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PROJECT TERMINATION

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FINAL REPORT

PROJECT B-277

INVESTIGATION OF RESIDUAL SURFACE DISINFECTION

By

W. R. TOOKE, JR. and T. W. KETHLEY

GRANT NUMBER EF 00327-02

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PERSISTENCE OF SURFACE DISINFECTANTS

by

W. R. Tooke, Jr. and T. W. Kethley

Introduction

Surface disinfectants can play an essential role in the prevention of reinfections arising from the spread of pathogenic organisms deposited on floors, walls, and furnishings. Technologists have recognized for many years the special value of a surface disinfectant which is capable of yielding a long-term antibacterial potential. Much research has been pursued on this subject, testing methods have been proposed, and products of varying merits have been developed.

We have not undertaken in our own laboratories extensive experimental evaluations of existing products or methods, but have reviewed carefully much of the literature in this field. The principal conclusions derived from this review were:

1. A working theory of residual surface disinfection in terms of mass transport phenomena has not been applied to the problem.

2. Much of the previous work has been directed towards demonstrating long periods of residual effects (days, weeks, months) and relatively slow killing times (usually hours).

The lack of a theoretical model has necessitated a largely empirical or trial and error approach to the formulation of residual surface disinfectants. The progress that has been made is a tribute to the "persistence" of the

investigators. Continued empirical research, at best, could be expected to yield only marginal returns or incremental improvements.

The prior emphasis on extended persistence and the toleration of slow kills may have tended to "miss the mark" with respect to some of the most acute needs for residual surface disinfection. We shall discuss this in more detail later.

The foregoing conclusions from our review of prior work provided the basis for our research program. First, we should endeavor to develop a physical or theoretical model of residual surface disinfection; then, validate and perfect the model if possible. And finally, we should direct major attention to systems offering a potential for rapid kills of pathogens -- sacrificing, if necessary, some of the extended persistence that is not truly essential to certain important unfulfilled needs.

In this paper we will briefly describe a conceptual model of the mass transfer of toxic agents from a treated surface into a deposited bacterial aggregate. The effects of certain materials properties and environmental variables will be discussed qualitatively. In the light of this concept, we shall consider some of the practical limitations that are necessarily imposed upon a workable system. Performance objectives will then be formulated, and a preliminary test method described.

Model of Mass Transfer

Disinfectant in liquid solution is applied to a surface as a thin layer of perhaps 1 mil (0.001 in.) in thickness. Bacteria may be deposited in, on, and above this layer in various forms or aggregations. These may include droplets of liquid serum or fine aerosols, alone or supported on dust, lint,

etc. In any case, it is reasonable to anticipate that the form which is the least intimately attached to the surface is most likely to be resuspended in the atmosphere. It is to this essentially unattached bacterial aggregate that we would direct primary attention. For the purpose of our model, we shall assume that this particle is not in effective physical contact with the surface. It may be suspended on a fiber of lint at distances of the order of 20 mils or more from the surface. Obviously, any effective contact of lethal agent with this bacterial aggregate can be established only through vapor phase mass transfer. The physical situation is depicted in Figure No. 1.

We shall assume that the disinfectant solution consists of active, relatively volatile toxicant(s) and inert, relatively non-volatile extender(s). Referring to Figure 1, the mass transport requirements may be described qualitatively as follows:

1. Active toxicant moves by liquid-liquid diffusion under a concentration gradient to the surface of the disinfectant film.

2. At the surface the toxicant vaporizes with a resulting large increase in volume (decrease in concentration).

3. Toxicant vapor diffuses away from the surface through the air under a partial pressure gradient. Its concentration (partial pressure) drops as it becomes diluted with an increasing volume of air.

4. At the surface of the bacterial aggregate, which we shall assume to consist of partially dried proteinaceous material, a portion of the toxicant vapor condenses or absorbs into the liquid phase. The concentration at the surface of the aggregate rises toward equilibrium with the surrounding vapor.

5. The condensed vapor moves under a concentration gradient by liquid-liquid diffusion into the interior of the bacterial aggregate.

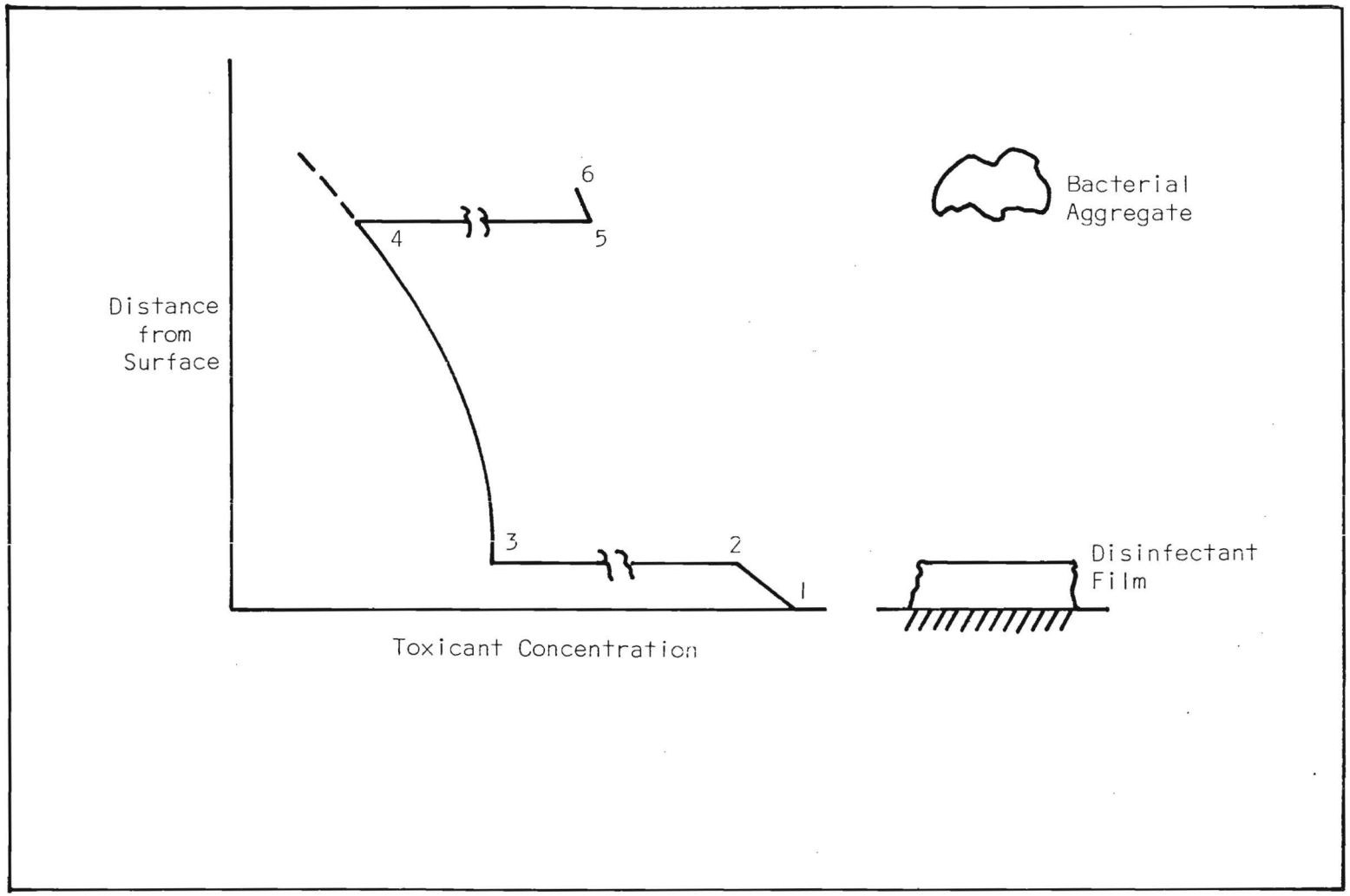


Figure 1. Concentration Profile.

6. The concentration of toxicant established within the bacterial aggregate depends upon:

(a) the partial pressure of toxicant in the surrounding vapor,

(b) the time of exposure, and

(c) the solubility of the toxicant in the bacterial aggregate.

If the biological activity of the attained concentration of toxicant is great enough, the bacterial cells are killed.

This is the essence of our model. We shall not undertake a rigorous mathematical analysis. However, it will be worthwhile to pursue the theory sufficiently to gain an appreciation of the relevant variables.

The theory of liquid-liquid diffusion is not well developed. The rate of steady state diffusion of solute through solvent (N_A) is given by

$$N_A = \frac{D_L}{Z_L} (c - c_i) \tag{1}$$

where D_L is the liquid-liquid diffusivity, Z_L is the film thickness and $(c - c_i)$ is the concentration gradient. Successful correlations of experimental data on dilue solutions have been attained using the Stokes-Einstein equation

$$D_L = \frac{T}{\mu F} \tag{2}$$

where T is the absolute temperature, μ is the solution viscosity and F is a function of the molecular volume of the solute.

Thus, we may observe from the two equations that the rates of transport of toxicant to the surface of the disinfectant film, and the penetration into

the bacterial aggregate are directly proportional to the absolute temperature and the concentration gradient and inversely proportional to the liquid film thickness, the solution viscosity, and the toxicant molecular volume.

The liquid-liquid transport in the two media (disinfectant film and bacterial aggregate) must be linked by interphase transport at each surface and vapor phase transport between the surfaces. The diffusion of a vapor (toxicant) through a stagnant gas layer (air) has been shown to follow the relation

$$N_A = \frac{D_v P}{RTZ_v p_{BM}} (p_i - p) \qquad (3)$$

where D_v is the diffusivity of toxicant vapor in air, P is the total pressure, p_i and p are the partial pressures of toxicant at the surface and at the extremity of the stagnant layer, Z_v is the thickness of the stagnant layer, and p_{BM} is a logarithmic means of the partial pressure of air between the extremities. R and T are the gas constant and absolute temperature. Gas diffusivities are frequently determined experimentally by application of Equation 3, or may be computed from kinetic theory.

Under steady state conditions, the diffusion rate of toxicant will be constant from the liquid surface to the extremity of the stagnant gas film, thus

$$N_A = \frac{D_L}{Z_L} (c - c_i) = \frac{D_v P}{RTZ_v p_{BM}} (p_i - p) \qquad (4)$$

(Liquid Phase) (Vapor Phase)

This equation states concisely the physical relationships involved. The magnitude of the driving forces for diffusion may be illustrated graphically by a curve representing the partial-pressure -- liquid-concentration equilibrium as shown in Figure 2. The position of the equilibrium curve depends upon the solubility of the toxicant in the liquid phase and its vapor pressure. Equilibrium conditions obtain at the phase boundaries. If point A represents the concentration, c, within the liquid at Z_L and the partial pressure, p, at Z_v, the gas extremity, and if point B is the composition at the interface (equilibrium), then the length of line AM represents the concentration driving force and the length of line BM represents the partial pressure driving force. The effects of the several variables may be inferred from the plot.

For a given system at stated film thicknesses (liquid and vapor), temperature and pressure, the position of the equilibrium line and the slope of line AB are fixed. In general, it may be shown that the mass flux, N_A, will increase with increasing liquid concentration, c, and decrease with increasing partial pressure at the extremity of the gas film, p. The concentration, c, might be altered by formulation design. The partial pressure, p, could be altered by changing the velocity and/or composition of air in a room. In either case, equilibrium conditions (c_i, p_i) and mass flux (N_A) would be changed in conformity with Equation 4.

If the toxicant is changed (a different compound), then both the diffusivities and the equilibrium curve will be altered. In general, a toxicant of higher vapor pressure will tend to increase the flux and partial pressure, p, at any given liquid concentration, c. Qualitatively, this effect may be seen to result in part from a leftward shift of the equilibrium curve, which increases both the concentration and pressure driving forces.

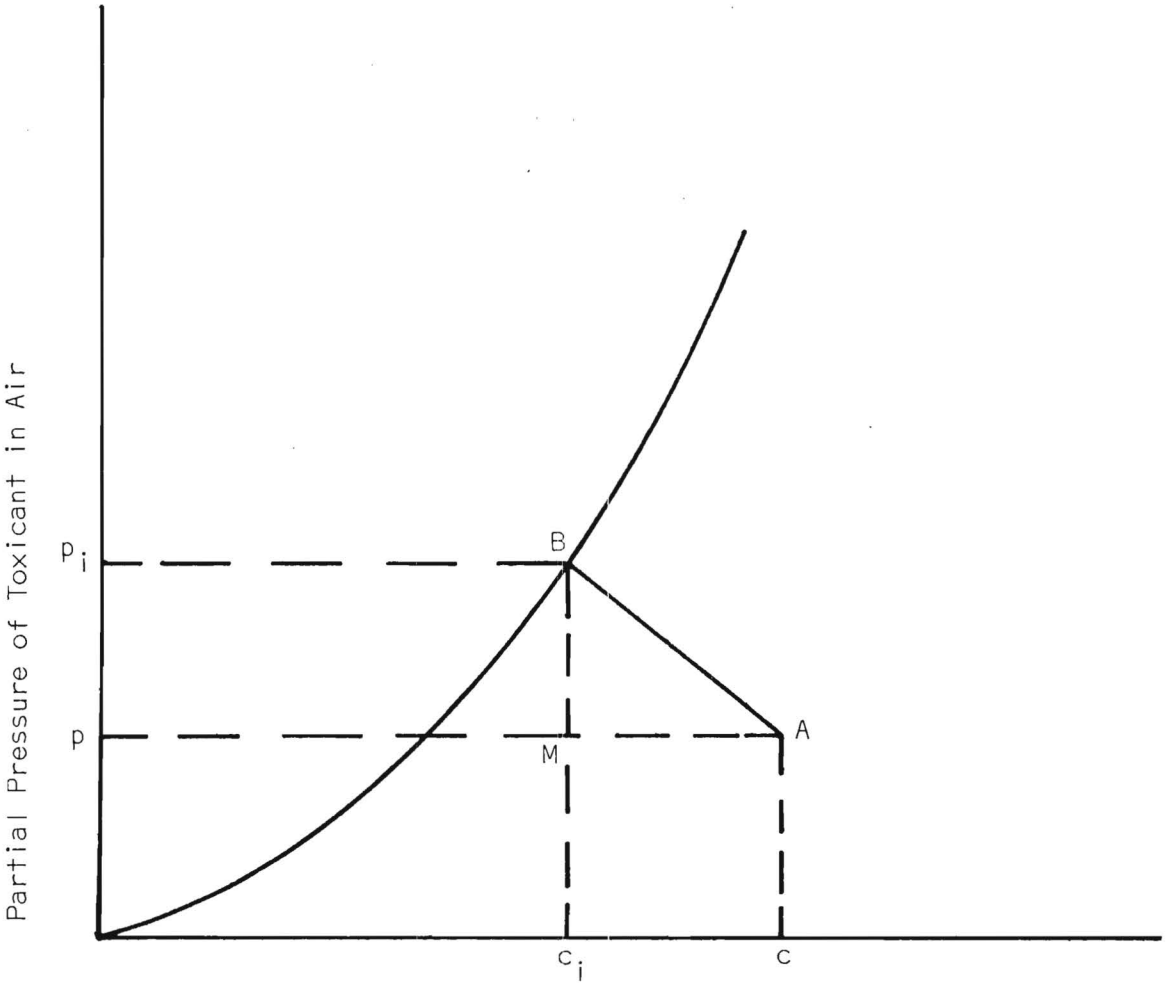


Figure 2. Driving Forces for Toxicant Evaporation.

Returning now to Figure 1, we see that, in principle, the mass transfer computations have permitted us to determine a partial pressure in the vapor phase at any level above the surface of the disinfectant film. A series of such computations defines the partial pressure profile.

The bacterial aggregate is immersed in a toxicant vapor having a partial pressure corresponding to its level above the surface of the disinfectant film. Diffusion through the surface of the bacterial aggregate and into its interior is the inverse process of the evaporation phenomena we have just described, and similar relationships are applicable. Again, we may make use of an equilibrium curve, Figure 3, to describe the situation. This time the point A is on the opposite side of an equilibrium curve and the partial pressure and concentration gradients are similarly defined. In this case we are dealing with unsteady state conditions with respect to the absorption of toxicant by the bacterial aggregate. Point A is not fixed, but moves in the direction of equilibrium as a function of time. As the bacterial aggregate saturates, the driving forces drop to zero. With appropriate assumptions, one may compute the concentration profile of toxicant within the bacterial aggregate at any time. Again, the relationships of Equation 4 are applicable.

We shall not undertake to pursue the theoretical model of residual surface disinfection beyond this point. The description is, admittedly, oversimplified; but it provides a concept of the effects of the relevant variables.

Practical Limitations

Surface Reservoir

Previous investigations have shown that in mopping operations with disinfectant solutions, approximately 0.04 ml of solution per square inch was

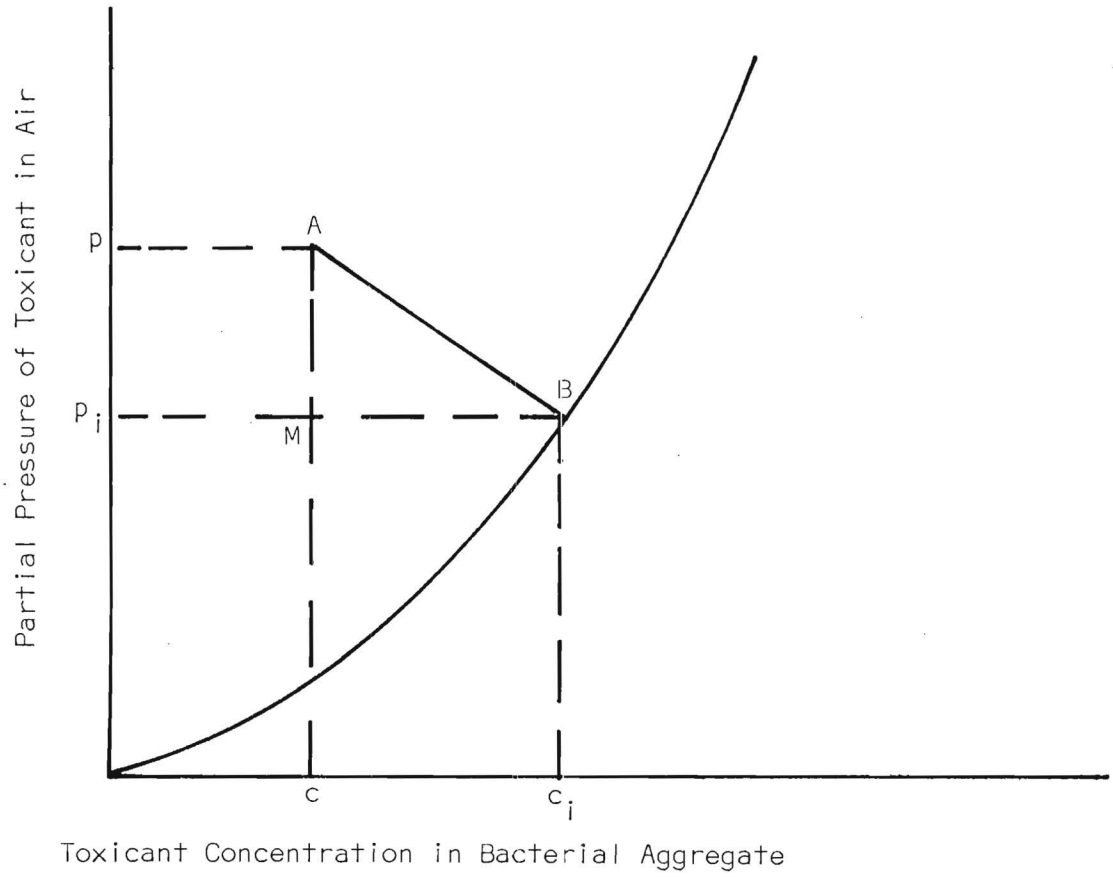


Figure 3. Driving Forces for Toxicant Absorption.

deposited. For an aqueous solution this would correspond to a film thickness of approximately 2.04 mils. Accordingly, it is not unreasonable to anticipate that the toxicant reservoir can be equivalent to about 1 mil in thickness.

Evaporation Rate Versus Persistence

The optimum disinfectant is that which maintains a uniform environment of lethal vapor above its surface for the maximum possible time period. This requires that the evaporation rate of toxicant per square foot of surface area be constant until the toxicant reservoir is depleted. Thus, the question is: "What is the depletion time for a 1 mil thick reservoir of toxicant at various constant evaporation rates?" The applicable equation is

$$\theta = \frac{W}{N_A} \tag{5}$$

where θ is the time in hours, W is the charge of toxicant in g/ft^2 and N_A is the evaporation rate in $g/ft^2/hr$. A plot of this equation, Figure 4, shows the limits of feasibility for persistence of 1 and 2 mil thick films. Obviously, for depletion times greater than 100 hours the evaporation rates must be vanishingly small. However, the possible persistence is limited only by the effectiveness of the toxicant at very low concentrations.

Evaporation Rate Versus Environmental Concentrations

A more serious limitation is imposed upon residual surface disinfectants in terms of concentration tolerances in the atmosphere of the human environment. We shall make several simplifying assumptions in estimating the possible concentrations as follows:

$$C = N_A t \frac{S}{V} \tag{6}$$

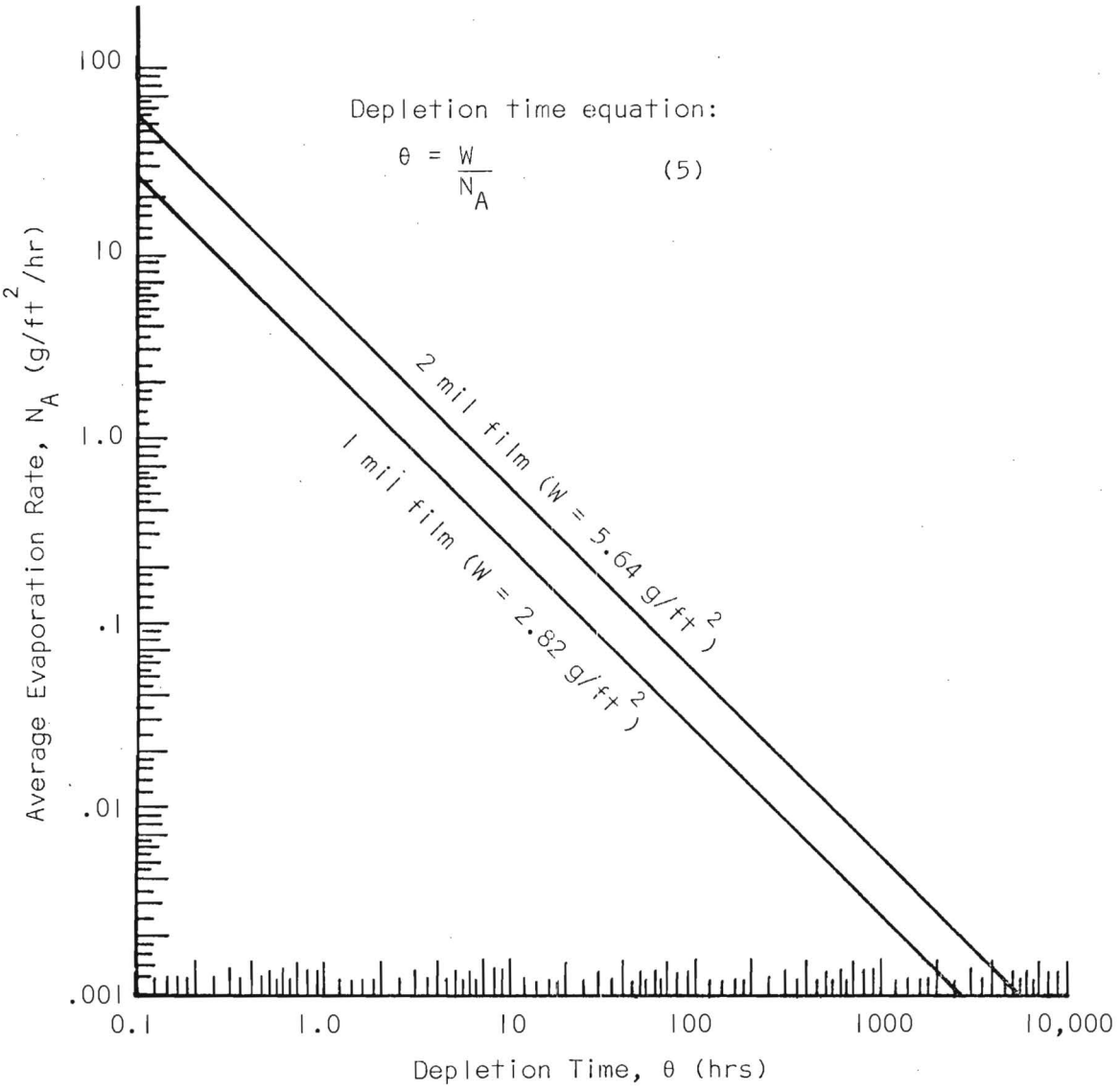
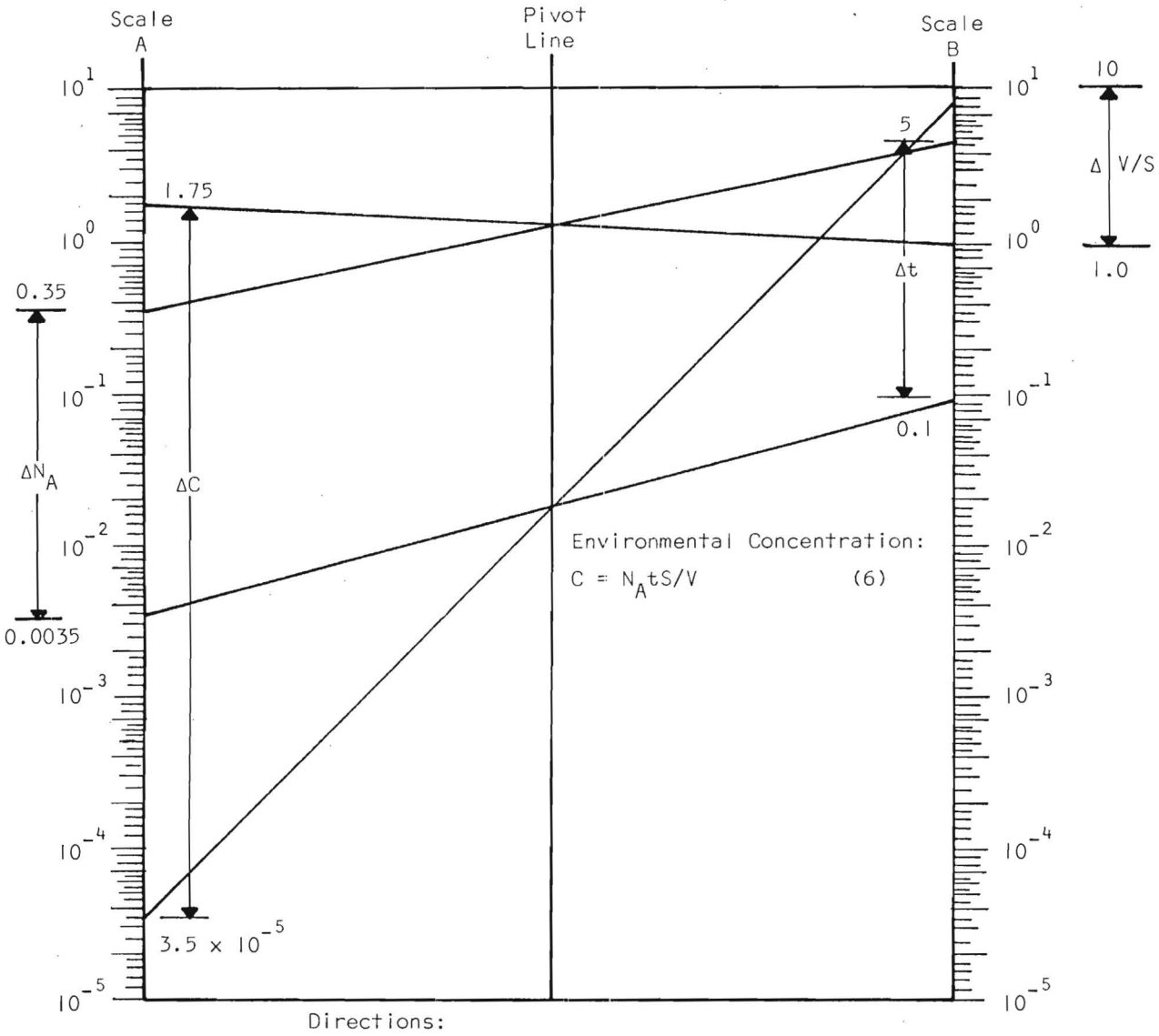


Figure 4. Evaporation Rates versus Depletion Times.

where C is the average equilibrium room concentration in g/ft^3, N_A is the average evaporation rate in $g/ft^2/hr$, t is the time in hours for a complete change of air by the ventilating system and S/V is the treated surface/volume ratio of the room. The assumptions are that during time, t, all evaporated material is uniformly dispersed throughout the room, and the amount exhausted is equal to the amount evaporated, i.e., equilibrium is established. It is interesting to study the relations of Equation 6 in nomographic form, Figure 5. We have established an upper limit for N_A of 0.35 $g/ft^2/hr$ based on minimum persistence requirements of about 8 hours. An arbitrary lower limit of 0.0035 $g/ft^2/hr$ (a reduction of two orders of magnitude) would appear to be most optimistic in anticipating 800 hours persistence. Air change rates of from 1 in 5 hours (t = 5 hr) to 10 per hour (t = 0.1 hr) provide a fairly realistic wide range. For the nomographic presentation in Figure 5, the S/V ratio is inverted, thus, V/S ratios are of interest from about 10 to 1. The former would correspond to a large high-ceiling room with only the floor treated; the latter to a small cubical room with walls, floors, ceiling, and furnishings treated. At the maximum limits of these constraints we find that vapor concentration could range from 3.5×10^{-5} g/ft^3 to 1.75 g/ft^3.

Human Environmental Aspects

The foregoing estimations show clearly that potential vapor concentrations in the human environment cannot be ignored. In designing a residual surface disinfectant, concentration levels must be low enough to preclude human toxic effects even for continuous exposure conditions. It is also necessary to anticipate that direct skin contact with the liquid disinfectant will occur. Possible irritant or toxic effects must be considered and minimized.



Directions:

(1) Connect values of N_A on Scale A and t on Scale B with a straight line,

(2) Connect located pivot point with V/S value on Scale B and,

(3) Read C on Scale A.

Figure 5. Nomographic Solution of Concentration Equation Showing Ranges of the Variables.

Of the several practical limitations, the human environmental factors are of overriding importance. While experimental aspects of these factors are beyond the scope of our investigation, we cannot emphasize too strongly the necessity for detailed study of this environmental problem.

Performance Objectives

We have already indicated that much of the previous work in this field has focused primary attention on persistence of disinfectant action with a corresponding sacrifice of rapid bacterial killing rates. Our efforts have been directed first toward achieving a desired rapid rate-of-kill, and then toward maintaining this rate for the maximum feasible time.

This approach is consistent with the needs of one of the most critical areas requiring persistent disinfection -- the surgical operating room. Here, the ability of a surface disinfectant to kill bacteria must be measured in minutes rather than hours if it is to be of any practical value. Ordinary disinfectants serve adequately for periodic cleanup of the area, but during the course of an operation the redispersion of freshly deposited active bacteria can be reduced only by a rapidly-acting residual disinfectant. Accordingly, we adopted general performance objectives as follows:

$$\text{Lethal Persistence} \geq 8 \text{ hr}$$

$$\text{Killing Time} \leq 30 \text{ min}$$

Naturally, we would hope to be able to greatly exceed these minimums, but a material fully meeting these objectives could be considered a candidate for further study.

Experimental Approach

In the future we shall seek to combine precise quantitative mass transfer and toxicity parameters to attain reliable predictions of residual disinfectant performance. However, this did not appear to be immediately feasible, and in any case, more direct experimental verification is required. Therefore, we chose first to develop a screening test that would be helpful for both theoretical confirmation and practical formulation guidance.

The test we shall describe is distinctive from prior methods primarily by the fact that the bacteria are not permitted to gain contact with the toxicant except by vapor phase transfer. Direct liquid-liquid or liquid-solid interphase transfer is precluded. We shall not attempt to justify or demonstrate direct dimensional similitude with "typical" or "representative" service conditions. Rather, we affirm that the test tends to evaluate the ability of a residual surface disinfectant to perform a task exceeding the difficulty of ordinary conditions. This provides a conservative approach for the development of formulation principles for residual surface disinfectants.

Screening Test for Persistent Disinfectants

The present screening test is a part of a larger testing procedure, which encompasses toxicity of compounds in aqueous solutions, estimations of evaporation rates in a gas-phase hydrocarbon analyzer, and direct determination of disinfectant losses from the test surface by weight loss and analysis of residues. From results of these examinations, candidate compounds and disinfectant compositions are selected for biological evaluation in the screening test.

In the actual screening test, a suspension of bacterial cells is prepared from a 44-48 hour culture of Staphylococcus aureus, grown in brain-heart infusion broth. A 2.0 ml portion of the culture is placed into a 100 ml dilution

bottle containing 0.1 per cent tryptone. A 0.6 ml portion of this suspension is removed with a serological pipette, and carefully spread over the surface of a suspended sheet of lens tissue.* This quantity of liquid is just sufficient to wet the entire sheet, which is approximately 4 x 6 in. The treated lens tissue is held suspended for 2 hours in order to equilibrate with ambient conditions. At the end of this time, 6 circular discs of 10 cm^2 area are die-cut from the sheet. Three of these serve as blanks or controls; the others are used in testing. Each disc contains approximately 10^6 viable cells of Staphylococcus aureus.

The candidate disinfectant solution is applied to a 9 cm diameter Whatman No. 1 paper. Exactly 0.25 ml of the disinfectant solution is pipetted to the center of the filter paper, which rests upon 16 mesh aluminum screen laid across an open petri dish. After the disinfectant-treated paper has been exposed to the atmosphere for the specified period of time (a minimum of 2 hours), another aluminum screen is placed on top of this paper, and the three test discs of bacteria-laden paper placed upon this screen. After 30 minutes exposure each test disc and each of the control discs are transferred to individual dilution bottles containing a solution of 0.1 per cent tryptone and 0.05 per cent poly(oxyethylene)sorbitan monooleate. Vigorous shaking decomposes the lens paper and almost all of the bacterial cells are freely suspended in the aqueous solution. Suitable dilutions, followed by preparation of pour plates with tryptone-glucose agar permit the accurate enumeration of the colony-forming cells remaining on the test discs as well as those upon the control discs.

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*Bioloid brand lens cleaning tissue, Will Corporation, No. 17471.

Although aseptic techniques are employed, such extraneous microbial contamination as does occur is easily distinguished because of the characteristic color and colony morphology of the test microorganism.

Test Data Analysis

Data from the test procedure are reported as an average per cent kill based on the colony counts from the exposed test discs as compared with the control discs. More specifically, $\%\ \text{Kill} = \frac{\text{Control-Test}}{\text{Control}} \times 100$.

If the kill resulting from a 30 minute exposure to the disinfectant is 90 per cent or more, it is anticipated that the disinfectant would demonstrate an even greater effectiveness in service. If this result is obtained after the disinfectant-treated filter paper has been previously exposed to the atmosphere for as long as 8 hours, then the minimum persistence objective will have been realized. In our evaluations, we plot Per cent Kill as a function of the disinfectant evaporation period and abstract two parameters as follows:

Toxic Intensity = Per cent Kill at 8 hr

Toxic Persistence = Maximum period for 90% kill

The procedure is illustrated in Figure 6, where are shown the results obtained employing p-chlorophenol as the phenolic toxicant, and ethylene glycol as the solvent. The Toxic Intensity of the pure compound is approximately 95 per cent kill at 8 hours; for the solvent system, approximately 99 per cent. The Toxic Persistence is approximately the same for both -- a 90 per cent kill is effected even after the disinfectant has been exposed for 40 hours. The subsequent kill histories are strikingly different. The biological effect of the pure compound remains constant at about 90 per cent kill, and abruptly falls away as the

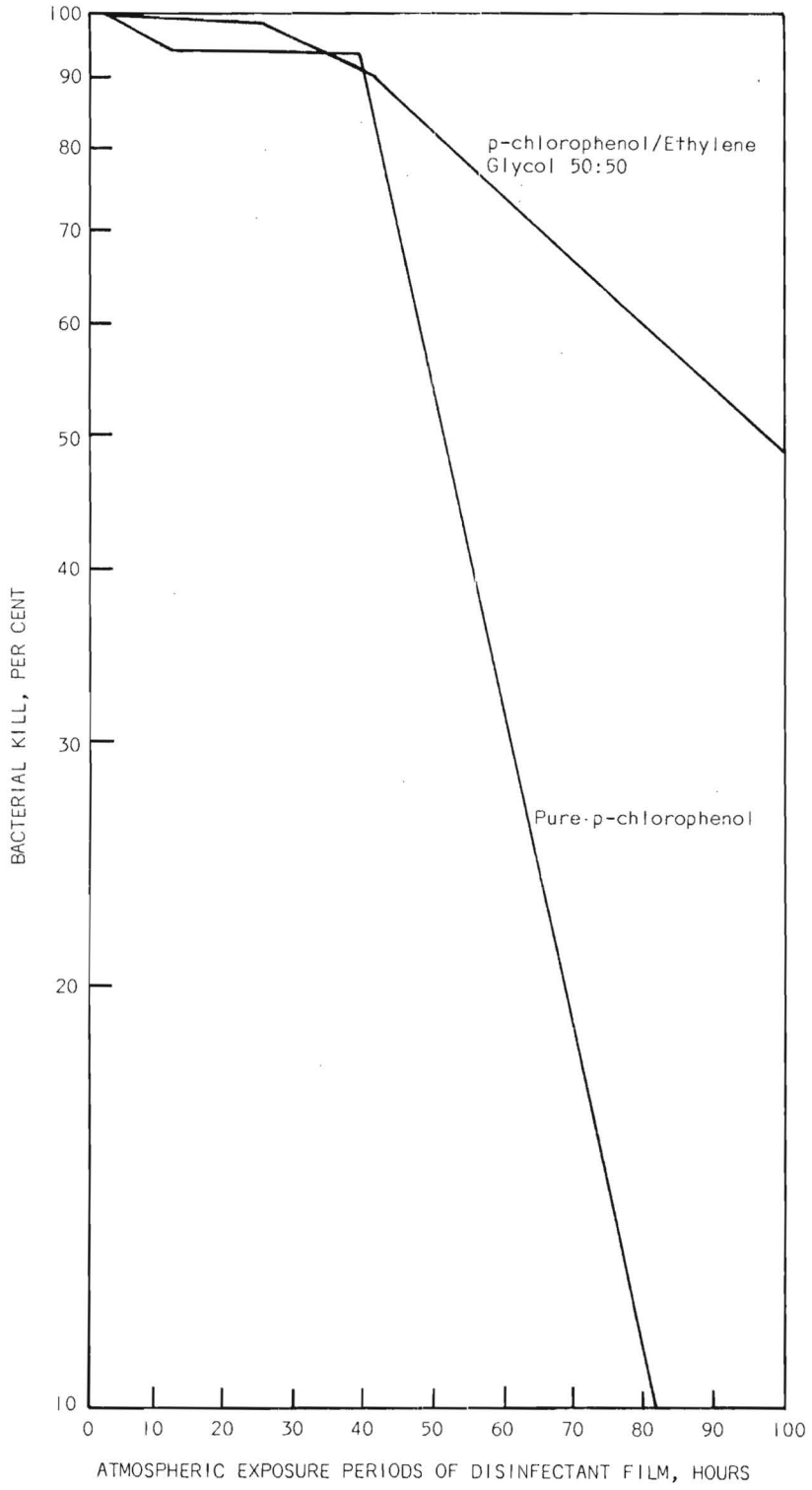

Figure 6. Disinfectant Performance Curves.

supply of phenolic is exhausted from the filter paper. On the other hand, the biological effectiveness of the solvent system drops more gradually; apparently the solvent retards the loss of phenolic, and in the final stages, the supply of phenolic is not exhausted, but the rate of evaporation is insufficient to effect substantial kills.

The information presented in Figure 6 is typical of our findings to date. Thus far, we have obtained satisfactory kills only with disinfectant systems incorporating the more volatile solid phenolics. Possibly we may find the test to be too stringent as compared with practical requirements. However, we have accumulated data on bacterial killing and persistence of effectiveness in this test which correlate very well with experimental mass transfer data on the disinfectants involved. Moreover, we have learned new approaches to formulation to enhance performance as measured by this test.

Reliability

We have found the test reproducible within reasonable limits for biological procedures, and not excessively affected by ordinary variations in ambient conditions (55% RH, 70° F). An exploration of the effect of excursions in temperature and humidity is presently being conducted.

Acknowledgments

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