Chemical Disinfection of Holding-Tank Sewage

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A number of chemical disinfectants were evaluated for their bactericidal and virucidal effectiveness in holding-tank sewage. It was found that the disinfection efficiencies of formaldehyde, benzalkonium chloride, cetylpyridinium chloride, and methylene blue were markedly improved if the pH of the sewage was raised from 8.0 to 10.5. When formaldehyde, benzalkonium chloride, and methylene blue were tested with either 2-week holding times with no sewage additions or 10-day holding times with daily sewage additions, disinfection effectiveness was maintained as long as the sewage pH was kept at 10.5 and the disinfectant concentration was kept at 100 mg/liter or more. Calcium hypochlorite, zinc sulfate, and phenol were found to be relatively ineffective disinfectants for holding-tank sewage.

Holding tanks and chemical toilets are widely used for small-scale waste containment and disposal in a variety of situations where on-site waste treatment systems are impractical, such as on aircraft, boats, and trains and in camps, parks, mobile homes, and construction sites. The trend toward increased use of such facilities and vehicles and a possible ban on waste discharges from vessels in navigable waters (3) indicates that the use of chemical toilets and holding tanks will continue to increase in the future. Although a variety of disinfectants and “sanitizers” are available for use in such systems, few studies have been reported in the literature on their disinfection efficacy in holding-tank or chemical-toilet wastes, particularly for enteric viruses. The purpose of this study was to evaluate a number of chemicals for their bactericidal and virucidal activity in holding-tank and chemical-toilet sewage and to develop conditions for their efficient use.

MATERIALS AND METHODS

Sewage. To simulate chemical-toilet and holding-tank sewage, which is generally stronger than typical domestic wastewater, a simulated sewage with a suspended-solids concentration of about 1,000 mg/liter was used in this study. The sewage contained per liter: 3.5 g (wet weight) of fecal material, 10 ml of urine, 0.25 g of toilet tissue, 0.4 ml of liquid hand soap, 100 ml of domestic raw sewage, and 885 ml of dechlorinated tapwater. The components were combined and blended for 1 min, and the mixture was used immediately.

Virus and virus assays. Type 1 poliovirus strain LSc was selected as representative of the enteric viruses potentially present in sewage and was used exclusively in this study. The virus was assayed in baboon kidney cell cultures by the plaque-forming-unit (PFU) method as previously described (4).

Bacteria and bacteria assays. The sewage used in this study had a heterogeneous population of bacteria which was largely contributed by the fecal material and domestic raw sewage it contained. Treated- and untreated-sewage samples were assayed for total viable bacteria on tryptone glucose yeast agar plates (1). Bacteria concentrations were expressed as colony-forming units (CFU) per milliliter.

Disinfectants. The following germicidal agents were used in this study: methylene blue, histological grade; phenol, certified grade; formaldehyde, reagent grade; and zinc sulfate, reagent grade (all the above from Fisher Scientific, Fair Lawn, N.J.); benzalkonium chloride (BAC) (Sterling Drug, Montvale, N.J.); calcium hypochlorite (HTH; Olin Chemical, Stamford, Conn.); and cetylpyridinium chloride (CPC), practical grade (MC/B Chemical, Norwood, Ohio). Those chemicals not already in a liquid form were prepared for use in experiments as concentrated stock solutions (wt/vol) in sterile distilled water.

Treatment of samples for bacteria and virus assay. Sewage samples taken for bacteria and virus assay were treated when they were obtained, either to remove or to neutralize the disinfectant they contained. Formaldehyde was neutralized with sodium bisulfite. Quaternary ammonium compounds and phenol were neutralized with Tween 80 and lecithin. Methylene blue and zinc were removed with Ionac C249 cation exchange resin, sodium form (Ionac Chemical Co., Birmingham, N.J.), and HTH was neutralized with sodium thiosulfate.

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RESULTS

Effect of disinfectant concentration and pH on bactericidal and virucidal activity. It has been known for some time that increasing pH above physiological levels improves the bactericidal activities of many quaternary ammonium compounds (2, 6). In addition, previous studies in our laboratory (Wallis et al., unpublished data) have shown that the bactericidal and virucidal activities of methylene blue and certain other dyes are improved at alkaline pH levels. Therefore, in the initial screening of chemical agents for germicidal activity in holding-tank sewage as a function of disinfectant concentration, the experiments were conducted at both the natural pH of the sewage (pH 8.0) and at pH 10.5. In these experiments, poliovirus was added to pH 8.0 raw sewage to give an initial virus concentration of about 10⁴ PFU/ml. The sewage was divided into 2 volumes, one of which was adjusted to pH 10.5 with Ca(OH)₂. Each volume was further divided into 100-ml samples, and disinfectants were added to samples to give the concentrations shown in Tables 1 and 2. One volume each of pH 8.0 and pH 10.5 sewage received no disinfectant and served as a control. The samples were held at 25°C in loosely capped bottles, and after 24 h, pH values were measured and bacteria and virus assays were made (Tables 1 and 2). HTH was highly bactericidal and virucidal throughout the entire concentration range tested at both pH 8.0 and 10.5. At pH 8.0, BAC, CPC, methylene blue, and formaldehyde demonstrated increasing bactericidal effectiveness with increasing concentration, whereas at pH 10.5 they were highly bactericidal at even the lowest concentrations tested. Formaldehyde demonstrated appreciable virucidal activity throughout the entire concentration range at both pH levels tested. BAC, CPC, and methylene blue were not highly virucidal at pH 8.0, but were effective at higher concentrations at pH 10.5. At both pH levels, phenol demonstrated no appreciable germicidal activity for the entire concentration range tested, and zinc sulfate was somewhat bactericidal only at the highest concentration tested.

### Table 1. Effect of disinfectant concentration and pH on bactericidal activity

| Conc (mg/liter) | Log₁₀ reduction in bacteria concentration* | BAC  | CPC  | Methylene blue | Phenol* | Formaldehyde | HTH  | Zinc sulfate* |
|----------------|--------------------------------------------|------|------|----------------|---------|--------------|------|--------------|
|                | pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 |
| 100            | 0 4.9 | 0.1  5.5 | 0  5.5 | 0  0 | 1.0  5.0 | 5.5  5.7 | 0.5  0.3 |
| 200            | 1.6 >5.5 | 0.2 >5.5 | 0 >5.5 | 0  0 | 2.8 >5.0 | 6.5 >5.7 | 0.6  0 |
| 400            | 1.9 >5.5 | 2.0 >5.5 | 0 >5.5 | 0.1 0 | 6.1 >5.0 | 6.5 >5.7 | 0.8  0 |
| 800            | >6.4 >5.5 | 4.8 >5.5 | 1.9 >5.5 | 0.1 0 | >6.8 >5.0 | 6.5 >5.7 | 0.9  0 |
| 1,600          | >6.4 >5.5 | >6.6 >5.5 | >6.2 >5.5 | 0.2 0 | >6.8 >5.0 | 6.5 >5.7 | 2.0  1.4 |

* Based on bacteria concentrations in control samples receiving no disinfectant. Control bacteria concentrations averaged 3 x 10⁶ colonies/ml at pH 8.0, and 2.5 x 10⁶ colonies/ml at pH 10.5.

* After 24 h, the pH levels in the samples initially adjusted to pH 10.5 had appreciably decreased.

### Table 2. Effect of disinfectant concentration and pH on virucidal activity

| Conc (mg/liter) | Log₁₀ reduction in virus concentration* | BAC  | CPC  | Methylene blue | Phenol* | Formaldehyde | HTH  | Zinc sulfate* |
|----------------|-----------------------------------------|------|------|----------------|---------|--------------|------|--------------|
|                | pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 pH 8.0 pH 10.5 |
| 100            | 0 0 | 0 0.1 0.1 | 0 0 | 0 >2.9 >2.4 | >2.1 >2.1 | 0 0.5 |
| 200            | 0 0 | 0 0.2 | 0 0 | 0 >2.9 >2.4 | >2.1 >2.1 | 0 0.4 |
| 400            | 0 0.2 | 0 0.5 | No data | 1.0 0 | 0 >2.9 >2.4 | >2.1 >2.1 | 0 0.3 |
| 800            | 0.2 0.6 | 0.1 2.1 | 0.4 1.6 | 0 0 | >2.9 >2.4 | >2.1 >2.1 | 0 0.3 |
| 1,600          | 0.1 >2.4 | 0.7 >2.1 | 0.6 1.6 | 0 0 | >2.9 >2.4 | >2.1 >2.1 | 0 0 |

* Based on virus concentrations in control samples receiving no disinfectant. Control virus concentrations averaged 8 x 10⁴ PFU/ml at pH 8.0 and 6 x 10⁴ PFU/ml at pH 10.5.

* After 24 h, the pH levels in the samples initially adjusted to pH 10.5 had appreciably decreased.
and exhibited no virucidal activity. The increased bactericidal and virucidal effectiveness observed for some of these agents at pH 10.5 was not simply due to the effects of high pH alone, because control sewage samples at the same pH showed appreciable levels of bacteria and persistence of virus.

A series of experiments was conducted to further investigate the effect of increasing pH on the bactericidal activities of formaldehyde, BAC, CPC, and methylene blue using a minimal disinfectant concentration. The virucidal effectiveness of formaldehyde was also evaluated because the results of the previous experiment indicated that it was effective for both bacteria and viruses at a minimal concentration. In experiments with formaldehyde, poliovirus was added to pH 8.0 raw sewage to give an initial concentration of about $3 \times 10^4$ PFU/ml. In all of the experiments, the pH 8.0 sewage was divided into four samples of 200 ml each, and the samples were adjusted to pH 9.0, 10.0, and 10.5 with Ca(OH)$_2$. The samples were further divided into 2 volumes of 100 ml each, and 1 volume was dosed with disinfectant to give a concentration of 100 mg/liter. The samples were held at 25°C in loosely capped bottles, and after 24 h pH levels were measured and bacteria and virus assays were made. The results of these experiments for formaldehyde, BAC, CPC, and methylene blue are shown in Tables 3, 4, 5, and 6, respectively. The bactericidal activities of 100-mg/liter concentrations of formaldehyde, BAC, and methylene blue at pH 10.0 and 10.5 were markedly improved over their activities at pH 8.0 or 9.0. CPC was highly bactericidal at pH 10.5 but not at pH 8.0, 9.0, or 10.0. At all pH levels tested, a 100-mg/liter concentration of formaldehyde was highly virucidal. At a 100-mg/liter concentration, CPC, BAC, and methylene blue were shown to be ineffective against viruses in the simulated sewage at either pH 8.0 or 10.5 (Tables 1 and 2). However, since this simulated sewage prepared for these studies contained an anionic detergent, the quaternary compounds would have been inactivated under these conditions.

Because a 100-mg/liter concentration of formaldehyde in raw sewage gave extensive virus inactivation in 24 h at pH levels ranging from 8.0 to 10.5, it could not be determined from the results of this experiment whether increasing pH enhanced virus inactivation. Therefore, an experiment similar to the one described in the previous paragraph was performed with formaldehyde at a concentration of 25 mg/liter. The results of this experiment (Table 7) indicate that the virucidal activity and the bactericidal

### Table 3. Effect of increasing pH on the bactericidal and virucidal effectiveness of 100 mg of formaldehyde per liter

| pH (at 0 time) | pH (after 24 h) | Log$_{10}$ bacteria and virus concentrations |
|---------------|----------------|--------------------------------------------|
|               | Control samples | Formaldehyde samples | Bacteria (CFU/ml) | Virus (PFU/ml) | Control samples | Formaldehyde samples | Bacteria (CFU/ml) | Virus (PFU/ml) |
| 8.0 | 8.3 | 8.4 | 7.78 | 4.85 | 5.90 | <2.70 |
| 9.0 | 8.7 | 9.0 | 7.70 | 4.88 | 4.23 | <2.70 |
| 10.0 | 10.1 | 10.0 | 7.65 | 4.81 | <0.70 | <2.70 |
| 10.5 | 10.4 | 10.5 | 6.11 | 4.85 | <0.70 | <2.70 |

### Table 4. Effect of increasing pH on the bactericidal effectiveness of 100 mg of BAC per liter

| pH (at 0 time) | pH (after 24 h) | Log$_{10}$ bacteria concentrations |
|---------------|----------------|----------------------------------|
|               | Control samples | BAC samples | Control samples (CFU/ml) | BAC samples (CFU/ml) |
| 8.0 | 8.2 | 8.5 | 7.08 | 5.95 |
| 9.0 | 8.9 | 8.9 | 7.29 | 6.11 |
| 10.0 | 9.85 | 9.85 | 6.51 | 2.32 |
| 10.5 | 10.4 | 10.5 | 6.53 | 1.30 |

### Table 5. Effect of increasing pH on the bactericidal effectiveness of 100 mg of CPC per liter

| pH (at 0 time) | pH (after 24 h) | Log$_{10}$ bacteria concentrations |
|---------------|----------------|----------------------------------|
|               | Control samples | CPC samples | Control samples (CFU/ml) | CPC samples (CFU/ml) |
| 8.0 | 8.36 | 8.46 | 7.56 | 7.27 |
| 9.0 | 8.71 | 8.94 | 7.56 | 7.05 |
| 10.0 | 9.73 | 9.90 | 7.09 | 7.0 |
| 10.5 | 10.15 | 10.36 | 6.11 | 0.70 |

### Table 6. Effect of increasing pH on the bactericidal effectiveness of 100 mg of methylene blue per liter

| pH (at 0 time) | pH (after 24 h) | Log$_{10}$ bacteria concentrations |
|---------------|----------------|----------------------------------|
|               | Control samples | Methylene blue samples | Control samples (CFU/ml) | Methylene blue samples (CFU/ml) |
| 8.0 | 8.6 | 8.0 | 7.23 | 6.73 |
| 9.0 | 9.0 | 9.0 | 7.70 | 5.85 |
| 10.0 | 9.9 | 9.85 | 6.46 | 3.90 |
| 10.5 | 10.3 | 10.2 | 6.98 | 1.0 |
activity of formaldehyde were markedly improved as the pH was increased in the alkaline range.

Effect of holding time on bactericidal and virucidal activity. To determine the ability of chemical disinfectants under alkaline conditions to sustain bactericidal activity for a prolonged period and to determine the degree and rate of virus inactivation with minimal disinfectant concentration, a series of experiments was conducted in which samples of pH 10.5 raw sewage containing 30- and 100-mg/liter concentrations of formaldehyde, BAC, methylene blue, or HTH were held at room temperature for 14 days and were periodically assayed for bacteria and virus. Raw sewage at pH 10.5, which contained no disinfectants, was used as a control. In these experiments, raw sewage which had been adjusted to pH 10.5 with Ca(OH)₂ was dosed with poliovirus to give an initial concentration of 10⁴ PFU/ml. The sewage was then divided into 100-ml volumes, and the disinfectants were added to these samples to final concentrations of 30 and 100 mg/liter. A volume of pH 10.5 sewage containing no disinfectant served as a control. The samples were held at 25°C in loosely capped bottles, and samples were taken periodically for virus and bacteria assay. The pH levels of the samples were maintained at 10.5 by periodic adjustment with Ca(OH)₂ if necessary (Tables 8 and 9).

Both the 30- and 100-mg/liter concentrations of BAC and methylene blue and the 100-mg/liter concentration of formaldehyde were capable of keeping bacteria concentrations well below those in control sewage for the entire 14-day period. However, some bacterial regrowth was apparent in the samples with 30 and 100 mg of BAC per liter by days 4 and 11, respectively. The bactericidal effects of BAC were more rapid at a concentration of 100 mg/liter than at 30 mg/liter, as indicated by the longer persistence of bacteria in the latter. Although both concentrations of formaldehyde and HTH were at first highly bactericidal and virucidal, extensive bacterial regrowth occurred in both HTH samples and in the 30-mg/l formaldehyde sample after several days. Virus inactivation rates in samples containing BAC and methylene blue were somewhat greater than that for control sewage but were relatively slow as compared with those for samples containing HTH or formaldehyde. Virus inactivation was somewhat more rapid with methylene blue than with BAC.

Effect of daily sewage addition on the

| Table 7. Effect of increasing pH on the bactericidal and virucidal effectiveness of 25 mg of formaldehyde per liter |
|---------------------------------------------------------------|
| pH (at 0 Time) | Control samples | Formaldehyde samples | Log₁₀ bacteria and virus concentrations | Control samples | Formaldehyde samples |
|----------------|---------------|---------------------|----------------------------------------|----------------|---------------------|
| 8.0            | 8.1           | 8.2                 | 7.70                                   | 4.38           | 6.45                |
| 9.0            | 8.9           | 9.0                 | 7.30                                   | 4.48           | 3.90                |
| 10.0           | 9.9           | 9.95                | 7.16                                   | 4.37           | 1.84                |
| 10.5           | 10.3          | 10.25               | 6.94                                   | 4.44           | <0.70               |

| Table 8. Effect of holding time on the bactericidal activity of formaldehyde, BAC, methylene blue, and HTH |
|---------------------------------------------------------------|
| Time (days) | Control sewage | Formaldehyde | BAC | Methylene blue | HTH |
|-------------|----------------|--------------|-----|---------------|-----|
|             | 30 mg/liter    | 100 mg/liter | 30 mg/liter | 100 mg/liter | 30 mg/liter | 100 mg/liter | 30 mg/liter | 100 mg/liter | 30 mg/liter | 100 mg/liter |
| 0           | 3.7            | <0.0         | 0   | 0.6           | 0   | 1.3           | <0.0           | 1.0           | 1.3           |
| 1           | 4.7            | 1.0          | <0.0| 0.0           | 0.0 | 2.5           | <0.0           | <0.0           | <0.0           |
| 2           | 5.9            | <0.0         | <0.0| 0.0           | 0.0 | 2.5           | <0.0           | <0.0           | <0.0           |
| 4           | 6.5            | 3.8          | <0.0| 2.6           | <0.0| <0.0          | <0.0           | 5.7           | 1.5           |
| 7           | >6.7           | 4.6          | <0.0| 2.5           | <0.0| <0.0          | <0.0           | 5.9           | 5.1           |
| 11          | >6.7           | 5.5          | <0.0| 3.5           | 0.3 | <0.0          | <0.0           | 6.0           | 5.6           |
| 14          | >6.7           | 4.8          | <0.0| 1.9           | 2.1 | <0.0          | <0.0           | 6.0           | 6.0           |
bactericidal activity of formaldehyde, methylene blue, and BAC. An experiment was conducted to determine the effects of incremental additions of sewage or sewage plus disinfectant on the ability of formaldehyde, methylene blue, and BAC to sustain bactericidal activity for a prolonged time period. Initially, 100-ml samples of raw sewage, adjusted to pH 10.5 with calcium hydroxide, were dosed with formaldehyde, methylene blue, or BAC to give concentrations of 1,000 and 100 mg/liter. The samples containing 1,000 mg of disinfectant per liter were given daily 100-ml volumes of fresh pH 10.5 sewage, and the samples containing 100 mg of disinfectant per liter were given daily 100-ml volumes of fresh pH 10.5 sewage with 100 mg of disinfectant per liter. The samples were held at 25 C and assayed for bacteria just prior to the daily sewage additions. Two types of pH 10.5 control sewage samples were used: one received no additional sewage and the other received a daily 100-ml volume of fresh pH 10.5 sewage. The experiment was conducted for 10 days, and a total of nine daily sewage additions were made. All samples were maintained at pH 10.5 by periodic adjustment with calcium hydroxide.

Bacterial growth was extensive in both control sewage samples, with concentrations near the 10^7 CFU/ml level throughout most of the experiment (Table 10). In all samples containing disinfectants, bacteria concentrations were substantially lower than those in controls. This was true of those samples initially containing 1,000 mg of disinfectant per liter and receiving daily doses of sewage and of those samples initially containing 100 mg of disinfectant per liter and receiving daily doses of sewage with

| Table 9. Effect of holding time on the virucidal activity of formaldehyde, BAC, methylene blue, and HTH |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Time (days)                     | Control sewage  | Formaldehyde    | BAC             | Methylene blue  |
|                                |                 | 30 mg/liter     | 100 mg/liter    | 30 mg/liter     |
|                                |                 |                 |                 |                 |
| 0                              | 5.0             | <1.7            | 4.6             | 4.6             |
| 1                              | 4.5             | <1.7            | 4.1             | 3.7             |
| 2                              | 3.9             | <1.7            | 3.0             | 2.0             |
| 4                              | 3.4             | <1.7            | 2.2             | 1.7             |
| 7                              | 2.7             | <1.7            | <1.7            | 1.7             |
| 11                             | <1.7            | <1.7            | <1.7            | 1.7             |
| 14                             | <1.7            | <1.7            | <1.7            | 1.7             |

| Table 10. Effect of daily sewage addition on the bactericidal activity of BAC, formaldehyde, and methylene blue |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Time (days)                                     | Control sewage  | BAC             | Methylene blue  |
|                                                |                 |                 |                 |
| Sample 1*                                       | Sample 2*       | Sample 1'       | Sample 2'       |
| 0                                               | 4.3             | 6.1             | 1.6             |
| 1                                               | 5.7             | 6.9             | 2.0             |
| 2                                               | 7.2             | 7.2             | 2.5             |
| 3                                               | 7.1             | 7.2             | 1.8             |
| 4                                               | 7.4             | 7.2             | 1.9             |
| 5                                               | 7.3             | 7.5             | 1.9             |
| 6                                               | 7.3             | 6.9             | 1.6             |
| 7                                               | 7.4             | 7.2             | 2.1             |
| 8                                               | 7.7             | 7.0             | 2.6             |
| 9                                               | 7.6             | 6.9             | 3.0             |
| 10                                              | 7.3             | 6.9             | 3.3             |

* No daily sewage addition.

a Daily addition of sewage.

Initial 1,000 mg/liter disinfectant concentration in sewage with daily addition of sewage only.

Initial 100 mg/liter disinfectant concentration in sewage with daily addition of sewage containing 100 mg of disinfectant per liter.
100 mg of disinfectant per liter. Although some bacterial growth occurred in a number of the disinfectant-containing samples as the experiment progressed, it was never extensive, and bacteria concentrations were kept well below the concentrations in control samples.

DISCUSSION

In raw sewage adjusted to pH 10.5, formaldehyde, methylene blue, and BAC were found to be effective disinfectants for periods of at least 14 days. In addition, the bactericidal effectiveness of these agents in raw sewage could be maintained for a period of at least 10 days when raw sewage was added daily, as long as the pH was maintained at about 10.5 and the disinfectant concentration was maintained at 100 mg/liter or more. HTH, although initially highly germicidal, had insufficient stability to be effective for more than a few days. The lack of stability of HTH, as well as its properties of corrosiveness in solution and strong oxidizing power which made it difficult to store and handle, detract from its suitability as a holding-tank or chemical-toilet disinfectant. Zinc sulfate and phenol demonstrated poor bactericidal and virucidal effectiveness in both pH 8.0 and 10.5 raw sewage at relatively high concentrations, and therefore appear to be unsuitable disinfectants for holding tanks and chemical toilets.

In general, the results of this study suggest that at pH 10.5, formaldehyde, methylene blue, BAC, and CPC would be suitable holding-tank and chemical-toilet disinfectants. Although the germicidal property of alkaline formaldehyde has been previously suggested (A. R. White, U.S. Patent 2, 077, 060, 1937), quantitative studies on its bactericidal and virucidal effectiveness were not reported. One possible advantage of formaldehyde over some other disinfectant chemicals for holding tanks and chemical toilets is that, upon dilution and at less alkaline pH levels, it can be biodegraded in natural aquatic environments and in biological wastewater treatment systems. Formaldehyde is capable of being used as a substrate by bacteria either directly at low concentrations (5) or after its chemical conversion by a variety of reactions with various naturally occurring chemicals (7) to either formate or hydroxymethyl derivatives. Thus, the use of formaldehyde as a holding-tank disinfectant would be particularly attractive in situations where biodegradability is essential.

Although the quaternary ammonium compounds BAC and CPC and the dye methylene blue were somewhat less efficient germicides than was formaldehyde, they were effective in pH 10.5 sewage at relatively low concentrations, and they may be useful in holding-tank and chemical-toilet situations where biodegradability is not a critical factor or where formaldehyde would not be sufficiently stable. In addition, these agents are not highly volatile or irritating to the skin or mucous membranes as is formaldehyde, thus making these agents safer and easier to handle. It should be noted that the germicidal properties of quaternary ammonium compounds such as BAC and CPC are adversely affected by soaps and anionic detergents. Therefore, it is likely that the soap in the simulated holding-tank sewage used in our experiments decreased the bactericidal and virucidal effectiveness of these agents. In fact, it has been shown that in the absence of high concentrations of soaps and anionic detergents the bactericidal and virucidal effectiveness of CPC is markedly improved at pH 10.5 (Wallis et al., unpublished data).

The observation that formaldehyde, the quaternary ammonium compounds BAC and CPC, and the dye methylene blue were more efficient germicides at pH 10.5 than at 8.0 has important practical application in the disinfection of holding-tank and chemical-toilet sewage, because the use of any of these disinfectants in combination with enough base to give a pH of 10.5 greatly improves their germicidal efficiency, thereby reducing the quantity of chemical required for disinfection. The enhanced germicidal effectiveness of these agents at alkaline pH levels may have practical applications other than sewage disinfection.

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