MATHEMATICAL MODEL FOR DETERMINING RATE OF KILL OF MICROBIAL AE--ETC(U)

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MATHEMATICAL MODEL FOR DETERMINING RATE OF KILL OF MICROBIAL AEROSOLS BY VAPOR-PHASE DISINFECTANTS

by

J. Bruce Harstad

CB Detection and Alarms Division

July 1981

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MATHEMATICAL MODEL FOR DETERMINING RATE OF KILL OF MICROBIAL AEROSOLS BY VAPOR-PHASE DISINFECTANTS

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- Bactericidal
- Decay rate
- Disinfectant
- Disinfection
- Formic acid
- Lactic acid
- Microbial
- Serratia marcescens
- Sterilization
- Vapour
- Vapor
- Water

**ABSTRACT**
A method for screening chemical vapors for decontaminating microbial aerosols was developed by applying Raoult's law. The method included a simple small-scale aerosol chamber system that was conveniently and rapidly operated by one person.

The vegetative bacterium Serratia marcescens (SM) was used for the test aerosol. The maximum decay rate of an SM aerosol in a disinfectant vapor at a given relative humidity (RH) was determined by determining the decay rate of an SM aerosol in the equilibrium vapor over a binary solution of disinfectant and water. Ternary solutions of disinfectant, water, and a nonvolatile nonelectrolyte were used to obtain any selected concentration of disinfectant vapor and water vapor. Relationships between (continued on reverse side)

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### Key Words
- Acid
- Bactericidal
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- Disinfectant
- Disinfection
- Formic acid
- Lactic acid
- Microbial
- Serratia marcescens
- Sterilization
- Vapour
- Vapor
- Water
20. ABSTRACT. Continued

The SM aerosol decay rate and the independent variable (equilibrium vapor) were reduced to equations by regression analysis and/or described graphically. It was possible to predict the SM aerosol decay rate over the whole vapor composition range from limited experimental data simply because the relationship between the SM aerosol decay rate and the equilibrium vapor is first order when vapor composition is expressed thermodynamically; i.e., in mole fractions.

Vapors tested included formic acid, propionic acid, lactic acid, glycolic acid, levulinic acid, ethylene glycol, propylene glycol, and methyl sulfoxide. The modeling experiment was done with formic acid vapor.
The work described in this report was authorized under Project 1L161102A71A. Research in Defense Systems; Scientific Area 1, Support of Biological Defense Materiel. This work was started in January 1975 and completed in June 1978. The experimental data are recorded in notebooks 8970, 9376, and 9558.

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MATHEMATICAL MODEL FOR DETERMINING RATE OF KILL OF MICROBIAL AEROSOLS BY VAPOR-PHASE DISINFECTANTS

1. INTRODUCTION

The purpose of this research was to develop a method for screening chemical vapors for decontaminating microbial aerosols.

The first task was to develop a technique for determining the decay rate of microbial aerosols as a function of disinfectant vapor concentration and relative humidity (RH). To accomplish this, a simple aerosol chamber system, which was small enough to fit in an exhaust-equipped hood and which could be conveniently and reproducibly operated by one person, was used.

The final part of the investigation concerned the development of methods for screening disinfectant vapors by applying Raoult’s law. Binary solutions of disinfectant and water were used to determine the maximum decay rate of a microbial aerosol in a disinfectant vapor at a given RH. Ternary solutions of disinfectant, water, and a nonvolatile nonelectrolyte were used to obtain any selected concentration of disinfectant vapor and water vapor. The relationships between the variables were reduced to equations by regression analysis and/or described graphically.

The project was discontinued before its conclusion; therefore, most of the graphs and equations are based on minimal data and some relationships were not completely defined.

2. MATERIALS AND METHODS

2.1 Test bacteria.

Serratia marcescens (SM) was used as the test organism because it is easily cultured and identified and because of its extensive use in the past as a simulant for vegetative bacterial agents. Cultures of SM were transferred weekly by inoculating 50 ml of nutrient broth (Difco) in a 250-ml Erlenmeyer flask with a few drops from a previous culture. The flask was incubated at 27° to 30°C in a rotary shaker-incubator for 18 hours and then stored at 4°C.

2.2 Small-scale aerosol chamber technique.

The aerosol technique devised for this study consisted of aerosolizing SM into an inverted three-neck 600-ml round bottom flask (aerosol flask) and then transferring a portion of this aerosol into a three-neck 5.6-liter flask (transfer
flask) from which assays of the viable aerosol population were made periodically to
determine the exponential decay rate according to the formula:

\[ C = C_0 e^{-kt} \]  

or

\[ \ln C = \ln C_0 - kt \]

where

\( C \) is the SM aerosol concentration per unit volume at any time \( t \)
\( t \) is the time in minutes
\( C_0 \) is the intercept or \( C \) at zero time
\( k \) is the proportionality constant that will be referred to here as the decay constant

The SM aerosol was produced in the aerosol flask by 12 squeezes of a
rubber bulb connected to a glass Vaponefrin nebulizer containing 2 ml of SM broth
culture. To eliminate any concentration effects due to settling, a 5-ml syringe was
connected to the nebulizer reservoir which allowed the mixing of the culture in the
nebulizer prior to aerosolization. No attempt was made to control the RH in the
aerosol flask which increased rapidly from ambient to 70% to 90% as a result of
aerosol production and the humidity of the ambient air. The aerosol flask was
equipped with an outlet filter to allow air to flow in or out to atmospheric
pressure and to collect the residual aerosol after completion of a test.

Two minutes after aerosolization, 100 ml of aerosol was withdrawn from the
aerosol flask with a syringe and transferred immediately into the transfer flask. Measured volumes of aerosol (5 to 100 ml, depending on the expected decay rate) were
withdrawn periodically from the transfer flask with a syringe and immediately
expelled into the intake tube of a 340-hole sieve sampler\(^1\) drawing room air at its
design rate of 28 liters per minute. SM particles in the aerosol samples were
impinged on the surface of tryptose agar (Difco) plates in the sampler. The plates
were incubated at 30°C for at least 24 hours and then the colonies were counted and
the regression equation \( C = C_0 e^{-kt} \) was determined by the method of least squares.
\( C \) was estimated from the colony counts by a statistical method called the positive
hole method by Andersen.\(^2\) This method converts the colony count to a particle
count; e.g., 339 colonies = 339 positive holes = 2008 particles.

Zero time was taken as the time when one-half the aerosol volume (50 ml)
had been expelled into the transfer flask. The entire 100 ml was introduced in
0.1 minute. In syringe-sieve sampling, \( t \) was the time when one-half the volume in
the syringe had been expelled into the sieve sampler. The time required to expel
the entire volume into the sieve sampler ranged from 0.02 minute for a 5-ml syringe
sample to 0.1 minute for a 100-ml syringe sample. The sieve sampler was turned on
just before the syringe sample was introduced and turned off 0.15 minute after the
sample was expelled.
In syringe-sieve sampling, the minimum value for t was 0.30 minute and the minimum interval between subsequent samples was 0.8 minute. The number of syringe-sieve samples per test ranged from eight in control (no disinfectant) tests to one in tests with decay constants k greater than nine. In the latter tests, the extra data point needed to compute k was the intercept, $D_0$, from control tests, nominally $2 \times 10^2$ SM particles per milliliter. The maximum volume of a syringe-sieve sample was 100 ml; therefore, the limit of the measurement system was $k = 33$ which represents the recovery of a single SM particle from a 100-ml sample of aerosol at $t = 0.30$ minute.

No dilution accompanied the withdrawal of samples from the transfer flask. The pressure balancer was simply a rubber glove or balloon hanging down inside the flask which expanded into the flask as samples were withdrawn from the flask. It also served as a stirrer although experience with several other methods indicated that stirring tends to increase the decay rate without improving the "goodness of fit" of the regression equation. Given the size and shape of the flask and the small aerosol particle size, adequate mixing was probably achieved by the slight temperature differences on the sides of the flask and the fact that it was moved slightly during each procedure involving it. In the tests, the flask was gently swirled momentarily (0.05 minute) before each sample withdrawal.

The RH of the air in the transfer flask was measured with narrow-range Aminco-Dunmore lithium chloride electric hygrometer sensing elements.

2.2.1 Glass syringes.

Only glass syringes greased with stopcock grease were used for aerosol sampling. Disposable plastic syringes were tried but all types and sizes gave erratic and much lower recoveries than glass syringes, possibly due to electrostatic charge effects.

2.3 Screening methods—application of Raoult's law.

2.3.1 Binary solutions of disinfectant and water.

The saturation concentration of a disinfectant vapor is the maximum amount which may coexist with water vapor in the air. Stated another way — the saturation concentration of a disinfectant vapor in air is a function of the RH. If each component obeyed Raoult's law, the disinfectant vapor saturation concentration would diminish linearly with an increase in the RH.

The bactericidal activity of a disinfectant vapor at saturation concentration was defined by determining the bactericidal activity of the equilibrium vapor over a binary solution of disinfectant and water. Maximum bactericidal activity of a disinfectant vapor at a given RH would be expected at a disinfectant vapor concentration at or near saturation. The bacterial aerosol decay constant $k$ at disinfectant vapor saturation concentration will be referred to here as $k_s = \text{maximum value of } k \text{ at a given RH.}$
The technique consisted of sealing 50 ml of the disinfectant solution in the transfer flask containing ambient air and allowing it to vaporize for at least 16 hours to its equilibrium or saturation vapor pressure at a constant temperature (nominally 25°C). The composition of the equilibrium vapor is a function of the partial pressures of the components in the vapor and the total vapor pressure. The partial pressure of a component of the vapor is defined as its mole fraction in the vapor times the total vapor pressure, assuming ideal gas behavior (Dalton's law).

The relationship between the composition of the vapor and that of the solution is described by Raoult’s law which states that for an ideal solution the partial vapor pressure of each component is equal to its mole fraction in the solution multiplied by the vapor pressure of the pure component; i.e.,

\[ p = x \rho^0 \]  

(2)

where

- \( p \) = partial vapor pressure
- \( \rho^0 \) = vapor pressure of the pure component
- \( x \) = mole fraction in the solution

Even for a nonideal solution, Raoult’s law applies to a component as its mole fraction approaches unity or a solution may show ideal behavior over a limited concentration range. In most solutions, there is some type of influence of one component on the other so that equation 2 does not hold. There may be a tendency for chemical action, forming a compound in solution, or a component becomes less volatile in the presence of the other due to the formation of nonvolatile complexes or there may be dissociation of the components in solution.

For a nonideal solution, the composition of the vapor can be estimated from vapor-liquid equilibrium data or from Raoult’s law in the form:

\[ p = \gamma x \rho^0 \]

where \( \gamma \) is the activity coefficient which is essentially a deviation factor. Deviations from Raoult’s law are often extreme as the concentration of a component approaches zero.

Except for lactic acid, the disinfectant vapor saturation concentrations were not assayed. However, estimates of the disinfectant vapor saturation concentrations would be useful information.

The disinfectant vapor saturation concentration was calculated from vapor pressure data given in the literature using the following form of the ideal gas law:

\[ X = M x \rho^0 / RT \]

(3)
where
\[ X = \text{disinfectant vapor saturation concentration in grams per liter} \]
\[ M = \text{gram molecular weight} \]
\[ R = \text{molar gas constant for ideal gases} = 0.08205 \]
\[ T = \text{thermodynamic temperature in degrees Kelvin (K)} = 273.15 + ^\circ C \]

If the activity coefficient \( \gamma \) is known, the expression becomes
\[ X = M x P_0 \gamma / RT \quad (4) \]

Vapor pressure \( P_0 \) at 25\(^\circ\)C was extrapolated or interpolated from a compilation by Daniel R. Stull\(^3\) or from the International Critical Tables\(^4\) using the approximate Clapeyron-Clausius equation which states that a plot of \( \ln P_0 \) against \( T^{-1} \) gives a straight line. For example, the table below, from the International Critical Tables, shows vapor pressure data for formic acid, one of the chemicals screened. Vapor pressure, \( P_0 \), is given in mmHg.

\[
\begin{array}{cccccccc}
   P_0 & 18.9 & 33.1 & 52.2 & 82.6 & 125.9 & 189.7 & 270.6 \ 
   ^\circ C & 10 & 20 & 30 & 40 & 50 & 60 & 71 & 80 & 90 & 100 & 100.75 \\
\end{array}
\]

According to the regression equation computed from the above data, \( \ln P_0 = 18.179 + 4309.8 T^{-1} \); the vapor pressure of pure anhydrous formic acid at 25\(^\circ\)C (298.15K) is 41 mmHg = 0.054 atm.

2.3.2 Ternary solutions.

Ternary solutions of disinfectant, water, and a nonvolatile nonelectrolyte were used to investigate a method for obtaining any selected concentration of disinfectant vapor and water vapor by mixing the appropriate mole fractions in the transfer flask and allowing evaporation to equilibrium vapor pressure at a constant temperature (nominally 25\(^\circ\)C).

3. RESULTS AND DISCUSSION

3.1 SM aerosol particle size distribution.

Table A-1 shows the particle size distribution of a control (no disinfectant) SM aerosol at 70\% RH and 24\(^\circ\)C as determined with a six-stage Andersen sampler, Model 0101.\(^2\) Contrary to the recommended procedure previously described, the aerosol in this particular test was stirred with a large magnetic stirring bar rotating at approximately 100 rpm. NMD is the number median diameter of the aerosol particle distribution. GSD is the geometric standard deviation of the distribution indicating the degree of heterogeneity of the aerosol. A perfectly homogeneous
aerosol has a GSD of 1; i.e., all of the particles are the same size. $r^2$ is the coefficient of determination of a regression line indicating the "goodness of fit" of the data points about the regression line. A perfect fit has an $r^2$ of 1.

SM aerosol particle size appeared to decrease exponentially with time. Regression analysis showed that the predicted NMD at zero time was 1.1 μm.

Sieve sampler data were obtained for comparative purposes. Andersen sampler recoveries were greater than those with the sieve sampler because the aerosol RH increases rapidly on passage through the Andersen stages favoring viable collection. With the Andersen sampler, the SM aerosol decay constant, $k$, was 0.0556; whereas, that with the sieve sampler was 0.0768.

The sieve sampler was used in this study instead of the Andersen sampler because it requires much less time and material and is easier to use.

3.2 Binary solutions of disinfectant and water.

The results with several binary solutions are summarized in table A-2. The data are limited to the listed RH's for each test disinfectant, thus graphing of $k_s$ versus RH was not feasible.

One of the formic acid solutions tested contained 90.6% formic acid by weight. Using 46.03 as the gram molecular weight of formic acid, the composition of this solution, expressed in mole fractions, was formic acid = 0.79 and water = 0.21. Solving equation 3, which assumes ideal behavior, the predicted formic acid vapor saturation concentration would be 0.080 gram per liter at 25°C. Similarly, the predicted RH of the equilibrium vapor would be 71% since, by definition, $RH = p/p^o$. However, formic acid and water are known to form an azeotropic mixture with a maximum boiling point. Such a condition is caused by negative deviations from Raoult's law; i.e., both components have partial vapor pressures less than those predicted by Raoult's law. Stated another way — the activity coefficients γ are less than 1. Reduction of literature data"15 shows that the equilibrium partial vapor pressures of formic acid and water over an aqueous solution containing 90.6% formic acid by weight are 0.039 and 0.0054 atm, respectively, at 25°C which converts to an activity coefficient of 0.91 for formic acid and 0.82 for water. Therefore, the predicted formic acid vapor saturation concentration according to equation 4 would be 0.073 gram per liter. The predicted RH of the equilibrium vapor would be 0.17 or 17%.

Bactericidal activity of formic acid vapor at saturation concentration was much greater at 17% RH than at 98% RH. However, the next section of this report, dealing with ternary solutions, shows the $k_s$ versus RH curve for formic acid vapor as having a maximum value of $k_s$ at about 75% RH.

Solutions of the two hydroxycarboxylic acids (lactic acid and glycolic acid) were nonideal in that they contained intermolecular esters in equilibrium. The lactic acid solution equilibrated was an aqueous solution 87.9% by weight total
lactic acid. Lactic acid solutions are composed of lactic acid, intermolecular esters, and water all in equilibrium. The esters are expressed in terms of equivalent quantities of lactic acid. Complete esterification would correspond to 125% lactic acid since 100 grams of a polymer \((C_3H_6O_2)_n\) by hydrolysis gives \((100)(90)/(72)\) grams = 125 grams of lactic acid. According to literature data, the approximate composition of an 87.9% by weight solution, expressed in mole fractions, is: monomeric lactic acid = 0.41, intermolecular esters = 0.08, and water = 0.51.

The lactic acid vapor saturation concentration was measured and found to be \(3.7 \times 10^{-5}\) grams per liter. Solving equation 3 and using 90.08 as the molecular weight of lactic acid, the vapor pressure \(p^0\) of pure anhydrous crystalline lactic acid would be \(2.5 \times 10^{-5}\) atm at 25°C. Literature data on the vapor pressure of lactic acid are sparse and in poor agreement because it is difficult to determine the boiling point of lactic acid at reduced pressure and impossible at atmospheric pressure because of the ease with which it undergoes self-esterification when heated. Values for \(p^0\) at 25°C extrapolated from the literature ranged from \(1 \times 10^{-5}\) to \(5 \times 10^{-5}\) atm.

The approximate composition of the glycolic acid solution equilibrated, expressed in mole fractions, was: monomeric glycolic acid = 0.34, intermolecular esters = 0.04, and water = 0.62.

The levulinic acid solution was actually multicomponent rather than binary in that the solution equilibrated was technical levulinic acid containing lactones. It is very difficult to obtain by distillation levulinic acid which does not contain traces of lactones and water. The approximate composition of the equilibrium solution, expressed in mole fractions was: levulinic acid = 0.87, lactones = 0.12, and water = 0.01.

Except for lactic acid, the disinfectant vapor saturation concentration \(X\) was calculated. These calculated values of \(X\), particularly those where the disinfectant liquid mole fraction \(x\) does not approach unity, are considered approximate. According to Chemical Engineers' Handbook, accurate vapor-liquid equilibrium data are difficult to obtain and much of the published data on activity coefficients are of doubtful quality. Chemical Engineers' Handbook also describes several graphical and mathematical methods based on thermodynamics for checking the accuracy of activity coefficients. Published data on vapor pressures would be expected to be more reliable than those for activity coefficients but should not be regarded as having the same degrees of accuracy as that for the most widely researched chemical, water.

3.3 Ternary solutions of formic acid, water, and glycerol.

Ternary solutions were tested because \(k_s\) was too large at most PH's to be determined with binary solutions of formic acid and water; i.e., dilution with a third component was necessary to obtain finite values of \(k\) at most RH's. Also, \(k\)
could be predicted over the whole vapor composition range from a minimum of experimental data if a functional relationship between \( k \) and the independent variable (equilibrium vapor) exists.

The results with ternary solutions, assuming ideal (Raoult’s law) solutions, are summarized in table A-3. Glycerol was used as the third component because of its mutual solubility, lack of bactericidal activity, and low vapor pressure (6.4 \( \times \) \( 10^{-7} \) atm at 25\(^\circ\)C) compared to formic acid and water.

The quantities listed in table A-3 or used in data reduction and regression analyses are:

\[
x_1 = \text{formic acid solution mole fraction}
\]
\[
x_2 = \text{water solution mole fraction}
\]
\[
x_3 = \text{glycerol solution mole fraction} = 1 - x_1 - x_2
\]
\[
x_1' = \text{formic acid vapor density (grams per liter) assuming an ideal solution}
\]
\[
x_1''/x_2 = \text{formic acid vapor density fraction} = x_1'/x_1 + x_2 + x_3
\]
\[
y = \text{vapor mole fraction assuming an ideal solution}
\]
\[
y_1 = \text{formic acid vapor mole fraction}
\]
\[
y_1' = \text{formic acid vapor mole fraction} = x_1p_1/x_1p_0 + x_2p_0 + x_3p_0
\]
\[
y_1'' = \text{formic acid vapor saturation mole fraction}
\]
\[
y_1^s = \text{formic acid vapor mole fraction when glycerol solution mole fraction} x_3 = 0
\]
\[
k = k_0 e^{b y_1}
\]
\[
k_0 = k \text{ for control (no disinfectant) SM aerosols}
\]
\[
k_s = k_0 e^{b y_1^s}
\]

3.3.1 Curve fitting.

Linear regression analysis (least square method) of the data at 43% RH (table A-3), assuming a simple exponential relationship between the variables of the form

\[
k = k_0 e^{b y_1}, \quad (5)
\]

showed that

\[
k \text{ at 43% RH} = 0.0211e^{18.3y_1} \quad (6)
\]
\[
r^2 = 0.999
\]
\[
k_s \text{ at 43% RH} = 1.4 \times 10^3
\]
Figure B-1 is a semilog plot of $k$ at 43% RH versus $y_1$ showing the fitted regression line 6. It is not possible to decide whether the relationship is statistical or functional from looking at the data either graphically or mathematically; additional evidence is needed. In the case of a functional relationship, there exists an exact mathematical formula relating the two variables, and the only reason that the observations do not fit the equation exactly is because of errors of measurement in the observed values of one or both variables. In the case of a statistical relationship, there is no exact mathematical relationship between the variables — only a statistical association. The distinction is important. If equation 5 describes a functional relationship, then $k$ should be determinable over the whole vapor composition range from a minimum of experimental data. Otherwise, the fitted equation could be reliably used only for interpolation and to summarize the data.

Alternate relations for describing the aerosol decay rate are:

\[ k \text{ vs } x_1 \text{ or } X_1 \]  \hspace{1cm} (7)

and

\[ k \text{ vs } X_1/X \]  \hspace{1cm} (8)

The regression equations describing relations 7 and 8, assuming a simple exponential relationship between the variables, are:

\[
\begin{align*}
\text{k at 43\% RH} & = 0.0371e^{34.8x_1} \\
 r^2 & = 0.984 \\
\text{k_s at 43\% RH} & = 0.0371e^{34.8(1-x_2)} \\
 & = 4.7 \times 10^5 \\
\end{align*}
\]

or

\[
\begin{align*}
\text{k_s at 43\% RH} & = 0.0371e^{34.8X_1} \\
\end{align*}
\]

since the density of formic acid vapor at 25°C according to equation 3 is 0.10 grams per liter and

\[
\begin{align*}
\text{k at 43\% RH} & = 0.0112e^{12.0X_1/X} \\
 r^2 & = 0.984 \\
\text{k_s at 43\% RH} & = 161 \\
\end{align*}
\]

All of the regression lines fit the data well. However, regression line 6 fits the data points better than either regression line 9 or regression line 11. The value of $k_s$ predicted by equation 9 appears inordinately high. The value of $k_s$ predicted by equation 11 is less than any observed or reported value for control SM aerosols.

Figure B-2 is a semilog plot of $k$ at 43% RH versus $x_1$, showing fitted regression line 9. Figure B-3 is a semilog plot of $k$ at 43% RH versus $X_1/X$. 

15
showing fitted regression line 11. The patterns of the observed values indicate that a curved line with a decreasing slope would fit the data better than regression line 9; whereas, a curved line with a positive curvature would fit the data better than regression line 11. The best fitting lines, assuming no particular functional or exact relationship between variables, were described by the following second degree exponential equations:

\[
\begin{align*}
k &\text{ at } 43\% \text{ RH } = 0.0237e^{52.2x_1} - 81.9x_1^2 \\
 & r^2 = 0.998 \\
k_s &\text{ at } 43\% \text{ RH } = 15
\end{align*}
\]

and

\[
\begin{align*}
k &\text{ at } 43\% \text{ RH } = 0.0237e^{5.53x_1/\Sigma X} + 9.28(x_1/\Sigma X)^2 \\
 & r^2 = 0.998 \\
k_s &\text{ at } 43\% \text{ RH } = 718
\end{align*}
\]

Figure B-4 is a graph showing fitted regression line 13.

Equation 12 must be an approximation useful only for interpolation because the predicted value of \(k_s\) is 15; whereas, the observed value of \(k\) at \(x_1 = 0.20\) is 31. This is impossible since \(k\) cannot be greater at \(x_1 = 0.20\) than at \(x_1 = 0.47\). Stated another way — differentiation of equation 12 shows that the regression line has a maximum at \(x_1 = 0.32\); whereas, the comparable value for the true regression line would be not less than 0.47. Figure B-5 is a graph showing fitted regression line 12. Figure B-6 shows additional evidence that a plot of \(\ln k\) at a constant RH versus \(X\) has a negative curvature from recent work with a single-phase system (unpublished) in which the disinfectant vapor concentration was directly measured by experiment.

3.3.2 Mathematical model.

It was concluded that the mathematical model for determining the rate of kill of a microbial aerosol by a disinfectant vapor at a given RH and temperature is equation 5; namely,

\[k = k_0 e^{b y_1}\]

and that the maximum value of \(k\) at a given RH and temperature is

\[k_s = k_0 e^{b y_1^s}\]
In contrast, the associations between

\[ k \text{ and } X_1 \]

and

\[ k \text{ and } X_1/X \]

are apparently statistical; i.e., the fitted regression lines could be reliably used only for interpolation. Also, it is unrealistic to conclude that a relation which describes the equilibrium vapor in terms of weight rather than number of molecules could be functional.

Table A-3 shows that \( k_s \) and \( b \) are functions of the RH. Figure B-7 is a semilog graph of \( k \) versus RH showing a maximum at about 75\% RH at 25°C. The slope coefficient \( b \) of regression equation 5 increases with an increase in RH as shown in Figure B-8. Thus, the bactericidal activity of formic acid vapor at 25°C is completely described at all RH’s >43%.

\section*{Conclusions}

The small-scale chamber technique would be useful for screening candidate disinfectant vapors. The equipment is inexpensive and generally available. Tests with binary and ternary solutions are easily and rapidly performed. The results would be expected to be highly reproducible because the variance associated with the independent variable (equilibrium vapor) would be inherently small. It is possible to predict the microbial aerosol decay rate over the whole vapor composition range from limited experimental data because the first-order relationship between the aerosol decay constant \( k \) and the equilibrium vapor is apparently functional when vapor composition is expressed thermodynamically; i.e., in mole fractions. In contrast, the relations used in the literature, namely, microbial aerosol decay rate at a constant RH versus disinfectant vapor density and microbial aerosol decay rate at a constant disinfectant vapor density versus RH are statistical and, therefore, useful only for interpolation.

\section*{Recommendations}

The following procedure is recommended for screening candidate disinfectant vapors.

1. A candidate vapor would first be tested against Bacillus subtilis var. niger spore aerosols of known particle size distribution. Spore aerosols are much more resistant to decay than SM aerosols. A vapor effective against spore aerosols would be expected to be much more effective against other microorganisms.

2. The initial test temperature would be 25°C or other constant temperature near room temperature.
5.3 The equilibrium vapor over a binary solution of disinfectant and water would be used to determine the maximum bactericidal activity of a disinfectant vapor at a given RH. Tests would be conducted at several RH's; e.g., 0.02, 0.05, 0.10, 0.30, 0.50, 0.70, 0.90, 0.95, and 0.98. These data would be reduced to a $k_s$ versus RH curve. Control tests would also be conducted over a wide range of RH's. These data would be reduced to a $k_0$ versus RH curve. The slope coefficient $b$ of the $\ln k$ versus $y_1$ regression equation at each RH would be determined from the values of $k_0$ and $k_s$. These data would be reduced to a $b$ versus RH curve. These curves completely describe the bactericidal activity of the disinfectant vapor over the whole vapor composition range. The predicted values of $k$ would be considered approximate being based on only two observations at a given RH; i.e., $k_0$ and $k_s$. However, the information would be adequate for screening purposes particularly if the curves were smoothed. The composition of an equilibrium vapor should be measured directly by experiment. It is possible, by the application of thermodynamics, to predict vapor composition over the whole range of concentration from limited vapor-liquid equilibrium data and also to check the accuracy of the predictions by applying thermodynamic checks such as those described in Chemical Engineers' Handbook. Also, published vapor-liquid equilibrium data might be used to predict vapor composition if the thermodynamic checks indicate sufficient accuracy.

5.4 Ternary solutions would be used if either $k_s$ was too large at a particular RH to be determined with binary solutions or more observations were desired for the determination of the slope coefficient $b$ of the $\ln k$ versus $y_1$ regression equation at a given RH.

5.5 Determination of bactericidal activity is not the sole criterion of a disinfectant vapor. The questions of toxicity to humans, carcinogenicity, corrosiveness, flammability, economy, ease of use, dissemination, and others must also be answered.
LITERATURE CITED


### APPENDIX A

**TABLES**

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<thead>
<tr>
<th>Recovery per ml, C</th>
<th>Sieve C = 24.9e^-0.0748t</th>
<th>Sieve C = 4.60e^-0.0556t</th>
<th>Andersen C = 1.00e^-0.0099t</th>
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<tr>
<td>Size distribution*</td>
<td>N = 8</td>
<td>N = 9</td>
<td>N = 3</td>
</tr>
<tr>
<td>Size distribution*</td>
<td>t² = 0.9984</td>
<td>t² = 0.9999</td>
<td>t² = 0.997</td>
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<tr>
<td>Mean D(MMD)</td>
<td>1.50</td>
<td>1.63</td>
<td>1.92</td>
</tr>
<tr>
<td>GSD = 1.43</td>
<td>0.89</td>
<td>0.99</td>
<td>0.99</td>
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* MMD = number median diameter in μm; GSD = geometric standard deviation; t = number of observations used to compute regression equation.

**Table A-1**: SM Aerosol Particle Size Distribution at 70% RH and 24°C and Comparison of Sieve and Andersen Sampler Recoveries

<table>
<thead>
<tr>
<th>SM aerosol particle</th>
<th>Recovery per ml, C</th>
<th>Sieve C = 24.9e^-0.0748t</th>
<th>Sieve C = 4.60e^-0.0556t</th>
<th>Andersen C = 1.00e^-0.0099t</th>
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<tr>
<td>min</td>
<td>4.63</td>
<td>1.06</td>
<td>2.73</td>
<td>2.73</td>
</tr>
<tr>
<td>6.0</td>
<td>1.72</td>
<td>2.10</td>
<td>2.73</td>
<td>2.73</td>
</tr>
<tr>
<td>8.1</td>
<td>1.30</td>
<td>2.37</td>
<td>2.73</td>
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<td>10.3</td>
<td>1.00</td>
<td>2.29</td>
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<td>2.73</td>
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<tr>
<td>12.8</td>
<td>0.82</td>
<td>2.01</td>
<td>2.73</td>
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<tr>
<td>13.9</td>
<td>0.72</td>
<td>1.94</td>
<td>2.73</td>
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<td>17.0</td>
<td>0.56</td>
<td>1.81</td>
<td>2.73</td>
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<tr>
<td>20.3</td>
<td>0.46</td>
<td>1.72</td>
<td>2.73</td>
<td>2.73</td>
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<td>23.8</td>
<td>0.35</td>
<td>1.63</td>
<td>2.73</td>
<td>2.73</td>
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<td>29.4</td>
<td>0.29</td>
<td>1.50</td>
<td>2.73</td>
<td>2.73</td>
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<td>Disinfectant, p°atm</td>
<td>Solution</td>
<td>Vapor</td>
<td>Tests^c</td>
<td>k_6</td>
</tr>
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<td>-----------------------</td>
<td>----------</td>
<td>-------</td>
<td>---------</td>
<td>-----------</td>
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<td></td>
<td>x^a</td>
<td>x^b</td>
<td>RH</td>
<td>Mean</td>
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<td>Formic acid, 5.4 x 10^{-2}</td>
<td>0.79</td>
<td>7 x 10^{-2}</td>
<td>17</td>
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<tr>
<td>Formic acid</td>
<td>0.015</td>
<td>1 x 10^{-3}</td>
<td>98</td>
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<tr>
<td>Propionic acid, 5.4 x 10^{-3}</td>
<td>0.99</td>
<td>2 x 10^{-5}e</td>
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<td>Lactic acid, 2.5 x 10^{-5}</td>
<td>0.41</td>
<td>3.7 x 10^{-2}</td>
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<td>Glycolic acid^d</td>
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<td>Ethylene glycol, 1.7 x 10^{-4}</td>
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<td>Propylene glycol, 3.1 x 10^{-4}</td>
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<td>Levulinic acid, 2.9 x 10^{-6}</td>
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<td>Methyl sulfoxide, 7.9 x 10^{-4}</td>
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<td>2 x 10^{-3}</td>
<td>1</td>
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<td>None^f</td>
<td>None</td>
<td>None</td>
<td>-1</td>
<td>16</td>
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^a Disinfectant mole fraction.
^b Approximate disinfectant saturation concentration calculated using equation 3 or 4.
^c Number of tests or fraction of tests in which sieve sampler SM recovery was zero.
^d Vapor pressure literature insufficient.
^e Measured, mean of seven samples.
^f Ambient air at 10% to 20% RH.
Table A-3. Decay Rate of SM Aerosols in the Equilibrium Vapors over Ternary Solutions of Formic Acid, Water, and Glycerol at 25°C

<table>
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<tr>
<th>(x_1)</th>
<th>(x_2)</th>
<th>(y_1)</th>
<th>(y_1^s)</th>
<th>RH(^b)</th>
<th>Test(^b)</th>
<th>(k^c)</th>
<th>(k_o)</th>
<th>(b^d)</th>
<th>(k_s)</th>
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<td>0.01</td>
<td>0.53</td>
<td>0.0318</td>
<td>0.607</td>
<td>0.43</td>
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<td>0.035</td>
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<td>0.02</td>
<td>0.53</td>
<td>0.0617</td>
<td>0.607</td>
<td>0.43</td>
<td>2</td>
<td>0.069</td>
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<tr>
<td>0.05</td>
<td>0.53</td>
<td>0.141</td>
<td>0.607</td>
<td>0.43</td>
<td>2</td>
<td>0.31</td>
<td>0.0211</td>
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<td>1400</td>
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<td>0.10</td>
<td>0.53</td>
<td>0.247</td>
<td>0.607</td>
<td>0.43</td>
<td>2</td>
<td>1.7</td>
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<td>0.20</td>
<td>0.53</td>
<td>0.397</td>
<td>0.607</td>
<td>0.43</td>
<td>7/8</td>
<td>31</td>
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<td>0.15</td>
<td>0.648</td>
<td>0.288</td>
<td>0.487</td>
<td>0.55</td>
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<td>0.0297</td>
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<tr>
<td>0.07</td>
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<td>0.255</td>
<td>0.77</td>
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<td>0.0586</td>
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<tr>
<td>0.02</td>
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<td>0.0353</td>
<td>0.0791</td>
<td>0.932</td>
<td>9</td>
<td>9.0</td>
<td>0.0967</td>
<td>128</td>
<td>2400</td>
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<tr>
<td>0.01</td>
<td>0.977</td>
<td>0.0175</td>
<td>0.0402</td>
<td>0.966</td>
<td>4</td>
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<tr>
<td>0.015</td>
<td>0.985</td>
<td>0.0258</td>
<td>0.0258</td>
<td>0.978</td>
<td>2</td>
<td>11</td>
<td>0.111</td>
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<td>0.005</td>
<td>0.988</td>
<td>0.00874</td>
<td>0.0203</td>
<td>0.983</td>
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<td>0.66</td>
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<td>0.002</td>
<td>0.9953</td>
<td>0.00349</td>
<td>0.00816</td>
<td>0.9931</td>
<td>3</td>
<td>0.21</td>
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<tr>
<td>0.001</td>
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<td>0.00174</td>
<td>0.00409</td>
<td>0.9966</td>
<td>3</td>
<td>0.18</td>
<td>0.118</td>
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<tr>
<td>0.0005</td>
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<td>0.000871</td>
<td>0.00204</td>
<td>0.9983</td>
<td>2</td>
<td>0.15</td>
<td>0.119</td>
<td>266</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\(^a\) Smoothed by regression analysis.

\(^b\) Number of tests or fraction of tests in which sieve sampler recovery was zero.

\(^c\) Geometric mean.

\(^d\) \(b = \text{slope coefficient of regression equation } S = (\ln k - \ln k_o)/y_1\).
APPENDIX B

FIGURES

Figure B-1. SM Aerosol Decay Constant $k$ at 43% RH and 25°C as a Function of Formic Acid Vapor Mole Fraction $y_v$

Figure B-2. SM Aerosol Decay Constant $k$ at 43% RH and 25°C as a Function of Formic Acid Solution Mole Fraction $x_1$
Figure B-3. SM Aerosol Decay Constant $k$ at 43% RH and 25°C as a Function of Formic Vapor Density Fraction, $X_1/\Sigma X$.

Figure B-4. SM Aerosol Decay Constant $k$ at 43% RH and 25°C as a Function of Formic Acid Vapor Density Fraction, $X_1/\Sigma X$. 

\[ k = 0.012 + 1.20X_1 \pm X \]

\[ k = 0.0217 + 0.53X_1 - 2X + 0.28X_1 \pm X^2 \]
Figure B-5. SM Aerosol Decay Constant $k$ at 43% RH and 25°C as a Function of Formic Acid Solution Mole Fraction $x_1$.

Figure B-6. SM Aerosol Decay Constant $k$ at 50% to 60% RH and 25°C as a Function of Lactic Acid Vapor Concentration $X$. The 95% confidence intervals are for a single (future) value of $k$ at a chosen value of $X$.
Figure B-7. SM Aerosol Decay Rate Constant $k_b$ at $25^\circ C$ as a Function of RH

Figure B-8. Slope Coefficient $b$ of Equation 5 at $25^\circ C$ as a Function of RH
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