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Representative food spoilage, indicator and pathogenic organisms were exposed to newly developed iodophors obtained from New Zealand and widely used representative domestic germicides. The organisms used in these experiments were Salmonella derby, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus lactis, Micrococcus luteus, yeast of the genus Candida, Escherichia coli, Streptococcus faecalis, and spores of Bacillus licheniformis.

In the majority of the trials, Chambers method of preparing and evaluating germicides was followed. All vegetative cells were exposed to varying concentrations of germicides in both distilled and hard (USDA 500 ppm $CaCO_3$) water for time periods varying from 15 to 300 seconds. Bacterial spores were exposed up to 20 minutes. The germicides tested included five iodophors (three New Zealand products); and for purposes of comparison a hypochlorite; and two quaternary ammonium compounds (QAC), one, a normal slightly alkaline preparation and the other a specially buffered highly acidic QAC preparation.

The results of germicide experiments showed generally similar effectiveness by iodophors and hypochlorite. Both were superior to the

QAC's when used at lower concentrations.

Yeast cells were significantly more resistant to the hypochlorite than to the iodophors, especially at lower concentration levels.

The only germicides that provided rapid destruction of spores were the hypochlorite and a New Zealand iodophor. Both produced complete destruction after a 20 minute exposure.

A procedure to more closely simulate actual use of germicides in food and dairy industries was attempted in this study. Special polished metal strips were inoculated with organisms and exposed to an iodophor, a hypochlorite, and a QAC for 15, 30, 60 and 300 seconds. Again the halogen compounds were superior to the QAC in destroying the bacterial cells.

Factors Affecting The Germicidal Activity Of Iodophor Germicides

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INTRODUCTION

For bactericides used on the farm and in dairy and food plants, restaurants and institutions, preparations of greatest interest include iodophors, hypochlorites, and quaternary ammonium compounds. Representative solutions from each group were employed in this study.

The pratical application of any of these preparations is dependent on a number of considerations, including bactericidal activity against various microorganisms, stability, compatibility with associated or added compounds, corrosive action on metals, toxic effect on skin surfaces, and cost (8).

This study was undertaken to compare newly developed iodophors obtained from New Zealand with five widely used domestic preparations, including, two iodophors, a hypochlorite and two QACs. The hypochlorite and QACs were included to provide comparative destruction data with the iodophors. The organisms tested are representatives of product spoilage types, pollution indicators and foodborne pathogens. The vegetative cells of bacteria and the yeast studied were exposed to different concentrations of germicides for time periods of 15, 30, 60, 120, and 300 seconds. Spores were treated for periods up to 20 minutes. All cells were tested in double distilled and USDA hard water (500 ppm $CaCO_3$) (10).

An attempt was made to develop a procedure that more closely simulated actual use of germicides in food and dairy industries. Most dairy processing equipment, pipe lines and vats, as well as much surface

areas in meat and poultry plants are constructed of metal. Using a modification of the method suggested by the American Association of Analytical Chemists for seeding penicillin cups (24), special polished stainless steel strips were inoculated with organisms and exposed to various concentrations of different germicides for varying time periods.

Properties of different germicides are discussed and their bactericidal activity is compared against a variety of organisms.

REVIEW OF LITERATURE

History

Germicides were first used as deodorants for controlling foul odors in garbage and sewage. The use of chlorine compounds and carbolic acid in the 19th century produced dramatic results, but it was not learned until later that the beneficial results were due to the destruction of infective microorganisms. These two compounds were later used for disinfecting hands, for treatment of hospital gangrene, in the treatment of wounds, and in antiseptic surgery. At this time the germ theory of human disease was not known and the importance of disinfection was not widely recognized.

Lister, in 1867, encouraged medical disinfection after publishing the successful results of experiments he had carried out to prevent infection by dressing wounds with lint soaked in phenol. His procedure was quickly adopted and practiced all over Europe.

When many of the modern methods of bacteriology were developed in 1881, by Robert Koch, the significance and potential of germicides and disinfection were finally recognized, and rapid progress followed.

IODINE

For more than 150 years, iodine has been used for various purposes in medicine. In 1816, it was used in medical practice for the treatment of goiter. It was later reported that iodine was effective against a variety of diseases. Tincture of iodine was first used for the treatment of wounds during the American Civil War. It was a 5% solution of iodine in diluted alcohol. The tincture was later changed by dissolving one ounce of iodine in one pint of alcohol. In 1873, Davaine found that iodine attenuated the virulence of anthrax bacilli and later showed that a high dilution of iodine placed in contact with anthrax-infected blood for one hour rendered the blood harmless when injected into guinea pigs.

Since the latter part of the nineteenth century, iodine has been used in various forms as an antiseptic for the skin, wounds, and mucous surfaces of the body; for the sterilization of air and of inanimate objects such as catgut and surgical instruments; as prophylactic and therapeutic agents in diseases, for the disinfection of drinking water and swimming pool water; and for sanitization in hospitals, food processing plants, and the dairy industry (22).

Chemical and Physical Properties

Iodine is solid at room temperature, but it has a high vapour pressure, and so sublimes readily. It is only slightly soluble in cold water but is much more soluble in boiling water. The solubility in water is greatly enhanced by the presence of iodide ions. Iodine is very soluble in organic solvents, where solutions are either violet

or brown in color. Chloroform, carbon disulfide, carbon tetrachloride, and benzene solutions of iodine produce a violet color, where as, in alcohol, ether, glycerin, and propylene glycol, a brown color is produced. The concentration of an iodine solution may be determined iodometrically by titration with a standard solution of sodium thiosulfate. Since iodine in dilute solution gives a characteristic yellowish color, this may be used for a semiquantitative estimation of iodine in solutions.

In water, iodine undergoes the following reactions:

(1) $I_2 + H_2O \rightleftharpoons HOI + I^- + H^+$ $\qquad K = 0.3 \times 10^{-12}$

Hypoiodous acid dissociates, forming hypoiodite ion:

(2) $HOI \rightleftharpoons H^+ + OI^-$ $\qquad K = 5 \times 10^{-13}$

Periodides are formed from iodine in the presence of iodide ion.

(3) $I_2 + I^- \rightleftharpoons I_3^-$ $\qquad K = 1.4 \times 10^{-3}$

Hypoiodous acid may decompose, yielding iodate and iodide.

$$3HOI \longrightarrow HIO_3 + 2H^+ + 2I^-$$

Iodine, at pH values below 6, exists principally in the form of I_2, and maximal germicidal action is observed within this range. As the pH is increased above 7.5 the germicidal activity is decreased. The decrease in activity is due to an increase in hypoiodous acid which has much less killing power than molecular iodine. In the presence of iodides there is always some periodide or triiodide formed which has very low germicidal activity. Because excess iodide takes up the free iodine into periodide, and thus reduces the germicidal activity of the solution, excess iodide should be avoided. The germicidal action of triiodine is 0.08 that of molecular iodine.

The iodide ion shows an insignificant degree of germicidal activity (17).

Some advantages of iodine include its high chemical activity which potentiates it as a germicide. It is effective against a wide variety of organisms including bacteria, spores, viruese, yeasts, rickettsiae, and protozoa. The concentration of iodine necessary for disinfection does not vary greatly with different organisms and the organisms are killed rather than held in a bacteriostatic condition (33). Iodine undergoes some depressant effect in the presence of organic matter, but it is not as great as chlorine. It is generally not affected by water hardness, and it has a low toxicity. Microorganisms do not appear to develop resistance to iodine.

Iodophors

Iodophors are mixtures of iodine with surface active agents (surfactants) which act as carriers and solubilizers for iodine and release free iodine when diluted in water. They exhibit maximum activity and stability in acid solution and the formulation is controlled by the use of phosphoric acid to maintain a preferential pH through the normal dilution range (22). Iodine was not used extensively before 1950 because of its undesirable properties, but with the introduction of iodophors a revolution occurred in the use of iodine for disinfecting and sanitation purposes.

The solubilizing agents or carriers of iodine are usually synthetic organic compounds called surfactants which are water-soluble and lower the surface tension of water markedly even in very small

concentrations. In solution the carriers are able to solubilize up nearly 30 percent of their weight of iodine, of which from 70 to 80 percent may be released as available iodine when the concentrated solution is diluted. A portion of the iodine is firmly bound in the complex and is irrecoverable (15). The iodine is bound in the form of micellar aggregates and upon dilution the micelles are dispersed as the linkage of iodine is progressively weakened.

Nonionic surfactants have been accepted as the compounds to use as carriers of iodine and are extensively used in the production of iodophors, although iodophors consisting of iodine plus cationic or anionic surface active carries can be obtained. Nonionic surfactants display their activity in hard water and under a wide range of pH conditions which usually affect ionic compounds. In general, they provide better solubility, stability, and other desirable properties than ionic surfactants (22).

Iodophors have an almost universal or all-purpose practical use. They possess quick antimicrobial action on a wide array of microorganisms, and are miscible with water in all proportions. They are relatively non-toxic, non-irritating, do not sensitize; nor do susceptible microorganisms become resistant to these compounds. They are stable, non-corrosive (except on silver), and are effective in cold and warm water solutions, in soft and hard water, and over a wide pH range (22). Iodophors are surface active and have strong wetting powers which assists them as detergents as well as germicides. They stain soiling matter such as milk solids and milk stone indicating the physical cleanliness of equipment.

Mechanisms of Action

The germicidal action of iodine on microorganisms is attributed to direct iodination and oxidation of proteins. Anson and Stanley (1) reported that iodine denatures tobacco mosaic virus by modifying sulfhydryl and tyrosine groups.

Green, Herbert and Subrahmanyan (14) showed that a few parts per million iodine could inactivate zymohexase, an enzyme that is an essential component of all glucose oxidation systems regardless of origin, and it may be the primary point of attack of iodine.

When bacteria are treated with iodine, the inorganic phosphate uptake and oxygen consumption by the cells immediately ceases. Bacterial cells that are exposed to radioiodine show very little change in the cell fraction, indicating that an oxidation of -SH groups rather than a substitution into tyrosyl moieties occurs (34).

Shikashio, Sandine and Elliker (27) studied the effects of iodine on several sulfhydryl group dependent enzymes. Their results show that in the case of alcohol dehydrogenase, lactic dehydrogenase and glucose -6- phosphate dehydrogenase, the mechanism of iodine inactivation is due, at least in part, to oxidation of essential sulfhydryl groups. On a molar basis, iodine was more effective than trichlorocide and quaternary ammonium compounds in inhibiting activity of the enzymes studied with the exception of catalase and glyceraldehyde -3- phosphate dehydrogenase. Catalase is a sulfhydryl independent enzyme and was not inactivated by iodine at pH 7.0; However, at pH 8.5 there was 60 percent inhibition, which supports the view that iodination of tyrosyl and

histidyl components is the mechanism by which iodine inactivates catalase.

CHLORINE

Chlorine was discovered in 1774, by a Swedish chemist, but it was not until 1810, that Sir Humphrey Davy, definitely proved chlorine to be an element and gave it the name which it now bears. Henry, first produced chlorine of lime ($CaOCl_2$) near the end of the 18th century. During the first half of the 19th century the disinfecting and deodorizing properties of chlorine of lime were first recognized. In 1851, Watt, prepared sodium hypochlorite by electrolyzing sodium chloride. Chlorinated lime was used for the treatment of sewage and for disinfecting and deodorizing hospitals as early as 1854 (22).

In 1881, Koch reported the first instance in which hypochlorites were employed for the specific purpose of destroying microorganisms, and in 1894, Trabue drew attention to the use of hypochlorites in purifying water supplies after which time they were extensively used in water treatment (25). Today chlorine is one of the most widely used chemical disinfectants.

Chemical and Physical Properties

Chlorine is a heavy, green-colored gas (23). It is a very strong oxidizing agent which is believed to potentiate its germicidal properties. Because it is a strong oxidizing agent chlorine is very corrosive to metals and readily attacks organic matter. It readily reacts with nitrogen compounds forming chloramines which have a germicidal activity

much less than that of free chlorine.

Hypochlorites are manufactured as powders containing calcium hypochlorite and sodium hypochlorite combined with hydrated trisodium phosphate and as liquids containing sodium hypochlorite (25).

Hypochlorous acid is formed by the reaction of molecular chlorine with water according to the following reactions:

(1) $Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$ $\qquad K = 5 \times 10^{-14}$

It is also formed in solutions of hypochlorite in water. Hypochlorous acid (HOCl) dissociates in water, the degree of dissociation being determined by the pH of the solution.

(2) $HOCl \rightleftharpoons H^+ + OCl^-$ $\qquad K = 2.95 \times 10^{-8}$

Oxygen may also be liberated from hypochlorous acid under certain conditions by the following reaction:

(3) $2HOCl \longrightarrow 2HCl + O_2$

Free oxygen formed from hypochlorous acid also can attribute to the germicidal effectiveness of hypochlorites (15).

The disinfecting efficiency of hypochlorites increases with increasing concentrations of hypochlorous acid. Hypochlorous acid concentration in turn increases with decreasing pH.

During chlorination of water some of the chlorine will be taken up by impurities forming compounds which are called combined available chlorine and they are much less effective as germicides than free available chlorine. A sufficient amount of chlorine must be added to satisfy the chlorine demand and leave a residual of free available chlorine. This is known as break-point chlorination (22).

Free available chlorine may be found in three forms in water:

elemental chlorine (Cl_2), hypochlorous acid (HOCl), and hypochorite ion (OCl^-).

The stability of free chlorine in solution is affected by chlorine concentration, presence and concentration of catalysts, pH of solution, presence of organic matter and ultraviolet irradiation. The pH seems to show the greatest influence. More concentrated solutions are less stable than weaker ones. The most stable free available chlorine solutions are those having the following characteristics: low chlorine concentration, absence or low contents of copper, cobalt, nickel, or other catalysts, high alkalinity, low temperature, absence of organic material, and storage in dark and in closed containers shielded from ultra violet light (22).

Mechanism of Action

It was reported by early investigators that the bactericidal effect of chlorine is due to inhibition of certain essential enzyme systems. The mechanism of this inhibition was believed to involve the powerful oxidative action of chlorine in bactericidal amounts to inhibit sulfhydryl enzymes and other enzymes sensitive to oxidation. Inhibition of essential enzymes in this way caused death of the bacterial cell. Inhibition of glucose oxidation is paralleled by the percentage of bacteria killed (19).

It was earlier believed that HOCl liberates nascent oxygen which in turn combined with cell protoplasm resulting in death. Chang (5) reported that chlorine is bactericidal even under conditions that exclude direct oxidation of microorganisms.

Benarde, Snow, Olivieri and Davidson (3) showed by using C^{14} labeled phenylalanine uptake as an indicator, that Escherichia coli immediately stops uptake when exposed to chlorine. Their results indicate that a break in protein synthesis was involved rather than inactivation of an enzyme system in the catabolism of glucose.

Hypochlorite solutions attack proteins and related compounds, forming derivatives that have chlorine linked to nitrogen. Alpha amino acids and hypochlorites react to form chloramino acids, which further break down to aldehydes or ketones, ammonia, carbonic acid, and sodium chloride (15).

Chlorine and organic compounds containing active chlorine inactivate at very low concentrations a considerable number of enzymes e.g., triosephosphoric dehydrogenase, succinic oxidase and acetic oxidase. In the case of triosephosphoric dehydrogenase it can be shown that chlorine irreversibly oxidizes some sulfhydryl groups which are essential for the activity of the enzyme. In general, those enzymes which are very sensitive to the action of chlorine are shown to depend for their activity upon the presence of sulfhydryl groups (32).

Friberg (11) studied the reaction of chlorine with bacteria in water disinfection and reported that 10 percent to 25 percent of free available chlorine used was found in the bacterial cell after a five minute exposure to 0.5 ppm free available chlorine. When more free available chlorine was added an increase of uptake was observed. An increase from 10 percent to 22 percent was also seen with an increase in pH from 6.5 to 8.2 respectfully.

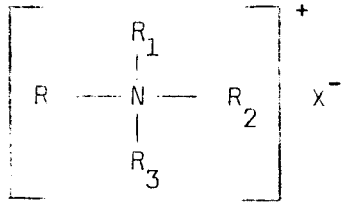
QUATERNARY AMMONIUM COMPOUNDS

Quaternary ammonium compounds (QACs) were first synthesized around the turn of the century. Some years later, Jacobs and his colleagues did a thorough study of the chemistry and germicidal properties of QACs (33). The potentialities of these compounds were not realized until 1935, when Domagk called the attention to their high bactericidal activities. Since then they have been widely used, but exaggerated germicidal performance was given to QACs by early investigators, who did not recognize the need for inactivators in the test subculture media (15).

Chemical and Physical Properties

QACs make up the groups known as cationic detergents, cationic wetting agents or cationic surface active germicides. They are organically substituted compounds in which the nitrogen atom has a covalence of five (33).

They have the general structure:



The R represents a long chain alkyl or polycyclic group which is hydrophobic. It is attached to the hydrophobic portion of the molecule. R_1, R_2, and R_3 represent hydrogen, alkyl, aryl or heterocyclic groups and x represents an anion (22).

When QACs are dissolved in water the cationic and anionic portion

of the molecule ionize in solution with the R group carrying the positive charge. The R group is always lipophilic which gives the molecule its high surface activity along with the ability to lower surface tension (33).

QACs are good wetting agents but are poor detergents. They are bacteriostatic and bactericidal for many gram positive and gram negative organisms. Gram negative organisms are less sensitive to QACs than gram positive. QACs are virtually ineffective against spores and fungi (15). Pseudomonas species also show particularly high resistance to these compounds (2).

QACs are presently used as sanitizers in dairies and food processing plants, and for sanitizing rinses for washed eating and drinking utensils (15).

Some advantages of QACs include their stability in long term storage, they are colorless and odorless, are non corrosive to metals and are non-toxic to the skin when properly used. They contain no phenol, iodine, active chlorine, mercury or other heavy metals.

Mechanism of Action

There have been several explanations for the germicidal action of QACs. It was first believed to be due to the high surface activity of these compounds. The germicidal action of QACs has more recently been attributed to protein denaturation, enzyme inhibition, and the disruption of cell membrane (15).

It has been shown that in very low concentrations, QACs precipitate proteins and dissociate conjugated proteins. Hotchkiss (17) reported

that QAC treated cells released nitrogen and phosphorous in the surrounding media. Gayle and Taylor (13) identified substances as being lysine and glutamic acid and later showed that treatment of cells with a QAC causes the leakage of amino acids. Knox, Auerbach, Zarudnaya, and Spirtes (19) showed that the death rate of E. coli parallel with the inhibition of certain metabolic reactions and that other reactions persist in the presence of the lethal amount of detergent. The detergents produce these effects of killing and inhibition proportional to the detergent bacterial ration, and not to the detergent concentration.

Lethal concentrations of QACs effect complete and irreversible inhibition of bacterial oxidase and dehydrogenase systems. These high concentrations also cause the cell membrane to disrupt releasing purines, pyrimidines, inorganic phosphorus, and amino acids (15). This effect is somewhat similar to that reported above by Hotchkiss. Dawson, Lominski, and Stern (6) used electron microscopy to determine the effects of a QAC on Staphylococcus aureus. They found that the QAC split lipoprotein complexes in the cell releasing autolytic enzymes which act within the cell. This reaction only occurred when low ratios of organisms to QAC were used. When the concentration of the QAC was increased, appreciable lysis did not occur. They postulated that the effect of the latter was due to the denaturation of the autolytic enzyme by the increased amount of QAC.

Quaternary ammonium compounds have proven to be very stable over long periods of time. Heineman (16) found that dilute solutions of a QAC showed no decrease in germicidal activity when tested after 14

months after their initial preparation. Lawrence (21) reports that a 10% solution of quaternary ammonium germicide stored at room temperature for ten years showed no evidence of a physical, chemical, or bacteriology change in property.

The QACs used in this study were accurately diluted to give a stock solution of 1000 ppm and the concentration of active ingredients was determined by the method of Furlong and Elliker (12). The solutions were checked again at the end of the study and showed no change in concentration.

MATERIALS AND METHODS - CHAMBERS PROCEDURE

Organisms used in Germicide Tests

The organisms used in this study were chosen because of their significance as spoilage types in dairy and food processing plants and as foodborne pathogens. They are also of great concern in hospitals, cafeterias, and resturants. Salmonella derby and Staphylococcus aureus were chosen to represent foodborne pathogens. Pseudomonas species are able to grow at refrigerated temperatures causing deterioration of milk and milk products and other foods. Bacillus licheniformis represents spore formers which show high resistance to germicides. Streptococcus lactis is a typical dairy contaminant and is useful because of its exceptional resistance to common germicides. Micrococcus luteus is representative types frequently found in a variety of foods. A member of the genus Candida was used as a typical food spoilage yeast. E. coli and Streptococcus faecalis are enteric bacteria used as indicators of fecal contamination in food and water.

Preparation of Cultures

Spores were obtained by inoculating two liters of Schaeffer's sporulation medium (26) with Bacillus licheniformis and incubating on a shaker for six days at 30 C. The culture was centrifuged, spores collected, resuspended in physiological saline, and recentrifuged. The suspension was washed three times in this manner and finally diluted in physiological saline to obtain the desired concentration for the germicide tests. The spore suspension was heat shocked at 80 C,

aseptically tubed adding enough spore suspension for each germicide test. Tubes of the suspension were stored at 4 C. Before use each tube was heat shocked again.

The other bacteria and the yeast were inoculated on tube slants prepared by using Standard Methods (SM) agar (31) incubated for 18 to 24 hours. Lactic agar (7) was used in the case of S. lactis. These inoculated tubes were stored at 4 C until ready for use and transferred once a month. To prepare for tests the isolate to be used was inoculated on another tube of SM agar, and incubated for 18 to 24 hours. The culture was washed from this slant with 0.25 M phosphate buffer and three milliliters were aseptically pipetted onto medicine bottle slants and incubated 18 to 24 hours. The number of bottles of culture required varied with each organism. The culture was washed from the slant with 10 ml sterile 0.25 M phosphate buffer into a sterile screw cap tube and violently agitated with a Vortex mixer and aseptically filtered through Whatman no. one filter paper. The filtrate was transferred into a sterile screw cap tube and placed on ice. One ml of this suspension was used to make a 1:100 dilution in phosphate buffer and the O.D. was measured at 440 nm. The suspension was diluted as necessary to obtain a predetermined O.D. for each culture in order to obtain the desired concentration.

GERMICIDES AND TITRATIONS

Germicides

Eight different germicides were used in this study: a sodium hypochlorite solution sold under the brand name X-4; two QACs, one, an

n-alkyl dimethyl dichlorobenzyl ammonium chloride, the other an n-alkyl dimethyl benzyl ammonium chloride n-alkyl dimethyl ethylbenzyl ammonium chloride with 30% phosphoric acid. Five iodophors were used: Mikroklene DF, a butoxypolypropoxy polyethoxy ethanol-iodine complex with 6.5% phosphoric acid, and a nonylphenoxypoly (ethylenenoxy) ethanol iodine complex with 6.75% phosphoric acid and 14.0% glycolic acid sold under the brand name Iodophor.[1]

Three of the iodophors used were obtained from New Zealand. They were Low Foam 601, Klenziodophor, and Mikroklene.[2]

Titrations

Each original germicide solution was diluted with double distilled water to make up stock solutions of approximately 1000 ppm. Titrations were carried out within 24 hours of each germicide test to determine the concentration of active ingredient in the stock solutions. It was observed that stock solutions were stable for periods up to two months, after which time new solutions were prepared. Available iodine of the iodophor stock solution was determined by titrating with standard sodium thiosulfate to a colorless end point. The iodometric method (29) was used to determine the amount of available chlorine in the hypochlorite stock solution.

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[1] Klenzade Products, Division of Economics Laboratory, Inc., St. Paul, Minnesota 55102.

[2] Economics Laboratory, New Zealand LTD. P.O. Box 10061, Hamilton.

The QACs used in this study were accurately diluted to give a stock solution of 1000 ppm and the concentration of active ingredients was determined by the method of Furlong and Elliker (12). The solutions were checked again at the end of the study and showed no change in concentration.

Final Germicide Solutions

The quantity of stock solution required for the different concentrations in parts per million (ppm) was calculated the day before each experiment. This was determined by the amount of active ingredients in the stock solutions. The germicide solutions were prepared immediately before each test.

The final germicide solutions were prepared by adding the appropriate amount of germicide to sterile, acid cleaned, 250 ml metal capped flasks. The volume was brought to 99 ml with sterile double distilled water for the soft water tests and 98.1 ml for the hard water tests. To obtain a water hardness of 500 ppm $CaCO_3$ (10) 0.9 ml of sterile hard water solution was added for the hard water tests.

Inactivators

Germicides were inactivated by using the method of Humphreys and Johns (17). Iodophor and hypochlorite inactivator was prepared by adding 360 mg sodium thiosulfate and 40 ml 0.25 M phosphate buffer to one liter of double distilled water. To insure immediate inactivation of the higher germicide concentrations used in the spore tests, 720 mg of sodium thiosulfate was used.

Quaternary ammonium compound inactivator contained 4.4 gms asolectin, 31.2 ml Tween 80 and 40 ml 0.25 M phosphate buffer per liter of double distilled water.

For both types of inactivators the pH was adjusted to 7.2 and the solutions were dispensed in bottles and autoclaved for 15 minutes. Two ml of the appropriate neutralizer were pipetted into petri plates to which one ml of germicide-organism mixture was added after the desired exposure period. The plates were slightly tilted to insure that all of the germicide solution would go directly into the neutralizer.

Test Method and Controls

The Chamber's method (4) of evaluating bactericidal agents was used in this study with several modifications. In each experiment, four different germicides were tested against one organism in hard and soft water. Two concentrations of each germicide were evaluated. Unless indicated otherwise, the cell suspension contained approximately 10×10^9 organisms per ml. When one ml of this suspension was added to 99 ml of germicide solution the density of organisms was approximately 100×10^6 per ml.

Germicide solutions containing the highest concentrations used in that test were used for inactivator controls. They were prepared exactly as the germicides used in the experiments. One ml of this solution was pipetted into a petri plate which contained two ml of appropriate inactivator. One ml of organism suspension whose concentration was dilute enough to provide a countable plate was immediately

added to this germicide-inactivator solution. One ml of this same diluted organism suspension was added to an empty plate. The number of colonies of the two plates were compared after 48 hours incubation. Noticeable differences would indicate that the germicide was not being effectively neutralized.

To test for inactivator toxicity, one ml of the mentioned dilute organism suspension was added to a plate containing two ml of inactivator and left to stand for as long as the longest exposure period for that test.

The bacteria and yeast used in this study were exposed to 12.5 and 25 ppm of germicides for periods of 15, 30, 60, and 300 seconds. The spores were exposed to 200 and 300 ppm for 30 seconds, 2, 5, 10, and 20 minutes. The pH values of all germicide-organism suspensions were determined immediately after the longest exposure period. All tests were conducted at room temperature.

MATERIALS AND METHODS - NEW METAL STRIP PROCEDURE

A procedure to more closely simulate actual use of germicides in food and dairy industries was attempted in this study. Much of the surface that products contact consists of metal, and it is of great importance that these areas are effectively sanitized. Special metal strips therefore were employed in this study.

Materials used included: Coplin staining jars; duplex staining dish; stainless steel forceps; autoclavable, wide mouth, linear polyethylene with polypropylene screw closure, 119 x 61 mm, 250 ml centrifuge bottles; Special 18 gauge, 1 x 3 inch, 304 No. 7 mirror finish metal strips with deburr edges and a 1/8 inch hole 3/8 inches from the end.

Organisms used in germicide tests

Five organisms were used in this study: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, yeast of the genus *Candida*, and *Salmonella derby*.

Germicides

Three germicides were used in each experiment: An iodophor sold under the brand name Iodophor, a nonylphenoxypoly (ethylenenoxy) ethanol iodine complex with 6.75% phosphoric acid and 14.0% glycolic acid; a sodium hypochlorite sold as X-4; and a quaternary ammonium compound, an n-alkyl dimethyl dichlorobenzyl ammonium chloride sold as Ster-Bac. Methods of Standardizing and titrating germicides were similar to those described above for the Chambers method.

Final Germicide Solution

The germicide solutions were made up in 250 ml metal cap flasks and each transferred into individual sterile coplin staining jars prior to each experiment. The iodophor was used at concentrations of 25 and 50 ppm, the hypochlorite at 50 and 100 ppm and the quaternary ammonium compound (QAC) at 50 and 200 ppm. These different concentrations were chosen because they represent those concentrations recommended for use and actual concentrations used in food and dairy processing plants.

Inactivators

Germicide inactivator was prepared and 99 ml was dispensed in wide mouth, screw cap centrifuge bottles and autoclaved for 15 minutes.

Test Methods

The organisms to be tested were inoculated to tube slants prepared by using standard methods (SM) agar and incubated for 18 to 24 hours. Potato dextrose agar was used for yeast. The culture was washed from this slant with 0.25 M phosphate buffer and three ml were aseptically pipetted onto 8 oz. bottle slants and incubated for 18 to 24 hours. The number of bottles of culture required varied with each organism. The culture was washed from these bottle slants with 10 ml sterile 0.25 M phosphate buffer into sterile screw cap tubes and violently agitated with a Vortex mixer. With the exception of the yeast, the cells were filtered through Whatman no. 1 paper. The filtrate was transferred into a sterile duplex staining dish capable of holding

20 metal strips. Sufficient filtrate was added to completely cover the metal strips.

The metal strips were individually wrapped in aluminum foil and sterilized with all other materials the day before the experiment was to be carried out.

The methods of the Association of Official Analytical Chemists (24) for inoculating polished stainless steel cylinders was followed. Using flamed stainless steel forceps, the metal strips were placed in the organism solution. After a 15 minute contact period the metal strips were removed using flamed forceps and placed individually in sterile petri plates. They were then placed in an incubator at 37 C and let dry for 30 minutes.

The inoculated metal strips were aseptically removed from the dish using flamed forceps and placed in Coplin jars containing the germicide solution. After the proper exposure periods, the strips were removed with flamed forceps and placed in the screw cap centrifuge bottles containing 99 ml of appropriate inactivator. Each bottle was vigorously shaken 50 times and one ml of this inactivator-organism solution was transferred into a petri plate and agar poured. The inoculated metal strips were exposed for time periods of 15, 30, 60, and 300 seconds.

Germicide Formulations

*Iodophor A - Mikroklene

*Iodophor B - Low Foam 601

*Iodophor C - Klenziodophor

Iodophor D - a nonylphenoxypoly (ethylenenoxy) ethanol iodine complex with 6.75% phosphoric acid and 14.0% glycolic acid sold under the brand name Iodophor.

Iodophor E - a butoxypolypropoxy polyethoxy ethanol-iodine complex with a 6.5% phosphoric acid sold as Mikroklene DF.

Hypochlorite - a sodium hypochlorite sold under the brand name X-4.

QAC A - an n-alkyl dimethyl benzyl ammonium chloride n-akyl dimethyl ethylbenzyl ammonium chloride with 30% phosphoric acid.

QAC B - an n-akyl dimethyl dichlorobenzyl ammonium chloride sold as Ster-Bac.

* These products were obtained from New Zealand, and their formulations are not known.

RESULTS

The germicidal effect of eight different preparations on Salmonella derby is shown in Table 1. The hypochlorite and iodophors showed similar bactericidal efficiency causing 100 percent destruction in nearly every case within 15 seconds at 12.5 ppm. Water hardness (500 ppm $CaCO_3$) had very little effect on these compounds. Iodophor B was less effective and required five minutes exposure for total destruction. Iodophor C showed no bactericidal activity in hard water even at higher concentrations (25 ppm) and exposure periods of five minutes.

QAC B at 50 ppm destroyed all Salmonella cells within 60 seconds in soft water but was significantly affected by the presence of hard water salts. QAC A was totally ineffective except at concentrations of 100 ppm in hard water after five minutes exposure. As can be seen in most of the tables, QAC A was more effective than QAC B in the presence of hard water salts (500 ppm $CaCO_3$).

Staphylococcus aureus (Table 2) showed comparatively high resistance in these studies to all germicides. The hypochlorite and iodophors showed similar destruction rates requiring, in most cases, the maximum exposure period (five minutes) for substantial reduction of viable S. aureus cells. Iodophor C showed no destruction when used in hard water.

QAC B at 25 ppm did not cause 100 percent destruction of S. aureus cells within five minutes but at much higher concentration (200 ppm) all cells were destroyed within 60 seconds. QAC A at 200 ppm also caused 100 percent destruction within 60 seconds but only when used in hard water.

Table 1. Destruction of Salmonella derby by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.40	0	0	0	0
	12.5	USDA**	6.70	TNC	0	0	0
	25	distilled	3.05	0	0	0	0
	25	USDA	6.40	0	0	0	0
Iodophor B	12.5	distilled	2.75	TNC	21	2	0
	12.5	USDA	3.30	TNC	TNC	TNC	0
	25	distilled	2.50	TNC	11	0	0
	25	USDA	2.70	TNC	TNC	TNC	0
Iodophor C	12.5	distilled	3.20	60	2	0	0
	12.5	USDA	6.90	TNC	TNC	TNC	TNC
	25	distilled	2.90	1	0	0	0
	25	USDA	6.25	TNC	TNC	TNC	TNC
Iodophor D	12.5	distilled	3.30	3	0	0	0
	12.5	USDA	6.70	79	2	0	0
	25	distilled	3.05	0	0	0	0
	25	USDA	4.80	0	0	0	0
Iodophor E	12.5	distilled	3.50	0	0	0	0
	12.5	USDA	6.70	2	0	0	0
	25	distilled	3.25	0	0	0	0
	25	USDA	6.70	2	0	0	0
Hypochlorite	12.5	distilled	5.60	0	0	0	0
	12.5	USDA	7.20	2	0	0	0
	25	distilled	6.25	0	0	0	0
	25	USDA	7.20	0	0	0	0
QAC A	50	distilled	3.00	TNC	TNC	TNC	TNC
	50	USDA	5.92	TNC	TNC	TNC	TNC
	100	distilled	2.80	TNC	TNC	TNC	TNC
	100	USDA	3.65	TNC	TNC	TNC	0
QAC B	50	distilled	4.50	11	3	0	0
	50	USDA	6.30	TNC	TNC	TNC	TNC
	100	distilled	5.20	0	0	0	0
	100	USDA	7.70	TNC	TNC	TNC	0

* Initial number of cells, 29.5×10^9 per ml.
** U.S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 2. Destruction of Staphylococcus aureus by iodophor, hypochlorite and QAC germicides.

Germicide	Conc.	Test	pH of germicide	Average number of surviving organisms*			
	ppm	water	solution	15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.25	TNC	117	1	0
	12.5	USDA**	6.25	TNC	TNC	114	81
	25	distilled	2.98	151	6	0	0
	25	USDA	6.35	TNC	118	15	6
Iodophor B	12.5	distilled	2.60	TNC	TNC	6	0
	12.5	USDA	3.50	TNC	TNC	TNC	TNC
	25	distilled	2.40	TNC	154	3	0
	25	USDA	2.70	TNC	TNC	TNC	21
Iodophor C	12.5	distilled	3.15	TNC	TNC	2	0
	12.5	USDA	6.28	TNC	TNC	TNC	TNC
	25	distilled	2.90	193	29	0	0
	25	USDA	6.15	TNC	TNC	TNC	TNC
Iodophor D	12.5	distilled	3.20	TNC	106	3	0
	12.5	USDA	6.70	TNC	TNC	18	8
	25	distilled	2.95	244	8	1	0
	25	USDA	5.20	TNC	78	3	2
Iodophor E	12.5	distilled	3.45	TNC	50	2	0
	12.5	USDA	7.10	TNC	54	21	20
	25	distilled	3.15	50	6	0	0
	25	USDA	6.99	27	14	0	0
Hypochlorite	12.5	distilled	5.20	149	14	4	4
	12.5	USDA	6.75	TNC	146	18	8
	25	distilled	5.90	210	3	0	0
	25	USDA	7.90	TNC	113	7	3
QAC A	25	distilled	3.25	TNC	TNC	TNC	TNC
	25	USDA	7.15	TNC	TNC	278	14
	200	distilled	2.45	TNC	TNC	TNC	126
	200	USDA	2.80	TNC	226	1	0
QAC B	25	distilled	4.70	TNC	TNC	2	0
	25	USDA	6.00	TNC	TNC	173	10
	200	distilled	5.10	27	2	0	0
	200	USDA	7.00	102	11	0	0

* Initial number of cells, 14×10^9 per ml.

** U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

The hypochlorite and iodophors proved to be very effective against Pseudomonas (Table 3). All iodophors, except Iodophor B and Iodophor C, showed 100 percent destruction within 15 seconds at the lowest concentration used (12.5 ppm). It took 30 seconds for complete destruction by Iodophor C at 12.5 ppm in soft water and again it was completely ineffective in hard water. Iodophor B showed a total kill after five minutes exposure in both hard and soft water at 12.5 ppm.

At lower concentrations (25 ppm) QAC B showed no destruction of Pseudomonas cells. Two hundred parts per million killed all cells within 60 seconds in soft water but no destruction was seen when used in hard water. At 200 ppm, QAC A showed total kill within 15 seconds. At 25 ppm in soft water a reduction inviable cells could be seen after 30 seconds exposure but no destruction was seen in hard water.

Streptococcus lactis (Table 4) showed considerable resistance to most of the germicides, particularly with two New Zealand iodophors, Iodophor C and Iodophor B. Iodophor C at 12.5 and 25 ppm showed total destruction against this organism after five minutes exposure in distilled water. Iodophor B was totally ineffective against S. lactis. Iodophor A, the other New Zealand product, was more effective in hard water causing 100 percent kill within 60 seconds at 12.5 ppm. It took longer exposure periods (five minutes) to cause complete destruction in soft water.

The two domestic iodophors showed similar destruction rates against S. lactis. Both compounds were most effective in hard water.

Table 3. Destruction of Pseudomonas aeruginosa by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.19	0	0	0	0
	12.5	USDA**	6.76	TNC	5	0	0
	25	distilled	2.90	0	0	0	0
	25	USDA	6.30	0	0	0	0
Iodophor B	12.5	distilled	2.56	60	5	4	0
	12.5	USDA	3.40	TNC	TNC	76	0
	25	distilled	2.35	15	0	0	0
	25	USDA	2.58	TNC	13	3	0
Iodophor C	12.5	distilled	3.05	12	0	0	0
	12.5	USDA	6.68	TNC	TNC	TNC	TNC
	25	distilled	2.80	0	0	0	0
	25	USDA	6.00	TNC	TNC	TNC	TNC
Iodophor D	12.5	distilled	3.10	0	0	0	0
	12.5	USDA	5.85	TNC	0	0	0
	25	distilled	2.90	0	0	0	0
	25	USDA	4.70	0	0	0	0
Iodophor E	12.5	distilled	3.39	0	0	0	0
	12.5	USDA	6.70	27	0	0	0
	25	distilled	3.10	0	0	0	0
	25	USDA	6.71	0	0	0	0
Hypochlorite	12.5	distilled	5.49	0	0	0	0
	12.5	USDA	6.55	26	0	0	0
	25	distilled	6.95	0	0	0	0
	25	USDA	7.91	0	0	0	0
QAC A	25	distilled	3.20	TNC	120	39	14
	25	USDA	6.98	TNC	TNC	TNC	TNC
	200	distilled	2.50	0	0	0	0
	200	USDA	2.70	2	0	0	0
QAC B	25	distilled	4.80	TNC	TNC	TNC	TNC
	25	USDA	6.39	TNC	TNC	TNC	TNC
	200	distilled	5.14	TNC	2	0	0
	200	USDA	7.80	TNC	TNC	TNC	TNC

* Initial number of cells, 31×10^9 per ml.

** U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 4. Destruction of Streptococcus lactis by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.45	TNC	TNC	TNC	0
	12.5	USDA**	7.49	TNC	TNC	0	0
	25	distilled	3.15	TNC	TNC	413	0
	25	USDA	6.90	TNC	1	0	0
Iodophor B	12.5	distilled	2.60	TNC	TNC	TNC	TNC
	12.5	USDA	3.00	TNC	TNC	TNC	TNC
	25	distilled	2.38	TNC	TNC	TNC	TNC
	25	USDA	2.50	TNC	TNC	TNC	TNC
Iodophor C	12.5	distilled	3.15	TNC	TNC	TNC	0
	12.5	USDA	6.81	TNC	TNC	TNC	TNC
	25	distilled	2.90	TNC	TNC	TNC	0
	25	USDA	6.00	TNC	TNC	TNC	TNC
Iodophor D	12.5	distilled	3.19	TNC	TNC	TNC	0
	12.5	USDA	6.09	TNC	62	0	0
	25	distilled	2.93	TNC	TNC	16	0
	25	USDA	4.18	TNC	TNC	13	0
Iodophor E	12.5	distilled	3.39	TNC	TNC	77	0
	12.5	USDA	7.20	TNC	TNC	6	0
	25	distilled	3.10	TNC	22	0	0
	25	USDA	6.72	TNC	59	0	0
Hypochlorite	12.5	distilled	6.50	TNC	2	0	0
	12.5	USDA	9.00	TNC	TNC	TNC	0
	25	distilled	7.80	6	0	0	0
	25	USDA	9.01	TNC	TNC	12	0
QAC A	25	distilled	3.15	TNC	TNC	TNC	TNC
	25	USDA	7.00	TNC	TNC	20	0
	200	distilled	2.50	TNC	TNC	TNC	TNC
	200	USDA	2.70	TNC	TNC	131	0
QAC B	25	distilled	4.93	TNC	0	0	0
	25	USDA	7.50	TNC	48	0	0
	200	distilled	5.25	0	0	0	0
	200	USDA	8.11	1	0	0	0

* Initial number of cells, 1.6×10^9 per ml.

** U.S. Department of Agriculture, 500 ppm ($CaCO_3$).

It took 60 seconds for Iodophor D to destroy all cells in hard water, whereas, five minutes were required for total reduction in soft water. Iodophor E at 12.5 ppm caused complete destruction in five minutes in both hard and soft water. It took a shorter exposure period (60 seconds) for 25 ppm to show the same effectiveness.

Hypochlorite at 12.5 ppm killed all S. lactis cells within 60 seconds in soft water but in hard water the time required for total destruction was five minutes at that same concentration. At 25 ppm in soft water all cells were destroyed within 30 seconds and again hypochlorite was affected by water hardness requiring five minutes exposure for total destruction.

QAC B was more effective than the iodophors against S. lactis requiring only 30 seconds for complete destruction at 25 ppm in soft water. QAC A was less effective showing no germicidal affect in soft water, even when used at concentrations of 200 ppm and exposure periods of five minutes. When used in hard water QAC A at 25 ppm caused reduction of viable S. lactis cells after 60 seconds exposure and complete reduction after five minutes.

All eight germicides tested were equally effective against Micrococcus luteus (Table 5). Complete destruction was observed within 15 seconds with all germicides. The effectiveness of Iodophor C, however, was significantly affected by the presence of water hardness.

Yeast of the genus Candida was exposed to germicides and the results are shown in Table 6. All iodophors were effective in destroying all yeast cells within 15 seconds at concentrations of 12.5 ppm in soft water. Iodophor C was almost totally inhibited in

Table 5. Destruction of Micrococcus luteus by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.24	0	0	0	0
	12.5	USDA**	6.61	0	0	0	0
	25	distilled	3.00	0	0	0	0
	25	USDA	6.31	0	0	0	0
Iodophor B	12.5	distilled	2.65	0	0	0	0
	12.5	USDA	3.11	7	2	0	0
	25	distilled	2.46	0	0	0	0
	25	USDA	2.60	0	0	0	0
Iodophor C	12.5	distilled	3.11	0	0	0	0
	12.5	USDA	6.73	TNC	TNC	TNC	TNC
	25	distilled	2.90	0	0	0	0
	25	USDA	6.15	TNC	TNC	TNC	4
Iodophor D	12.5	distilled	3.20	1	0	0	0
	12.5	USDA	4.21	2	0	0	0
	25	distilled	2.97	0	0	0	0
	25	USDA	6.21	0	0	0	0
Iodophor E	12.5	distilled	3.41	0	0	0	0
	12.5	USDA	7.05	0	0	0	0
	25	distilled	3.15	0	0	0	0
	25	USDA	6.75	0	0	0	0
Hypochlorite	12.5	distilled	5.81	TNC	4	0	0
	12.5	USDA	7.59	0	0	0	0
	25	distilled	6.90	1	0	0	0
	25	USDA	8.88	0	0	0	0
QAC A	50	distilled	2.71	21	1	0	0
	50	USDA	4.45	0	0	0	0
	200	distilled	2.31	0	0	0	0
	200	USDA	2.40	0	0	0	0
QAC B	50	distilled	5.02	0	0	0	0
	50	USDA	7.00	0	0	0	0
	200	distilled	5.45	0	0	0	0
	200	USDA	8.20	0	0	0	0

* Initial number of cells, 2×10^9 per ml.

** U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 6. Destruction of a yeast of the genus Candida by iodophor, hypochlorite and QAC germicides.

Germicide	Conc. ppm.	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.14	0	0	0	0
	12.5	USDA**	6.80	18	1	0	0
	25	distilled	2.90	0	0	0	0
	25	USDA	6.30	0	0	0	0
Iodophor B	12.5	distilled	2.40	0	0	0	0
	12.5	USDA	3.02	254	4	0	0
	25	distilled	2.71	1	0	0	0
	25	USDA	2.60	TNC	0	0	0
Iodophor C	12.5	distilled	3.06	0	0	0	0
	12.5	USDA	6.80	TNC	TNC	TNC	TNC
	25	distilled	2.80	0	0	0	0
	25	USDA	6.00	TNC	TNC	TNC	5
Iodophor D	12.5	distilled	3.10	0	0	0	0
	12.5	USDA	3.30	110	0	0	0
	25	distilled	2.90	0	0	0	0
	25	USDA	4.00	5	0	0	0
Iodophor E	12.5	distilled	3.31	0	0	0	0
	12.5	USDA	7.30	0	0	0	0
	25	distilled	3.10	0	0	0	0
	25	USDA	6.80	0	0	0	0
Hypochlorite	12.5	distilled	5.50	TNC	TNC	TNC	0
	12.5	USDA	8.00	TNC	TNC	TNC	0
	25	distilled	6.50	TNC	TNC	56	0
	25	USDA	8.10	TNC	TNC	TNC	0
QAC A	50	distilled	3.20	TNC	TNC	TNC	TNC
	50	USDA	7.00	TNC	TNC	TNC	2
	200	distilled	2.65	TNC	TNC	TNC	TNC
	200	USDA	3.50	TNC	TNC	4	0
QAC B	50	distilled	5.60	152	15	1	0
	50	USDA	8.00	TNC	TNC	TNC	3
	200	distilled	5.90	20	0	0	0
	200	USDA	8.81	37	0	0	0

* Initial number of cells, 3×10^8 per ml.

** U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

hard water. The other iodophors were to a much lesser extent affected by the presence of water hardness. The hypochlorite was particularly ineffective against the yeast cells requiring five minutes exposure for total destruction. Possibly, the larger cell mass of yeast created a greater chlorine demand that had to be satisfied before the chlorine could destroy the yeast cells. QAC B at 50 ppm in soft water also required five minutes exposure for complete destruction. QAC A was less effective showing germicidal action only in hard water.

Iodophor D and Iodophor E showed similar destruction rates against Escherichia coli (Table 7) requiring 30 seconds for total destruction at 12.5 ppm in soft water. Iodophor A showed the same results. Iodophor C and Iodophor B was less effective against this organism. The former required 60 seconds for 100 percent destruction at 12.5 ppm in soft water and the latter destroyed all cells after a five minute exposure.

At 12.5 ppm, hypochlorite in soft water killed all cells within 60 seconds. In hard water the same concentration required only 30 seconds for complete destruction.

QAC B at 50 ppm in distilled water caused a reduction in the number of viable E. coli cells after 60 seconds exposure. It showed no destruction when used in hard water. QAC A at 200 ppm destroyed all cells within 60 seconds in distilled water, only 30 seconds was required for that same concentration to effect total destruction in hard water.

Table 7. Destruction of Escherichia coli by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.51	TNC	0	0	0
	12.5	USDA**	7.31	TNC	TNC	150	0
	25	distilled	3.20	3	0	0	0
	25	USDA	6.91	TNC	TNC	2	0
Iodophor B	12.5	distilled	2.59	TNC	TNC	TNC	0
	12.5	USDA	3.00	TNC	TNC	TNC	0
	25	distilled	2.39	TNC	TNC	TNC	0
	25	USDA	2.50	TNC	TNC	TNC	1
Iodophor C	12.5	distilled	3.25	TNC	TNC	0	0
	12.5	USDA	6.69	TNC	TNC	TNC	TNC
	25	distilled	2.89	TNC	3	0	0
	25	USDA	6.25	TNC	TNC	TNC	TNC
Iodophor D	12.5	distilled	3.10	TNC	0	0	0
	12.5	USDA	6.59	TNC	264	0	0
	25	distilled	3.01	23	0	0	0
	25	USDA	4.10	TNC	0	0	0
Iodophor E	12.5	distilled	3.31	TNC	0	0	0
	12.5	USDA	7.61	TNC	0	0	0
	25	distilled	3.20	3	0	0	0
	25	USDA	6.90	TNC	1	0	0
Hypochlorite	12.5	distilled	7.90	TNC	27	0	0
	12.5	USDA	7.70	1	0	0	0
	25	distilled	7.75	0	0	0	0
	25	USDA	8.60	0	0	0	0
QAC A	50	distilled	3.00	TNC	TNC	TNC	TNC
	50	USDA	6.20	TNC	TNC	TNC	TNC
	200	distilled	2.60	TNC	TNC	0	0
	200	USDA	2.65	TNC	0	0	0
QAC B	50	distilled	8.18	TNC	TNC	29	7
	50	USDA	8.39	TNC	TNC	TNC	TNC
	200	distilled	5.22	0	0	0	0
	200	USDA	7.45	TNC	17	3	0

* Initial number of cells, 8×10^9 per ml.
** U.S. Department of Agriculture, 500 ppm ($CaCO_3$).

Streptococcus faecalis (Table 8) showed some resistance to all iodophors. Hypochlorite at 12.5 ppm was totally effective against this organism causing complete destruction within 15 seconds in both distilled and hard water. QAC B at 50 ppm was fairly effective against *S. faecalis* causing complete destruction after 60 seconds. At higher concentrations (200 ppm) it only required a 15 seconds exposure period for total destruction. QAC A was less effective requiring at least 60 seconds for total kill. The germicidal effect against *S. faecalis* was increased by the presence of water hardness (500 ppm $CaCO_3$).

Spores of *Bacillus licheniformis* were exposed to very high concentrations (200 and 300 ppm) of germicides and its high resistance is shown in Table 9. Only two of the germicides tested exerted any distructive effect on the spores. Hypochlorite at 200 ppm destroyed all spores within 20 minutes in soft water and substantially reduced the number of surviving cells in 10 minutes. No reduction was observed when hypochlorite was used in hard water. The only iodophor that showed any destruction of spores was Iodophor C. Two hundred parts per million in soft water destroyed all cells within 20 minutes and a reduction in the number of viable cells was noted after a two minute exposure. Similar destruction rates were observed when Iodophor C was used in hard water, this being the only case where this product was not neutralized by hard water salts (500 ppm $CaCO_3$). The QACs were completely ineffective against the spores.

Table 8. Destruction of Streptococcus faecalis by iodophor, hypochlorite and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*			
				15 sec	30 sec	60 sec	300 sec
Iodophor A	12.5	distilled	3.31	TNC	TNC	11	0
	12.5	USDA**	7.03	TNC	5	2	0
	25	distilled	3.05	TNC	TNC	0	0
	25	USDA	6.60	TNC	0	0	0
Iodophor B	12.5	distilled	2.70	TNC	TNC	27	0
	12.5	USDA	3.40	TNC	TNC	TNC	2
	25	distilled	2 50	TNC	TNC	254	0
	25	USDA	2.41	TNC	TNC	247	0
Iodophor C	12.5	distilled	3.00	TNC	TNC	0	0
	12.5	USDA	6.70	TNC	TNC	TNC	TNC
	25	distilled	2.79	TNC	4	0	0
	25	USDA	6.19	TNC	TNC	TNC	TNC
Iodophor D	12.5	distilled	3.00	TNC	TNC	7	1
	12.5	USDA	5.80	TNC	20	1	0
	25	distilled	2.80	TNC	72	0	0
	25	USDA	3.92	TNC	21	0	0
Iodophor E	12.5	distilled	3 30	TNC	TNC	261	1
	12.5	USDA	7.15	11	0	0	0
	25	distilled	3.00	TNC	TNC	1	0
	25	USDA	6.90	TNC	17	0	0
Hypochlorite	12.5	distilled	5 09	0	0	0	0
	12.5	USDA	6.59	0	0	0	0
	25	distilled	6.20	0	0	0	0
	25	USDA	8 10	0	0	0	0
QAC A	50	distilled	2.90	TNC	TNC	TNC	TNC
	50	USDA	6 65	TNC	165	0	0
	200	distilled	2 50	TNC	TNC	TNC	TNC
	200	USDA	2.70	TNC	TNC	13	0
QAC B	50	distilled	5.55	TNC	TNC	TNC	TNC
	50	USDA	8.90	33	20	0	0
	200	distilled	5.60	223	0	0	0
	200	USDA	8.90	0	0	0	0

* Initial number of cells, 1.6×10^9 per ml.

** U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 9. Destruction of Bacillus licheniformis spores by iodophor, hypochlorite and QAC germicides.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving organisms*				
				30 sec	2 min	5 min	10 min	20 min
Iodophor A	200	distilled	2.22	TNC	TNC	TNC	TNC	TNC
	200	USDA**	2.22	TNC	TNC	TNC	TNC	
Iodophor B	200	distilled	1.73	TNC	TNC	TNC	TNC	TNC
	200	USDA	1.80	TNC	TNC	TNC	TNC	TNC
Iodophor C	200	distilled	2.11	TNC	286	39	11	0
	200	USDA	2.39	TNC	120	39	13	6
Iodophor D	200	distilled	2.29	TNC	TNC	TNC	TNC	TNC
	200	USDA	2.41	TNC	TNC	TNC	TNC	TNC
Iodophor E	200	distilled	2.38	TNC	TNC	TNC	TNC	TNC
	200	USDA	2.61	TNC	TNC	TNC	TNC	TNC
Hypochlorite	200	distilled	8.23	TNC	TNC	TNC	16	0
	200	USDA	8.80	TNC	TNC	TNC	TNC	TNC
QAC A	300	distilled	2.30	TNC	TNC	TNC	TNC	TNC
	300	USDA	6.99	TNC	TNC	TNC	TNC	TNC
QAC B	300	distilled	7.00	TNC	TNC	TNC	TNC	TNC
	300	USDA	7.20	TNC	TNC	TNC	TNC	TNC

* Initial number of cells, 3×10^6 per ml.

** U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Tables 10 through 14 show the destruction rates of an iodophor, a hypochlorite and a quaternary ammonium compound against organisms inoculated on polished metal strips.

Iodophor (Table 10) at 25 ppm substantially reduced the number of viable E. coli cells after 300 seconds. At 50 ppm, the time required for similar destruction was reduced to 60 seconds.

Hypochlorite at 50 ppm showed similar results as the iodophor used at that same concentration. At 100 ppm, hypochlorite caused substantial destruction of E. coli cells after 30 seconds and total destruction was seen after 300 seconds.

Quaternary ammonium compound (Table 10) at 50 ppm was ineffective against E. coli. At higher concentrations (200 ppm), significant destruction was obtained only in distilled water after 300 seconds.

Iodophor (Table 11) at 50 ppm caused 100 percent destruction of Pseudomonas aeruginosa after 60 seconds when used in distilled water. Hypochlorite was similarly effective against this organism. QAC showed no germicidal activity against Pseudomonas.

Iodophor and hypochlorite (Table 12) caused similar destruction rates against Staphylococcus aureus causing complete kill after 300 seconds. The QAC also effected 100 percent kill against S. aureus at 200 ppm after five minutes exposure in distilled water. The same concentration in hard water was not effective.

Yeast cells (Table 13) were effectively destroyed by iodophor and hypochlorite after 60 seconds. QAC caused 100 percent destruction when used at higher concentrations (200 ppm) in distilled water.

Iodophor at 50 ppm (Table 14) destroyed all Salmonella cells after a five minute exposure in distilled water. It was less effective in hard water. Hypochlorite at 100 ppm destroyed all cells after five minutes exposure in distilled and hard water. The QAC showed substantial reduction of S. derby cells only when used at 200 ppm in distilled water. QAC was totally ineffective in hard water.

Table 10. Destruction of Escherichia coli on metal strips.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving cells			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	distilled	3.10	TNC	318	274	6
	25	USDA	4.15	TNC	TNC	TNC	2
	50	distilled	2.70	TNC	TNC	6	2
	50	USDA	3.38	TNC	TNC	37	10
Hypochlorite	50	distilled	6.91	TNC	8	4	4
	50	USDA	7.51	TNC	TNC	53	4
	100	distilled	8.14	17	2	1	0
	100	USDA	8.43	19	6	2	0
QAC B	50	distilled	8.27	TNC	TNC	TNC	256
	50	USDA	8.85	TNC	TNC	TNC	TNC
	200	distilled	7.55	TNC	TNC	431	16
	200	USDA	8.60	TNC	TNC	TNC	TNC

Number of cells per ml in suspension used to inoculate strips, 23×10^8.

Table 11. Destruction of Pseudomonas aeruginosa on metal strips.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving cells			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	distilled	2.95	TNC	TNC	TNC	61
	25	USDA	4.24	TNC	TNC	TNC	22
	50	distilled	2.70	TNC	55	0	0
Hypochlorite	50	distilled	7.30	59	19	62	0
	50	USDA	7.70	TNC	105	58	0
	100	distilled	8.18	12	8	20	0
	100	USDA	8.70	186	22	3	0
QAC B	50	distilled	7.29	TNC	TNC	TNC	TNC
	50	USDA	8.60	TNC	TNC	TNC	TNC
	200	distilled	6.85	TNC	TNC	TNC	TNC
	200	USDA	8.80	TNC	TNC	TNC	TNC

Number of cells per ml in suspension used to inoculate strips, 6×10^9.

Table 12. Destruction of Staphylococcus aureus on metal strips.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving cells			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	distilled	3.00	TNC	TNC	274	0
	25	USDA	4.80	TNC	TNC	TNC	TNC
	50	distilled	2.70	TNC	TNC	373	62
	50	USDA	3.49	TNC	TNC	TNC	0
Hypochlorite	50	distilled	7.70	TNC	TNC	13	0
	50	USDA	8.20	TNC	TNC	237	0
	100	distilled	8.50	225	95	21	0
	100	USDA	8.80	171	31	15	0
QAC B	50	distilled	7.09	TNC	TNC	TNC	TNC
	50	USDA	8.83	TNC	TNC	TNC	TNC
	200	distilled	6.87	TNC	TNC	TNC	0
	200	USDA	8.33	TNC	TNC	TNC	TNC

Number of cells per ml in suspension used to inoculate strips, 29×10^8.

Table 13. Destruction of yeast of the genus Candida on metal strips.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving cells			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	distilled	2.90	TNC	8	20	6
	25	USDA	4.31	TNC	TNC	TNC	0
	50	distilled	2.62	TNC	14	17	0
	50	USDA	3.38	116	9	0	0
Hypochlorite	50	distilled	7.35	TNC	4	0	0
	50	USDA	7.50	151	4	2	0
	100	distilled	8.25	25	5	0	0
	100	USDA	8.61	8	1	0	0
QAC B	50	distilled	7.80	TNC	TNC	TNC	51
	50	USDA	8.60	TNC	TNC	TNC	39
	200	distilled	6.85	TNC	TNC	130	0
	200	USDA	8.59	TNC	TNC	148	10

Number of cells per ml in suspension used to inoculate strips, 6×10^8.

Table 14. Destruction of Salmonella derby on metal strips.

Germicide	Conc. ppm	Test water	pH of germicide solution	Average number of surviving cells			
				15 sec	30 sec	60 sec	300 sec
Iodophor E	25	distilled	2.90	TNC	TNC	122	3
	25	USDA	4.00	TNC	TNC	TNC	25
	50	distilled	2.70	TNC	TNC	245	0
	50	USDA	3.11	TNC	TNC	TNC	16
Hypochlorite	50	distilled	7.25	TNC	76	206	5
	50	USDA	7.90	TNC	TNC	TNC	12
	100	distilled	7.81	219	164	14	0
	100	USDA	8.40	TNC	TNC	39	0
QAC B	50	distilled	7.85	TNC	TNC	TNC	TNC
	50	USDA	8.65	TNC	TNC	TNC	TNC
	200	distilled	6.61	TNC	TNC	TNC	30
	200	USDA	8.60	TNC	TNC	TNC	TNC

Number of cells per ml in suspension used to inoculate strips, 14×10^8.

RANGE OF GERMICIDAL ACTIVITY

At low concentrations (12.5 ppm) iodophor A, shown in figure 1, totally destroyed most bacteria within 15, 30, and 60 seconds. Staphylococcus aureus and Streptococcus faecalis were the only bacteria not totally destroyed within 300 seconds.

Figure 2 shows the inefficiency of iodophor B at 12.5 ppm to effectively destroy cells. It was only effective against Micrococcus luteus and Candida, causing total destruction within 15 seconds in distilled water. Iodophor C (Figure 3) at low concentrations was effective against most cells when used in distilled water but was totally ineffective in the presence of hard water (500 ppm $CaCO_3$). This was also noted when this compound was used in higher concentrations. Iodophor D and E, figure 4 and 5, showed similar destruction rates at low concentrations killing all cells in most cases within 15, 30, and 60 seconds. Staphylococcus aureus, Streptococcus aureus, and Streptococcus faecalis were more resistant at these concentrations. The latter was more susceptible in the presence of hard water.

Hypochlorite as shown in figure 6 showed high destruction rates at low concentrations (12.5 ppm) of all bacteria except Staphylococcus aureus and yeast cells.

Figure 7 shows the inefficiency of the basic preparation of quaternary ammonium compound as compared with the iodophors and hypochlorite. QAC A, not shown, was totally ineffective at 25 ppm.

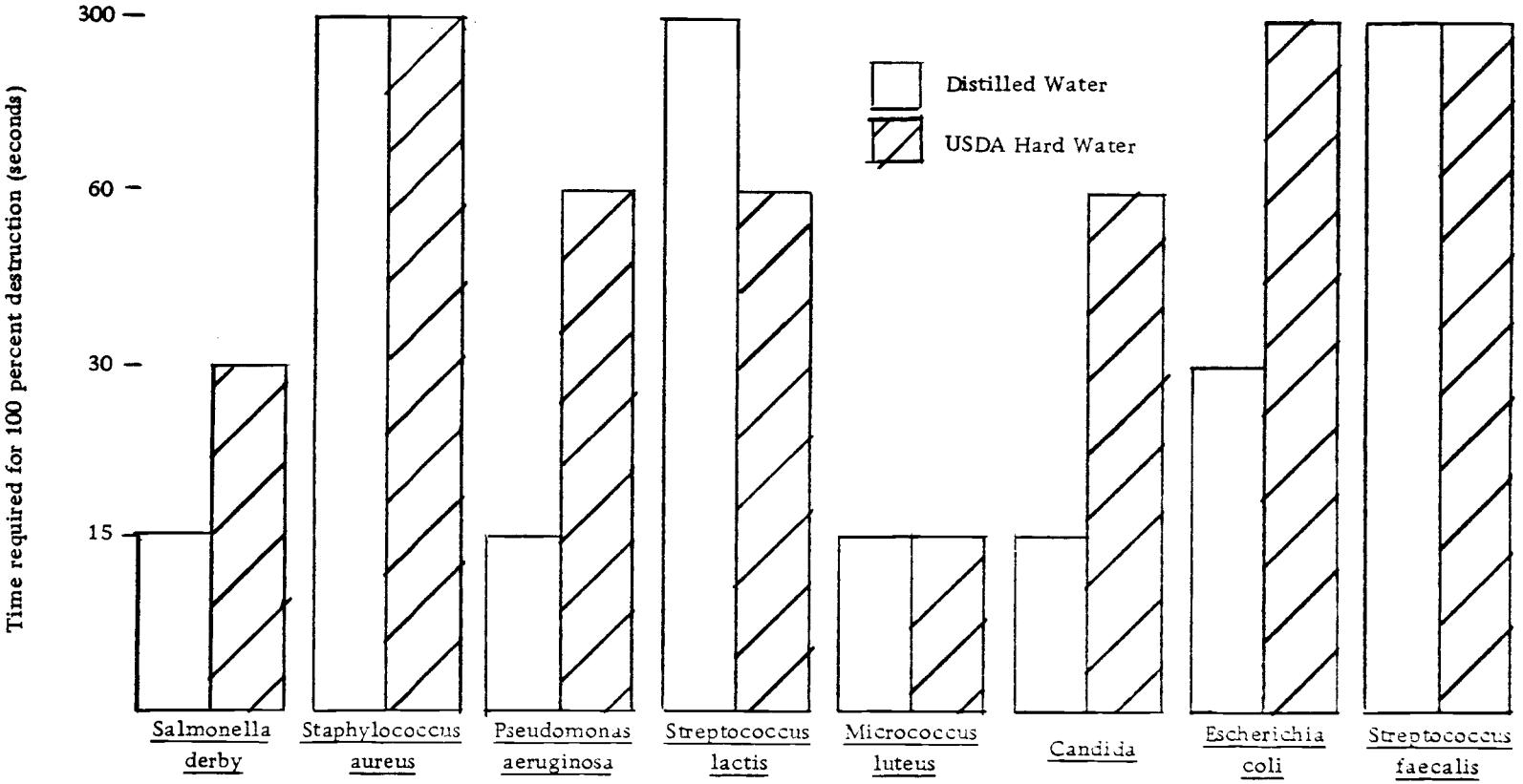

Figure 1. Relative destruction rates of 12.5 ppm iodophor A against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

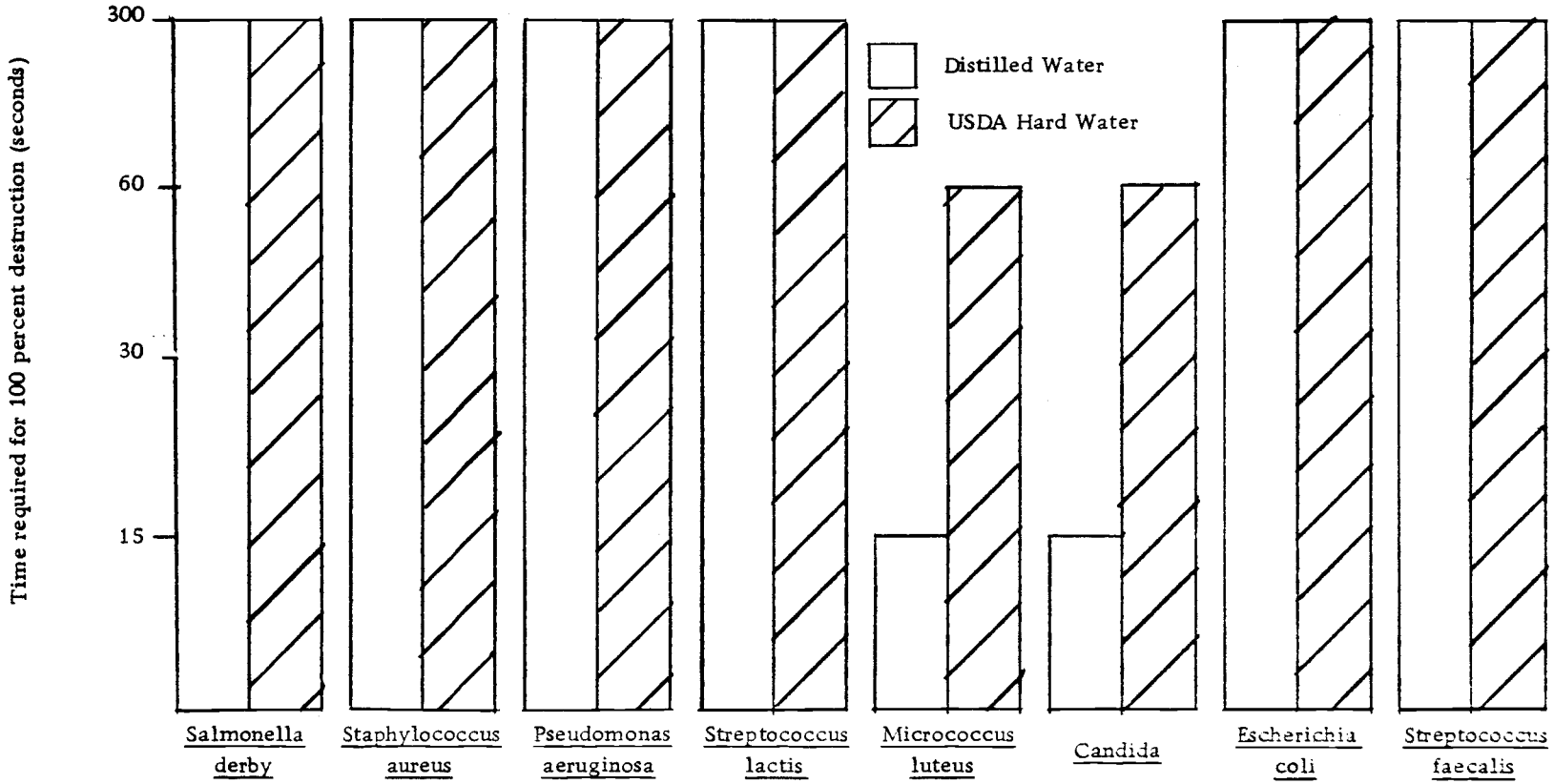

Figure 2. Relative destruction rates of 12.5 ppm iodophor B against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

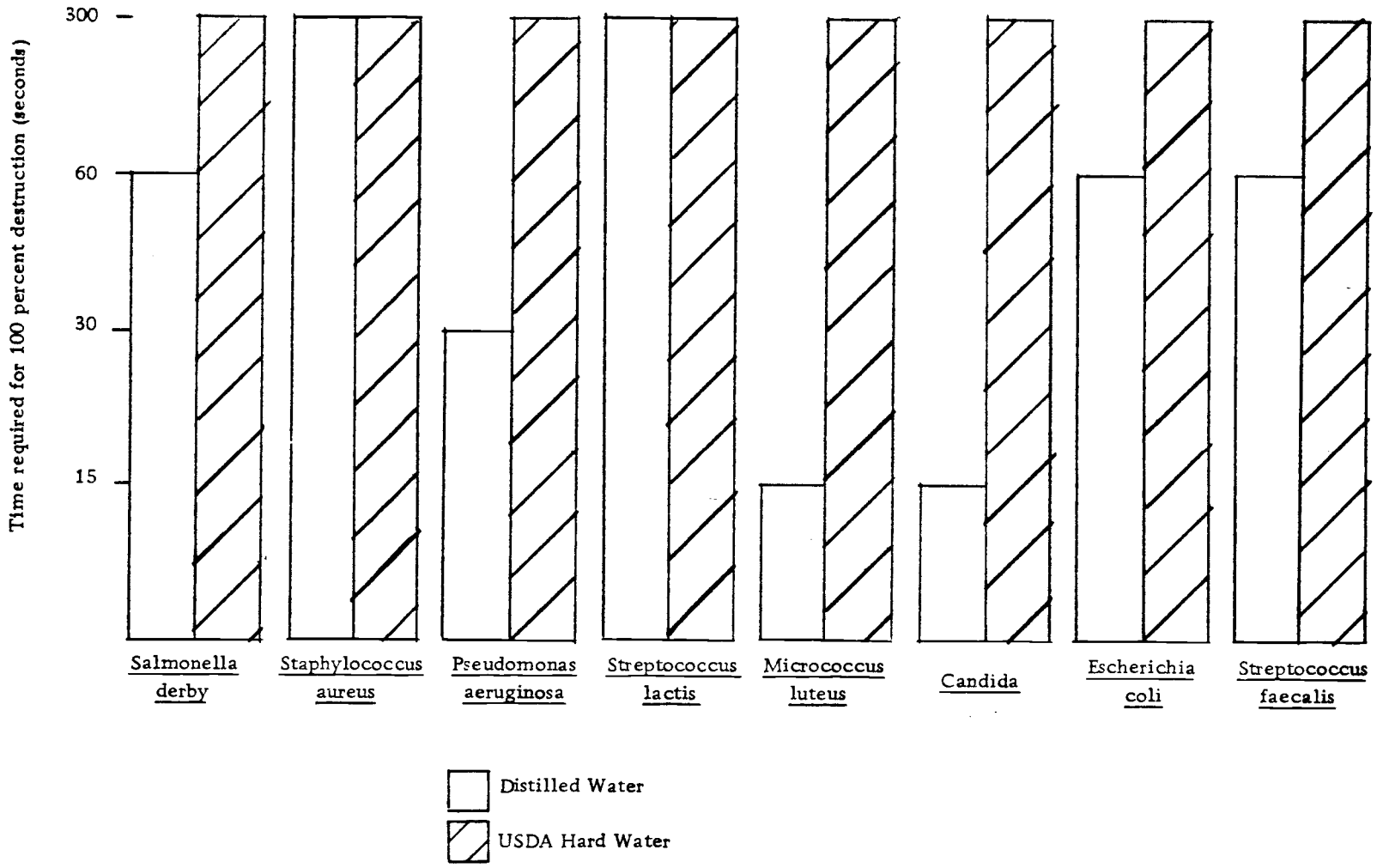

Figure 3. Relative destruction rates of 12.5 ppm iodophor C against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

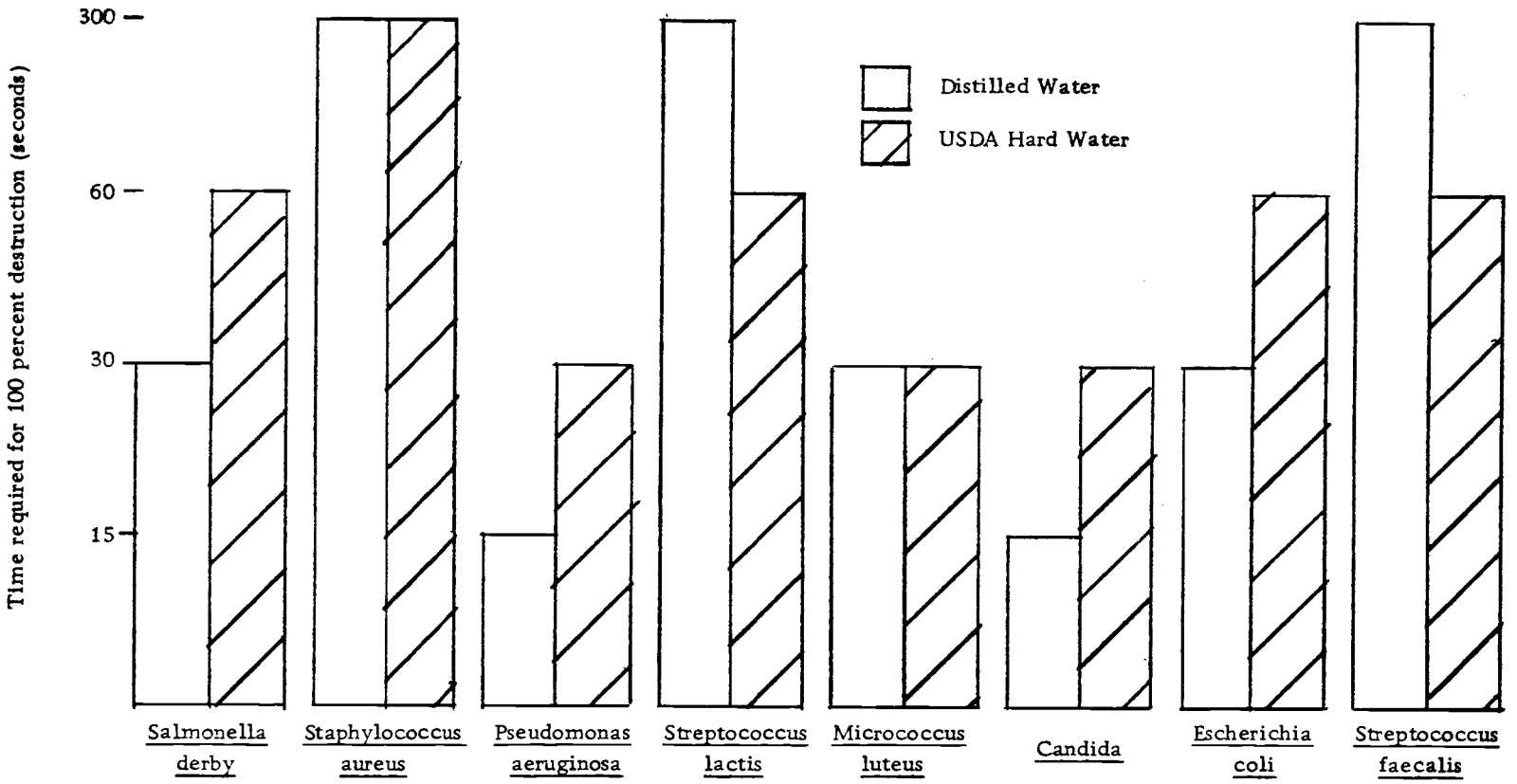

Figure 4. Relative destruction rates of 12.5 ppm iodophor D against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

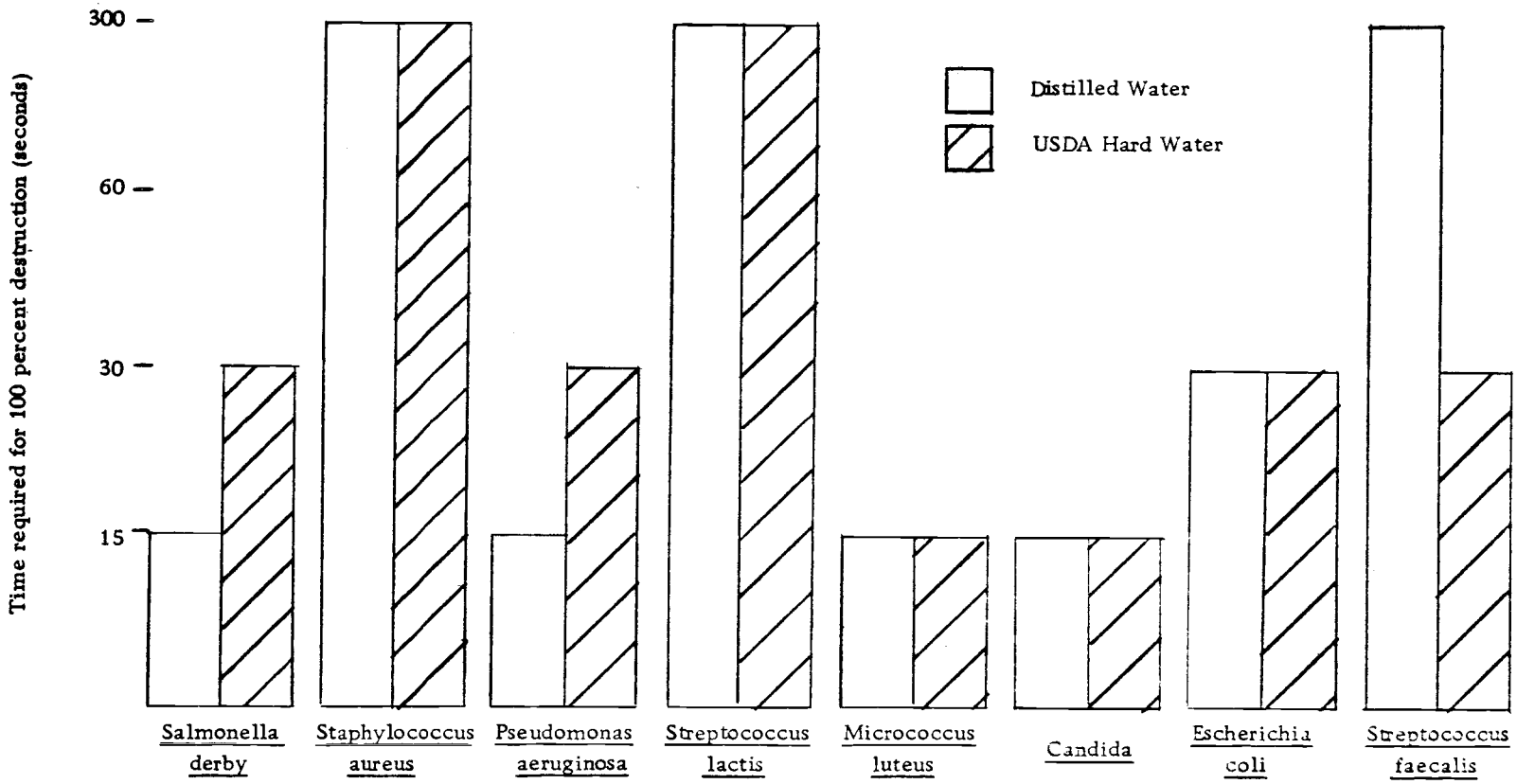

Figure 5. Relative destruction rates of 12.5 ppm iodophor E against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

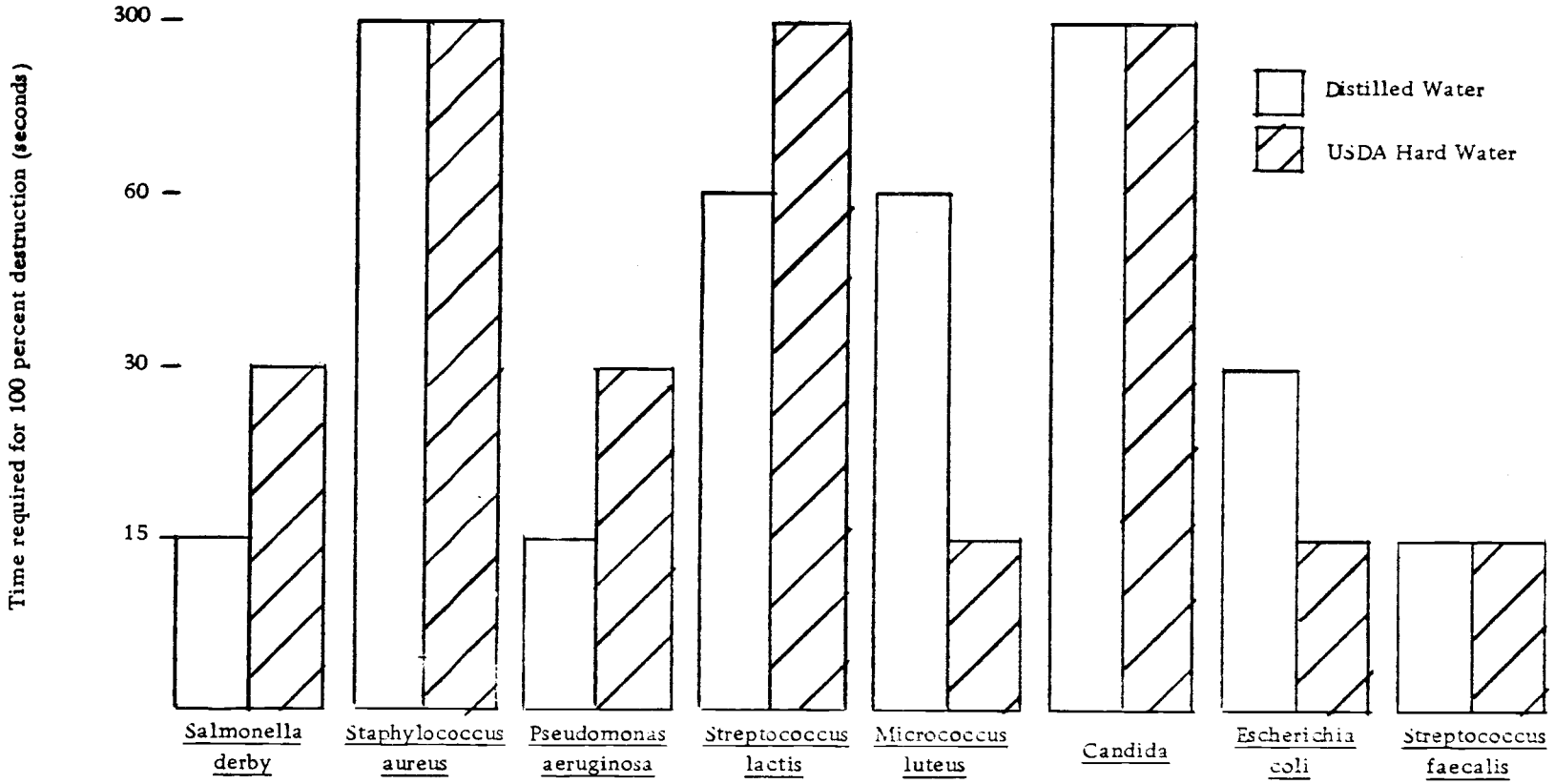

Figure 6. Relative destruction rates of 12.5 ppm hypochlorite against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

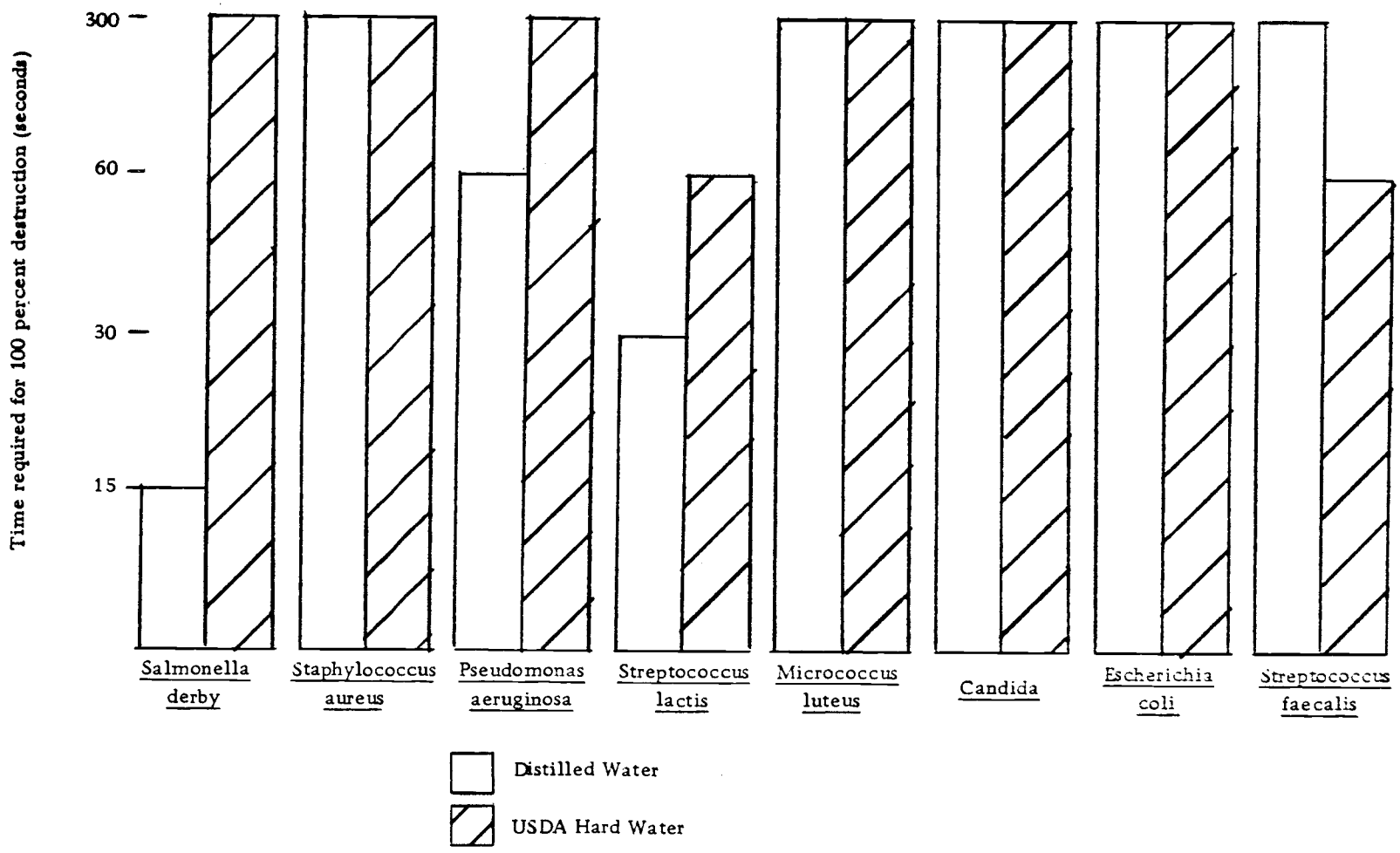

Figure 7. Relative destruction rates of 25 ppm QAC B against vegetative cells in distilled and USDA hard water. (Survival rates beyond 300 seconds not determined)

DISCUSSION

The eight different germicides used in this study consisted of widely used commercial products and therefore were representative of common types available on the market. Three of the iodophors tested (Iodophor A, Iodophor B, and Iodophor C) were obtained from manufacturers in New Zealand. The two domestic iodophors (Iodophor D and Iodophor E) showed high destruction rates in all tests. Both were very effective against all vegetative cells except Staphylococcus aureus which was the most resistant bacterial culture tested. Iodophor A showed a similar bactericidal activity. Iodophor C was also effective when used in distilled water but in almost every test this compound was totally ineffective in USDA hard water (500 ppm $CaCO_3$). The least effective of the iodophors used was Iodophor B. The iodophors were more effective against Streptococcus faecalis when used in USDA hard water. Streptococcus lactis in some cases showed similar results. Reasons for the differences in domestic and New Zealand iodophors are not readily apparent. Since iodine content and pH in use dilution of the different iodophor preparations approximate each other, the difference in germicidal activity may be related to nonionic surface active agent complexes with iodine and consequently rates of release of iodine in use-dilution.

The hypochlorite was very effective against most vegetative cells causing complete destruction in less than five minutes of all bacterial cells except Staphylococcus aureus. The iodophors exhibited a consistently greater rate of destruction of yeast cells than the hypochlorite. Possibly, the larger cell mass of yeasts creates a greater chlorine demand that had to be satisfied before the chlorine could

destroy the yeast cells. The iodophor in low concentration may be highly effective against yeast because of the rapid rate of destruction of specific enzymes such as glucose-6-phosphate dehydragenase presumably by oxidation of sulfhydryl groups. Hypochlorite and Iodophor C were the only germicides tested that were effective against spores. At 200 ppm in distilled water, both caused complete destruction after a 20 minute exposure period.

Quaternary ammonium compounds (QACs) were the least effective germicides used in this study. At higher concentrations (25, 50 and 200 ppm) the QACs, in most cases, showed significant bactericidal activity only at the highest concentration. Two QACs were tested, QAC B a basic quaternary ammonium compound and QAC A a highly acidic preparation. QAC B was significantly affected by the presence of water hardness (500 ppm $CaCO_3$), particularly against the gram-negative bacteria Pseudomonas, a food spoilage organism, and Escherichia coli an indicator of fecal contamination. Salmonella, a very important foodborne pathogen also showed resistance to QAC B when used in USDA hard water. At 50 ppm it was totally ineffective and at 100 ppm, five minutes exposure was required to kill all Salmonella cells. The results on Salmonella are considered highly significant because of the importance of this type of organism and related species in food products. Some recommendations have been made to use QACs for sanitization in meat and poultry processing plants and it is apparent that the hypochlorite and iodophors should provide more efficient destruction of Salmonella and related species than the QAcs.

The same pattern of destruction against bacteria was observed as has been reported in other investigations. In general, the more active iodophors showed the same order of activity as the hypochlorite used. QACs were affective primarily against the gram positive organisms and relatively ineffective against gram negative types of bacteria. Reasons for these differences have never been fully explained by research to date.

As reported by Soike, Miller, and Elliker (27), the pH of germicide solution markedly affects rates of destruction of different organisms by quaternary ammonium compounds. The pH levels in this study for USDA hard water and distilled water differed up to four pH units. This could account for the varying results seen with the QAC compounds. QAC A demonstrated similar results reported by Elliker (8) that most quaternary ammonium compounds tend to demonstrate greater germicidal activity in alkaline than in acid solution against certain gram positive bacteria. In almost every test this compound showed a higher germicidal rate in USDA hard water. QAC A was overall less effective than QAC B. Possibly the greater activity of acid QAC in hard water solution was related to the affect on hard water ions which ordinarily tend to neutralize QAC activity against microorganisms.

Such information does enable adjustment of quaternary solutions for greater activity in specific applications where a particular type of organism may present a problem in destruction.

SUMMARY

Representative organisms that are important as food spoilers, indicators of fecal contamination, and pathogens were selected for this test. All organisms were exposed to various concentrations of iodophors, hypochlorite, and quaternary ammonium compounds in both distilled and USDA hard water (500 ppm $CaCO_3$). The exposure time varied for vegetative cells from 15 to 300 seconds and the spore suspensions were tested up to 20 minutes.

The germicides used in this study represent widely used products available on the market. Three of the iodophors tested were obtained from New Zealand and represent products that are widely used for sanitation purposes in that country.

The iodophors exhibited high destruction rates against the vegetative cells. Iodophor B was the least effective of these compounds. Staphylococcus aureus was particularly resistant to all iodophors and Streptococcus faecalis was somewhat less resistant. Iodophor C was significantly affected by the presence of hard water salts (500 ppm $CaCO_3$) and in every case was totally ineffective when used in such solutions. The other iodophors were much less affected by water hardness and when tested against Streptococcus faecalis appeared to have been potentiated when used in hard water. Similar results were also seen with Streptococcus lactis.

The hypochlorite showed similar high destruction rates against most vegetative cells causing complete destruction in less than five minutes of all cells except Staphylococcus aureus. It was less

effective than the iodophors in destroying yeast cells. This could possibly be due to the larger cell mass requiring a higher chlorine demand.

Two quaternary ammonium compounds were tested. QAC B was a basic preparation and QAC A was highly acidic. They were the least effective of the germicides studied. QAC A was less effective than QAC B and showed greater bactericidal activity when used in USDA hard water. QAC B was adversely affected by water hardness.

Hypochlorite and Iodophor C were the only germicides tested that caused significant destruction of spores. At 200 ppm in distilled water, both caused complete destruction after 20 minutes exposure.

A procedure to more closely simulate actual use of germicides in food and dairy industries was developed in this study. Special polished metal strips were inoculated with organisms and exposed to an iodophor, a hypochlorite, and a quaternary ammonium compound. The iodophor and hypochlorite provided appreciable destruction in almost every case after a five minute exposure period. The QAC, used at much higher concentrations (200 ppm), was significantly less effective against organisms tested in these trials. Results with the new technique appear to offer promise of a superior method of evaluating germicides for sanitization of food handling equipment and utensils.

With improvements, this procedure for evaluating germicides could be of significance to the food and dairy industries. It simulates more closely actual operating conditions for plant equipment and utensils where surfaces have to be cleaned and sanitized. Possible improvements would be to standardize the number of organisms used in

the inoculating suspension and to develop a better method of removing the organisms from the strips.

This method also has the advantage of using concentrations of germicides actually employed to sanitize metal surfaces of equipment and utensils and therefore, provides a more accurate picture of effects of germicides under actual operating conditions. Tests run on lower levels than are used in plant practice always leave some doubt as to efficiency of germicides under actual use conditions.

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