AN ABSTRACT OF THE THESIS OF

Suzanne Springer for the Master of Science Degree

in Biology presented in August, 1987

Title: Disinfectant Effectiveness, Alcide Versus Hypochlorite

Abstract Approved: John W. Parish

Research was conducted to compare germicidal effectiveness of the new disinfectant Alcide, which contains chlorine dioxide (ClO_2) as the active ingredient, to a standard 50 ppm hypochlorite (bleach) solution. Two gram-negative bacteria, Escherichia coli and Salmonella typhimurium, were used. Bacterial concentrations ranged between 10^6 and 10^9/ml and there were varying organic conditions of zero, 5 percent and 10 percent loads. The study found a 1:50 aqueous dilution of Alcide to be equivalent to a standard 50 ppm dilution of bleach, both with and without organic load. It did not find the Alcide to be superior to the bleach in the presence of organic material though past studies have indicated that the active ingredient of Alcide, ClO_2, would not be as hampered in its effectiveness as the hypochlorite in the bleach. Using the Alcide full strength as recommended by the manufacturer, it would be expected to perform much better than the bleach at 50-200 ppm, but at a far higher cost.

DISINFECTANT EFFECTIVENESS, ALCIDE VERSUS HYPOCHLORITE

A Thesis
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by
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John Parrish
Approved for Major Department

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INTRODUCTION

A survey in 1977 found that in sixteen American hospitals there was a total of 224 products being used for disinfecting animate and inanimate objects. There was an average of 14.5 different formulations used within each hospital (Lennette 1980). Housekeeping disinfectants are expected to destroy vegetative bacteria, tubercle bacilli, and some lipid and nonlipid viruses (Perkins 1969). This type of disinfectant is considered to be an intermediate disinfectant, or chemical germicide, as opposed to a high-level disinfectant which will also kill resistant bacterial spores, or a low-level disinfectant which will kill only vegetative bacteria and lipid viruses (Perkins 1969; Lennette 1980). In choosing a disinfectant method and product, three things that must be considered are the microbial load, the concentration of the chemical germicide, and the environment of the microbe (Anderson and Sobieski 1980). Though the need for safe and effective chemical disinfectants is essential to a hospital in order to minimize infection hazardous to patients, the testing of these disinfectants is a complex and expensive process that few clinical microbiology laboratories can afford (Lennette 1980). It is even recommended that while hospitals should use cleaning and disinfection procedures that have been found to be reliable, routine microbiological samplings on in-house inanimate surfaces are only necessary when an outbreak of hospital-acquired disease occurs. Testing done with disinfectants by various groups does show occasional uncertainty because results gathered from data collected *in situ* sometimes conflict with that obtained in laboratory testing procedures (Block 1977).

Chemical analysis is not an effective method to use for determining which disinfectant would be best in a situation. Many factors can influence how cidal a chemical will be, so analytical data must be supplemented with bacteriological testing (Block 1977). There are rather definite standards for comparing methods of killing bacteria in drinking water and waste water, but such clear-cut guidelines are lacking for methods of testing the usefulness of different disinfectants, including chlorine disinfectants, in a clinical or industrial setting (Block 1977). Some of the factors that make testing difficult to standardize are 1) resistance to germicides that might be related to growing conditions of stock cultures, 2) workers using a variation from the testing ratio of culture size to germicide strength, 3) addition of an organic load in the actual use situation, 4) some chemicals rapidly kill a high percentage of test organisms at first but then kill the survivors at a slower rate, and 5) end points are sometimes difficult to establish (Block 1977).

Some criteria that Block suggested (1977) should be included in a disinfectant evaluation procedure are:

1) standardization of the apparatus and equipment, media composition, and reactions, 2) routine management of all stock and test cultures, 3) procedures for the exact concentration of the germicides to be tested, 4) accurate measurements of volumes of germicides and test cultures, 5) exact temperature control, and 6) accurate timing.

The general categories of chemical disinfectants are: phenols, mercuric compounds, chlorine and iodine compounds, alcohols, detergents, and gases of several kinds (Anderson and Sobieski 1980). Block (1977) lists basic methods accepted by the Association of Official Analytical Chemists (AOAC) for the testing of disinfectants:

1. The AOAC Phenol Coefficient Method - Used to test disinfectants that do not exert bacteriostatic effects that cannot be neutralized by one of four specified media or overcome by suitable transfer procedures.

2. The AOAC Use-Dilution Method - Confirms phenol coefficient results to determine maximum dilution effectiveness for practical disinfection.

3. The AOAC Method for Testing of Germicidal Spray Disinfectants - This method tests sprays used as spot disinfectants.

4. The AOAC Method for Testing Water Disinfectants for Swimming Pools - Tests the acceptability of products used to disinfect swimming pool water.

5. The AOAC Method for Determining Tuberculocidal Activity - Determines the maximum tuberculocidal dilution of disinfectants used on inanimate surfaces.

6. The AOAC Available Chlorine Germicidal Equivalent Concentration Method - Determines available chlorine germicidal equivalent concentrations with products offered for use as germicidal rinses for previously cleaned non-porous surfaces where speed of action is important, and is an index to activities equivalent to available chlorine as hypochlorite. (Available chlorine is a measurement of oxidizing capacity and is expressed in terms of the equivalent amount of elemental chlorine.) The pattern of acceptance by the Public Health Department (PHS Publication Number 934-1962) of germicides other than hypochlorite is based on activities equivalent to accepted concentrations of available chlorine as provided by hypochlorites.

It is the sixth method that was used as the basis for the testing in this study which focused on two disinfectants, the standard hypochlorite (bleach) and an experimental solution which also contains a chlorine compound as the germicidal ingredient.

Chlorine used as a disinfectant can be in the form of liquid chlorine (Cl_2), hypochlorite (OCl^-), chlorine dioxide (ClO_2), inorganic chloramines, or organic chloramines. Chlorine compounds containing 0.1 to 0.5 percent (1000 to 5000 ppm) concentration of active ingredient are considered to have an intermediate disinfectant status (Lennette 1980). Past studies have shown that chlorinated water at 0.15 to 0.25 ppm will kill, in 15 to 30 seconds, most intestinal and respiratory pathogens.

An advantage of using chlorine disinfectants is that they are stable even in a low pH environment. A major disadvantage associated with them is that they are inactivated by organic compounds. Chlorine has a tendency to acquire electrons and therefore it is a very strong oxidizing agent. It will react readily with many inorganic reducing substances and also organic substances (Block 1977). Once the reaction with these substances has occurred, the disinfecting ability of the chlorine is lost (Perkins 1969; Block 1977; Anderson and Sobieski 1980; Lennette 1980). Since bacteria are usually found in the hospital or laboratory setting along with organic loads such as blood, vomitus, feces, etc., this is a serious drawback to disinfection. Many times workers will not preclean the area before applying the disinfectant, so this high organic content detracts from ideal control. However, it has been reported that if the organic matter contains proteins, the chlorine reacts and forms chloramines which are also antibacterial (Block 1977). The reaction with organic substances is dependent on the concentration of the free available chlorine, which may be in the form of elemental chlorine (Cl_2), hypochlorous acid (HOCl) or hypochlorite ion (OCl^-) (Block 1977). Available chlorine in standard hypochlorites is usually equal to the amount of chlorine that was used to prepare the solution (Block 1977).

Studies of how chlorine kills bacteria have resulted in several theories. These include the idea that the chemical combines with the cell's protoplasm (Chang 1944). Another says that the chemical destroys the cell membrane chemically or mechanically, and that the cell contents diffuse out of the cell after the membrane is altered by chlorine (Block 1977). It has also been suggested that germicidal action from chlorine compounds might be a combination of factors (Perkins 1969; Block 1977). It seems to be the most popular consensus, however, that chlorine compounds kill bacteria by the denaturation of proteins when hydrogen bonds within enzymes are destroyed and essential reactions in the cell are inhibited (Green and Stumpf 1946; Perkins 1969; Block 1977).

The manufacture and sale of disinfectants is big business. In 1963, for example, one study compared twenty-seven new chlorinated compounds and four of them were found to be excellent when tested on Escherichia coli (Block 1977). Many of the compounds are not reported in the literature until they have been tested thoroughly and have been introduced on the market.

The chlorine compound present in Alcide (Alcide Corporation, Farmingdale, New York), a newly-marketed disinfectant tested in this study, is chlorine dioxide. Chlorine dioxide used as a disinfectant equals that of chlorine in its effectiveness as a bacterial germicide according to Ridenour and Ingols (1947). However, the chlorine dioxide is an extremely reactive compound and so is not manufactured and shipped in ready-to-use form. In the past, it has been shipped in components consisting of a solution of chlorine and a solution of sodium chlorite which are then mixed along with water at the site where the mixture is to be used (Block 1977). Alcide also comes in two separate bottles, but the components are somewhat different from those generally used in chlorine dioxide disinfectants. It consists of a base and an activator, which are mixed with water at 1:1:10, respectively. The base contains chlorite and the activator contains an organic acid which, after reacting with the chlorite, produces chlorous acid which slowly converts to the cidal chlorine dioxide as well as chloride and chlorate ions (Alcide Corp. 1987).

The purpose of this study was to compare the biological effectiveness of Alcide with a standard hypochlorite solution against representative bacteria in a nonorganic environment and environments with a 5 and 10 percent organic load. Household bleach contains a solution of 5 percent sodium hypochlorite, which is the equivalent USP solution.

Public Health Officials generally recommend that in restaurants, previously cleaned surfaces should be disinfected with a chemical solution which has germicidal ability equivalent to 100-200 ppm available chlorine in areas where it is certain that it will not be diminished in available chlorine to below 50 ppm (Block 1977). The test used to determine equivalency to a particular concentration of available chlorine employs a comparison of bacterial growth after timed exposure to the test disinfectant. The unknown germicide must show equal absence of growth in the same exposure time as the standard chlorine level to be considered equivalent. Standardized culture suspensions used for the test organisms are recommended to be approximately 2.0 x 10^8/ml. These cultures are then diluted to 0.01 times the concentration in the actual testing procedure. Plate counts are made from the dilution tubes at various times in the tests. Results are recorded as the log of the number of survivors, both for the standard disinfectant and the unknown which is being tested.

The bacteria used in this study were _Escherichia coli_ and _Salmonella typhimurium_. _S. typhimurium_ and _E. coli_ are both found in the intestines of humans and other animals and many are pathogenic for humans. These two bacteria are facultative, gram-negative bacilli which ferment glucose. The genus _Salmonella_ is an important cause of food- and water-borne gastroenteritis, in addition to causing typhoid fever. *E. coli* is the primary causative agent of traveler's diarrhea and infections of the urinary tract (Lennette 1980). These bacteria have historically been used as test organisms for disinfectants because of their resistance compared to other bacteria (Tonney et al. 1928; Perkins 1969; Block 1977; Lennette 1980; Blaser et al. 1986). It has been theorized that the relative resistance of these gram-negative organisms is due to their outer membrane acting as some sort of barrier (Russell et al. 1986).

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains were obtained from cultures isolated at the Kansas Department of Health and Environment laboratories. Fresh working suspensions were prepared by inoculating 5 ml tubes of Trypticase soy broth with bacteria removed from agar slants. Tube contents were vortex mixed and incubated at 37 degrees Celsius for 4 to 5 hours. These cultures provided concentrations of organisms between 10^7 and 10^{11} cells/ml. Counts of colony-forming units (CFUs) were made by preparing serial 10 fold dilutions and plating 0.1 ml quantities of the 10^{-6}, 10^{-7}, and 10^{-8} dilutions in duplicate or triplicate on Trypticase soy agar plates. Colonies were counted after 48 hours incubation at 37 degrees Celsius. Disinfectant testing used bacterial concentrations of approximately 10^6, 10^7, 10^8, and 10^9 CFUs/ml for each set of trials. These suspensions were prepared by transferring 0.1 ml aliquots from the appropriate concentration of bacteria into tubes containing disinfectant at the beginning of the timed trial.

Procedure for Evaluating Disinfectant Effectiveness

The incubated, 4-5 hour old, working suspensions, which ranged from 10^{11} to 10^9 organisms/ml, and the three, serial, 10 fold dilutions of this suspension, were all used in a set of experimental trials. At zero time, 0.1 ml of bacterial suspension was inoculated into the 10 ml of disinfectant and immediately vortexed. As each minute passed during a

10-minute period, 0.1 ml aliquots were removed with a pipette and inoculated onto a D/E neutralizing agar (see Neutralization below) plate. Two bacterial concentrations were tested at a time, with separate pipettes used for the removal of samples from each tube. The experimental and control plates were incubated in an inverted position at 37 degrees Celsius for 48 hours before counting colonies. If an organic load was to be used for the trial, the disinfectant had either 0.5 ml or 1.0 ml less water in its final volume which was made up with calf serum to produce an organic load at either 5 percent or 10 percent. Ten ml of disinfectant or disinfectant plus serum was used for each respective concentration of bacterial inoculum tested in the trial. All sets of trials were done in duplicate (data later shown in Figures 2, 4 through 10, 13, 15, and 18). Several sets, however, were done in triplicate (Figures 14, 16, and 17). Four Alcide trials (Figures 1, 3, 11 and 12) were done only once, with no duplication because the decision was made to repeat the experiment with a higher concentration of Alcide.

Chlorine Determinations

Fresh stock solutions of bleach disinfectant for each trial were prepared from the manufacturer's information on chlorine content of sodium hypochlorite. Deionized water was used for the dilutions.

Alcide disinfectant was mixed according to the manufacturer's instructions and also diluted with deionized water to the desired concentration just prior to each trial. The Alcide concentration containing 50 ppm of chlorine compound was obtained by diluting the product 1:500 (low concentration Alcide). This dilution was not found to be satisfactory for comparison to the bleach because it was so much less effective. Tests were done using The Association of Official Analytical Chemists Available Chlorine Germicidal Equivalent Concentration Method (Block 1977) to find the dilution of Alcide which would be the germicidal equivalent without organic load. Experimentation with several concentrations found that a 1:50 (high concentration Alcide) dilution of Alcide would have the same cidal effect on _E. coli_ as the 50 ppm bleach, when no organic load was present. This method used 30- and 60-second exposure periods, plating the bacteria as described earlier, and then analyzing results as positive or negative, looking for the concentration of Alcide which gave data equivalent to the bleach.

Neutralization

D/E agar media (Difco Laboratories, Detroit, Michigan), which contains 0.6 percent sodium thiosulfate as the effective neutralizing ingredient, was used to neutralize chlorine at the end of each exposure period. Neutralizers have been recognized since 1952 as an important component in experiments with disinfectants which are testing for cidal action and rate of kill (Engley and Dey 1970). They are used to stop the disinfecting action of the chlorine compound by reacting with it. This enables the experimenter to control the disinfectant exposure period by transferring bacteria to the neutralizing media at a predetermined time. D/E media will allow growth of both organisms used in this study equal to the Trypticase soy agar control media (Engley and Dey 1970). Experiments done as part of this study agreed with Engley and Dey that the D/E and control media supported the bacterial growth to equal numbers (data not shown).

RESULTS

Developmental Experiments

Bleach

Previous published studies used a range of bleach dilutions for comparison purposes to evaluate microbial control. For this study three dilutions of bleach, 5000 ppm, 500 ppm, and 50 ppm, were tested to determine which concentration would be most appropriate. An *E. coli* suspension of 10^7 CFUs/ml was killed in less than 10 seconds using 5000 ppm and 500 ppm. The 50 ppm level gave positive results for growth; that is, surviving organisms were found after 10, 20, and 30 seconds of exposure. Inasmuch as previous studies with organic loads had shown that the organic material would extend the killing time, the 50 ppm was chosen as the suitable concentration. Using too high a concentration for the standard would have killed everything immediately even with an organic load. The 50 ppm level would give a sampling window, a time frame that would allow evaluation of the disinfectant with and without an organic load.

Alcide

As mentioned in Materials and Methods, two concentrations of Alcide were used in this experiment. The lower concentration Alcide data is shown in Figures 1, 3, 4, 11, 12, and 13. It is the lower concentration that, though it was equal to the bleach in ppm, was found not to be equivalent to bleach in its germicidal effectiveness. This fact can be illustrated by comparing the action of bleach (Figure 17) with the action of low concentration Alcide (Figure 13) on a 10^7/ml population of E. coli when no organic load was present. The bleach killed all the test organisms in 2 minutes and the low concentration Alcide reduced them by 4 logs within 8 minutes, but never reduced the population to zero. The data for low concentration Alcide will not be discussed further but are presented as part of the complete set of results for this experiment.

After the low concentration Alcide was found not to be effective enough to be compared to bleach, other Alcide dilutions were tested on a 10^7 CFUs/ml suspension of E. coli. A 1:50 dilution was still found to have viable bacteria after 30-second exposure but none after 60-second exposure. These were the same results obtained with bleach (data not shown), and thus it was concluded that this was the germicidally equivalent dilution. This concentration (high concentration Alcide) is the one used for the remainder of the studies which are discussed in the following section of this paper.

Experimental Results

S. typhimurium

Figures 1 through 8 and Figure 18 show data collected using S. typhimurium as the test organism. Figure 2 illustrates that the high concentration Alcide with no organic load killed all populations tested except those containing 10^9 CFUs/ml within 3 minutes and the 10^9 CFUs/ml population was completely killed in 10 minutes.

Figures 7 and 8 show that adding 5 or 10 percent organic load to the Alcide provided about equal protection for the bacteria, causing a 3 or 4 log reduction within 3 or 4 minutes. Almost all the organisms that were still viable at 3 or 4 minutes survived the rest of the 10-minute exposure.

Figure 18 illustrates that bleach with no organic load killed all the _S. typhimurium_ within one minute.

Figures 5 and 6 show that adding 5 or 10 percent organic load to the bleach protected the bacteria, with the 10 percent level giving somewhat better protection. At the end of the 10-minute time period, none of the original concentrations had been completely killed.

E. coli

Figures 9 through 17 present the data collected using _E. coli_ as the test organism. Figure 14 shows that all populations except the 10^7 CFUs/ml were killed completely within one minute using the high concentration Alcide with no organic load.

Figures 15 and 16 illustrate that adding 5 or 10 percent organic load to the Alcide protected the bacteria about equally, and no population was competely killed in the 10-minute exposure period.

Data for bleach with no organic load are shown in Figure 17. All the E. coli were killed within 2 minutes in that case.

Figures 9 and 10 illustrate that with a 5 percent or 10 percent organic load added to the bleach, the E. coli populations were reduced gradually, but only the two lowest CFUs/ml populations with the bleach plus 5 percent organic load were completely killed.

Comparisons

Comparing the results using high concentration Alcide and bleach on S. typhimurium and E. coli, there were two population sizes, 10^6 CFUs/ml and 10^7 CFUs/ml, that were present for every set of trials conducted. Table 1 and the corresponding Figures 19 and 20 present the results for the 10^6 CFUs/ml populations. Table 2 and corresponding Figures 21 and 22 present results for the 10^7 CFUs/ml populations. Information on these figures has been plotted in earlier figures, but is repeated here in such a way that comparisons can be made more easily.

Table 1 - Comparison of effects of high concentration Alcide (1:50 aqueous dilution) and bleach (50 ppm) on the 10^6/ml concentrations of _E. coli_ and _S. typhimurium_ under all conditions of organic load

Organic Load	Disinfectant	Bacteria	Log Reduct.	Time Lapse/ Max. Reduct.
*None	Bleach	_E. coli_	6	1
		S. typhimurium	6	1
	High Alcide	_E. coli_	6	1
		S. typhimurium	6	1
**5%	Bleach	_E. coli_	6	8
		S. typhimurium	5.5	10
	High Alcide	_E. coli_	5.5	10
		S. typhimurium	4	10
***10%	Bleach	_E. coli_	5.5	9
		S. typhimurium	3.5	6
	High Alcide	_E. coli_	5	10
		S. typhimurium	3	1

* Data not shown

** Figure 20

*** Figure 19

As listed in Table 1, the bleach and high concentration Alcide with no organic load killed all the bacteria completely in one minute.

With a 5 percent organic load (Figure 20), the *E. coli* had a greater reduction than *S. typhimurium* in both the bleach and high concentration Alcide, with a 5 log versus a 5.5 log reduction and a 5.5 log versus a 4 log reduction, respectively. The 5 percent organic load provided protection for all the populations, extending killing time as well as retaining some viability in most cases.

Adding 10 percent organic load also protected the populations; however, *S. typhimurium* was more resistant than the *E. coli*. The bleach plus 10 percent organic load reduced the *E. coli* by a log of 5.5 and *S. typhimurium* by 3.5; the high concentration Alcide plus 10 percent organic load reduced the *E. coli* by a log of 5 and the *S. typhimurium* by a log of 3. In all cases, the disinfectant plus 10 percent organic load had slightly better protection than the 5 percent.

Differences can be seen in the exposure times that had passed when maximum reductions had been reached. Using disinfectant plus 10 percent organic load on *S. typhimurium*, no more organisms were killed after 6 minutes with the bleach, and maximum reduction had been reached after only one minute with the high concentration Alcide. The lower decreases seen in these trials were reached relatively early in the 10-minute exposure time; in other words, the disinfectant plus 10 percent organic load did not continue to gradually decline the populations further, as they did on the *E. coli* over a 9- or 10- minute period.

Table 2 - Comparison of effects of high concentration Alcide (1:50 aqueous dilution) and bleach (50 ppm) on the 10^7/ml concentrations of _E. coli_ and _S. typhimurium_ under all conditions of organic load

Organic Load	Disinfectant	Bacteria	Log Reduct.	Time Lapse/ Max. Reduct.
*None	Bleach	_E. coli_	6	3
		S. typhimurium	6	1
	High Alcide	_E. coli_	6	2
		S. typhimurium	6	1
**5%	Bleach	_E. coli_	5	8
		S. typhimurium	5.5	7
	High Alcide	_E. coli_	4	5
		S. typhimurium	4	5
***10%	Bleach	_E. coli_	5.5	7
		S. typhimurium	3	3
	High Alcide	_E. coli_	4.7	10
		S. typhimurium	4	8

* No figure given

** Figure 21

*** Figure 22

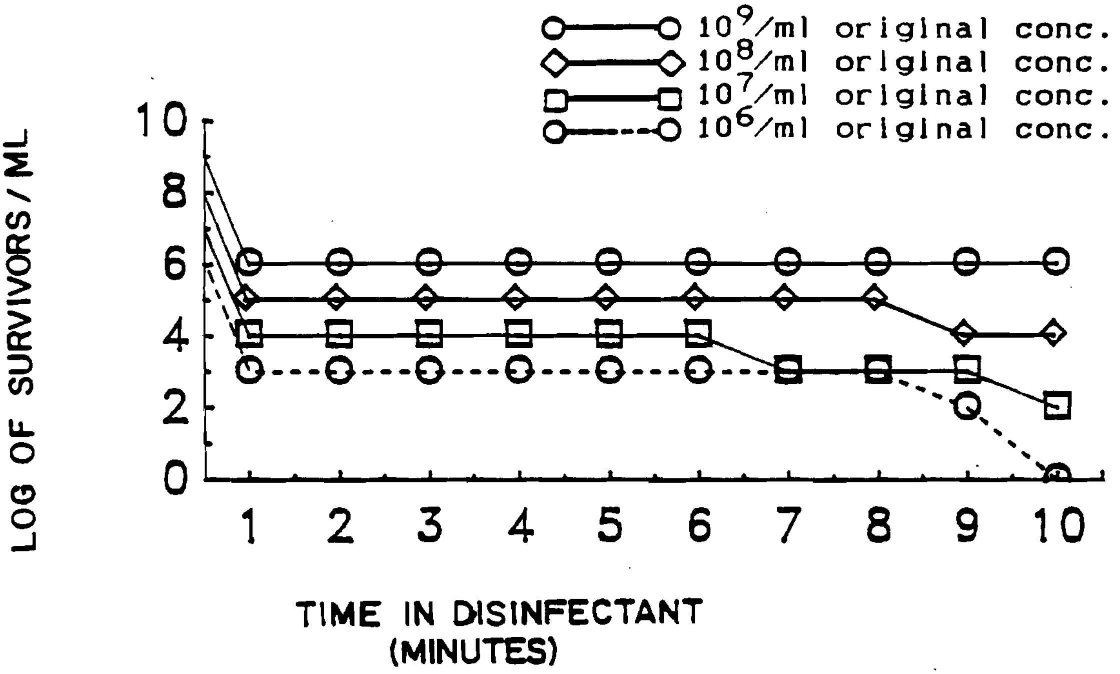
Table 2 shows that bleach and high concentration Alcide both killed all the E. coli and S. typhimurium when no organic load was present. The 10^7 E. coli population took longer to kill than the 10^6 E. coli (Table 1) under these conditions, requiring 3 minutes with the bleach and 2 minutes with the high concentration Alcide.

Adding a 5 percent organic load (Figure 21) to the bleach or Alcide protected the populations somewhat, allowing log reductions of 5 for E. coli and 5.5 for S. typhimurium in bleach; a 4 log reduction for both E. coli and S. typhimurium in Alcide. Killing times were also extended, with the maximum kill requiring 7 or 8 minutes for the bleach and 4 minutes for the Alcide.

The 10 percent organic load (Figure 22) added to bleach and Alcide protected better than the 5 percent in only one case (bleach and S. typhimurium). The bleach performed better than the Alcide with 5 percent loads on E. coli and S. typhimurium, and the 10 percent load on E. coli; but high concentration Alcide performed better than bleach with 10 percent load on the S. typhimurium. Killing times for 10 percent were similar to the 5 percent with the exception of the 3 minutes for the S. typhimurium's relatively small 3 log reduction.

Figure 1. Surviving *S. typhimurium* vs. time in low concentration Alcide (1:500 aqueous dilution) with no organic load.

Figure 2. Surviving *S. typhimurium* vs. time in high concentration Alcide (1:50 aqueous dilution) with no organic load.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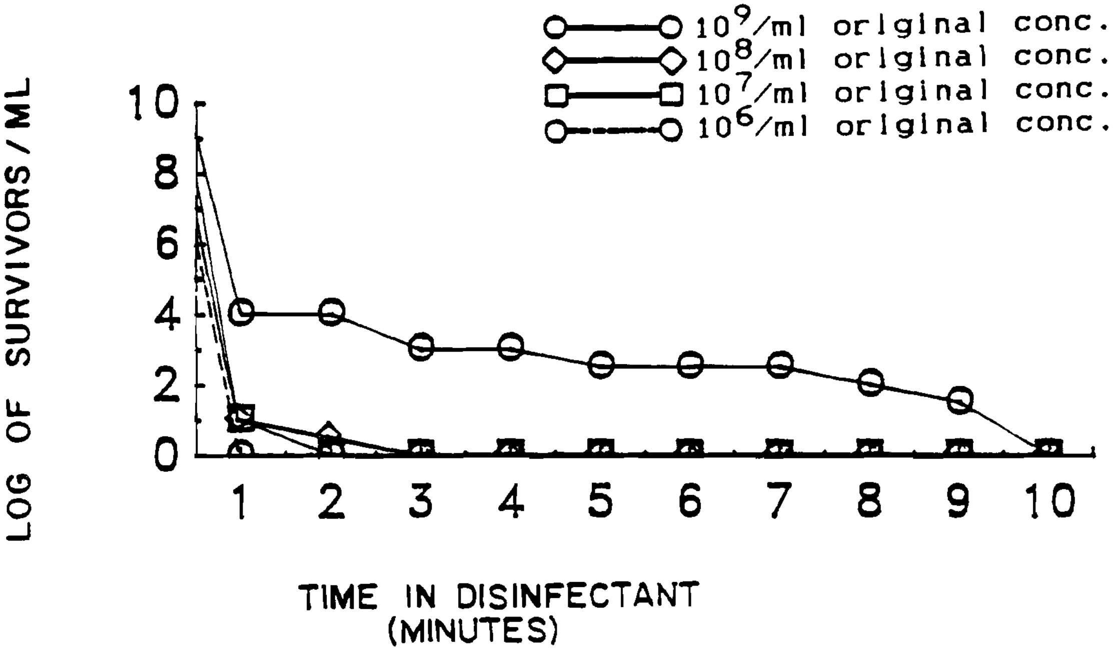
(MINUTES)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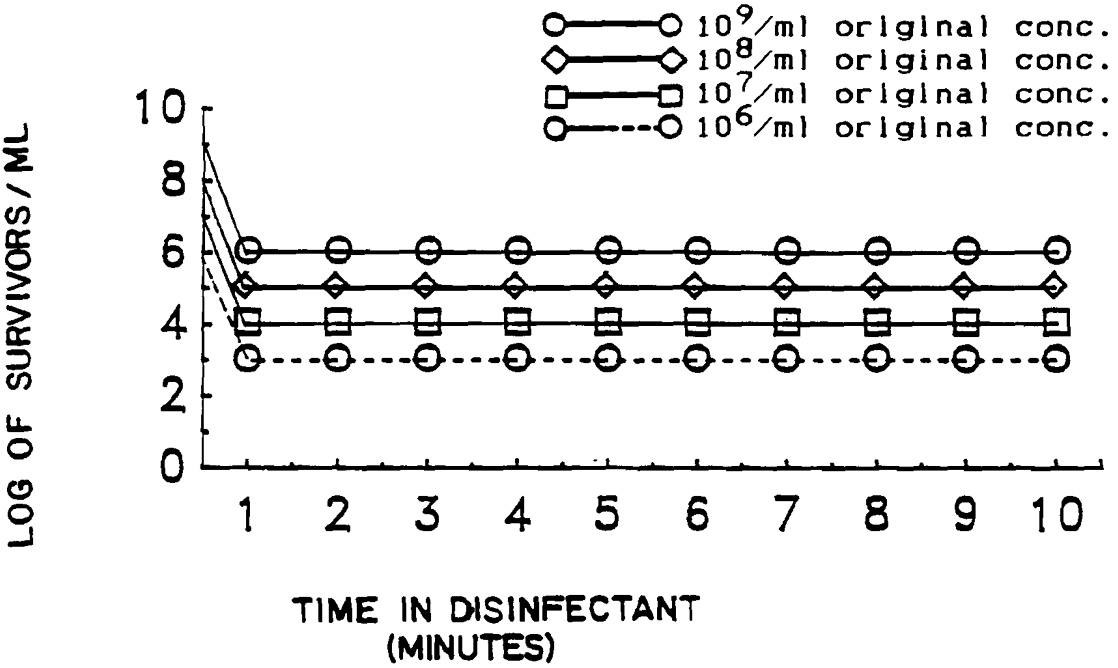
TIME IN DISINFECTANT
(MINUTES)

Figure 3. Surviving *S. typhimurium* vs. time in low concentration Alcide (1:500 aqueous dilution) with percent organic load.

Figure 4. Surviving *S. typhimurium* vs. time in low concentration Alcide (1:500 aqueous dilution) with percent organic load.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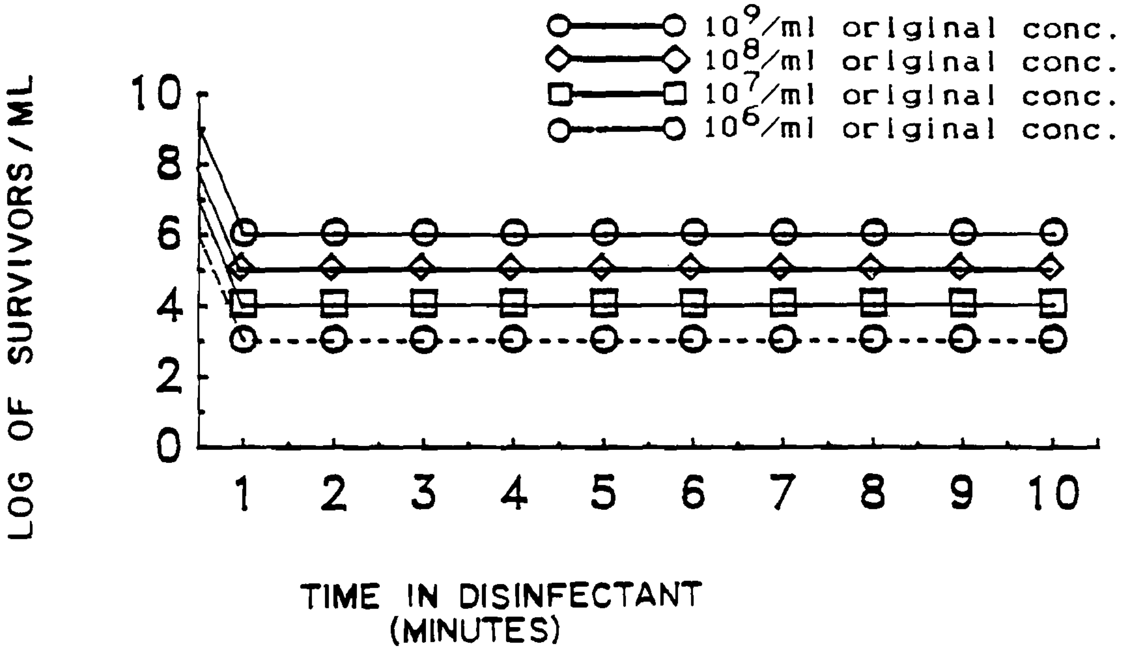
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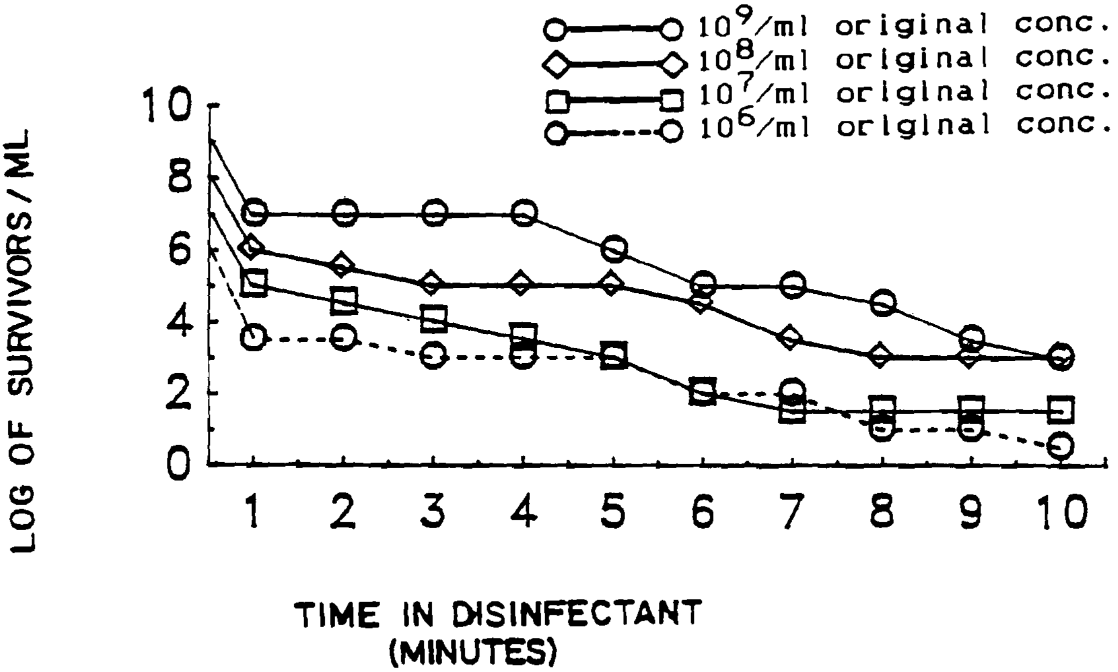
TIME IN DISINFECTANT
(MINUTES)

Figure 5. Surviving *S. typhimurium* vs. time in bleach (50 ppm) with 5 percent organic load.

Figure 6. Surviving *S. typhimurium* vs. time in bleach (50 ppm) with 10 percent organic load.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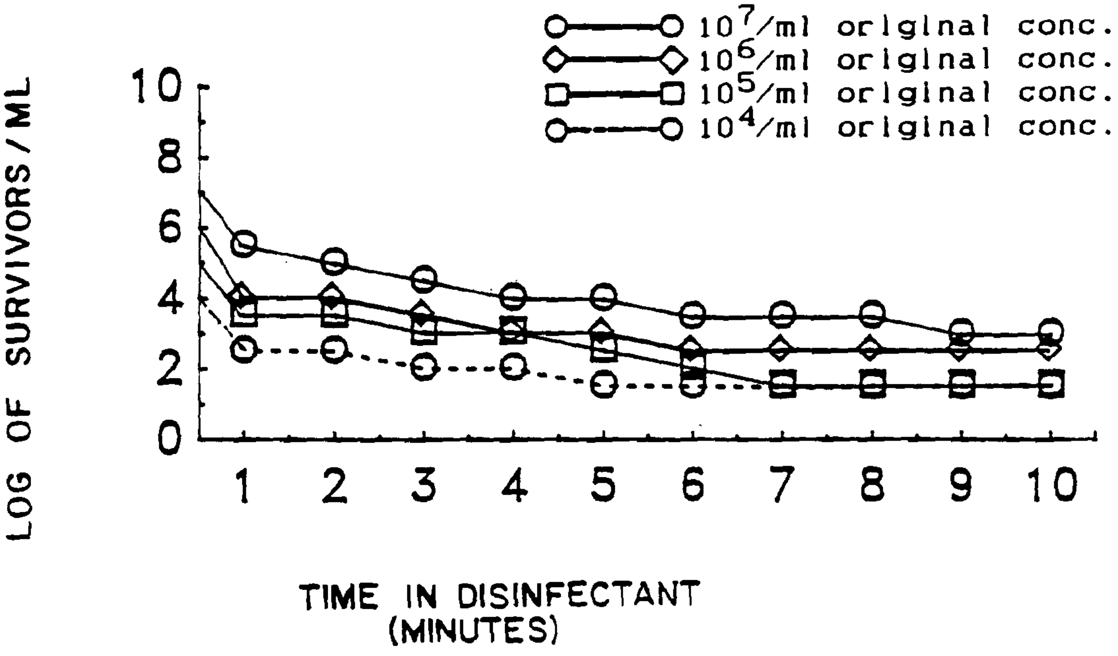
TIME IN DISINFECTANT
(MINUTES)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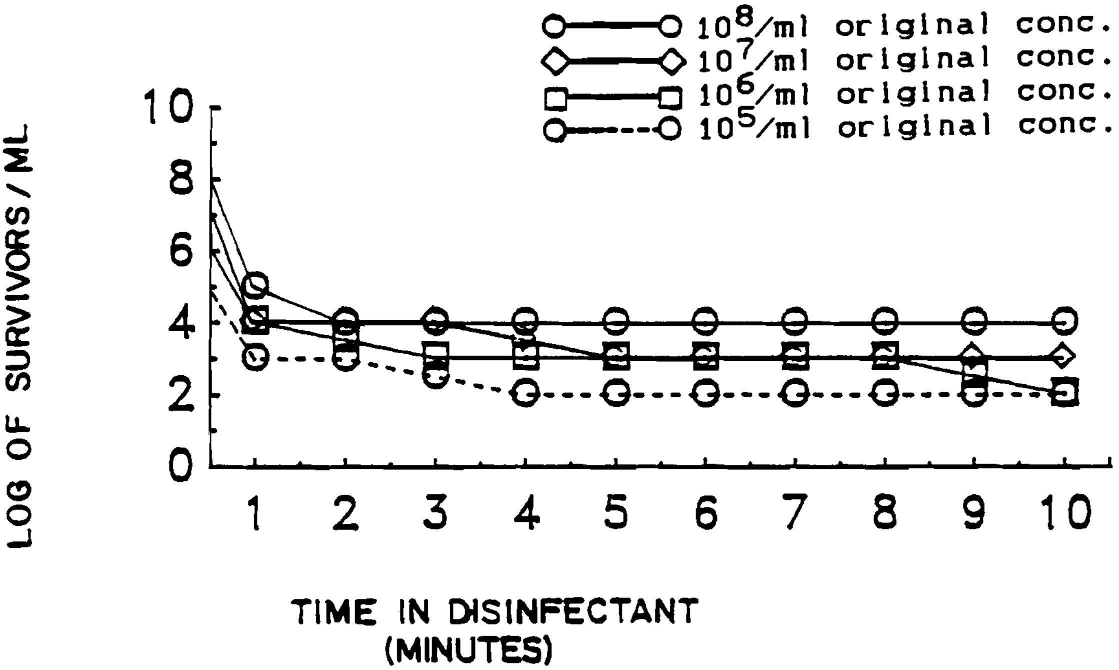
TIME IN DISINFECTANT
(MINUTES)

Figure 7. Surviving S. *typhimurium* vs. time in high concentration Alcide (1:50 aqueous dilution) with 5 percent organic load.

Figure 8. Surviving S. *typhimurium* vs. time in high concentration Alcide (1:50 aqueous dilution) with 10 percent organic load.



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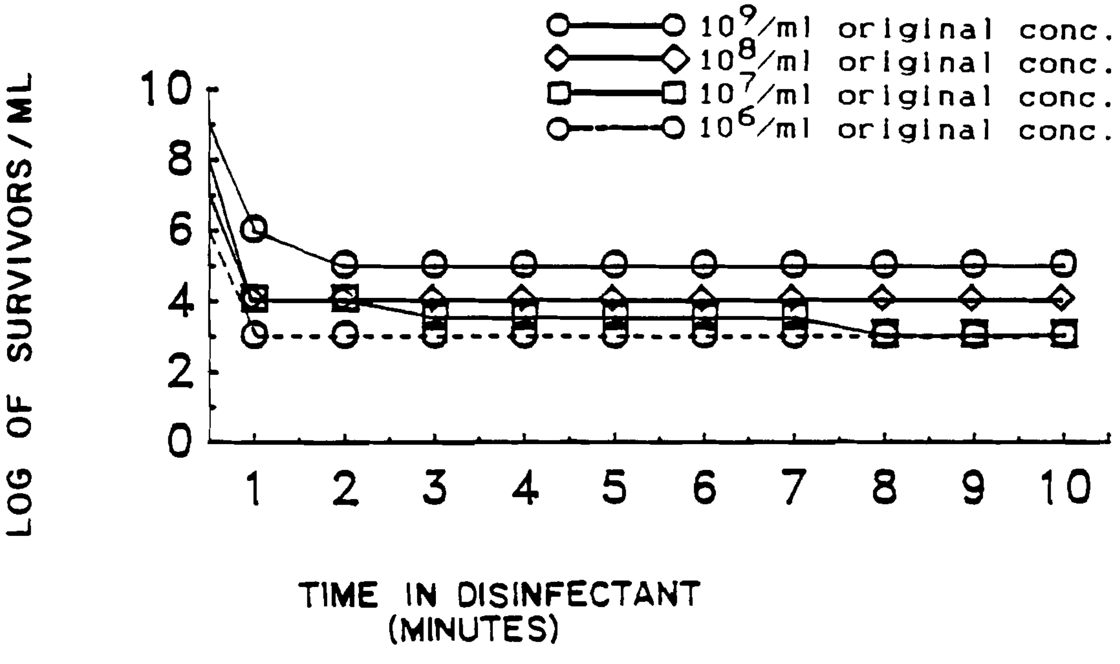
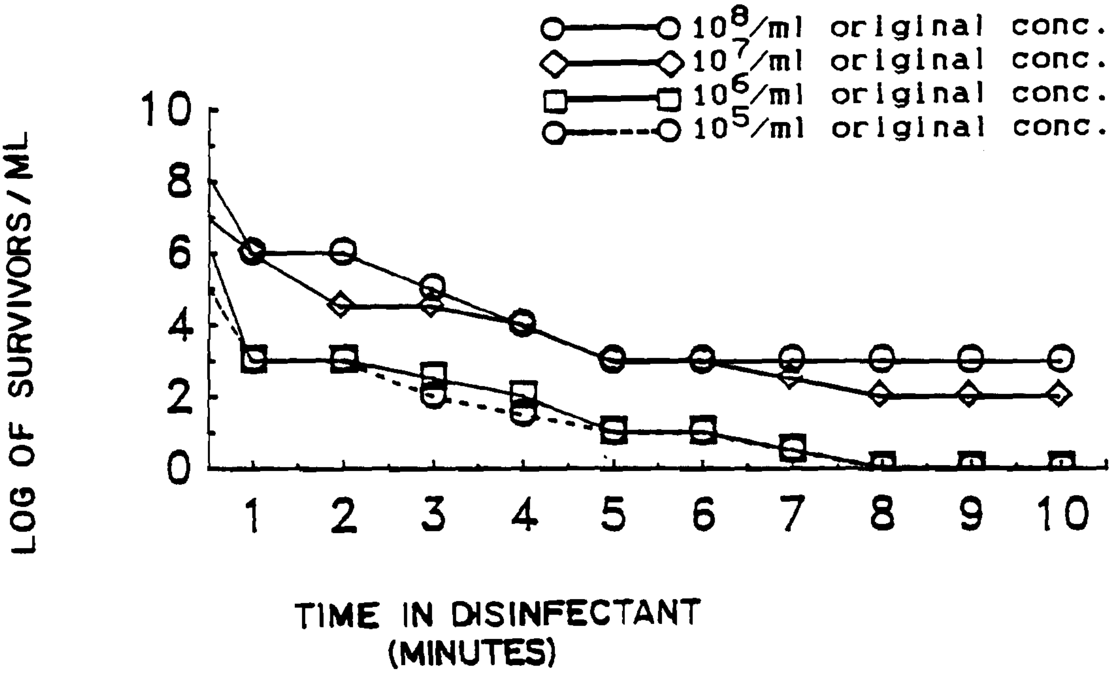
10
TIME IN DISINFECTANT (MINUTES)



10^9/ml original conc.
10^8/ml original conc.
10^7/ml original conc.
10^6/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)

Figure 9. Surviving *E. coli* vs. time in bleach (50 ppm) with 5 percent organic load.

Figure 10. Surviving *E. coli* vs. time in bleach (50 ppm) with 10 percent organic load.



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10^7/ml original conc.
10^6/ml original conc.
10^5/ml original conc.
LOG OF SURVIVORS / ML
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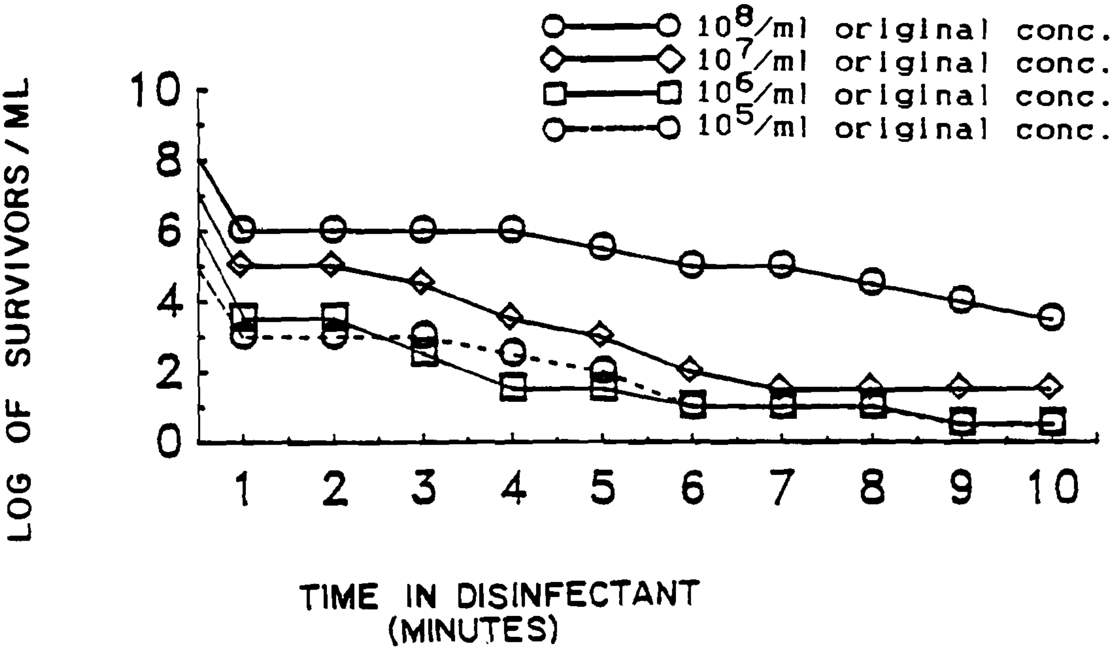
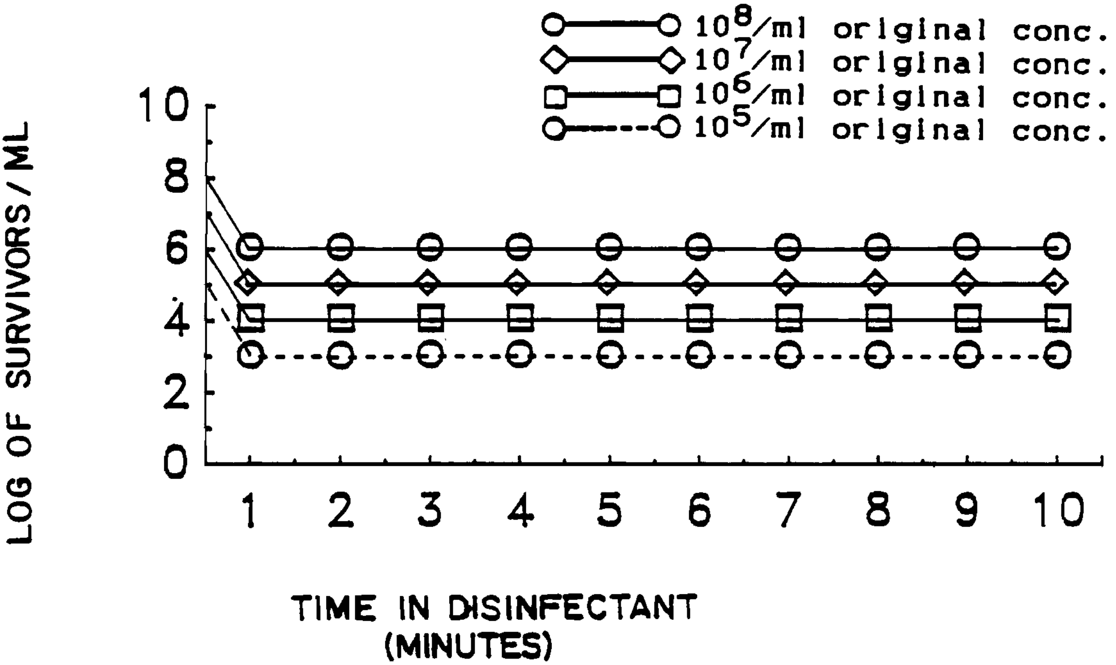
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)



10^8/ml original conc.
10^7/ml original conc.
10^6/ml original conc.
10^5/ml original conc.
LOG OF SURVIVORS / ML
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)

Figure 11. Surviving *E. coli* vs. time in low concentration Alcide (1:500 aqueous dilution) with 5 percent organic load.

Figure 12. Surviving *E. coli* vs. time in low concentration Alcide (1:500 aqueous dilution) with 10 percent organic load.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10^6/ml original conc.
10^5/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)

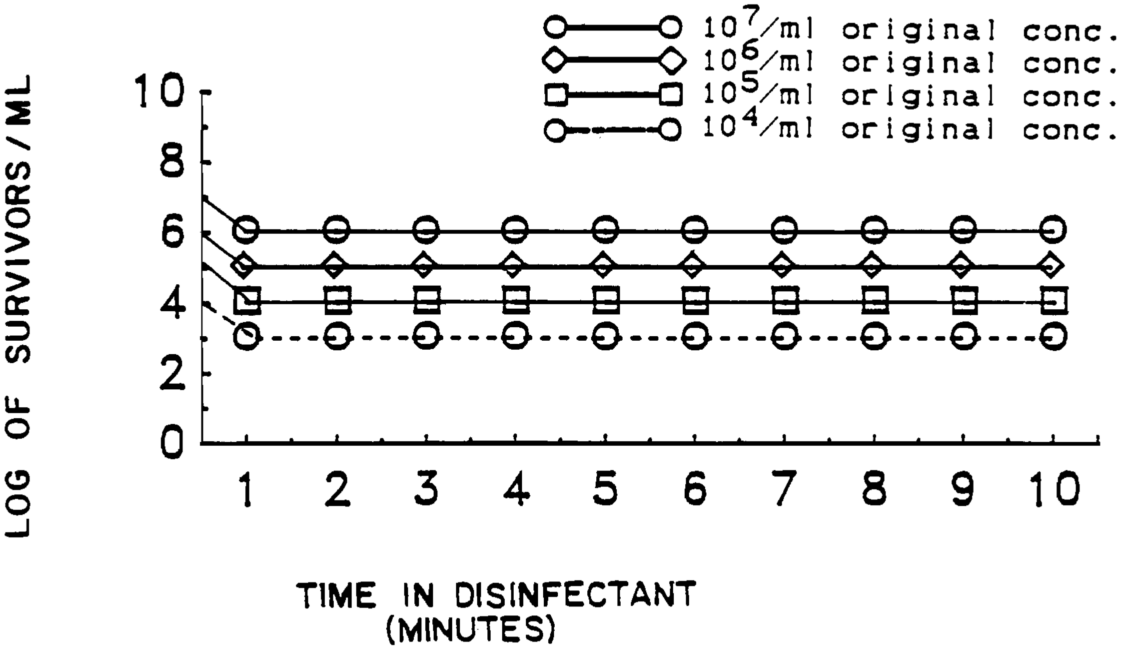
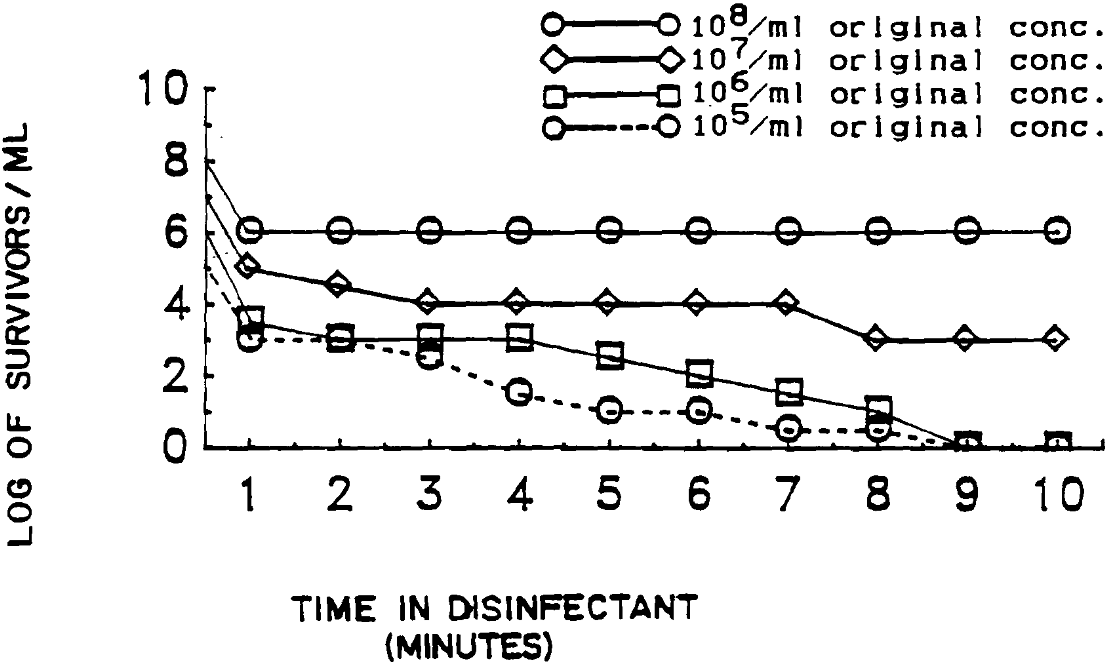

10^7/ml original conc.
10^6/ml original conc.
10^5/ml original conc.
10^4/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)

Figure 13. Surviving _E. coli_ vs. time in low concentration Alcide (1:500 aqueous dilution) with no organic load.

Figure 14. Surviving _E. coli_ vs. time in high concentration Alcide (1:50 aqueous dilution) with no organic load.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10⁷/ml original conc.
10⁶/ml original conc.
10⁵/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)

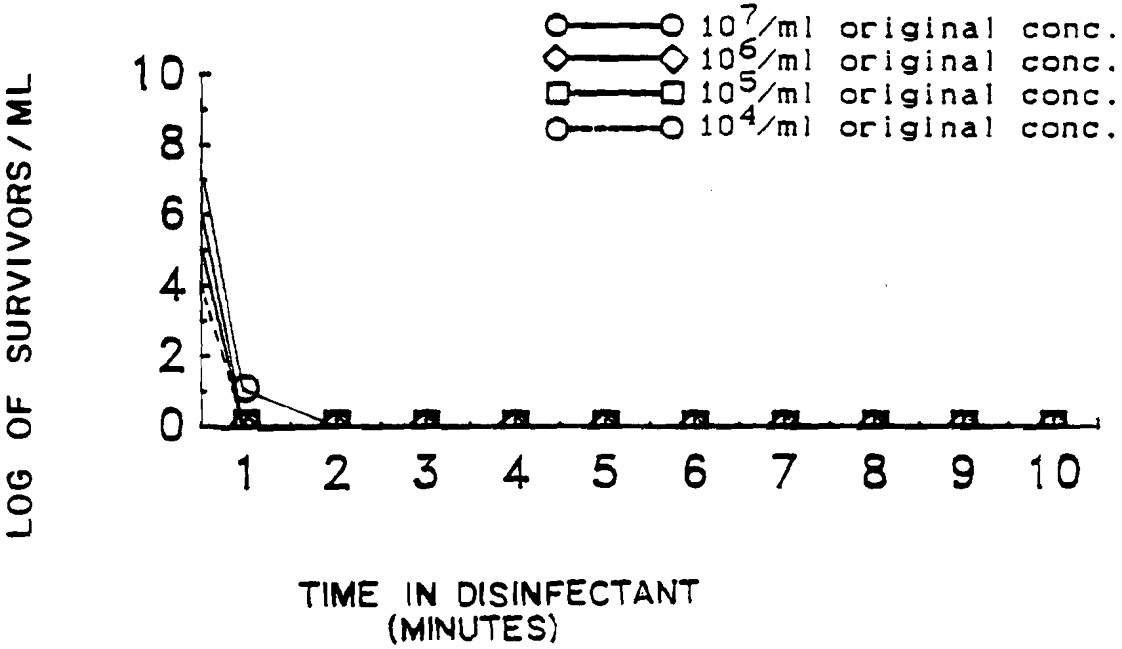
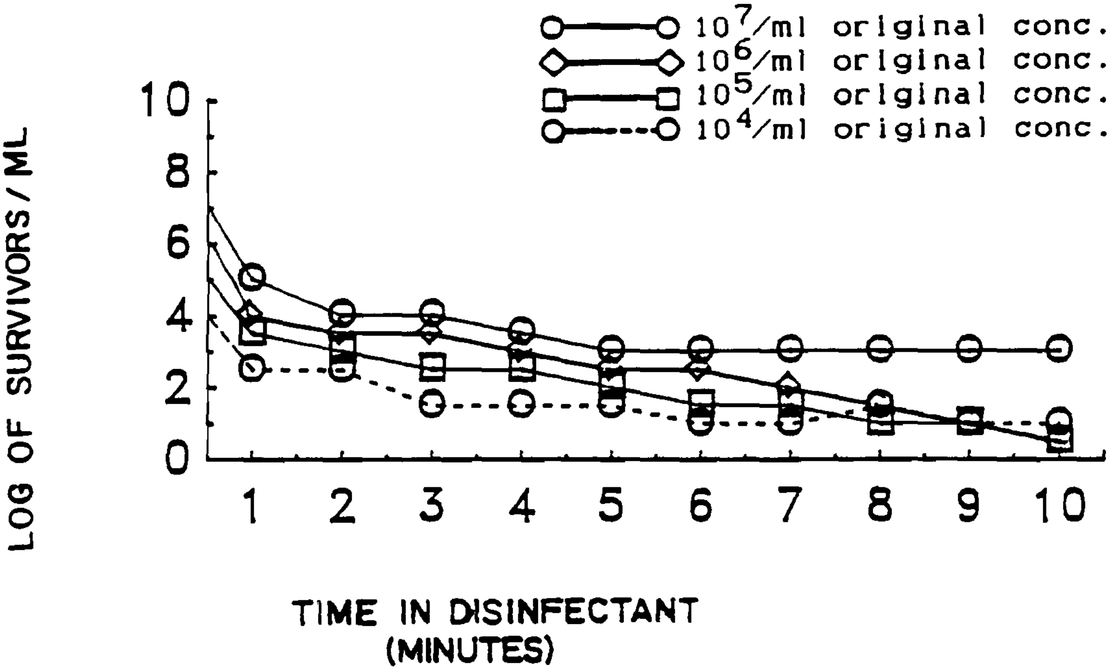
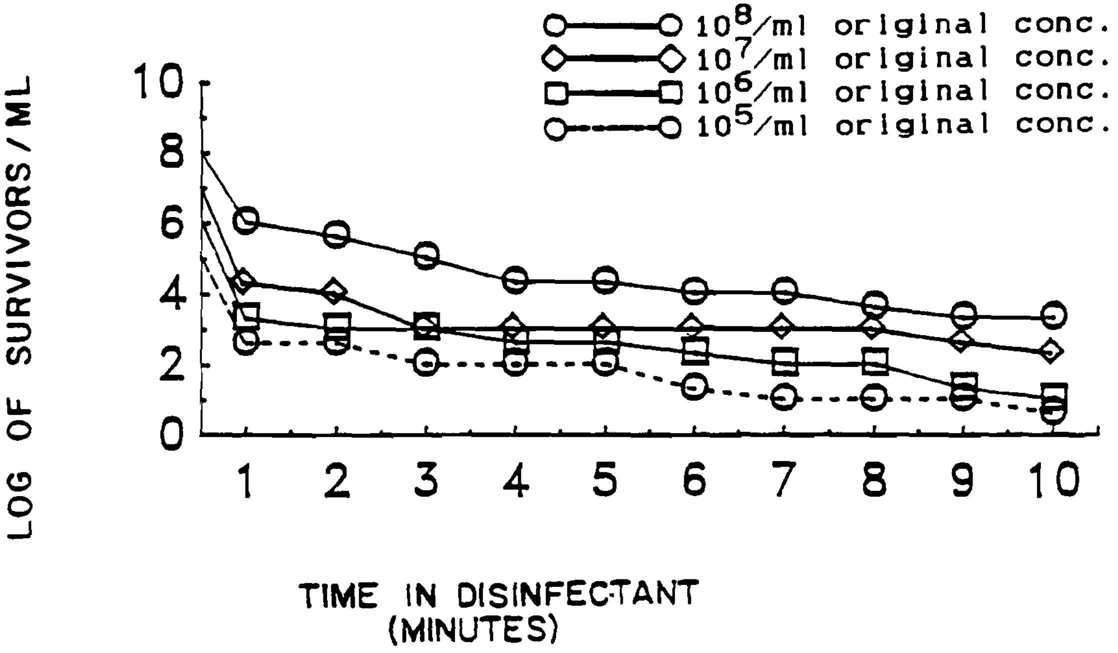

10⁷/ml original conc.
10⁶/ml original conc.
10⁵/ml original conc.
10⁴/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)

Figure 15. Surviving *E. coli* vs. time in high concentration Alcide (1:50 aqueous dilution) with 5 percent organic load.

Figure 16. Surviving *E. coli* vs. time in high concentration Alcide (1:50 aqueous dilution) with 10 percent organic load.



10⁷/ml original conc.
10⁶/ml original conc.
10⁵/ml original conc.
10⁴/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)



10⁸/ml original conc.
10⁷/ml original conc.
10⁶/ml original conc.
10⁵/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
10
1
2
3
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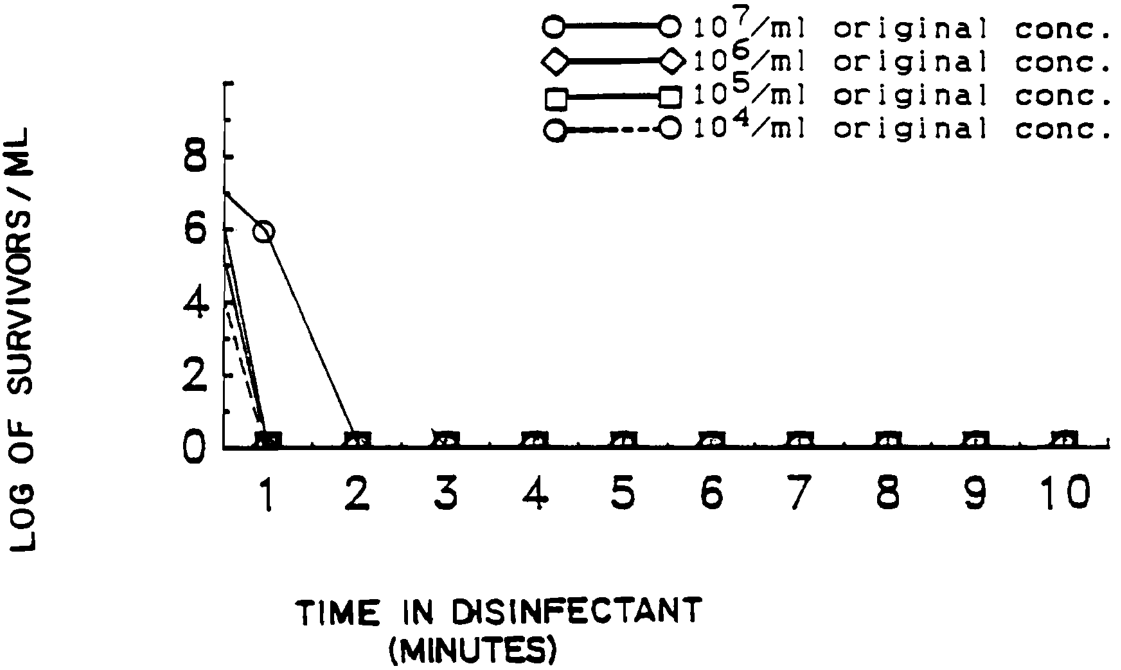
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)

Figure 17. Surviving *E. coli* vs. time in bleach (50 ppm) with no organic load.

Figure 18. Surviving *S. typhimurium* vs. time in bleach (50 ppm) with no organic load.



10^7/ml original conc.
10^6/ml original conc.
10^5/ml original conc.
10^4/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
1
2
3
4
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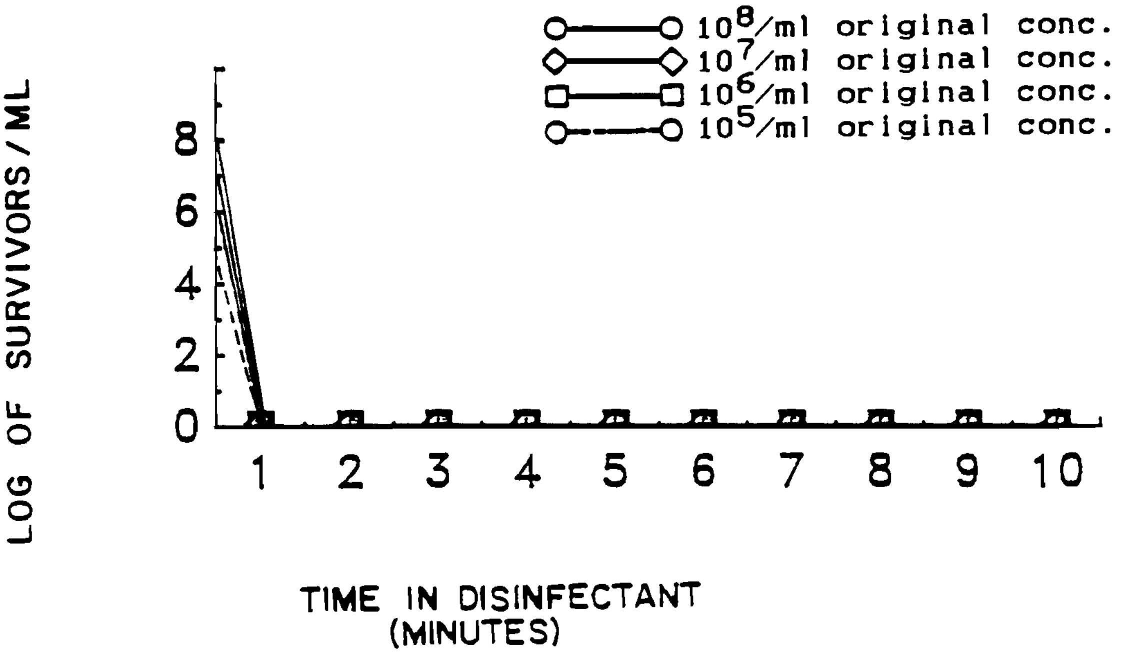
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)



10^8/ml original conc.
10^7/ml original conc.
10^6/ml original conc.
10^5/ml original conc.
LOG OF SURVIVORS / ML
0
2
4
6
8
1
2
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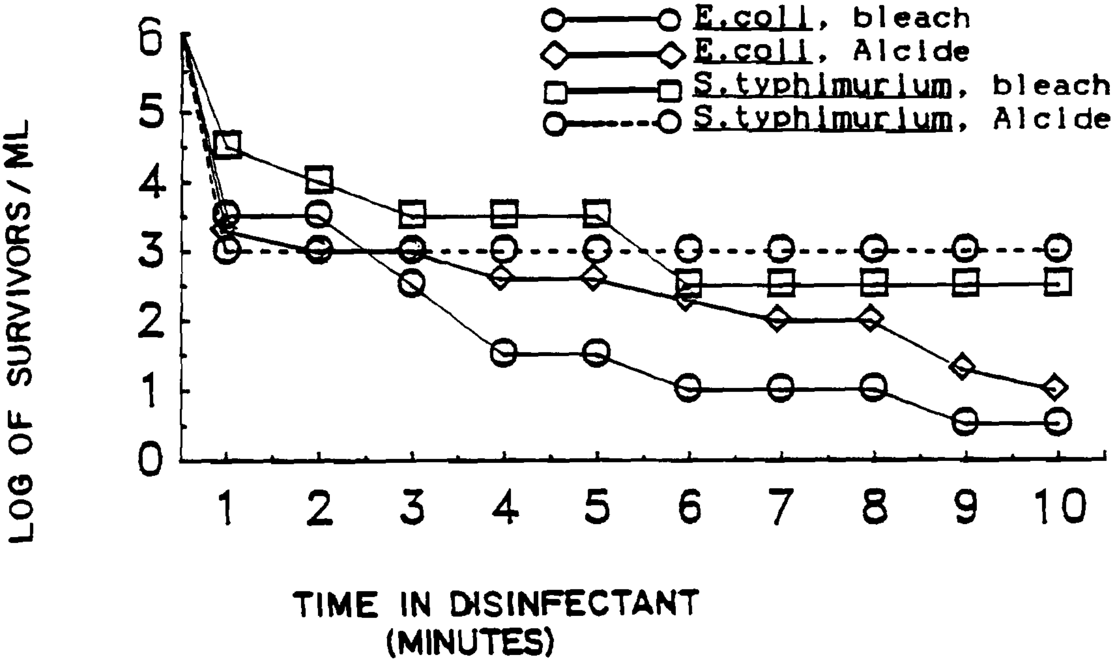
5
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)

Figure 19. Comparison of surviving *S. typhimurium* and *E. coli* from original 10^6/ml concentrations after being exposed to high concentration Alcide (1:50 aqueous dilution) or bleach (50 ppm) with 10 percent organic loads.

Figure 20. Comparison of surviving *S. typhimurium* and *E. coli* from original 10^6/ml concentrations after being exposed to high concentration Alcide (1:50 aqueous dilution) or bleach (50 ppm) with 5 percent organic loads.


E.coli, bleach
E.coli, Alcide
S.typhimurium, bleach
S.typhimurium, Alcide
LOG OF SURVIVORS / ML
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)

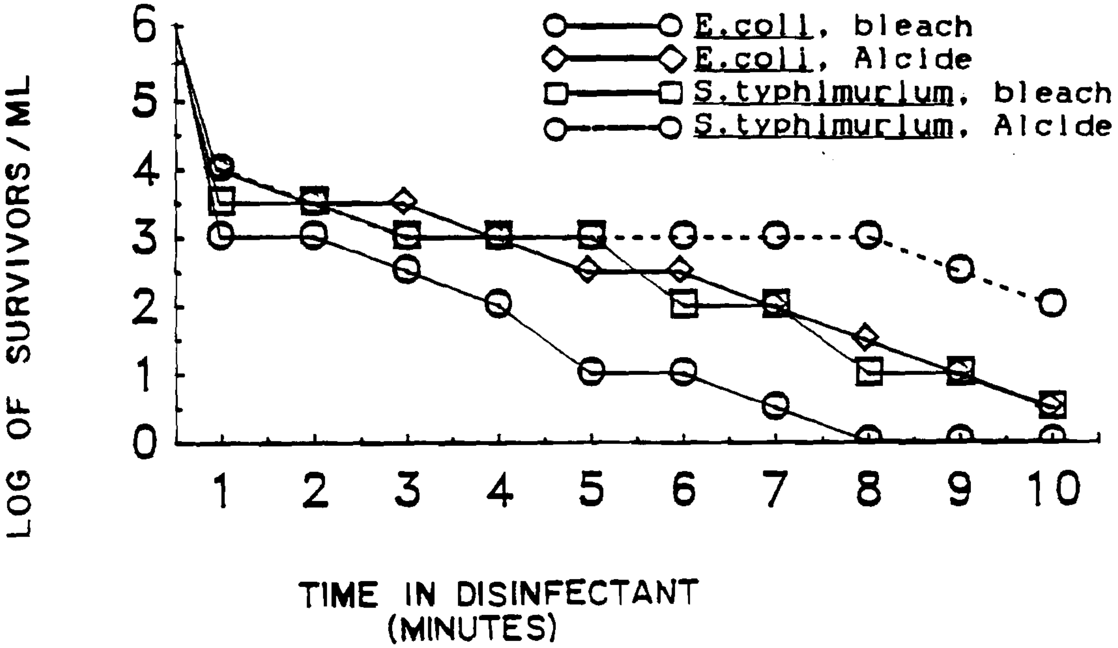
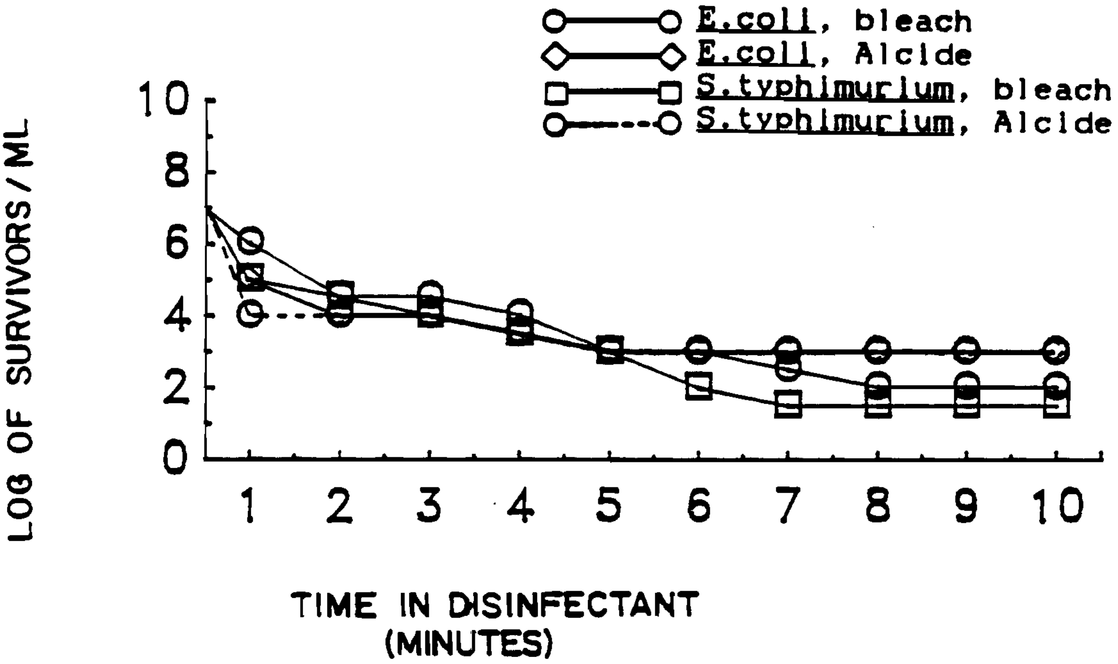
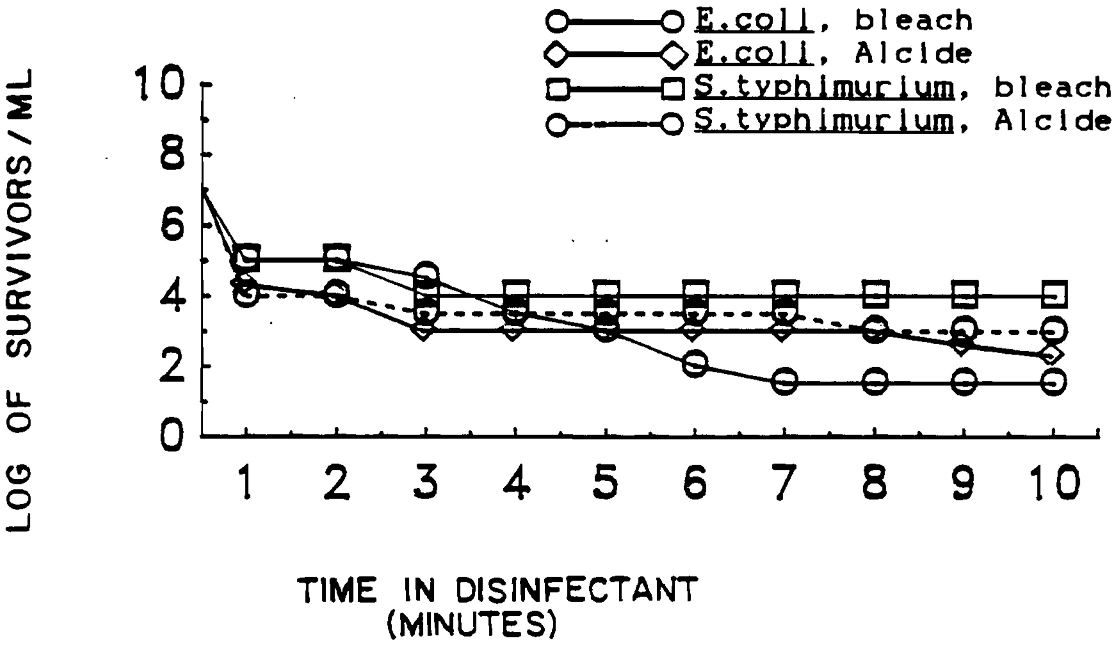

E.coli, bleach
E.coli, Alcide
S.typhimurium, bleach
S.typhimurium, Alcide
LOG OF SURVIVORS / ML
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT (MINUTES)

Figure 21. Comparison of surviving S. typhimurium and E. coli from original 10^7/ml concentrations after being exposed to high concentration Alcide (1:50 aqueous dilution) or bleach (50 ppm) with 5 percent organic loads.

Figure 22. Comparison of surviving S. typhimurium and E. coli from original 10^7/ml concentrations after being exposed to high concentration Alcide (1:50 aqueous dilution) or bleach (50 ppm) with 10 percent organic loads.


E.coli, bleach
E.coli, Alcide
S.typhimurium, bleach
S.typhimurium, Alcide
LOG OF SURVIVORS / ML
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
TIME IN DISINFECTANT
(MINUTES)


E.coli, bleach
E.coli, Alcide
S.typhimurium, bleach
S.typhimurium, Alcide
LOG OF SURVIVORS / ML
10
8
6
4
2
0
1
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5
6
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8
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10
TIME IN DISINFECTANT
(MINUTES)

DISCUSSION

Conclusions

With no organic load OCl^- and ClO_2 are equivalent in their antimicrobial control, as seen in Figures 2, 14, 17, and 18. In comparing the germicidal equivalents of bleach and high concentration Alcide in the presence of organic load, however, there is no data here that would suggest Alcide is any better than bleach. The lower concentration of Alcide was so much less effective that it should be discounted (Figures 1, 3, 4, 11, 12, and 13). This recommendation is made in spite of the manufacturer's information of percentage chlorine compound. Their information states the content of chlorine at about 2.5 percent. Therefore, a dilution factor of 1:500 which was used to make the low concentration Alcide would indicate that it should have the same amount of available chlorine (50 ppm) as the 1:1000 dilution of 5 percent bleach.

Previous research supports the Alcide manufacturer's claim (Spiegelman et al. 1986) that ClO_2 should be a superior disinfectant to hypochlorite with an organic load (Ridenour and Ingols 1947; Ridenour and Armbruster 1949; Longley et al. 1982). Considering that the germicidal equivalents found in the study used a dilution of 1:1000 of the full-strength bleach and a 1:50 dilution of the full-strength Alcide, the manufacturer's recommended dilutions must be further investigated.

A brochure describing handling and use of Alcide (1987) instructs the worker to use the disinfectant at full strength. Bleach is recommended by Public Health officials to be used within a range of 50 to 200 ppm. Comparing the recommended use dilutions, it can be expected from this study that Alcide would perform much better than the bleach since it would be 50 times stronger than our germicidally equivalent test solution, and the bleach would be the same dilution or up to 4 times the strength of our standard test (AOAC recommended) bleach solution. The purchaser of the disinfectant has to consider the germicidal effectiveness first and foremost (Engley 1980). However, cost cannot be ignored. The Alcide Corporation's price list (1987) states the cost as $30 per 3-gallon yield for a $10/gallon figure for small purchases, and $1850 for a 660-gallon yield which would be about $2.80/gallon for quantity purchases. Clorox bleach in the Topeka, Kansas, Dillon Stores is $1.04/gallon. Diluted to 50 ppm, this would be a cost for bleach of $.00104/gallon, or $.00416/gallon if used at 200 ppm. If larger quantities of bleach were purchased directly from a manufacturer, it could probably be obtained at a cheaper cost per gallon.

Using the hypothesis that Alcide at full strength would kill the bacteria with 10 percent load, it is assumed that bleach probably would perform as well if used full strength. But, as stated earlier, the criteria for bleach use is that it be used at a concentration that would prevent it from being further diluted to below 50 ppm by organic materials in the environment. There are problems caused by using a higher concentration of bleach. It has a strong odor, it may discolor contacted surfaces, and it has a corrosive effect on metals (Block 1977; Engley 1980). Alcide also can have a corrosive effect on metals, if not rinsed off those surfaces, according to the manufacturer's information.

Recommendations for Further Research

A number of questions remain that warrant further investigation. It would be useful to know the ppm of available chlorine in these disinfectants when found by an accepted method of determination. These data could be collected at various times during the 10-minute exposure periods with each set of conditions and could help with interpretation of germicidal effects observed in the testing.

Another question relates to the comparison of actions of ClO_2 and OCl^- in environments with organic load. As stated earlier, ClO_2 does not readily react with organic material (Ingols and Ridenour 1948; Longley et al. 1982) to form combined chlorine compounds, and OCl^- does (Block 1977). The hypochlorite ion forms chloramines which are bactericidal (Harvill et al. 1942; Chang 1944; Wattie 1944; Shull 1981), but are much slower acting (Hudson et al. 1983; Wolfe et al. 1984; Means et al. 1986). Past studies show that where the hypochlorite ion reacts with organic material there is an immediate formation of chloramines (Ward et al. 1984). The question related to this comparison is, why did the ClO_2 in the Alcide not perform better than the OCl^- in the bleach with organic loads? In other words, why did the bleach continue to have a germicidal effect though much was immediately "tied up" in chloramine compounds, which should have taken much longer than 10 minutes to be effective (Ward et al. 1984)? Available chlorine tests could possibly give some insight into this question, as they would indicate whether or not there was still free hypochlorite ion to be bactericidal. If there were no free hypochlorite ion, then it might indicate that the chloramines were as germicidal as the OCl^-.

In many cases there was little difference shown here in the effects of adding either a 5 percent or 10 percent organic load. Perhaps the 5 percent reacted with all of the chlorine that was present and therefore the 10 percent would not have a greater protecting effect on the bacteria. This could not have been the case if the population reduction that continued to occur with the bleach and high-concentration Alcide plus serum over the 10-minute period was due to free available chlorine still remaining in the solution. Wolfe found the breakpoint weight ratio of chlorine to organic material to be 7.6:1 (1984). Further experimentation should take into account this breakpoint data and include calculated ratios which would be of value for additional testing.

The effect of pH on the actions of the disinfectants was not considered in this study. Past research indicates that there is great variation in the percentage of OCl^- and HOCl which are being produced from the bleach, depending on the pH of the system (Chang 1944; Block 1977; Longley et al. 1982). At a pH of 5.6, there is a 96.8 percent HOCl and 3.2 percent OCl^-; at pH of 7.5, 48.9 percent HOCl and 51.1 percent OCl^- ; and at pH of 9.0, 2.9 percent HOCl and 97.1 percent OCl^-, all of these for 20 degrees Celsius (Chang 1944; Block 1977; Lippy 1984). If the pH of the bleach in this study was lowered during testing, then the Alcide was compared to less OCl^- than was originally present in the alkaline hypochlorite solution. Further experiments might either control the pH of the system or monitor it throughout the experiment so that its impact on the results obtained from bleach could be examined.

The pH question could also be applicable to the study of the mechanisms involved in the cidal effects of the different types of chlorine. HOCl is thought to be more effective than OCl^-, and this is due to the fact that it can penetrate the bacterial wall easier, having no charge (Lippy 1986). It has been found that the enzyme that is affected by chlorine is probably one involved in the process of glucose oxidation at a point in the fermentation cycle

(Green and Stumpf 1946; Stumpf and Green 1946). Once inside the cell, perhaps the two types of chlorine would be equally effective. The germicidal differences among the various types of chlorine compounds may not be due to their action but to their penetration abilities. In addition to the popular enzyme interaction hypothesis, there is also evidence that chlorine compounds have been found to react with deoxyribonucleic acid and this could explain some germicidal properties (Dugan 1978; Shull 1981). The study of germicidal modes of action has many aspects that warrant further investigation.

Though *S. typhimurium* and *E. coli* are widely accepted indicator organisms for testing intermediate disinfectants, further tests using other types of bacteria could be valuable in testing Alcide. Laboratory strains that have been subcultured probably have different resistances, superior and inferior, than environmental bacteria (Ward et al. 1984; Wolfe et al. 1984), and would be variables in these proposed studies. One explanation of the differences is that resistance can be obtained by the presence of transmissable R-factor plasmids (Ward et al. 1984). Studies of resistance of different types and strains of bacteria would be useful in judging the effectiveness of disinfectants and also would perhaps explain some contradictory data that has been collected in experiments using them.

SUMMARY

The purpose of this study was to test the effectiveness of Alcide disinfectant, using a standard hypochlorite solution (bleach) for comparison. The test bacteria, *Escherichia coli* and *Salmonella typhimurium*, were exposed to disinfectant with no organic load or a 5 or 10 percent load, for a period of 10 minutes. The data showed a 1:50 dilution of Alcide solution is germicidally equivalent to a 1:1000, 50 ppm, dilution of 5 percent hypochlorite solution, both with and without organic load conditions. The Alcide Corporation recommends using its product full strength. The recommended use dilution for bleach is a range of 50 ppm to 200 ppm, which is one to four times the dilution used in this experiment. Alcide would be expected to perform much better than bleach at these use dilutions.

LITERATURE CITED

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Alcide Corporation. 1987. New L. D. disinfectant-fast-acting, hard-surface disinfectant from Alcide. Alcide Corp. 2 pp.

Alcide Corporation. 1986. Price list. Alcide Corp. 1 p.

Alcide Corporation. 1987. Technology profile. Alcide Corp. 7 pp.

Anderson, D. A., and R. J. Sobieski. 1980. Introduction to microbiology, ed. 2. C. V. Mosby Co., St. Louis, Mo. 518pp.

Blaser, M. J., P. F. Smith, W. L. Wang, and J. C. Hoff. 1986. Inactivation of Campylobacter jejuni by chlorine and monochloramine. Appl. Environ. Microbiol. 51(2):307-311.

Block, S. S. 1977. Disinfection, sterilization and preservation, ed. 2. Lea and Febiger, Philadelphia, Pa. 1049pp.

Chang, S. L. 1944. Destruction of micro-organisms. J. Am. Water Works Assoc. 36(11):1192-1207.

Dugan, P. R. 1978. Use and misuse of chlorination for the protection of public water supplies and the treatment of wastewater. ASM News. 44(3):97-102.

Engley, F. B., Jr., and B. P. Dey. 1970. Unpublished report presented at CSMA proceedings of 56th mid-year meeting. University of Missouri School of Medicine, Columbia, Mo.

Engley, F. B., Jr. 1980. Disinfection. Personal communication. 19 pp.

Green, D. E., and P. K. Stumpf. 1946. The mode of action of chlorine. J. of Am. Water Works Assoc. 38(11):1301-1305.

Harvill, C. R., J. H. Morgan, M. C. Hagar, and A. R. Todd. 1942. Maintenance of chlorine residual in the distribution system. J. of Am. Water Works Assoc. 34(12): 1797-1806.

Hudson, L. D., J. W. Hankins, and M. Battaglia. 1983. Coliforms in a water distribution system: a remedial approach. J. Am. Water Works Assoc. 75(11):564-568.

Ingols, R. S., and G. M. Ridenour. 1948. Chemical properties of chlorine dioxide in water treatment. J. Am. Water Works Assoc. 40(11):1207-1227.

Lennette, E. H., A. Balows, W. J. Husler, Jr., and J. P. Truant. 1980. Manual of clinical microbiology, ed. 2. Am. Soc. Microbiol., Wash. D. C. 1044 pp.

Lippy, E. C. 1986. Chlorination to prevent and control waterborne diseases. J. Am. Water Works Assoc. 78(1):49-52.

Longley, K. E., and AWWA Disinfection Committee. 1982. Disinfection. J. Am. Water Works Assoc. 74(7):376-380.

Means, E. G. III, T. S. Tanaka, D. J. Otsuka, and M. J. McGuire. 1986. Effects of chlorine and ammonia application points on bactericidal efficiency. J. Am. Water Works Assoc. 78(1):62-69.

Perkins, J. J. 1969. Principles and methods of sterilization in health sciences, ed. 2. Charles C. Thomas, Springfield, Ill. 560 pp.

Public Health Service Publication No. 934. 1962. Food service sanitation ordinance and code. Food Service Sanitation Manual. 54 pp.

Ridenour, G. M., and E. H. Armbruster. 1949. Bactericidal effect of chlorine dioxide. J. Am. Water Works Assoc. 41(6):537-550.

Ridenour, G. M., and R. S. Ingols. 1947. Bactericidal properties of chlorine dioxide. J. Am. Water Works Assoc. 39(6):561-567.

Russell, A. D., S. A. Hammond, and J. R. Morgan. 1986. Bacterial resistance to antiseptics and disinfectants. J. Hosp. Infect. 7:213-225.

Shull, K. E. 1981. Experience with chloramines as primary disinfectants. J. Am. Water Works Assoc. 73(2):101-104.

Spiegelman, S. N., and C. J. Giambrone. 1986. Comparative bactericidal activity of hypochlorites and Alcide LD 10:1:1 disinfectant in the presence of organic loads. Report presented at ASM Annual meeting. Alcide Corp., Farmingdale, N. Y. 14 pp.

Stumpf, P. K., and D. E. Green. 1946. A note on an enzymatic method of estimating chlorine. J. Am. Water Works Assoc. 38(11):1306-1308.

Tonney, F. O., F. E. Greer, and T. F. Danforth. 1928. The minimal "chlorine death points" of bacteria. Am. J. Public Health. 18:1259-1263.

Ward, R. N., R. L. Wolfe, and B. H. Olson. 1984. Effect of pH, application technique, and chlorine-to-nitrogen ratio on disinfectant activity of inorganic chloramines with pure culture bacteria. Appl. Environ. Microbiol. 48(3):508-514.

Wattie, E., and C. T. Butterfield. 1944. Relative resistance of *Escherichia coli* and *Eberthella typhosa* to chlorine and chloramines. Public Health Reports. 59: 1661-1671.

Wolfe, R. L., N. R. Ward, B. H. Olson. 1984. Inorganic chloramines as drinking water disinfectants: a review. J. Am. Water Works Assoc. 76(5):74-88.