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Project Summary

Inactivation of Microbial Agents by Chemical Disinfectants

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Drinking water disinfection kinetics are used to evaluate *Escherichia coli*, poliovirus, and *Giardia lamblia* cysts with regard to their relative resistance to inactivation under a variety of physical and chemical conditions. The report explains the concept of C·t product (the product of residual disinfectant, C, in mg/L and contact time, t, in minutes) and reviews the effects of temperature and pH on C·t values. The limitations and dangers of extrapolating C·t values beyond the range of experimental data are also discussed.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The primary purpose of drinking water disinfection is to control waterborne diseases by inactivating the pathogenic microorganisms in the water. Disinfection is the final (and sometimes only) engineering process barrier to the entry of viable pathogens into the water distribution system.

After chlorine began to be used as a drinking water disinfectant around the turn of the century, interest in its biocidal effectiveness brought about disinfection research. Information on the kinetics of disinfection was soon developed. Since the early 1970's, concern about chemical by-products of chlorination has resulted in a higher level of research activity involving alternative disinfectants, including chloramine, ozone, and chlorine dioxide. The early disinfection research was focused on inactivation of bacteria. Viruses were studied later. Most recently, inactivation of *Giardia* cysts has been the topic of much research work.

This report presents a comprehensive review of disinfection research. The concepts of disinfection kinetics that were developed by early researchers and later modified are used in this report to evaluate *Escherichia coli*, poliovirus, and *Giardia* cysts with regard to their relative resistance to inactivation under a variety of physical and chemical conditions. The document explains the concept of C·t product (the product of residual disinfectant, C, in mg/L and contact time, t, in minutes) and reviews the effects of temperature and pH on C·t values. The limitations and dangers of extrapolating C·t values beyond the range of experimental data are also discussed.

Disinfection Kinetics

Inactivation of microorganisms can be considered to have the characteristics of a first-order chemical reaction, with the microorganism and the disinfectants constituting the reactants. This concept was expressed as Chick's Law and is written as

$$\log N/N_o = -K \cdot t \qquad (1)$$

where N_o = the original number of organisms

N = the number of organisms remaining at time t

t = the contact time

K = a proportionality constant

Ideally, plots of log N/N_0 versus t for various contact times should provide a straight line (first-order kinetics). In actual experiments, first-order kinetics are often not observed throughout the entire range of experimental conditions, but rather during only a portion of the experiment. Thus survival curves may depart from the ideal (Figure 1 a) and show (1) an initial lag period before first-order kinetics are observed (Figure 1 b), (2) a rapid initial decline in population (Figure 1 c), or (3) multiple kinetics sometimes referred to as "tailing off" (Figure 1 d). Experimental disinfection data commonly fail to follow first-order kinetics strictly (Figure 1 a). Other disinfection kinetic models have been proposed, but they are not reviewed in this report.

When the biocidal efficacy of disinfectants are compared, the major considerations are disinfectant concentration and time needed to inactivate a certain proportion of the population of exposed organisms. The C·t concept can be expressed as

$$k = C^n \cdot t \qquad (2)$$

where C = disinfectant concentration, mg/L

n = a constant, also called the coefficient of dilution

t = the contact time (minutes) required to inactivate a specified percentage of microorganisms

k = a constant for a specific microorganism exposed under specific conditions

To apply Equation 2 to disinfection data, the results are used from a number of individual experiments performed with different disinfectant concentrations under identical experimental conditions. Disinfectant concentrations (C) and times (t) needed to attain the specified degree of inactivation (e.g., 99%) are plotted on double logarithmic paper. Such plots should produce a straight line with a slope of n. When n = 1, the C·t value remains constant regardless of disinfectant concentration, and disinfectant concentration and exposure time are of equal importance. If n exceeds 1, disinfectant concentration is more important than contact time, and C·t values required for a specified kill decline as C increases. On the other hand, when n is less than 1, contact time is more important than disinfectant concentration, and C·t values for a specified kill increase as C increases and t decreases.

The value of n is an important factor in determining the degree to which extrapolation may be valid beyond the range of experimental observations. In addition, evaluating n is valid only if the experimental data follow Chick's Law (Equation 1), which is often not the case. Values of n have been evaluated, and results generally fall in the range of 0.5

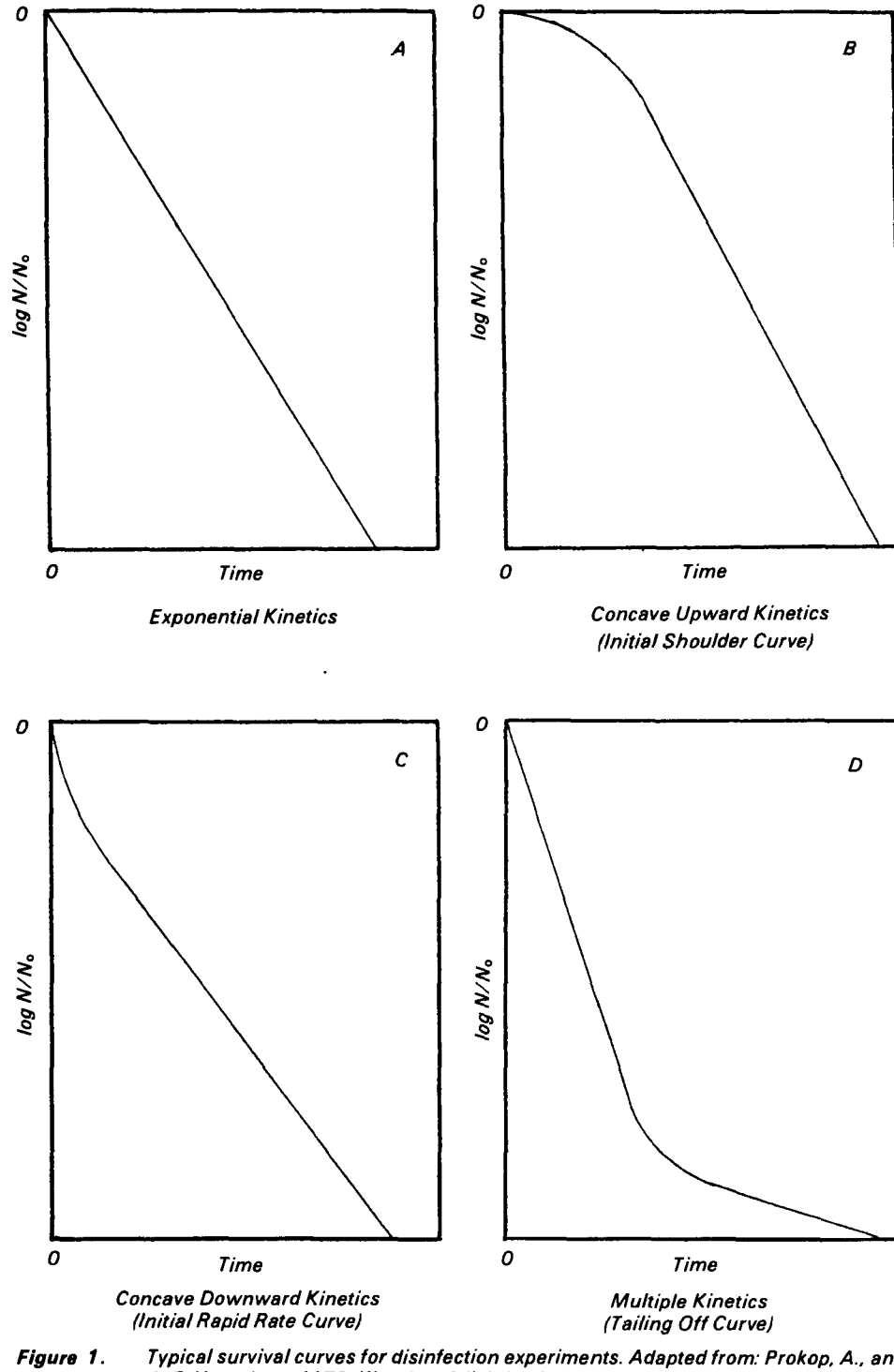

Figure 1. *Typical survival curves for disinfection experiments. Adapted from: Prokop, A., and A. E. Humphrey. 1970. Kinetics of disinfection. In:* Disinfection, *M. A. Bernarde, ed., Marcel Dekker, Inc., N.Y. pp. 61-83.*

to 2. Because of uncertainties about n, extrapolation of data from specific C and t conditions to other values under the assumption that n = 1 would be of questionable validity. Tables of n values for free chlorine, chloramine, chlorine dioxide, and ozone are given in the full report.

Temperature Effects

Effects of temperature change on disinfection efficacy have been evaluated by a number of investigators. Disinfection rates are generally increased by a factor of 2 to 3 as temperature increases by 10°C. This coefficient is referred to as the Q_{10} value. Reported Q_{10} values for viruses are usually in the range of 2 to 3. A slightly wider range of Q_{10} values is found for disinfection studies in which ozone and chlorine dioxide have been used. Some concerns have been expressed that as temperature approaches 0°C, disinfection rates might decrease by a much greater factor than would be indicated by Q_{10} values. However, no aspect of physical laws that govern chemical diffusion and reaction rates in aqueous media would support such a concept. Thus the common rule of a 2- to 3-fold increase in inactivation rates per 10°C increase in temperature seems fairly well substantiated.

Characteristics of Disinfectants and Microorganisms

Disinfectants

The disinfectants reviewed in this report (free chlorine, chloramines, chlorine dioxide, and ozone) have individual characteristics that influence both the results of laboratory tests and their performance in the field. These characteristics are reviewed here briefly.

Free chlorine exists in aqueous solution as HOCl and OCl^-. HOCl is a much more effective biocide than OCl^-, so the efficacy of free chlorine is pH-dependent.

Chloramines are formed when chlorine and ammonia react. Chloramines are generally much less effective than free chlorine, with equivalent inactivation times about 25- to 100-fold higher for monochloramine than for equivalent concentrations of free chlorine. Chloramine efficacy is also pH-dependent. Application of chloramine laboratory results to field conditions is fraught with uncertainty. Most laboratory studies have been done with preformed chloramine, whereas treatment plant practice would result in at least some contact with free chlorine before chloramines are formed, even when the order of addition is ammonia first and chlorine second. Chloramine disinfection as practiced in the field may be more effective than laboratory results would suggest, but the extent of this improvement would be site-specific and would need to be evaluated on a plant scale at each site.

Chlorine dioxide efficacy is less subject to the influence of pH than either free chlorine or chloramine. Chlorine dioxide is a more effective disinfectant at pH 9 than at pH 6. This is a reversal of the behavior shown by free chlorine and chloramine. Because it is present in water as an undissociated dissolved gas, chlorine dioxide is more easily lost through volatilization than free chlorine or chloramine. This behavior could affect the kinetics of disinfection experiments with long exposure times, especially at higher water temperatures.

Ozone, like chlorine dioxide, is present in water as a dissolved gas, must be prepared onsite, and cannot be stored. Ozone is subject to losses by volatilization during disinfection experiments. The volatility and high reactivity of ozone make it very difficult to maintain a stable concentration during experiments and in actual practice. For these reasons, C·t values for ozone tend to be less precise than C·t values for the other disinfectants.

Microorganisms

Waterborne pathogens of concern can be divided into three groups: bacteria, viruses, and protozoan cysts. They encompass a wide diversity of sizes, life cycles, and other biological characteristics, including resistance to chemical disinfectants. Note that even within different isolates of the same species, resistance to disinfection can vary. Furthermore, differences in disinfection resistance have been observed between organisms that were cultured in the laboratory and those found naturally in the environment. Finally, differences exist in relative resistance to various chemical disinfectants. Whereas organism A might be more resistant to chlorine than organism B, the opposite might be observed for chloramine or chlorine dioxide.

Application of the C·t Concept to Disinfection Practice

In 1962, Watson's Law ($k = C^n \cdot t$) was used as a basis for a procedure for making recommendations on disinfection practice. The C·t value recommendations were based on constant C·t values, making an implicit assumption that n = 1. This use of the C·t concept may have been the first to relate disinfection laboratory data to recommended field practice.

C·t values were used to compare biocidal efficiencies in 1980, but the background of the concept was not explained, and no attempt was made to extrapolate to other values for either C or t from those calculated from available data.

The use of C·t values to interpret disinfection data has become more prevalent in the 1980's. The 99% inactivation level has been used for calculating C·t values in most studies, probably because it is the level at which exponential kinetics ($N/N_0 = K \cdot t$) are usually best approximated. If exponential kinetics were followed, and if C·t values for 99% inactivation were known, C·t values for other levels of inactivation could easily be calculated. The ideal is not often observed, though. Problems associated with initial lags (Figure 1 b) and tailing off (Figure 1 d) make it difficult to calculate C·t values for conditions not directly observed in experiments. These difficulties should be noted when applying data from the following section of this report.

Inactivation of Microorganisms

Bacteria

Though pathogenic bacteria are among the target organisms for disinfection, little information is available on their inactivation. Most of the research related to bacteria has focused on indicator organisms. Studies in the 1940's did not reveal substantial differences in disinfection resistance between bacterial pathogens and members of the coliform group. Thus data for *E. coli* should indicate the degree of disinfection needed for the pathogenic bacteria.

Two factors that can influence disinfection results are the relative resistance of laboratory-grown cultures versus that of natural organisms and protection of bacteria by particulate matter. Cell cultures grown in the laboratory are more easily inactivated. Bacteria that are within particles of feces or other organic matter or that are attached to activated carbon particles are not inactivated as readily as bacteria that are not associated with such particulate matter.

In the full report, data show ranges of C·t values for 99% inactivation of *E. coli* by free chlorine, chloramine, chlorine dioxide, and ozone. With free chlorine, the range of experimental conditions for which data are available is somewhat reduced for *E. coli*. The reason is that at low pH and high temperature (pH 6, 25°C), inactivation proceeds so fast that C·t measurements are difficult to attain with confidence. C·t values for free chlorine are given for pH 6 and 10, and for 5° and 15°C. The mean C·t for 99% inactivation at 5°C and pH 6 was 0.045 mg/L · minutes. For chloramine at 5°C and pH 7, the mean C·t was 22 mg/L · minutes. Chloramine data are given for pH 7 and 9, and for 5°, 15°, and 25°C. The mean C·t for chlorine dioxide at 5°C and pH 6.5 was 0.6 mg/L · minutes, a higher value than that observed for free chlorine. This level contrasts with the relative efficacy of free chlorine and chlorine dioxide for poliovirus and *Giardia* cysts. In both of the latter cases, chlorine dioxide is the more powerful disinfectant. Chlorine dioxide data span a temperature range of 5° to 25°C and a pH range of 6.5 to 7. A mean C·t value of 0.2 mg/L · minutes was obtained for 99% inactivation of *E. coli* by ozone at pH 7.2 and 1°C. Ozone data are also available at 12°C and pH 7.0.

For the four disinfectants, the n values were generally near 1 when *E. coli* was the target organism. The ranges of C·t values were relatively narrow for experiments conducted at the same pH and temperature using different disinfectant concentrations. This result suggests that C·t values for *E. coli* are relatively reliable.

Viruses

The most extensive research on virus inactivation has been done with members of the enterovirus group because the viral agents responsible for waterborne disease (Hepatitis A virus, rotavirus, Norwalk virus, etc.) were identified only recently. Methods for laboratory growth and enumeration of the pathogenic viruses are difficult or not yet available. Most of the disinfection data presented in this report are for poliovirus.

Factors involved in viral resistance to disinfection are their natural or innate resistance, aggregation into virus clumps, and association with particulate materials. Research results suggest that viral aggregation or clumping can cause deviation from exponential inactivation kinetics, particularly the tailing off curves (Figure 1 d). The protective effects of particulate matter are similar for viruses and bacteria. The best protection is offered by virus-particle complexes associated with human fecal material. Viral clumps in fecal particles are most likely to be highly protected from inactivation.

The viral C·t data base is largest for poliovirus 1. For 99% inactivation with free chlorine, C·t values averaged 1.1 and 2.0 mg/L · minutes for two different researchers. For 5°C and pH 10, C·t averaged 10.5 mg/L · minutes. Data are available for 5° and 15°C, and for pH 6 and 10.

In contrast to these values for free chlorine, the 99% inactivation value for chloramine at 5°C and pH 9 averaged 1420 mg/L · minutes, indicating that chloramine is a very weak viral disinfectant. Chloramine data are available at pH 9 and 5°, 15°, and 25°C.

Chlorine dioxide was as effective as free chlorine, with a mean C·t of 3.6 mg/L · minutes for 99% inactivation at 5°C and pH 7. Data are available for pH 7 and 9, and for 5° to 25°C. Ozone was the most effective agent. A 99% inactivation was attained at 5°C and pH 7.2 with a mean C·t of 0.2 mg/L · minutes. Ozone data are available for 5°, 10°, and 20°C, and for pH 7.0 or 7.2.

A limited number of other data are also presented in the full report. Overall, the C·t values for poliovirus 1, rotavirus, and bacteriophage f_2 are similar. Laboratory studies done with preformed chloramine indicate that all of these viruses are extremely resistant to chloramine. The apparent biocidal efficiency of chloramine as it is used in waterworks practice would be higher because of the free chlorine that is present for a short time.

Protozoan Cysts

The inactivation of *Endomoeba histolytica* cysts by chlorine and other disinfectants was studied extensively during the 1940's and 50's, mainly because of concerns about waterborne transmission of amoebic dysentery in military forces operating in areas where this disease was prevalent. These studies established conclusively that the cysts of *E. histolytica* were very resistant to inactivation. The appearance of giardiasis as an important waterborne disease in the United States stimulated disinfection research on the inactivation of cysts of the etiologic agent *Giardia lamblia*. A method for determining cyst viability by *in vitro* excystation was developed, but problems developed in obtaining *G. lamblia* cysts, and deficiencies occurred in the excystation procedure. Thus most disinfection research is currently conducted using *G. muris* cysts (a species infective for mice) as a model for *G. lamblia* cysts. This approach seems to work well. A comparative study of excystation and mouse infectivity for measurement of chlorine-exposed *G. muris* cysts indicated that the results were similar for both methods. *Giardia lamblia* has a complex life cycle. The conversion from the active trophozoite to the inactive, resistant cyst occurs in the lower portion of the intestinal tract. The cysts do not multiply and are relatively inert in the environment, excysting to form the trophozoite stage only after ingestion by the host.

Because the cysts are relatively large (ovoid bodies 8 to 12 by 7 to 10 μm in diameter), protection from inactivation by association with particulate matter may be less important for them than for smaller, more easily occluded pathogens. Little information has been developed on this subject.

The largest data base available for *Giardia* is from disinfection research with *G. muris* cysts. The available data for cysts of the human pathogen *G. lamblia* also are included in the report.

Mean C·t values for 99% inactivation of *G. lamblia* cysts by free chlorine range from 65 to greater than 150 mg/L · minutes for 5°C and pH 6. Data are available for 5°, 15°, and 25°C, and for pH 6, 7, and 8. Data on *G. muris* cover the range of 3° to 25°C, and pH 5 to 9. At 5°C and pH 6, a C·t of greater than 150 mg/L · minutes has been reported. However, researchers have also obtained a C·t of 68 mg/L · minutes for 3°C, pH 6.5, and 99% inactivation.

Chloramine data are available for *G. muris* in a temperature range of 3° to 18°C and a pH of 6.5 to 8.5. A mean C·t of 463 mg/L · minutes was obtained for 99% inactivation at 3°C, pH 6.5, but the chloramine was not preformed. At 18°C and pH 7, a C·t of 184 mg/L · minutes was obtained when chloramine was not preformed. In contrast, at 15°C and pH 7, a mean C·t of 848 mg/L · minutes resulted from use of preformed chloramine.

Data for 99% inactivation of *G. muris* by chlorine dioxide are available for 5° and 25°C at pH 7 and 9. At 5°C and pH 7, the mean C·t is 11.2 mg/L · minutes. This value is about one order of magnitude lower than those for free chlorine

and chloramine, and it suggests that chlorine dioxide is a powerful cysticidal agent.

Ozone disinfection data are available for *G. muris* and *G. lamblia* at pH 7 from 5° to 25°C. At pH 7 and 5°C, the mean C·t value was 1.9 mg/L · minutes for 99% inactivation of *G. muris* and 0.6 mg/L · minutes for *G. lamblia.* Ozone appears to be somewhat more effective against cysts than chlorine dioxide.

A summary of the comparative efficiency of free chlorine, chloramine, chlorine dioxide, and ozone for inactivation of specific bacteria, viruses, and protozoan cysts appears in Table 1. Ozone shows the highest efficiency, inactivating 99% of all types of microorganisms at very low C·t values. Chloramine shows the lowest efficiency. Chloramine C·t values for viral agents are particularly high. Free chlorine at pH 6 to 7 and chlorine dioxide at pH 7 are approximately equivalent for poliovirus 1 inactivation. Free chlorine appears to be considerably more effective than chlorine dioxide for inactivation of rotavirus, bacteriophage f_2, and *E. coli,* whereas chlorine dioxide appears to be much more effective than free chlorine for *G. muris* cysts. The data in Table 1 also show the relative variability in resistance among and within the groups of microorganisms. The general pattern of greater resistance of cysts compared with viruses, and of viruses compared with bacteria is evident for free chlorine, chlorine dioxide, and ozone. Although cyst C·t values for preformed chloramines at 5°C are not yet available, the available values at 15°C suggest that cysts may be more sensitive to preformed chloramine than the viruses.

The bacteriophage f_2 C·t values suggest that the use of this virus to indicate virus inactivation is questionable. On the other hand, poliovirus appears to be a relatively good indicator, since it is substantially more resistant to free chlorine and chlorine dioxide than rotavirus and bacteriophage f_2. Rotavirus, however, appears to be somewhat more resistant to preformed chloramine than poliovirus 1.

Finally, *G. muris* cysts appear to be somewhat more resistant than *G. lamblia* cysts to free chlorine and ozone. Also, considerable uncertainty exists with regard to C·t values for 99% inactivation of cysts by free chlorine. Values derived from studies by different investigators show substantial variation.

Table 1. *Summary of C·t Value Ranges for 99% Inactivation of Various Microorganisms by Disinfectants at 5°C*

	Disinfectant			
Micro-organism	*Free Chlorine, pH 6 to 7*	*Preformed Chloramine, pH 8 to 9*	*Chlorine Dioxide, pH 6 to 7*	*Ozone, pH 6 to 7*
E. coli	*0.034-0.05*	*95-180*	*0.4-0.75*	*0.02*
Polio 1	*1.1-2.5*	*768-3740*	*0.2-6.7*	*0.1-0.2*
Rotavirus	*0.01-0.05*	*3806-6476*	*0.2-2.1*	*0.006-0.06*
Bacterio-phage f_2	*0.08-0.18*	--	--	--
G. lamblia *cysts*	*47->150*	--	--	*0.5-0.6*
G. muris *cysts*	*30-630*	--	*7.2-18.5*	*1.8-2.0*

All of the C·t values discussed above are based on 99% inactivation of the microorganisms. As indicated, the nature of inactivation curves prevents extrapolation from the 99% inactivation C·t values to obtain reliable C·t values for other levels of inactivation (e.g., 50%, 90%, 95%, 99.9%, etc.). The curves nearly always show either an initial shoulder, tailing off, or other more complex configurations (see Figure 1). Extrapolation from initial shoulder curves on the 99% inactivation level (assuming exponential inactivation rates*) will underestimate C·t values for less than 99% inactivation and overestimate C·t values for greater than 99% inactivation. Extrapolation from initial rapid rate and tailing off curves will usually have the opposite effect, depending on the point at which the inactivation rate begins to decrease. Thus extreme caution must be used if any attempt is made to extrapolate C·t data to other inactivation percentages.

Conclusions

1. The C·t values compiled provide a basis for comparing the effectiveness of different disinfectants for inactivation of specific microorganisms and for comparing the relative resistance of different microorganisms to specific disinfectants. In some cases, the C·t values derived from exposure to different concentrations of the same disinfectant under specific pH and temperature conditions show little variation, and in other cases, a wide range of C·t values occurs. Discerning the reasons for widely differing values is almost always difficult, whether considering the results from only one investigation or from several. These factors make it difficult to pinpoint disinfection requirements. C·t values must be used cautiously to evaluate disinfection practice or to establish disinfection criteria for use in the field, and appropriate safety factors must be incorporated into the C·t values.
2. Some major problems in applying the results of C·t values to developing disinfection requirements are as follows: a) the failure of disinfection data to follow the exponential rates described by the empirical C·t equation, b) differences in disinfection resistance between different isolates of the same species and between different species within groups (bacteria, viruses, cysts), c) state-of-the-microorganism effects such as aggregation, prior growth conditions, and protective effects that cannot be factored into the values, d) influence of experimental conditions (mixing intensity, disinfectant concentration variations, etc.) on inactivation rates, e) problems relating to the relevance of laboratory data to field conditions.
3. Because of the limited data available, uncertainties still remain regarding disinfection requirements for *Giardia lamblia* cysts. Most of the data available for *G. lamblia* cysts indicate lower requirements than do the

*A straight line extended from 100% survival at time 0 through the 99% inactivation time point.

more extensive data available for the model *G. muris* cysts. These uncertainties are very important because this pathogen is the most resistant of all waterborne pathogens of concern. Additional research is in progress using *G. muris* cysts and chloramines and free chlorine at low temperatures. Other research indicates that an alternative to the excystation method for determing *G. lamblia* and *G. muris* viability may soon be available. Such an alternative would facilitate disinfection research on this species. The method would still involve microscopic observation and therefore would not result in improved detection of viability at low cyst concentrations (greater than 99% inactivation).

4. For some disinfectants (mainly chloramines), utilities should perhaps be required to demonstrate the efficacy of their disinfection practices for controlling pathogens of concern. This alternative approach may well be warranted because of the extreme dependence of chloramine disinfection efficiency on field conditions that cannot all be taken into account in developing overall C·t values.
5. For inactivation by free chlorine, pH is a very important factor because of the rapid decrease in the more effective disinfectant chemical species (HOCl) that occurs over a pH range of 7 to 8. Many natural waters fall into this pH range. Monitoring of pH and subsequent pH modification may be advisable in some cases to enhance disinfection efficiency, particularly at low temperatures.

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The complete report, entitled "Inactivation of Microbial Agents by Chemical Disinfectants," (Order No. PB 86-232 568/AS; Cost $11.95, subject to change) will be available only from:

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