EFFECT OF A HEXACHLORAPHENE DETERGENT ON THE MICROBIAL POPULATION OF THE HANDS OF FOOD HANDLERS

F. J. POST AND J. L. BALZER
Environmental Health Laboratories, School of Public Health
University of California, Los Angeles

A handwashing study was conducted to determine whether a 3% hexachloraphene hand detergent could materially reduce the normal bacterial population on the hands of food handlers. Total count, staphylococcus count, and total gram negative bacteria count showed significant reductions when the hexachloraphene detergent was used. No decline occurred when three other hand detergents were used. It was suggested that the gram negative bacteria count may be a better indicator of transient organisms on the hands than the total count which includes a greater proportion of resident bacteria.

Transient organisms, as defined by Price (15), collected fortuitously from the environment on the hands of food handlers are considered to be important because of the possibility that food poisoning or disease organisms may be among them (8). These organisms are considered relatively easy to remove by conscientious washing with good detergents (4). The resident flora, however, are refractory to removal and require a thorough scrubbing such as the surgical scrub used by physicians. Indeed much of our knowledge of handwashing detergents and procedures has resulted from studies of this technique.

The substance synthesized by Gump (6) and named hexachloraphene [bis (2 hydroxy -3, 5, 6 trichlorophenyl) methane] or G-11 has been shown to be actively bacteriostatic and germicidal in vitro (12, 16, 21, 26). Shemano and Nickerson (22), and Isikow and Gump (9) and others have shown by the use of radioactive tracers that this substance is deposited on the skin and remains for a considerable period of time. Studies on handwashing (19) have shown that hexachloraphene in combination with various skin detergents markedly reduces the resident and transient flora on the hands and keeps them at low levels as long as the detergent is used.

In view of the often demonstrated effectiveness of hexachloraphene in reducing transient and resident bacteria on surgeons hands, it was thought that a hexachloraphene detergent might bring about a reduction in the bacterial population of the hands of culinary workers also. To determine whether this could be brought about on hands which are constantly in food and dishwater was the purpose of this study.

Materials and Methods

Choosing a technique which could be effectively used under field conditions proved to be a problem. The Price (15) technique in which the hands are washed and the washings plated on nutrient media, with certain modifications, has been most often used by investigators (2, 7, 13, 14, 25). The variation of Quinn et al. (18) is considered to be the most informative; however, because of its complexity and requirements for time and detailed and faithful performance, it was not successful as a field technique in this instance. The four basin technique of Lawrence (11) was used.

To each of four sterile 3.5-liter stainless steel basins was added 1 liter of sterile distilled water and a sterile piece of gauze. The subjects rinsed both hands in basin 1 and rubbed vigorously to wrist level with the sterile gauze for 30 to 40 seconds. The gauze was left in the basin. The subject then picked up the gauze from basin 2 and the amount of test detergent recommended by the manufacturer for normal use was dispensed onto the hand and gauze. The subject vigorously rubbed his hands with this detergent-pad for 50 to 60 seconds. If, during this time more water was needed for lather, the cloth was dipped into the basin and scrubbing resumed. Then the cloth was dropped into the basin. No other hand contact with this basin was permitted. The subject then rinsed in basin 3 for 30 seconds, using the contained gauze cloth to aid in removal of the lather. This procedure was repeated in basin 4. No visible detergent remained on the hands after rinsing in the last basin.

Immediately after the subject finished rinsing, samples were drawn from the four basins as follows: The gauze pad was swirled around the basin with the tip of a sterile 10-ml pipette and then squeezed against the side of the basin. This was repeated. A 10-ml sample was then pipetted into a 25-ml sterile tube. When cationic or hexachloraphene detergents were used, 10 ml of a sterile neutralizing solution was added to the tube prior to sampling. (Azolactin (lecithin) (0.25% solution) was used to neutralize the cationic detergent (17, 3) and 1% Tween 80 was used to neutralize hexachloraphene (23). When neutralizers were used, a duplicate sample without neutralizer was run in parallel from basins

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1 and 3 for comparison. No neutralizers were used with the anionic detergents.

Pour and surface plates for 5 groups of organisms were made within 1 hr using duplicate 1- and 0.1-ml portions of each basin sample. Total counts were determined by nutrient agar (Difco) pour plates, coliform counts and a total deoxycholate count (coliforms plus all other colonies) by deoxycholate agar (Difco) pour plates, staphylococci on Chapman-Stone agar (Difco) surface plates, and enterococci using KF agar (10) surface plates. The last test was discontinued after consistently negative results. Colonies were counted after 48 hours incubation at 36°C. On plates with more than 300 colonies estimates were made according to the procedure in Standard Methods for the Examination of Dairy Products (24).

The various populations are reported on the basis of total bacteria removed (i.e. the sum of 4 basins). Determinations were made on the contents of all basins since preliminary studies showed that no prediction could be made as to the contents of which basin or basins would yield the highest counts and also on the theory that the sum of the counts in the contents of all basins used should be more indicative of the actual number removed.

The detergents used in this study were a standard anionic liquid yellow soap², a pure white neutral medium titered bar soap³, a cationic detergent bar⁴, and a liquid detergent⁵, containing 3% hexachlorophene as the active ingredient.

Four subjects from the dietary section of the University of California Hospital, Los Angeles volunteered for the study. One worker was a dishwasher, one a waitress-short order girl, one a chef, and one a tray line worker. Each subject was given a small pocket or purse size container of the test detergent. During the study the only comment received on any of the detergents concerned the hexachlorophene - pHisoHex. All subjects thought it cleaned the hands well but expressed a dislike for the product. Their reason was that it felt unnatural when compared to the expected slippery feeling associated with more familiar detergents. Most, however, did not seem to mind after becoming used to the new feeling. There were no irritation complaints.

Cade (1) suggested that transient organisms could be distinguished from resident organisms since the former are removed more readily and should result in much higher counts in the first few basins. Results of an attempt to duplicate this with ten basins or bowls is presented in Figure 1. One attempt with cloth swatches accomplished this fairly well, but one attempt using hand friction failed to show any uniform decline. Counts of each basin for the four culinary workers showed no reasonable pattern of decline - some went up, then down, some went down, then up and in general were very erratic. This method of distinguishing resident and transient flora could not be reliably used based on the data from these experiments.

Results of the handwashings are presented in Figures 2, 3, 4 and 5 as the logarithmic average of all test days during the use of a given detergent. It is felt that this method of presentation fairly repre-
sents such data. Since the number of coliforms on many days was below the minimum necessary for a count, these figures are plotted as actual count. Chapman-Stone agar counts are reported as staphylococci since gram stains of random representative colonies indicated only this cell form. Random colonies appearing yellow or yellow plus gelatinase activity were picked for coagulase testing. None were recovered using the Difco dehydrated plasma tube test.

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The total counts and staphylococcus counts very likely represent both transient and resident organisms and while it would seem reasonable in many instances that the former could occur in higher numbers than the latter, exact figures of each are not determinable. Thus these counts could not be used to distinguish the two groups of flora.

Originally only coliforms were to be counted on deoxycholate agar as indicators of possible fecal contamination, or at least as transient organisms. Reference to Figures 2, 3, 4, and 5 however, show that these counts are extremely erratic, only occasionally following the total count pattern. Explanation for this is very likely to include consideration of their presence in food handled by the workers, personal hygiene, and relatively low level environmental contamination. In any event, the occurrence of these organisms was sufficiently low and erratic in enough instances to make interpretation of detergent effect questionable. At the same time the coliform counts were made, it was noted that many other colonies were present on the deoxycholate medium. Since growth on this medium is restricted to gram negative bacteria, although not all of this group will grow, the coliform count and this count were combined to make a total deoxycholate count. This count of gram negative bacteria is considered by the authors to be much more representative of, if not composed solely of, transient organisms. Further study is being pursued on the possibilities of this point.

As can be seen in Figures 2, 3, 4, and 5 all workers presented a fairly uniform pattern of total count, staphylococcus count and total deoxycholate count during the period of establishing the “normal” level of contamination with the anionic yellow soap. Individual variation between workers existed but all total counts averaged about 10^7 and two were above 10^8. The staphylococcus and total deoxycholate

![Figure 1](https://example.com/figure1.png)

Figure 1. Number of bacteria removed from the hands in each of ten bowls using two methods of washing.

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![Figure 2](https://example.com/figure2.png)

Figure 2. Test period logarithmic averages of total counts, staphylococcus counts and total deoxycholate counts and counts of coliforms from the hands of the chef, male.

Table 1. Percent Reduction of Bacterial Counts on the Hands of Culinary Workers Using pHisoHex

<table>
<thead>
<tr>
<th>Worker</th>
<th>Total count</th>
<th>Staphylococci</th>
<th>Deoxycholate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chef</td>
<td>96.4</td>
<td>94.2</td>
<td>98.6</td>
</tr>
<tr>
<td>Waitress-short-order girl</td>
<td>98.0</td>
<td>96.7</td>
<td>58.1</td>
</tr>
<tr>
<td>Dishwasher-tray-line girl</td>
<td>97.4</td>
<td>97.9</td>
<td>30.0</td>
</tr>
<tr>
<td>Tray-line girl</td>
<td>70.0</td>
<td>37.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Percent reduction based on “normal” contamination level during the period of establishing the “normal” level of contamination with the anionic yellow soap. Individual variation between workers existed but all total counts averaged about 10^7 and two were above 10^8. The staphylococcus and total deoxycholate...
counts were somewhat lower but followed essentially the same pattern.

Introduction of the hexachloraphene detergent brought a marked reduction in the average counts (see Table 1). Reduction was most marked for the total count. In those cases, Figure 2 and 3, where a return to the control soap was made the average total and staphylococcus counts essentially returned to their former values. This is advanced as evidence that the “normal” level of contamination for these two parameters has real meaning. The total deoxycholate count cannot be as easily interpreted. Percent reduction varied from 98.6 to 0 (this last actually was an increase) and the influence of hexachloraphene was not as apparent. Coliforms could not be interpreted satisfactorily because of the large number of zero readings and the high variability. The other test detergents did not bring about significant changes from the control soap which further supports the validity of the apparent effect of hexachloraphene. It should also be noted that reductions with the hexachloraphene detergent occurred in spite of its use only during working hours.

Tentative interpretation of these results is that the hexachloraphene detergent leaves a deposit on the skin as Fahlberg et al. (5) and Gump and Cade (7) have shown and that continuous use of this detergent reinforces this deposited material. This deposited material amply demonstrated with surgical scrub studies, prevents the growth of resident flora resulting in a marked reduction of those counts which include these organisms. The transient organisms represented by gram-negative bacteria, are usually readily removed by washing and normally do not have a great opportunity to grow on the skin of the hands. Indeed, these gram-negative organisms are rarely included among the resident bacteria (20) and they may very likely be found on the hands only when fortuitously obtained from the environment. Even then they tend to die rapidly by dessication (20). If this is indeed the case then gram-negative bacterial counts of the hands would be expected to vary considerably and be lower than the total count. A glance at the Figures confirms this. Furthermore, since most of these would be removed with washing and re-added from the environment, the influence of hexachloraphene would not be as pronounced as on the resident flora, especially with the frequency of hand washing reported here. Part of the explanation for this lies in the fact that hexachloraphene is considered primarily a bacteriostat but may kill with time. Thus its effect on the transient organisms should be less pronounced than on the resident organisms. Figures 2, 3, 4, and 5, and Table 1 indicate that this probably happened, although the Chef (see Figure 2) showed a marked reduction. His hands were commonly in contact with foods known to have high counts of gram negative bacteria (raw hamburger, chicken, etc.). This may account for the very high initial counts and possibly for

**Figure 3.** Test period logarithmic averages of total counts, staphylococcus counts and total deoxycholate counts and counts of coliforms from the hands of the waitress - short order girl.

**Figure 4.** Test period logarithmic averages of total counts, staphylococcus counts and total deoxycholate counts and counts of coliforms from the hands of the dishwasher-tray line girl.

**Table 1.**
some of the apparent reduction during use of hexachloraphene when contamination may not have been so high. Table 1 indicates a high degree of variability with this group of organisms. Indeed there was actually a slight increase in numbers on the hands of the tray line worker (see Figure 5). This person also showed the least reduction of the four workers in the other counts. Suggested explanations include: failure to use the hexachloraphene, use of hand creams, etc. that neutralize or remove the deposited material, or continuous contamination by a wide variety of organisms of the transient type. The exact reason was not determined.

The deoxycholate count is not considered to be a count of all gram-negative bacteria present and thus is somewhat limited. However, it is suggested that this count is highly indicative of the group of gram-negative bacteria and the transient organisms in general. However, it must be noted that certain environmental sources such as the nose and mouth may not have as high gram-negative as gram-positive counts, thus these organisms would not satisfactorily measure this source of contamination. Virtually all other bacterial sources have a large compliment of gram-negative bacteria. Further studies on this subject with other hexachloraphene hand detergents will include a count more fully representative of the gram-negative bacteria.

Figure 5. Test period logarithmic averages of total counts, staphylococceous counts, total deoxycholate counts and counts of coliforms from the hands of the tray line girl.

CONCLUSIONS

Hexachloraphene, a bacteriostatic and bactericidal agent, is deposited on the hands of users of the commercial detergent pHisoHex. In a study of four culinary workers this detergent brought about a marked decrease in the total bacterial population and total staphylococci (including both resident and transient organisms) and affected the total gram-negative bacteria (as determined by counts on deoxycholate agar) erratically. The gram-negative bacteria are thought to more accurately reflect environmental contamination than do the other two counts which include a far greater proportion of resident bacteria. However, more work needs to be done on this point. It is concluded that hexachloraphene can bring about a considerable reduction of resident flora on the hands of culinary workers and appears to have some effect on the transient flora, although this point needs further clarification.

REFERENCES

14. Pohle, W. D. Germicide activity of resin soap and fatty acid-resin soap is indicated by handwashing experiments. Oil and Soap, 18:2-7.1941.
17. Public Health Service. Environmental Aspects of In-
EFFECT OF ACIDIFICATION ON STABILITY AND BACTERICIDAL ACTIVITY OF ADDED CHLORINE IN WATER SUPPLIES

HELEN HAYS, P. R. ELLIKER AND W. E. SANDINE

Department of Microbiology
Oregon Agricultural Experiment Station, Corvallis
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Studies were conducted to determine the effect of acidification on the bactericidal activity and stability of added chlorine in water supplies. The destruction of P. fluorescens, P. fragi and A. metalcaligenes by 5 and 10 ppm available chlorine at different pH levels was determined. Results indicated that bactericidal efficiency of hypochlorite solutions was greatly increased when the dilution water was acidified below pH 6.0. Studies to determine the effect of acidification of the water on the ability of the available chlorine indicated that at various pH levels from pH 4.0 to 9.0 there is little loss of available chlorine within 24 hrs.

Several studies (4, 6, 7, 8, 9) have demonstrated the need for bactericidal treatment of food plant water supplies to destroy bacteria that enter and cause spoilage in products like cottage cheese. In many instances chlorination of such water just before use at a level of 5 to 10 ppm available chlorine would suffice to destroy spoilage bacteria that would enter the food product from that source. There is considerable evidence also, however, that spoilage bacteria in some water supplies are not completely eliminated by this treatment, possibly because some waters are so highly buffered at an alkaline pH that chlorine compounds added to them cannot accomplish rapid bactericidal action. To overcome this difficulty, acidification of cottage cheese wash water was recommended in earlier reports (4, 9).

Furthermore, the time required for 99.99 per cent destruction of several Pseudomonas and Alcaligenes species by 3 and 5 ppm available chlorine was shown to be greatly increased at low temperatures and high pH (3). In view of the wide spread interest in many parts of the country in systems for combined acidification and chlorination of plant waters, it was decided to conduct studies on bactericidal activity of chlorine over a wide pH range, using representative spoilage bacteria. Also, since no information appeared to be available on stability of low concentrations of chlorine at such pH levels, a number of stability determinations were included.

METHODS

Pseudomonas fluorescens IM, Pseudomonas fragi, and Alcaligenes metalcaligenes were used as representative types of bacteria present in water supplies which cause spoilage in dairy products. The organisms were grown 24 hr on bottle slants of tryptone-yeast-extract-glucose agar, removed by washing with sterile phosphate-buffered water and filtered through Whatman No. 2 filter paper. The method of preparing the cultures and the technique of evaluating bactericidal activity were those described by Chambers (2). Calcium hypochlorite was used as the chlorinating agent. A sufficient number of trials also was carried out with sodium hypochlorite to demonstrate similarity in results with these two types of compounds. The available chlorine was determined by the standard thiosulfate titration (1). The germicide was buffered with 0.01 M solutions of appropriate buffer systems; pH 4.0 and 5.0 with sodium acetate-acetic acid, pH 6.0 and 7.0 with

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