

A Practical Approach to Evaluation of the Germicidal Efficiency of a General Purpose Military Disinfectant

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ABSTRACT

ROGERS, M. R. (U. S. Army Quartermaster Research and Engineering Center, Natick, Mass.), J. T. MAHER, AND A. M. KAPLAN. A practical approach to evaluation of the germicidal efficiency of a general purpose military disinfectant. *Appl. Microbiol.* 9:497-501. 1961.—The bactericidal activity of a general purpose disinfectant consisting of 25% sodium-*o*-phenylphenolate and 75% sodium-4- and 6-chloro-2-phenylphenolate was evaluated by a simulated in-use, surface-square dilution method. Common floor (asphalt, rubber, and unglazed tiles) and wall (stainless steel tile, ceramic tile, and painted wood) surfaces of various porosities and compositions were selected to simulate actual-use conditions.

The method used consisted of inoculating the surfaces of 1-in. square sections of floor and wall covering with a test organism, air-drying the inoculated surface, applying the disinfectant, allowing it to act for 10 min, and recovering the survivors by plating. Confirmatory results of the standard phenol coefficient and use-dilution tests indicated 700 ppm of the disinfectant to be a safe use concentration. The in-use surface-square dilution studies have shown that this is a more than adequate safe concentration for stainless steel, both glazed and unglazed ceramic tile, and nonwaxed surface of asphalt tile. However, concentrations ranging between 2,500 and 6,000 ppm for plastic-fortified rubber tile, 1,500 and 2,000 ppm for waxed asphalt tile, and 2,000 ppm for painted wood were required to achieve 99.9% reduction of either *Salmonella choleraesuis* or *Salmonella schottmuelleri*. These results indicate that a disinfectant concentration derived from the Association of Official Agricultural Chemists use-dilution test cannot always be relied upon to provide a dependable index to actual safe use-dilution when a disinfectant is supplied to certain wall or floor surfaces.

A previous study of the development of a general purpose disinfectant (GPD)¹ for military use (Mizuno, Rogers, and Kaplan, 1958 and 1959), was limited to reporting the destruction of the bacteria in the liquid

¹ GPD consists of 25% sodium-*o*-phenylphenolate and 75% sodium-4- and 6-chloro-2-phenylphenolate.

portion of the contents of field latrine buckets. Because this disinfectant was intended to be a general military "household" disinfectant, most of the present investigation is concerned with disinfecting commonly used floor and wall surface materials. These surfaces were selected to simulate, as far as practical, actual-use conditions. Surfaces of various porosities and compositions were used to determine the relative efficacy of the phenol coefficient and use-dilution confirmation tests as indices for practical disinfection.

It is now accepted that the phenol coefficient alone cannot be relied upon as a guide in preparing a safe use-dilution of phenolic disinfectants. The "use-dilution" procedure (Stuart, Ortenzio, and Friedl, 1953) was developed to check the validity of the phenol coefficient values and employs the use of stainless steel carriers. The present study concerns the development of a procedure in which commonly used floor and wall surfaces are substituted for the stainless steel carriers used in the "use-dilution" method.

Several methods have been proposed for a simple and reliable procedure to determine quantitatively the sanitary condition of various inanimate surfaces after disinfection. These procedures included various swabbing techniques, agar-contact methods, rinsing processes, tracer techniques, and in-use testing. Examples of swabbing techniques are described by the American Public Health Association (1949), Barnes (1952), Cain and Steele (1953), Higgins (1950), Buchbinder et al. (1947), Tiedeman et al. (1948), and Angelotti et al. (1958). Agar-contact methods were used by Barton, Gorfein, and Carlo (1954), Guiteras, Flett, and Shapiro (1954), Hammer and Olson (1931), Walter and Hucker (1941), and Angelotti and Foter (1958). Rinsing techniques were used by Angelotti and Foter (1958), American Public Health Association (1953), and Stedman, Kravitz, and Bell (1954*a, b* and 1955*a, b*). The isotope-tracer techniques were used by Ridenour (1952), Ridenour et al. (1952), Armbruster and Ridenour (1952), and Ridenour and Armbruster (1953). In-use testing of bactericidal agents in hospitals was described by Kundsinn and Walter (1961). Unquestionably, each of these methods had its advantages and disadvantages; therefore, it is important to select the procedure that best serves the purpose of the individual

(Walter, 1955). The procedure described in this study is an attempt to adapt the rinsing techniques to assist in obtaining more reliable information from the "use-dilution" method in evaluating the efficiency of the GPD.

MATERIALS AND METHODS

General procedure. The method of the present study, designated as the surface-square dilution method, consists of (i) inoculating the surfaces of 1-in. square sections of floor and wall coverings with a test organism, (ii) air-drying the inoculated surface, (iii) applying GPD, (iv) allowing 10 min for reaction, and (v) recovering the survivors by plating.

During the initial phase of this study, tests were run to determine a cell concentration in a broth suspension that would withstand drying, and allow countable controls without the necessity of further dilution. The number of organisms on the control plates was counted when 0.01 ml of an 18-hr undiluted broth culture was diluted with 1 ml of broth, and a 0.01-ml aliquot of the resultant suspension was used as the surface inoculum. Using this technique, reproducibility was not as satisfactory as with an undiluted broth culture. Although the latter procedure was adopted as standard in this study, other preliminary tests were run in which squares were plated directly by pouring tryptone glucose extract agar over the squares. However, the method of rinsing the squares in buffered distilled water and plating an aliquot of the latter was superior, because it permitted more thorough swirling and agitation.

Test organisms. Because the germicidal action of a general household disinfectant must be nonspecific, different species of vegetative pathogens of epidemiological significance were chosen as test organisms. *Salmonella schottmuelleri* (ATCC 9282) was selected as a representative of the paratyphoid group for its superior resistance to drying (Klarmann, Wright, and Shternov, 1953). *Salmonella choleraesuis* (ATCC 10708) was also used in accordance with the Association of Official Agricultural Chemists (AOAC) procedure for the use-dilution confirmation test. Since comparable destruction of both organisms was found at the same disinfectant concentration, *S. choleraesuis* was used only for part of this study.

Test surface materials. Six different 1½- by 1½-in. squares of precleaned carriers were used: stainless steel, unglazed ceramic tile, glazed ceramic tile, rubber tile (plastic fortified), asphalt tile, and painted wood.² Each surface was lacquered around the edges so that the exposed surface measured 1 by 1 in. Ceramic and stainless steel surfaces were sterilized by subjecting them to 82 C for 2 hr. The rubber and asphalt tiles and the painted wood were exposed to a 30-w ultra-

violet germicidal lamp for approximately 1 hr. The lamp was used because heat sterilization without altering the original characteristics of the tiles is not possible.

Culture techniques. *S. schottmuelleri* and *S. choleraesuis* were cultured in nutrient broth consisting of 0.5% GB³ beef extract, 0.5% sodium chloride, and 1% Bacto-peptone⁴ for 18 to 24 hr at 37 C; next, a 0.01-ml aliquot of an undiluted broth suspension was spread uniformly on each square, using a 4-mm sterile platinum loop. All squares were then air-dried at room temperature in Petri dishes with the covers slightly open. Although this procedure did permit contamination, the use of a selective medium did not allow growth of airborne contaminants. Considerable variability was noted in the day-to-day drying times of the inoculum on unlike surfaces, and daily variations, though not as marked, likewise occurred for like surfaces.

The time for complete drying of the inoculum with the same square varied daily according to the temperature and humidity. When the relative humidity was low, the disinfecting solution tended to dry more rapidly than when the humidity was higher. All squares were used immediately when completely dry as shown by visual inspection. The ambient temperature and relative humidity were recorded.

Applying GPD concentrations. Various concentrations of the GPD were made up in distilled water, 0.04 ml of each concentration added to each of a pair of dry, seeded squares, and the GPD and organisms mixed thoroughly over the surface, using a platinum loop. The quantity of disinfectant (0.04 ml) used was the amount found by Klarmann et al. (1953) to be deposited on a unit floor area during a standardized mopping operation. All disinfectant levels were tested in duplicate. Sterile buffered distilled water⁵ was used on one square as a control instead of the GPD. The GPD dilutions were permitted to act for 10 min. Each square was then aseptically transferred to a 600-ml beaker containing 20 ml of sterile buffered distilled water and swirled for 1 min in an attempt to achieve homogeneity of cell distribution.

Plating and counting. Aliquots of 1 ml of the resultant suspension were plated in brilliant green agar, a highly selective medium recommended for the isolation of *Salmonella*, excepting *Salmonella typhosa* (Difco Manual, 1953). Dilution plate counts from the control were also made. Plates were incubated at 37 C for at least 48 hr, and colonies were counted with a colony counter.

Originally, 10 ml of a 1% aqueous Lecithin⁶ solution

³ General Biochemicals, Inc., Chagrin Falls, Ohio.

⁴ Difco Laboratories, Inc., Detroit, Mich.

⁵ Dissolve 34.0 g KH₂PO₄ in 500 ml distilled water, adjust to pH 7.2 with 1 M NaOH and make up to 1 liter with distilled water, add 1.25 ml of this stock buffer to 1 liter of distilled water and dispense.

⁶ Distillation Products Industries, Rochester, N. Y.

² Three coats of semigloss Olive Drab Enamel FS-TT-E-529 (no antimicrobial preservative) on balsa wood.

TABLE 1. *Effective concentrations for 99.9% reduction of test organisms after exposure for 10 min*

Surface	Concn	RH (%) / temp (F)	<i>Salmonella choleraesuis</i> colony count†		Per cent reduction†
			controls	test	
	ppm				
Stainless steel tile.....	350	36/81	4.4×10^5	5.0×10^2	99.886
Ceramic tile (unglazed).....	150	22/81.5	1.7×10^5	0	100.0
Ceramic tile (glazed).....	150	37/—*	3.0×10^5	0	100.0
Asphalt tile (waxed surface).....	1,500-1,750	21/83	3.4×10^5	1.9×10^2	99.944
Asphalt tile (nonwaxed surface).....	500-650	43/78	6.6×10^5	2.6×10^2	99.961
Rubber tile (plastic fortified).....	5,500-6,000	30/81.5	1.3×10^6	4.0×10^1	99.997

* Unknown.

† Values in these columns refer to highest concentration of GDP listed.

TABLE 2. *Ranges of effective concentrations of different batches of general purpose disinfectant (GDP) required to obtain 99.9% reduction of Salmonella schottmuelleri after exposure for 10 min*

Surface	<i>S. schottmuelleri</i>		
	A	B	C
	ppm	ppm	ppm
Stainless steel tile.....	150-200	150-200	150-200
Ceramic tile (unglazed) ..	150-200	150-200	150-200
Ceramic tile (glazed).....	150-200	150	150
Asphalt tile (waxed surface).....	1,750-2,000	—*	—*
Asphalt tile (nonwaxed surface).....	650	500	500
Rubber tile (plastic fortified).....	6,000	2,500-3,000	2,000
Painted wood.....	—*	—*	1,800-2,000

A = Small batch, Economics Laboratory, St. Paul, Minn.

B = Same as A except this material was dry because of tight seal and non-use, whereas A was black and tacky due to frequent exposure to atmosphere (2½ years old when first used).

C = Preproduction sample no. 3, Scientific Oil Compounding Company, Chicago, Ill., 28 October 1957 (2 weeks old when first used).

* — = Not tested.

and 10 ml of Tween 20⁷ were incorporated into the medium to inactivate the carried-over GDP. Although Lecithin-Tween mixtures are routinely used to inactivate quaternary ammonium compounds, it was also found effective in inactivating the phenols in the GDP (Wilson and Mizuno, 1951; Mizuno et al., 1958 and 1959). It was found, however, that the dilution of the squares in the diluent, coupled with the high organic load of the medium, was sufficient to inactivate any carry-over, even at high GDP concentrations.

RESULTS

Recommended concentrations. The actual concentration of the GDP recommended for use as a result of these studies is 2,000 ppm for general disinfection of most inanimate surfaces except the type of rubber

⁷ Polyoxyethylene sorbitan monolaurate, Atlas Powder Company, Wilmington, Del.

tile used in these studies. Because the overwhelming majority of Army buildings have floor surfaces other than rubber tile, disinfection of rubber tiled floors could be eliminated from consideration for disinfection. This 2,000 ppm concentration will satisfactorily reduce bacteria on commonly used floor and wall surfaces, with a reasonable margin of safety (Tables 1 and 2).

Porosity. Several factors affecting bactericidal activity have been encountered throughout the course of this study, including surface tension, composition and porosity of the test squares, humidity, and temperature.

Although it was not an expressed purpose of this study to correlate disinfection with porosity, it was noted that, although the same disinfectant concentration resulted in comparable destruction on both glazed and unglazed ceramic surfaces, the concentration required to disinfect the waxed surface of asphalt tile was more than twice that required to disinfect the nonwaxed surface of asphalt tile (Tables 1 and 2). In contrast, Stedman et al. (1955b) claimed, "the practice of waxing surfaces daily does not alter markedly the pattern of disinfectant efficiency obtained on the unwaxed porous surface." The reasons for this discrepancy are not known, but it was noted that the inoculum tended to concentrate in spherical droplets on waxed surfaces, which probably led to a more concentrated inoculum resisting disinfection. This may be due also to the clumping together of masses of organisms which would interfere with the penetration of the GDP to all of the organisms present. The medium itself also exerts a protective action.

Other factors affecting bactericidal activity. Among the observations of significance which were encountered include the following: (i) The presence of organic material appreciably reduces the bactericidal properties of the phenolics (Stuart et al., 1953; Klarmann and Wright, 1954). (ii) It is also obvious that a 4-mm loop, as used in this study, does not carry the same volume each time a surface is inoculated. Thus, when using the same concentrations of disinfectant, different amounts of broth will likely result in replicates having different numbers of survivors. This may account in part for the erratic disinfecting action that is occasionally demonstrated. According to other investigators

(Stedman et al., 1954a), squares disinfected with strong concentrations showed, on occasion, a relatively large number of survivors, even though subsequent weaker concentrations gave a higher percentage reduction. The use of replicate tests for calculating an average percentage reduction enhanced the reliability of the results. (iii) It has also been demonstrated that aging and atmospheric exposure have no effect on the germicidal capacity of the laboratory-prepared GPD, and a pilot-plant sample does not differ in germicidal capacity, except in the case of plastic-fortified rubber tile (Table 1). This exception is probably the result of incomplete dissolution of the original laboratory-prepared sample at concentrations of the magnitude of 2,000 ppm and greater. (iv) During the course of these studies, no visible change could be detected in the surfaces evaluated when using the GPD at concentrations as high as 14,000 ppm.

DISCUSSION

The conventional method of arriving at the maximal safe use-dilution, presumed to be equivalent in efficiency to 5% phenol, is to multiply the phenol coefficient found (for *S. typhosa* or *Staphylococcus aureus*) by 20 to determine the number of parts of water in which one part of the disinfectant is to be incorporated. In addition, the phenol coefficient must be confirmed by the AOAC "Use-dilution" method. When the use-dilution can not be substantiated by this method, the highest dilution that will kill in the "use-dilution" procedure should be used as the guide to the highest dilution for use in practical disinfection.

According to the AOAC Use-Dilution procedure (Stuart et al., 1953), the maximal safe use-dilution for GPD was found to be 1:1,400 when using *S. choleraesuis* as the test organism and 1:660 when using *S. aureus* as the test organism. The 1:1,400 dilution confirms the validity of the phenol coefficient of 71 obtained with *S. typhosa* as the test organism since 1:1,400 is for all practical purposes the same as 1:1,420 ($20 \times 71 = 1,420$), 700 ppm, or 0.07%. The present studies have

shown that this is more than an adequate safe dilution to be applied to stainless steel, to both glazed and unglazed ceramic tile, and to the nonwaxed surface of asphalt tile. However, concentrations ranging between 2,500 and 6,000 ppm for plastic-fortified rubber tile, 1,500 and 2,000 ppm for waxed asphalt tile, and 2,000 ppm for painted wood were required to achieve in 10 min a 99.9% reduction of either *S. choleraesuis* or *S. schottmuelleri*.

The data reported in Table 2 were analyzed for statistical significance. Assuming a percentage reduction of 99.9, the probability that this is a chance occurrence and therefore not real is given in Table 3. In all except for one stainless steel test, the probabilities were very small.

The wide variation in the concentration of GPD required to reduce effectively microbial contamination on surfaces of different porosity indicates to some extent the inadequacies of test methods which do not attempt to duplicate the actual-use conditions in some reasonable manner. The use-dilution tests assist in providing phenol-coefficient confirmatory information on the safe use-dilution of chemical disinfectants which will provide a reasonable margin of safety for disinfecting inanimate surfaces. However, in some instances, relying on confirmatory "use-dilution" data can also provide an erroneous index of the true disinfecting power of a product, as noted with rubber tile in this study. From a practical standpoint, one must assume that the variation in the effective concentration of the disinfectant on various surfaces can become further complicated by ineffective janitorial procedures, which cannot be relied upon to reduce the amount of interfering organic matter to a low level. The frequency and quality of ordinary janitorial services in most public buildings, including hospitals, cannot be relied upon to provide the degree of cleanliness under which most disinfectants are evaluated in accordance with the procedures specified by the AOAC. Consequently, it is evident that there is a need for modifying the "use-dilution" test method to closely approach duplication

TABLE 3. Statistical significance of results reported in Table 2

Surface	A				B				C			
	Table 2 value		Next higher dilution		Table 2 value		Next higher dilution		Table 2 value		Next higher dilution	
	ppm	P	ppm	P	ppm	P	ppm	P	ppm	P	ppm	P
Stainless steel tile.....	150	1.00*	200	<0.001	150	<0.001	200	<0.001	150	<0.001	200	<0.001
Ceramic tile (unglazed).....	150	<0.025	200	<0.001	150	<0.025	200	<0.001	150	<0.025	200	<0.001
Ceramic tile (glazed).....	150	<0.001	200	<0.001	150	<0.001	200	—	150	<0.001	200	—
Asphalt tile (waxed).....	1,750	<0.001	2,000	<0.001	—	—	—	—	—	—	—	—
Asphalt tile (unwaxed).....	650	<0.001	850	<0.001	500	<0.025	—	—	—	—	—	—
Rubber tile (plastic fortified).....	6,000	<0.001	6,500	<0.001	2,500	<0.001	3,000	<0.001	2,000	<0.001	2,500	<0.001
Painted wood.....	—	—	—	—	—	—	—	—	2,000	<0.001	2,500	<0.001

A, B, C, see legend of Table 2. — = Not tested. P = Level of probability.

* Not significant.

of use conditions, as well as to obtain more representative data on the effect of a disinfectant formulation on the more common inanimate surfaces used in routine disinfecting practices in order to provide a more realistic safe use-dilution. One possible approach to this problem is to supplement the stainless steel ring carriers used in the AOAC "use-dilution" test (Stuart et al., 1953) with specified dimensional pieces of the more common surface materials, such as the various tile surfaces used in this study, and subject them to disinfection to simulate the actual conditions of practice.

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