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Research and Development

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# Project Summary

# Influence of Phosphate Corrosion Control Compounds on Bacterial Growth

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The influence of two phosphate corrosion control compounds on the growth and survival of coliform and other heterotrophic bacteria was investigated. The compounds studied included Shan-No-Corr* (a sodium, zinc-metaphosphate) and Virchem 932 (a zinc-orthophosphate).

The investigation was conducted in three parts:

A. Growth of *Citrobacter freundii, Enterobacter cloacae,* and *Klebsiella pneumoniae* was studied in pure culture (laboratory) investigations in the presence of various concentrations of Shan-No-Corr (0.1 to 2.0 mg/L of water, as product) and in the presence of Virchem 932 (0.01 to 1.0 mg/L of water, as zinc). In some experiments $Fe_2O_3$ (100 $\mu$g/L), an iron corrosion product, was also added.

B. Field investigations were conducted in three water distribution systems. Two interconnected systems received Shan-No-Corr and the third Virchem 932. Total coliform, total heterotrophic bacteria, and 16 different physicochemical parameters of the water were measured, twice weekly, before and after the addition of the corrosion control compounds. Statistical correlations were sought among observed changes in bacterial counts and the various physicochemical water parameters.

C. Model system studies were conducted by adding coliform (*C. freundii*) and other heterotrophic bacteria to a model water distribution system, establishing a steady-state bacterial population, adding the phosphate corrosion control compounds, and then comparing the growth of the bacteria before the addition of the phosphate compounds to growth after the additon.

Results presented no evidence that the corrosion control compounds stimulated coliform growth and some evidence that they might inhibit growth. Growth of the other heterotrophic bacteria might be stimulated by the compounds, although the evidence is not conclusive.

*This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

## Introduction

One major problem facing water utility companies is the control of pipe corrosion. Each year, hundreds of millions of dollars are spent by utility companies to maintain and replace pipes damaged by corrosion.

Different treatment methods exist to prevent pipe corrosion in water distribution systems. The most common treat-

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use

ments are those that rely on the addition of various inorganic compounds that will form a protective film on the walls of the pipes. Included in this group are carbonates, silicates, and phosphates

Phosphate corrosion inhibitors are quite popular and have been used for over 30 years. They come in three principal forms orthophosphates, bimetallic phosphates, and molecularly dehydrated polyphosphates

### *Objectives of the Project*

Due to a lack of information on the influence of phosphate addition (for corrosion control) on microbial growth and the potential public health significance, the objective of this project was to determine the influence of phosphate addition for corrosion control in water distribution systems on the survival and growth of coliform and other heterotrophic bacteria. Correlations between bacterial growth, phosphate addition, and various physicochemical parameters (i e., pH, temperature, chlorine levels) of the water were also investigated

## Materials and Methods

The influence of phosphate corrosion control compounds on the growth of coliforms and other heterotrophic bacteria was determined by comparing bacterial growth in the presence and absence of the compounds Investigations were performed on three levels:

A Pure culture investigations in the laboratory.

B Field investigations in three, small, public groundwater distribution systems.

C Model system investigations in the laboratory

This investigation utilized two commercially available phosphate corrosion control compounds. Shan-No-Corr, a sodium, zinc-metaphosphate manufactured by the Shannon Chemical Company, Malvern, PA, and Virchem 932, a zinc-orthophosphate manufactured by Technical Products Corporation, Portsmouth, VA

### *Pure Culture Investigations*

Experiments were performed in the laboratory to determine the influence of various concentraitons of Shan-No-Corr and Virchem 932 on the growth of pure cultures of *C. freundii* (ATCC 8090), *E. cloacae* (ATCC 13047), and *K. pneumoniae* (ATCC 13883).

Coliforms for the studies were cultured for 24 hr in tryptic-soy broth (Difco). Serial dilutions of 1 mL of this culture were done in sterile saline (0.85% w/v, pH 7.0) to obtain a final dilution of $10^{-6}$. A 1-mL dose of this suspension (approximately 1,000 to 6,000 cells/mL) was then added to 99 mL of sterile, dechlorinated tap water (in a 250 mL flask) amended with a phosphate corrosion control compound. The tap water was batch dechlorinated with activated carbon and sterilized by membrane filtration. Shan-No-Corr concentrations of 0 1, 0 2, 0.3, 0.5, 1.0, 1.5, and 2.0 mg/L (as product) were tested. In the case of Virchem 932, the concentrations tested were 0 01, 0.1, 0.3, 0.5, 0.75, and 1.0 mg/L (as zinc). In both studies, tap water without the phosphate corrosion control compound was used as a control. Various chemical characteristics of each batch of tap water (total and free chlorine, pH, alkalinity, turbidity, sparged and nonsparged total organic carbon, total, filtrable, and dissolved solids, nitrogen as $NO_2$-$NO_3$, zinc, orthophosphate, and total phosphate) were determined, prior to dechlorination, according to Standard Methods (1985) The flasks were incubated at 25°C and the coliforms were counted after 24, 48, and 168 hr by the spread plate procedure using one-tenth-strength tryptone-glucose-yeast agar as the plating medium, an incubation time of 48 hr for *C. freundii* and *E. cloacae* and 168 hr for *K. pneumoniae*, and an incubation temperature of 35°C.

Since the phosphate corrosion control compounds interact with iron corrosion products, it was of value to determine if there were any synergistic effects between the iron corrosion products and the phosphate compounds on coliform growth. To test this, pure culture experiments, as described above, were conducted with the addition of 100 $\mu$g of iron (as $Fe_2O_3$)/L of water. An additional control, consisting of tap water, $Fe_2O_3$, and no phosphate compound was added.

### *Field Investigations*

A field study was undertaken to determine the influence of Shan-No-Corr and Virchem 932 on the growth of coliforms and other heterotrophic bacteria in operating water distribution systems.

Three, small, public, water distribution systems in Chester County, PA, were used in the field investigations:

A. Bradford Glen: supplies groundwater chlorinated at a dose of 1.0 mg/L. The system consists of 100% ductile iron pipes and approximately 250 services. The system was started in 1975 and portions are under construction as houses are being built.

B. Spring Run: supplies groundwater chlorinated at a dose of 1.0 mg/L. The system consists of 30% ductile iron/70% asbestos/cement pipes and approximately 220 services. The system was installed during the period 1972-1975.

C Federal Drive: supplies groundwater chlorinated at a dose of 1.0 mg/L. The system consists of 100% ductile iron pipes and 67 services. The system was installed during the period 1972-1975.

### Sampling

The systems were sampled, at least twice a week, at or near the source water, at system midpoint, and at the end. At each sampling site, four 900-mL serial water samples were collected in clean, sterile polypropylene bottles containing 0.2 mL of a 20% (w/v) sodium thiosulfate solution added before autoclaving. The sodium thiosulfate was added to neutralize any residual chlorine present in the water. All samples were protected from sunlight and transported with noncontaminating artificial coolants to the laboratory for immediate processing. A pre-additive, 1985 (prior to addition of either Shan-No-Corr or Virchem 932) sampling phase of 2 months for Bradford Glen and Spring Run and 3 months for Federal Drive was performed to determine the background bacteriological and chemical profile of the systems. After the pre-additive sampling phase, the phosphate corrosion control compounds were added and sampling (1985 post-additive phase) continued until the winter. Sampling was stopped during the winter, although the phosphate compounds were added throughout the winter. Sampling resumed the following spring (1986 post-additive phase).

### Addition of Phosphate Corrosion Control Compounds

After the pre-additive, 1985 sampling phase, Shan-No-Corr was added to Bradford Glen and Spring Run (treated as one system because of an interconnect) water at a rate of 3.0 mg/L (as product) for 4 months (for passivation) and then reduced to 1.0 to 1 5 mg/L (for maintenance). Virchem 932 was added to Federal Drive water at a rate of 1.0 mg/L (as zinc) for 1 month (for passivation) and then reduced to 0.3 mg/L (for maintenance). The pH of all systems was adjusted to pH 6.5 to 7.0, according to manufacturer recommendations, with commercial caustic soda (50% NaOH)

## Bacteriological Analysis

The following bacteriological tests were performed on the samples:

A. Portions of each sample (100 mL) were analyzed for total coliforms by the membrane filter procedure (Standard Methods, 1985) using mENDO LES medium (Difco) and 24 hr incubation at 35°C. Typical coliform colonies were verified by growth and gas production in lauryl tryptose broth (Difco) and brilliant green bile broth (Difco). Approximately 23% of verified isolates were identified by the API Rapid E system (Analytab Products).

B. Heterotrophic plate counts were performed on 5 to 20 mL portions of each sample by the membrane filter procedure (Standard Methods, 1985) using R2A medium and 168 hr incubation at 28°C. After incubation, the colonies were counted and categorized by color (red, orange, yellow, and other). For comparative purposes, every fifth sample was analyzed (total counts only) by the spread plate procedure (Standard Methods, 1985) (1 mL of sample, R2A medium, and 168 hr incubation at 28°C) and by the standard pour plate procedure (Standard Methods, 1985).

## Chemical Analysis

Various physicochemical parameters of the water were determined (Standard Methods, 1985). Free and total chlorine and temperature were measured in the field while turbidity; pH; alkalinity; total, filtrable, and dissolved solids; orthophosphate, total phosphate (acid-hydrolyzable); non-sparged and sparged total organic carbon; nitrite-nitrate nitrogen; zinc; and calcium were measured in the laboratory.

## *Model System Investigations*

A model water distribution system was constructed as the third component of this research

The model system was a re-circulating system (Figure 1) and consisted of three asbestos/cement and three ductile iron pipe segments (4-inch diameter, 3 feet in length). Each pipe segment was supplied with Bradford Glen well water from a 10-gallon reservoir. Polyvinyl chloride tubing connected each pipe section to its own individual reservoir. For each pipe type, one segment served as a control, one received Shan-No-Corr, and the third received Virchem 932. The system was inoculated with heterotrophic bacteria to establish a steady-state population of approximately $10^4$ to $10^5$ cells/mL. The heterotrophic population consisted of coliform and non-coliform bacteria isolated from the distribution systems They were added at approximately the same ratio (coliform:non-coliform) observed in the distribution systems The model system was run at 25°C and at a flow rate (0.3 ft/sec) sufficient to induce turbulent flow and insure complete mixing of the water.

At the conclusion of the last experiment, various parts of the model system were sampled for coliforms Areas sampled included the inner wall surface of the ductile iron and asbestos-cement pipes, reservoirs, and the polyvinyl chloride tubing. Sampling was accomplished by swabbing each area with two sterile cotton swabs and then placing one swab into a tube of lauryl tryptose broth (Difco) and the other into tryptic-soy broth (Difco). In the case of the pipes, a sterile stainless steel spatula was used to scrape the pipe wall and the collected material was placed into the broths. Use of the spatula was necessitated by the rough surface of the pipe walls. Swabbing with a cotton swab would have resulted in loss of the cotton from the swab. Tubes demonstrating growth after 48 hr were examined for coliforms by standard procedures (Standard Methods, 1985).

The model system was generally sampled daily following the same protocol used in the field investigations. The influence of the following physicochemical parameters on the growth of the bacteria were tested:

A. Phosphate corrosion control compounds.

B. As in A but with the pH increased from approximately 6.2 (natural pH of the water) to 8.5.

C. Phosphate corrosion control compounds along with an assimilable carbon source (50 $\mu$g of total organic carbon as glucose/L of water).

Each experiment was run until a steady-state (or predictable rate of growth) heterotrophic population (coliform and non-coliform) was attained. Prior to the beginning of each new experiment, the heterotrophic population within the system was adjusted (if necessary) to the starting point of $10^3$ to $10^4$ cells/mL.

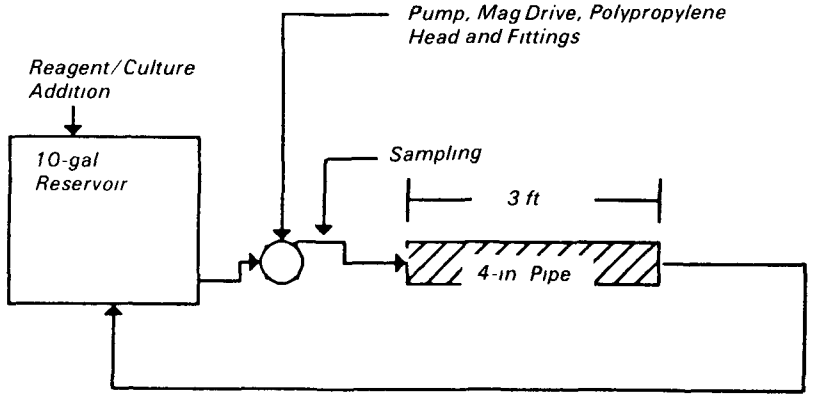


*Figure 1. Diagram of model system.*

## *Analysis of Data*

Analyses were performed using the BMDP Statistical Software available on Prime computer systems, and no data were treated as outliers.

### Pure Culture Investigations

The equivalence of repeated runs with the same compound - organism set was tested by one-way Analysis of Variance (ANOVA), grouped by run. At the same time the Levene test for equal variances was performed The significance of grouped means was tested over the range of concentrations by multiple linear regression, and against the control by Student's t-test.

### Field Investigations

Field data were tested by both parametric and non-parametric methods. Correlations of bacteriological and physicochemical parameters over the test period were sought using multiple linear regression for all systems combined or simple linear regression for individual systems. Spearman and Kendall rank correlations were also performed for the same data sets. The significance of correlations was tested using, where appropriate, tables of critical values for the appropriate r- or t-transforms

Equivalence of parameters for the pre-additive and post-additive periods was tested by a one-way ANOVA grouped by period and the significance of differences further tested by a separate-variance t-test. In addition, simple linear regressions on the two data sets were also performed and tested by appropriate methods. Both direct and $LOG_{10}$ bacterial counts were used in these analyses.

## Results and Discussion

### *Pure Culture Investigations*

Growth of *C. freundii*, *E. cloacae*, and *K. pneumoniae* was followed in the presence of various concentrations of Shan-No-Corr and Virchem 932 in dechlorinated, filter-sterilized tap water. In the presence of Shan-No-Corr there was no significant (Student's t-test and multiple linear regression analysis) pattern of growth inhibition or stimulation when compared to the control after 24, 48, 168 hr of incubation The addition of 100 $\mu$g/L of iron (as $Fe_2O_3$) did not change the results.

In the presence of Virchem 932 growth of *E. cloacae* was not significantly (Student's t-test) different from the control. *C. freundii* showed a significant (Student's t-test) decrease in growth (Table 1) after 48 and 168 hr of incubation in the presence of 0.3 mg/L and above of Virchem 932. At concentrations less than 0.3 mg/L or after 24 hr of incubation, there was no significant difference in growth. *K. pneumoniae* showed a significant (Student's t-test) decrease in growth (Table 2) after all three incubation times and generally at Virchem 932 concentrations of 0.3 mg/L and above. The addition of 100 $\mu$g/L of iron to the tap water enhanced the inhibitory action of the Virchem 932 with all three coliforms significantly (Student's t-test) inhibited.

Chemical analysis of the tap water used in the pure culture investigations indicated that the different batches of tap water used were uniform and did not vary greatly in their chemical properties.

### *Field Investigations*

Investigations were performed in three water distribution systems comparing coliform and heterotrophic bacterial counts prior to the addition of phosphate corrosion control compounds to counts obtained after the addition of the compounds. In Bradford Glen (BG), Spring Run (SR), and Federal Drive (FD) the mean total coliform counts per 100 mL sample for the period prior to the addition of the corrosion control compounds (pre-additive 1985) were 2.14, 1 19, and 1 88, respectively (Figure 2). Mean heterotrophic bacterial counts per mL were 6.43 for BG, 7 29 for SR, and 5 33 for FD (Figure 3) After the addition of Shan-No-Corr to BG and SR and Virchem 932 to FD (post-additive 1985), there was no significant change (Student's t-test) in the mean total coliform counts (Figure 2). However, the mean heterotrophic counts increased significantly (Student's t-test) in BG and SR (Figure 3). There was no significant change in FD. The corrosion control compounds were added over the 1985-1986 winter and sampling resumed in the spring of 1986 (post-additive 1986) Mean total coliform counts obtained in 1986 were significantly lower (Student's t-test) in BG and FD when compared to the pre-additive 1985 counts (Figure 2). Although lower, the count in SR was not significantly different from the 1985 count. Mean heterotrophic counts obtained in 1986 were significantly (Student's t-test) increased in all three systems when compared to the pre-additive 1985 counts (Figure 3).

Statistical analyses revealed little evidence that the corrosion inhibitors stimulate coliform growth in water

***Table 1.*** *Growth of* Citrobacter Freundii *in the Presence of Various Concentrations of Virchem 932*

| *Time (h)* | *Conc (mg/L)* | *Mean Cell Count ± S D (per ml)** | *Level of Significance#* |
|---|---|---|---|
| *24* | *0.0* | *404 0 ± 691 20* | -- |
| | *0.01* | *180.6 ± 275.38* | *NS* |
| | *0.1* | *252 7 ± 442 10* | *NS* |
| | *0.3* | *28.6 ± 19 32* | *NS* |
| | *0.5* | *7 7 ± 10.46* | *NS* |
| | *0.75* | *7.3 ± 7.20* | *NS* |
| | *1.0* | *4.3 ± 4.14* | *NS* |
| *48* | *0 0* | *12.3 ± 19.38 E4* | -- |
| | *0.01* | *31.9 ± 56 34 E4* | *NS* |
| | *0.1* | *8.2 ± 14.98 E4* | *NS* |
| | *0.3* | *0 2 ± 0 28 E4* | *<0.02* |
| | *0.5* | *5 7 ± 10 03 E2* | *<0.05* |
| | *0.75* | *34 5 ± 35 41 E2* | *<0 05* |
| | *1.0* | *4.3 ± 3.60 E2* | *<0 05* |
| *168* | *0.0* | *6 6 ± 1 61 E5* | -- |
| | *0.01* | *6 5 ± 1.40 E5* | *NS* |
| | *0.1* | *7.0 ± 2 57 E5* | *NS* |
| | *0.3* | *3 9 ± 3 14 E5* | *<0 02* |
| | *0.5* | *1.8 ± 2.61 E5* | *<0 001* |
| | *0.75* | *2.9 ± 3.69 E5* | *<0 001* |
| | *1 0* | *0 5 ± 0 54 E5* | *<0 001* |

**Mean of 4 runs, 3 plates/run*
*#Student's t-test; NS, not significant (p >0.05)*

**Table 2.** *Growth of* Klebsiella Pneumoniae *in the Presence of Various Concentrations of Virchem 932*

| Time (h) | Conc (mg/L) | Mean Cell Count ± S.D. (per ml)* | Level of Significance# |
|---|---|---|---|
| 24 | 0 0 | 96 1 ± 71.03 E3 | -- |
| | 0.01 | 105 5 ± 81 13 E3 | NS |
| | 0 1 | 19.4 ± 6.47 E3 | <0.01 |
| | 0 3 | 6.0 ± 3.39 E3 | <0.002 |
| | 0 5 | 5 7 ± 7 93 E3 | <0 002 |
| | 0 75 | 1 2 ± 91 00 E3 | <0.05 |
| | 1 0 | 1.1 ± 0 36 E3 | <0.002 |
| 48 | 0 0 | 93.8 ± 20.38 E4 | -- |
| | 0.01 | 131 0 ± 58 06 E4 | NS |
| | 0 1 | 97 0 ± 17 28 E4 | NS |
| | 0 3 | 38 2 ± 38 01 E4 | <0.002 |
| | 0 5 | 6.7 ± 4.72 E4 | <0.002 |
| | 0.75 | 1 9 ± 1 69 E4 | <0.001 |
| | 1 0 | 29.2 ± 42 85 E4 | <0.001 |
| 168 | 0 0 | 8 5 ± 1 06 E5 | -- |
| | 0 01 | 12 6 ± 3 21 E5 | <0 005 |
| | 0.1 | 8 1 ± 1 15 E5 | NS |
| | 0 3 | 5.9 ± 1.96 E5 | <0 002 |
| | 0 5 | 6.6 ± 3 61 E5 | NS |
| | 0 75 | 5 4 ± 3 44 E5 | <0 05 |
| | 1 0 | 5 5 ± 2 77 E5 | <0.01 |

*Mean of 3 runs, 3 plates/run
#Student's t-test, NS, not significant (p >0 05)

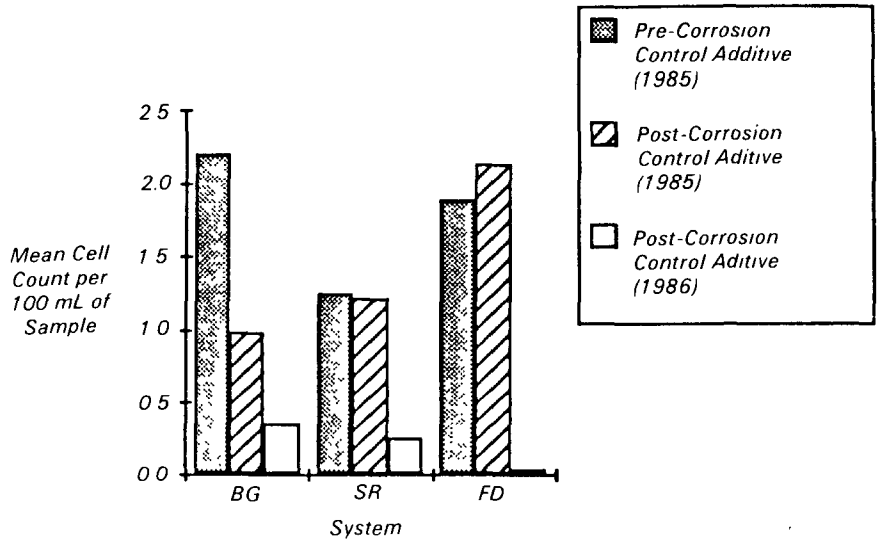


**Figure 2.** *Mean total coliform counts*

distribution systems. In addition, the observed increase in heterotrophic counts, after the addition of the inhibitors, could not be conclusively correlated to the phosphate compounds While strong, consistent correlations do exist between the heterotrophic counts and indicators of the presence of the inhibitors (e.g , total phosphates and zinc), they are tempered by other strong correlations in uncontrolled water parameters such as nitrogen levels

The API Rapid E system identified 23% of the isolated coliforms. *C. freundii, E. cloacae,* and *K. pneumoniae* accounted for 94% of the identified isolates prior to the addition of the phosphate corrosion control compounds and 81% of the isolates after the addition.

The spread plate procedure using R2A medium and 28°C for 168 hr proved far superior to the standard pour plate procedure in recovering heterotrophic bacteria. Counts were 10 to 13 times higher with the spread plate procedure than with the pour plate method.

## Model System Investigations

Six experiments were conducted with the model system and consisted of the following treatments of the water:

A. Passivation dosages of Shan-No-Corr and Virchem 932.

B. Maintenance dosages of Shan-No-Corr and Virchem 932.

C. Same as B.

D. Maintenance dosages of Shan-No-Corr and Virchem 932; pH of water adjusted to 8.0

E. Maintenance dosages of Shan-No-Corr and Virchem 932 plus 50 $\mu$g of carbon as glucose per L of water.

F. Same as E.

The model system was inoculated at the beginning of each experiment (if necessary) with non-coliform heterotrophs and *C. freundii* to achieve an approximate total population of $10^4$ to $10^5$/mL (*C. freundii* population approximately $10^1$ to $10^2$/100 mL). All experiments were run for 2 to 5 weeks. Experiment A, which ran for 5 weeks, was re-inoculated with *C. freundii* (to a level of $10^4$/100 mL) after approximately 2 weeks.

In all experiments we were able to establish the desired non-coliform heterotrophic population, however, we were unable to establish a coliform population that was detectable by the standard membrane filtration procedure. The various treatments did not appear to have any influence on the survival and growth of the established heterotrophic population.

Swabbing various parts of the model system (pipes, tubing, reservoirs) isolated bacteria. However, none of the isolated bacteria were coliforms

## Conclusions and Recommendations

1 There is little evidence that either Shan-No-Corr or Virchem 932

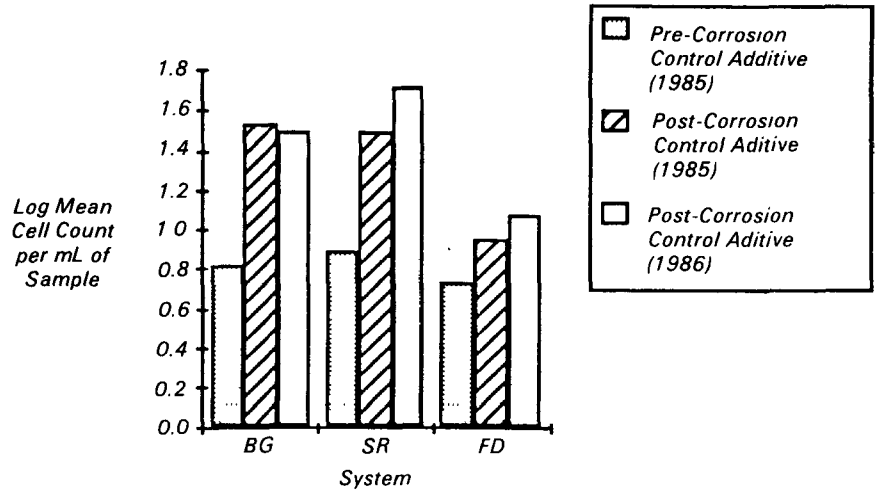


***Figure 3.*** *Log mean total heterotrophic plate counts*

stimulate coliform growth or survival in water distribution systems. However, the pure culture investigations suggest that coliform growth is inhibited by Virchem 932.

2. While field studies with Shan-No-Corr and Virchem 932 resulted in increased heterotrophic counts, this increase could not be conclusively correlated to the presence of the phosphate corrosion control compounds. Strong, consistent correlations do exist but are tempered by other strong correlations in uncontrolled physicochemical parameters such as nitrogen.
3. The field portion of this investigation was probably not of sufficient duration to get a good picture of the relationship between the phosphate corrosion control compounds and bacterial growth. Yearly sampling over a 5-year (minimum) period would probably be more reasonable.
4. It might be of value to analyze sampling data of water companies that add phosphate corrosion control compounds to their water. Many companies have been adding these compounds for decades. A comparison of pre- and post-additive data from such companies might reveal a relationshp between bacterial growth and the presence of these compounds.

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***Donald J. Reasoner*** *is the EPA Project Officer (see below).*
*The complete report entitled "Influence of Phosphate Corrosion Control Compounds on Bacterial Growth," (Order No. PB 87-198 297/AS; Cost: $13.95, subject to change)*
*will be available only from:*
*National Technical Information Service*
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