Factors Affecting the Activity of Phenolic Disinfectants

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ABSTRACT

Ortenzio, L. F. (U. S. Department of Agriculture, Washington, D. C.), C. D. Opalsky, and L. S. Stuart. Factors affecting the activity of phenolic disinfectants. Appl. Microbiol. 9:562–566. 1961.—Low challenge phenol coefficient and high challenge use-dilution tests were made on a neutral coconut oil soap emulsion of o-phenylphenol and aqueous solutions of sodium o-phenylphenate prepared in the laboratory from the phenol using a stoichiometric amount of NaOH as well as with increasing amounts of excess NaOH. The phenol had considerably greater activity in both test methods when emulsified with the neutral soap than when converted to the phenate and dissolved in water. Use dilution test results against Salmonella choleraesuis with both the phenol and the phenate were within the range which would have been predicted from the Salmonella typhosa coefficient results employing the conventional conversion multiple of 20 to determine the maximal number of parts of water to which one part of germicide could be added. With the emulsified phenol this was also true where Staphylococcus aureus was employed in both procedures. With the aqueous solution of the phenate the maximal safe use-dilution by the phenol coefficient found for S. aureus and the same conventional conversion procedure was roughly five times higher than the maximal safe use-dilution found by the use-dilution method. Results with aqueous solutions of the phenate to which increasing amounts of excess NaOH were added showed no significant differences in the phenol coefficient method with either S. typhosa or S. aureus. In the use-dilution method, significant decreases in activity were found as the excess NaOH was increased to 4% with both S. choleraesuis and S. aureus. Although the pH values of aqueous solutions of the phenate were raised as the amount of free NaOH was increased, the decreases in pH observed as the dilution with water was increased were such that only small differences existed at the high critical killing dilutions found in the low challenge phenol coefficient method, whereas rather large differences existed at the lower critical killing dilutions in the high challenge use-dilution method.

Although the effects of alkali on the activities of phenolic germicides have been studied and discussed by Schaffer and Tilley (1930), Ordal, Wilson, and Borg (1941), Klarmann (1957), and others, the magnitude of these effects in terms of practical disinfecting values is not fully appreciated. Most of the data available in the literature illustrating these effects have been compiled in phenol coefficient, dilution tube low challenge type tests and experiments show that procedures of this type often fail to detect changes in activity which may be critical in practical applications proposed for such products. The AOAC (1960) use-dilution method, which is a relatively high challenge type test method providing results similar to those that can be expected in practical applications, has been found, fortunately, to provide a dependable index for detecting such changes.

These studies were made to illustrate the differences which may be commonly encountered between phenol coefficient or dilution tube, low challenge test evaluations and use-dilution, high challenge test evaluations on phenolic germicides due to the alkali factor. For the illustration desired, the o-phenylphenol-sodium o-phenylphenate-sodium hydroxide complex provides adequate data. The selection of this specific phenol-phenate-hydroxide complex should not be construed as an attempt to point out specific advantages or disadvantages over any other phenol-phenate-hydroxide complex in the preparation of disinfectants or as evidence that all other phenol-phenate-hydroxide complexes will behave in an identical manner.

MATERIALS AND METHODS

In the first study, direct comparisons were made between the phenol coefficient values against Salmonella typhosa and Staphylococcus aureus and the use-dilution test results against Salmonella choleraesuis and Staphylococcus aureus for o-phenylphenol as determined from tests on the sodium salt in the absence of any excess sodium hydroxide and from tests on a neutral coconut oil soap emulsion.

A stock solution of the sodium salt was prepared by reacting 90.4 g of o-phenylphenol with 20.9 g of sodium hydroxide in distilled water and bringing the volume to 200 ml for a 50% solution of sodium o-phenylphenate free of excess alkali. The weights deviated slightly from the stoichiometric amounts to compensate for impurities in the chemicals. Each milliliter of this solu-
tion provided 0.5 g of the salt. This was employed for preparing all test solutions by distilled water dilution.

A stock neutral soap emulsion of o-phenylphenol was prepared by dissolving 10 g of o-phenylphenol in 20 ml of ethyl alcohol and sufficient amounts of a neutral liquid potassium soap of cocoanut oil preparation to provide 17.5 g of anhydrous soap, emulsified by vigorous stirring and brought to a 100-ml volume with distilled water. Each milliliter of this emulsion provided 0.1 g of the phenol. This was employed for preparing all test solutions by distilled water solution. The results of the tests by the phenol coefficient procedure and the use-dilution procedure are summarized in Table 1.

The results in this table show a phenol coefficient of 44.4 against *S. typhosa* for the emulsified phenol as compared to 30.2 when the determination was made on the phenate. This represents approximately a 45% increase in activity for the phenol over the phenate. With *S. aureus* the increase in activity in this test method from 20.9 to 34.6 is on the order of 65%. The maximal safe use dilutions as determined by the use-dilution method using *S. choleraesuis* conform very closely to the maximal safe use dilutions which might be calculated from the *S. typhosa* coefficient results using the factor of 20 as a multiple to determine the number of parts of water in which one part of the phenol could be incorporated to disinfect. The maximal safe dilution found from the phenate with this organism in the use-dilution method was 1:600 as compared to a coefficient calculated dilution of 1:604. With the phenol the safe dilution found in the test was 1:800 as compared to 1:888 determined by the coefficient calculation.

The results on the phenate with *S. aureus* by the use-dilution procedure are quite different from those found in the phenol coefficient method. They are also quite different from the results obtained with the neutral soap emulsified phenol in the use-dilution test. With the phenol-soap emulsion an effective dilution of 1:750 was found. This is approximately equivalent to the safe use dilution calculated from the *S. aureus* coefficient using the 20 conversion factor of 1:692. However, the safe use dilution of 1:85 as determined on the phenate by the use-dilution method is approximately 1/6 that for the phenol in the use-dilution test and only 1/5 that which would be indicated by the phenol coefficient tests, with *S. aureus* using the conversion factor of 20.

The use-dilution values listed in Table 1 were determined and confirmed by probit grid analyses of percentage kill results at three separate dilutions between the no response and total response levels, a procedure which has been described by Ortenzio and Stuart (1961). Thirty or more tests were employed as a basis for determining the percentage kill figures at each dilution. The probit grid assay sheet is shown in Fig. 1.

This figure illustrates clearly the wide differences in the magnitude of the responses obtained against *S. aureus* with o-phenylphenol in a neutral soap emulsion and with sodium o-phenylphenate. However, the magnitude of the differences between the emulsified phenol and the phenate against *S. choleraesuis* is not nearly as great, although the slopes of the assay lines are distinctly different. These results indicate that killing by o-phenylphenol and sodium o-phenylphenate is probably the result of different modes of action and that sodium o-phenylphenate possesses what has been termed by Klarmann, Shternov and Gates (1934) as “quasi-specific” killing characteristics, insofar as different species of bacteria are concerned, not shown by the parent substance, o-phenylphenol.

In a second study, comparisons were made between the phenol coefficient values found against *S. typhosa* and *S. aureus* with sodium o-phenylphenate in the absence of any excess sodium hydroxide and in the presence of 1.0, 2.0, 3.0 and 4.0% added sodium hydroxide on the weight of the sodium o-phenylphenate. Use-dilution tests were made on the same solutions using *S. choleraesuis* and *S. aureus* as the test organisms.

### Table 1. Phenol coefficient and use-dilution test data for o-phenylphenol as calculated from determinations on sodium salt and a neutral soap-emulsion

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Phenol coefficient values found against</th>
<th>Maximal safe dilution for use in disinfecting found in AOAC (1960) use-dilution method against</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhosa</em></td>
<td>30.2</td>
<td>1:600</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>20.9</td>
<td>1:85</td>
</tr>
<tr>
<td><em>S. choleraesuis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Phenylphenol as the Na salt...</td>
<td>44.4</td>
<td>1:800</td>
</tr>
<tr>
<td>o-Phenylphenol solubilized in CH₃OH and emulsified with K soap of cocoanut oil...</td>
<td>34.6</td>
<td>1:750</td>
</tr>
</tbody>
</table>

**FIG. 1. Probit grid assay of use-dilution data on o-phenylphenol tests as the sodium salt as compared to a neutral soap emulsion.**
A stock 50% solution of sodium o-phenylphenate was prepared on a stoichiometrical basis as previously described and separated into five equal aliquots. No excess sodium hydroxide was added to the first aliquot, 1.0% was added to the second, 2.0% to the third, 3.0% to the fourth, and 4.0% to the fifth. These five solutions were employed in preparing all distilled water dilutions necessary for the phenol coefficient and use-dilution tests. The results of this study are summarized in Table 2.

These results show that within the limits of experimental error there are no differences in the phenol coefficient results with either S. typhosa or S. aureus with sodium o-phenylphenate in the absence or presence of excess sodium hydroxide up to 4.0%. The phenol coefficients found against S. aureus although somewhat lower than those found with S. typhosa are high enough in all instances to indicate substantial value in practical disinfecting operations against this organism. The results obtained in the use-dilution tests are markedly different. With S. choleraesuis the maximal safe dilution for disinfecting was found to be 1:530 for sodium o-phenylphenate in the absence of any excess sodium hydroxide. This dilution is almost identical to the maximal safe use dilution of 1:532 indicated by the S. typhosa coefficient using the conversion multiple factor of 20. However, the maximal safe use dilution in the use-dilution method decreases significantly as the percentage of excess sodium hydroxide increases to 4.0% so that conversions of the S. typhosa phenol coefficient values using the constant multiple of 20 could not be expected to provide solutions effective in disinfecting if 1.0, 2.0, 3.0, or 4.0% excess sodium hydroxide was present. In the presence of 1.0% excess sodium hydroxide, the conversion factor of 20 would have to be reduced to 14, with 2.0% to 10, with 3.0% to 9.4, and with 4.0% to 9.0. Thus, a decrease by more than \( \frac{1}{2} \) is shown in practical disinfecting activity against gram negative enteric bacteria due to the presence of excess sodium hydroxide which goes undetected by the phenol coefficient testing method.

In the absence of any excess sodium hydroxide the “quasi-specific” effect for bacterial species previously shown for sodium o-phenylphenate shows up clearly in the use-dilution method results. It is not apparent in tests by the phenol coefficient method. The maximal safe use dilution for sodium o-phenylphenate found for S. aureus in the use-dilution method falls far short of the dilution of 1:368 which would be indicated as a possible safe dilution using the S. aureus coefficient and the conversion multiple of 20.

As the free sodium hydroxide is increased to 4.0%, the maximal safe use dilution for disinfecting against S. aureus decreases at a greater rate than was the case with S. choleraesuis. With 1.0% excess sodium hydroxide, the decrease is approximately 30%, with 2.0% excess sodium hydroxide, 33%; with 3%, 60%; and with 4%, 70%. As with the gram-negative enteric bacteria, this decrease in practical disinfecting efficiency against gram-positive staphylococci due to free sodium hydroxide is not detected by phenol coefficient tests using S. aureus.

The use-dilution data listed in Table 2 was derived by probit grid-dilution-percentage kill figure analyses using 30 or more carrier exposures at three dilutions between the no response and total response levels with all five preparations followed by confirmatory testing at the dilution indicated by the 99% kill-dilution intercept or the 95% confidence limit end point.

The probit assays for all five preparations and both test organisms are shown in Fig. 2. The assay lines in

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<table>
<thead>
<tr>
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<th>Maximal safe dilution for use in disinfecting found in AOAC (1960) use-dilution method against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella/choleraesuis typhosa</td>
<td>26.6/2.0%</td>
<td>1:530/1:75</td>
</tr>
<tr>
<td>Salmonella/choleraesuis aureus</td>
<td>18.4/2.0%</td>
<td>1:390/1:52</td>
</tr>
<tr>
<td>Salmonella/choleraesuis choleraesuis S. aureus</td>
<td>26.6/2.0%</td>
<td>1:260/1:50</td>
</tr>
<tr>
<td>Sodium o-phenylphenate + 1.0% excess NaOH</td>
<td>26.6/2.0%</td>
<td>1:250/1:30</td>
</tr>
<tr>
<td>Sodium o-phenylphenate + 2.0% excess NaOH</td>
<td>26.6/2.0%</td>
<td>1:240/1:22</td>
</tr>
<tr>
<td>Sodium o-phenylphenate + 3.0% excess NaOH</td>
<td>24.4/4.0%</td>
<td>1:22/1.0</td>
</tr>
</tbody>
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**FIG. 2.** Probit grid assays of use-dilution data obtained with sodium o-phenylphenate in the absence of excess NaOH and with increasing amounts of excess NaOH.
this figure illustrate graphically the marked reductions in activity with both test organisms as the amount of excess sodium hydroxide is increased. With *S. choleraesuis* the most pronounced decreases are shown with 1.0 and 2.0% excess sodium hydroxide. The further increases to 3.0 and 4.0% do not result in further decreases in activity of equal magnitude. With *S. aureus* on the other hand the decreases in activity are more pronounced as the excess sodium hydroxide is increased from 2 to 3% and from 3 to 4%.

In a third study, the pH values of the distilled water test dilutions made from the following five stock solutions were determined: sodium o-phenylphenate, sodium o-phenylphenate plus 1.0%, 2.0%, 3.0%, and 4.0% excess sodium hydroxide. It is believed that the decreasing pH values with dilution in each of these preparations accounts in part for the failure of the relatively low challenge phenol coefficient test to provide an index to the obvious practical deficiencies of sodium o-phenylphenate preparations in disinfecting against staphylococci and the inactivating effects of small amounts of free sodium hydroxide in practical disinfecting operations against both gram-negative enteric bacteria and gram-positive cocci. The results of these determinations are shown in Fig. 3.

In studying the results shown in Fig. 3 it should be remembered that the effective 10-min killing dilutions in the low challenge phenol coefficient method range from approximately 1:1,000 to 1:2,500 considering both test organisms. This represents a concentration range of 0.1 to 0.04% sodium o-phenylphenate on the base scale. As compared to this the 10-min killing dilution range in the relatively high challenge use-dilution procedures ranges from a dilution of 1:530 to 1:240 or a concentration range of approximately 0.2 to 0.4% on the base scale with *S. choleraesuis* and a dilution of 1:75 to 1:22 or a concentration range of 1.33 to 4.54% with *S. aureus*.

The pH of dilutions where the concentrations of sodium o-phenylphenate range is from 0.1 to 0.04% fall between 10.4 with sodium o-phenylphenate in the absence of excess alkali and 11.1 with sodium o-phenylphenate plus 4.0% excess alkali. This is substantially lower than the pH range of 10.9 to 11.55 covering the 10-min killing dilutions with *S. choleraesuis* in the relatively high challenge use-dilution test where excess alkali showed measurable inactivating effects (dilutions 1:240 to 1:390). Also it is much lower and much narrower than the range of 10.98 to 11.88 and above covering the concentrations found necessary to kill *S. aureus* in the relatively high challenge use-dilution procedure. The drop in pH with simple distilled water dilution with sodium o-phenylphenate in the absence of excess alkali due to hydrolysis is on the order of 0.7 pH.

It should be noted that while the pH level of test dilutions of sodium o-phenylphenate are raised as the amount of free sodium hydroxide is increased dilution in distilled water tends to counteract this increase so that dilutions of none of the test solutions providing concentrations of 0.0625% sodium o-phenolate or less, covering concentrations up to 4.0% of free sodium hydroxide had pH values higher than a 2.0% solution of sodium o-phenylphenate in the absence of excess alkali. Thus, it appears that in a low challenge type test where relatively high dilutions or low concentrations of germicide can kill the test organism the inactivating effects of the alkali ion can be obscured by dilution induced pH decreases; whereas, in a high challenge type test where relatively high concentrations or low dilutions of germicide are necessary to kill the test organism, the inactivating effect of the alkali ion will be clearly recorded. The curves in Fig. 3 clearly suggest also that this inactivating effect could be expected to be more pronounced as the level of challenge is increased by such factors as increased test organism resistance, increased test organism and organic load, or increases in carrier surface to test solution volume.

**DISCUSSION**

It should be acknowledged that in the interest of simplicity the factors of free alkali ions resulting from the hydrolysis of the neutral cocoanut oil soap, the specific activity of the neutral soap itself on the test organisms and the ratio of the neutral soap to o-phenylphenol have been ignored in these studies. They cannot, of course, be ignored by formulators of phenolic disinfectants. The results presented do, it is believed, illustrate the very wide differences in results which may be encountered with commercial products carrying phenolphenate-alkali complexes. They also clearly point up
the failure of low challenge phenol coefficient testing as a measure of the deficiencies which may be encountered with such products in practical disinfecting operations. It should be emphasized that the percentages of excess sodium hydroxide referred to here were added to the experimental stock solutions on the basis of weight of the sodium o-phenylphenate present and represent very small amounts of excess alkali; well within the range which could be expected in technical grades of commercial phenates.

Conclusions

Phenol coefficient or low challenge dilution tube testing procedures may fail to detect activity deficiencies in disinfectants which contain phenol-phenate-alkali complexes such as o-phenylphenol-sodium o-phenylphenate-sodium hydroxide. Use-dilution or relatively high challenge testing procedures provide a reasonably accurate measure of deficiencies found in such systems insofar as test organism specificity and the inactivating effects of excess alkalinity are concerned.

Evidence indicates that phenates may kill bacteria in a manner entirely different from the parent phenols and that this different mode of action may result in the demonstration of "quasi-specific" characteristics by the phenate not shown by the parent phenol.

It seems apparent from the high challenge use-dilution test results with the phenate and increasing concentrations of excess sodium hydroxide that the inactivating effect of excess alkali in phenolic type formulations can be expected to be more pronounced with gram positive cocci than with gram negative enteric bacteria.

The failure of the phenol coefficient, tube dilution low challenge test to show practical deficiencies in phenate preparations due to the presence of excess alkalinity can be attributed largely to decreases in pH in the higher aqueous dilutions effective in such methods.

Literature Cited


