103

Detergent solutions were made with formula no. 3 and formula no. 4 (1 oz per gal.) and tested as above, except that the washing time was extended to 2 hours

TABLE 1  
Average number of bacteria per sq cm remaining on microscope slides

WASHING TIME IN MINUTES	NO. 1 pH 11.5	NO. 2 pH 11.5	NO. 3 pH 10.0	NO. 4 pH 10.0
1	8,000	17,500		
2	4,500	12,000	15,000	42,500
3	250	9,500		
4	65	8,500	0	1,500
5	0	5,000		
6			130*	3,000
8			25*	3,000
10	13,000	2,500	0	1,750
15			0	2,250
20			0	1,500
30			0	2,250
45			0	670
60			0	800
90			0	825
120			0	850

\* Identified as *Bacillus subtilis*, not *Staphylococcus aureus*.

to insure that no recontamination would occur because of slow saponification. The pH of both solutions was 10.0.

It was found that formula no. 4 (without rodicide A) is not bactericidal, although there was a drop in the bacterial count as the dishwashing time increased. Formula no. 3 (containing rodicide A) produced a very marked sharp drop in the bacterial counts. The slide removed after 4 minutes' washing time was sterile, and the slides removed after 6 and 8 minutes' washing time were free from *Staphylococcus aureus*, although they were contaminated with *Bacillus subtilis*. All slides removed after 8 minutes were sterile. Table 1 summarizes the results of both tests.

<sup>4</sup> A product formulated by Rhodes Chemical Corporation, consisting of cetyl dimethyl ethyl ammonium bromide and an alkylated aryl polyether alcohol in aqueous-alcoholic solution.

<sup>5</sup> The same alkylated aryl polyether alcohol used in the two previous formulas.

The artificial soil was not sterilized before contamination with *Staphylococcus aureus*, and this organism was probably not the only contaminant, since *Bacillus subtilis* could easily have been introduced in preparing the soil. Complete removal of the soil from the slides without complete emulsification could account for the sterility of the slides in 4 minutes. As emulsification of the soil proceeded, *Staphylococcus aureus* and *Bacillus subtilis* could have been released from larger fat particles in which they were entrapped. The germicidal detergent, formula no. 3, killed *Staphylococcus aureus* more readily than *Bacillus subtilis*, which could account for the contamination with the latter organism of the slides removed after 6 and 8 minutes' washing time. Sporeforming organisms such as *Bacillus subtilis* are far more resistant to quaternary ammonium compounds than *Staphylococcus aureus* (Green and Birkeland, 1941; Hoogerheide, 1945; Du Bois and Dibblee, 1946).

#### SUMMARY

When cation-active agents are used as bactericides in detergent compositions, it is essential that the detergent be emulsifying but not saponifying. If the alkalinity of the detergent is sufficiently high to saponify fat, the resulting soap will inactivate the cation-active agent and render the solution completely ineffective germicidally.

#### REFERENCES

- Du Bois, ADRIEN S., AND DIBBLEE, DIANA 1946 Death-rate study on a high molecular quaternary ammonium compound with *Bacillus metiens*. *Science*, **103**, 734-735.
- GILCREAS, F. W., AND O'BRIEN, J. E. 1941 Laboratory studies of method for cleansing of eating utensils and evaluating detergents. *Am. J. Pub. Health*, **31**, 143-150.
- GREEN, T. W., AND BIRKELAND, J. M. 1941 The germicidal action of cetyl pyridinium chloride on bacterial spores. *J. Bact.*, **41**, 34.
- HOOGHEIDE, J. C. 1945 The germicidal properties of certain quaternary ammonium salts with special reference to cetyl-trimethyl-ammonium bromide. *J. Bact.*, **49**, 277-289.
- SNELL, FOSTER DEE 1932 Detergency of alkaline salt solutions. II. Lowering of interfacial tension. *Ind. Eng. Chem., Ind. Ed.*, **24**, 1051-1057.
- SNELL, FOSTER DEE 1933 Detergency of alkaline salt solutions. III. Deflocculating and emulsifying power. *Ind. Eng. Chem., Ind. Ed.*, **25**, 162-165.