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# Project Summary

# Predicting the Inactivation of *Giardia lamblia*: A Mathematical and Statistical Model

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**The 1986 amendments to the Safe Drinking Water Act (SDWA) require the U.S. Environmental Protection Agency (EPA) to promulgate primary drinking water regulations (a) specifying criteria under which filtration would be required, (b) requiring disinfection as a treatment technique for all public water systems, and (c) establishing maximum contaminant levels (MCLs) or treatment requirements for control of *Giardia lamblia*, viruses, *Legionella*, heterotrophic plate count bacteria, and turbidity. Because the *Giardia lamblia* organism is one of the most resistant to chlorine disinfection, the proposed Surface Water Treatment Rule (SWTR) specifies "C·t" values (the product of concentration of disinfectant in mg/L and disinfectant contact time in minutes) for 99.9% inactivation of *Giardia* cysts. Many factors influence *G. lamblia* reaction kinetics including temperature, pH, chlorine concentration, and inactivation level. A model is developed to describe these interactions and to predict C·t values based on specific model inputs. A strategy is proposed that uses the model to provide conservative C·t values for regulatory purposes.**

***This Project Summary was developed by EPA's Risk Reduction Engineering 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

EPA has proposed surface water treatment technique requirements to fulfill the SDWA requirements for systems using surface waters. Additional regulations specifying disinfection requirements for systems using ground water sources will be proposed and promulgated at a later date.

Under the proposed SWTR, all community and noncommunity public water systems would be required to treat their surface water sources to control *G. lamblia*, enteric viruses, and pathogenic bacteria. The minimum required treatment for each surface water would include disinfection. In addition, unless the source water is well protected and meets certain water quality criteria (limits on total or fecal coliforms and turbidity), required treatment would also include filtration. The treatment provided, in any case, would be required to achieve a 99.9% removal and/or inactivation of *Giardia* cysts, and at least 99.99% removal and/or inactivation of enteric viruses. Unfiltered systems would be required to demonstrate that disinfection alone achieve the minimum performance requirements. Filtered systems that meet certain turbidity removal and disinfection performance criteria and that comply with design and operating criteria specified by the State would be considered to be in compliance with these requirements.

To demonstrate a water system is achieving a specified percent inactivation, the system would monitor disinfectant residual(s), disinfectant contact time(s), pH, and water temperature, and apply these data to determine if its "C·t" [the product of disinfectant concentration (mg/L) and disinfectant contact time (minutes)] value equaled or exceeded the C·t value specified in EPA's rule or Guidance Manual. Because the *G. lamblia* organism is one of the most resistant to chlorine disinfection to be found in water, much interest and effort has been devoted to determining its C·t values. The C·t values necessary to achieve 99.9% inactivation of *Giardia* cysts by various disinfectants and under various conditions are specified in EPA's SWTR, and C·t values recommended for filtered systems, depending on the appropriate level of inactivation, are specified in the Guidance Manual associated with the SWTR.

Many factors influence *G. lamblia* reaction kinetics. Much effort was made to develop an adquate model to describe *G. lamblia* reactions with free chlorine and then to estimate the model parameters. This Project Summary presents an overview of the model development and parameter estimation process resulting from this project.

## The C·t Concept

In comparing the biocidal effectiveness of disinfectants, major considerations are the disinfectant concentration and time needed to attain inactivation of a certain proportion of the population exposed under specified conditions. The C·t concept in current use is based on the following empirical equation:

$$K = C^n t \qquad (1)$$

where

- K = constant for a specific microorganism exposed under specific conditions
- C = disinfectant concentration in mg/L
- t = the contact time required for a fixed percent inactivation in minutes

It is based on the van't Hoff equation used for determining the nature of chemical reactions in which the value of n determines the order of the chemical reaction.

The application of this equation to disinfection studies requires multiple experiments where the effectiveness of several variables, such as pH, temperature, and the disinfectant concentration, are examined to determine how they affect the inactivation of microbial pathogens. The value of n is a very important factor in determining the degree to which data extrapolated from disinfection experiments is valid.

Destroying pathogens by chlorination depends on a number of factors, including water temperature, pH, disinfectant contact time, degree of mixing, turbidity, presence of interfering substances, and concentrations of chlorine available. The pH, especially, has a significant effect on inactivation efficiency because it determines the species of chlorine found in solution.

The effect of temperature on disinfection efficiency is also significant. For example, virus destruction by chlorine indicates that contact time must be increased two to three times when the temperature is lowered 10°C. Disinfection by chlorination can inactivate *Giardia* cysts, but only under the most favorable conditions. Researchers have concluded that (1) these cysts are among the most resistant pathogens known, (2) disinfection at low temperatures is especially difficult, and (3) treatment processes before disinfection are important.

Studies on the use of *in vitro* excystation to determine cyst viability have shown that greater than 99.8% of *Giardia* cysts can be killed by exposure to 2.5 mg/L of chlorine for 10 min at 15°C and pH 6, or after 60 min at pH 7 or 8. At 5°C, exposure to 2 mg/L of chlorine for 60 min killed at least 99.8% of all cysts at pH 6 and 7. The same percentage of cysts were killed by 8 mg/L at pH 6 and 7 after 10 min as were killed by 8 mg/L at pH 8 after 30 min. C·t values for 99% inactivation of *G. lamblia* by free chlorine at different temperatures and pH values are shown in Table 1. The higher C·t values indicated inactivation rates decreased at lower temperatures and at higher pH values.

## Animal Infectivity Studies

Much *Giardia* inactivation data are based on excystation techniques because few *Giardia* cyst inactivation data are available based on the use of animal infectivity as a measure of cyst viability. Some investigators compared mouse infectivity and excystation for determining the viability of *G. muris* cysts exposed to chlorine and reported that both methods gave similar result recent experiment used Mongo gerbils to determine the effect chlorine on *G. lamblia* cysts. In a s of experiments, cysts were exposed various time periods to free chl concentrations ranging from 0.4 to mg/L at 0.5, 2.5, and 5.0°C and pH and 9. Each of five gerbils was fed $10^4$ of the chlorine-exposed cysts subsequently examined for evidenc infection. Since the test animals had received a dose of 5 x $10^4$ cysts since infectivity studies unchlorinated cysts showed approximately 5 cysts usually consti an infective dose, the follo assumptions were made depending the infectivity patterns occurring in animals receiving chlorine exposed c If all five animals were infected, th was assumed that the C·t had prod less than 99.99% inactivation; i animals were infected, the C·t produced greater than 99.9 inactivation. It is impossible to deter the exact level of inactivation for t results. If, however, one to four ani were infected, it was assumed tha level of viable cysts were five per ar and that 99.99% of the original population has been inactivated.

Table 2 summarizes data for different experimental condit examined. Column 3 shows the ran chlorine concentrations in mg/L to w cysts were exposed before being fe the gerbils, and Column 7 shows number of experiments which yielde infected gerbils out of 5. Column 4 sh the mean cyst exposure times Column 5 contains mean C·t values are the product of the chlo concentration and cyst exposure time

In addition to the animal infect several other data sets were consid as a data base for a log linear mod *Giardia* cysts. The studies on w these data sets were based characterized (Table 3). The first que to arise is the statistical compatibili the data sets. Because of the size o data set and the fact that it is base animal infectivity, set 2 data considered in all combinations. approach used was to construc indicator random variable to move regression intercept or slope compensate for data set differences. significance of the indicator ran variable would support the hypothes different regression surfaces, incompatibility of the data sets ch The indicator random variable created in such a way as to al

***Table 1.*** *C·t Values for 99% Inactivation of* Giardia lamblia *Cysts by Free Chlorine*

| Temp (°C) | pH | Range: Disinfectant Concentration (mg/L) | Range: Time (min) | Range: C·t | Mean C·t | No. of Exp. |
|---|---|---|---|---|---|---|
| 5 | 6 | 1.0-8.0 | 6-47 | 47-84 | 65 | 4 |
| | 7 | 2.0-8.0 | 7-42 | 56-152 | 97 | 3 |
| | 8 | 2.0-8.0 | 72-164 | 72-164 | 110 | 3 |
| 15 | 6 | 2.5-3.0 | 7 | 18-21 | 20 | 2 |
| | 7 | 2.5-3.0 | 6-18 | 18-45 | 32 | 2 |
| | 8 | 2.5-3.0 | 7-21 | 21-52 | 37 | 2 |
| 25 | 6 | 1.5 | < 6 | < 9 | < 9 | 1 |
| | 7 | 1.5 | < 7 | < 10 | < 10 | 1 |
| | 8 | 1.5 | < 8 | < 12 | < 12 | 1 |

***Table 2.*** *C·t Values for 99.99 % Inactivation Based on Animal Infectivity Data*

| pH | Temp °C | Range of Concentration (mg/L) | Range of Mean Cyst Exposure Time (min) | Range of Mean C·t Values from Data | Range of Predicted C·t Values | Number of Observations |
|---|---|---|---|---|---|---|
| 6 | 0.5 | 0.56-3.93 | 39-300 | 113-263 | 136-192 | 25 |
| 6 | 2.5 | 0.53-3.80 | 18-222 | 65-212 | 107-151 | 15 |
| 6 | 5 | 0.44-3.47 | 25-287 | 50-180 | 93-134 | 26 |
| 7 | 0.5 | 0.51-4.05 | 75-600 | 156-306 | 205-295 | 14 |
| 7 | 2.5 | 0.64-4.23 | 55-350 | 124-347 | 169-235 | 14 |
| 7 | 5 | 0.73-4.08 | 47-227 | 144-222 | 156-211 | 15 |
| 8 | 0.5 | 0.49-3.25 | 132-593 | 159-526 | 294-410 | 22 |
| 8 | 2.5 | 0.50-3.24 | 54-431 | 175-371 | 233-324 | 21 |
| 8 | 5 | 0.48-3.67 | 95-417 | 200-386 | 209-299 | 15 |

***Table 3.*** *Characterization of* G. lamblia *Free Chlorine Inactivation Studies Used in Predictive Models*

| Data Set | Cyst Source | Viability Assay | Comments |
|---|---|---|---|
| 1 | Symptomatic human | Excystation | Conventional survival curves based on multiple samples. End point ~ 0.1% survival |
| 2 | Gerbils, adapted from infected humans (CDC isolate) | Gerbil infectivity (10 animals/sample) | No survival curves. Endpoint sought ~ 0.01% survival |
| 3 | Symptomatic and nonsymptomatic humans | Excystation | Conventional survival curves based on multiple samples. End point ~ 0.1% survival |
| 4 | Gerbils adapted from infected humans (several isolates used) | Excystation | Conventional survival curves based on multiple samples. End point ~ 0.1% survival |

differentiate between data set 2 and other data sets considered and to move the regression intercept not the slope. The indicator random variable is defined as follows:

$$z = \begin{cases} 0 & \textit{data set 2} \\ 1 & \textit{data set 2} \end{cases} \quad (2)$$

Therefore the model to be used was defined as follows:

$$t = R\,I^{a}C^{b}pH^{c}temp^{d}10^{ez} \quad (3)$$

or

$$\log t = \log R + a \log I + b \log C + c \log pH + d \log temp + ez \quad (4)$$

In equations 2 and 3, when $z = 0$, equation 2 is defined over data set 2, and

$$t = R\,I^{a}\,C^{b}\,pH^{C}\,temp^{d} \quad (5)$$

where

| | | |
|---|---|---|
| t | = | time to a given level of inactivation |
| I | = | ratio of organisms remaining at time t to organisms at time zero |
| C | = | concentration of chlorine in mg/L |
| pH | = | log of the hydrogen ion concentration |
| temp | = | temperature in degrees C |
| R,a,b,c,d | = | Regression constants |

When z = 1 equation 3 is defined over the remaining data and

$$t = (R \cdot 10^{e})\,I^{a}\,C^{b}\,pH^{c}\,temp^{d} \quad (6)$$

For data sets 1 and 2, the indicator random variable for the intercept variable was not significant (p-value = 0.3372). All other data bases considered had a significant indicator random variable at the 0.05 level of significance. A formal test for differences of intercept and/or slope between data sets 1 and 2 was conducted and no difference was detected. Thus, statistical analysis supports the use data sets 1 and 2 for extending the model development and the parameters in equation 2 were reestimated using these data.

## Model Development

With the use of the indicator random variable approach parameters for the predicting equations were reestimated resulting in the following equation:

$$t = 0.12\,I^{-0.27}\,C^{-0.81}\,pH^{2.54}\,temp^{-0.15} \quad (7)$$

multiplying equation 7 by C yields

$$C{\cdot}t = 0.12\,I^{-0.27}\,C^{0.19}\,pH^{2.54}\,temp^{-0.15} \quad (8)$$

Equation 8 is used to calculate C·t values.

Table 4 summarizes the parameter estimates and diagnostic statistics for the equation. The fit of the model was good, with the regression variables explaining 86% of the variation in log (t).

The 95% confidence intervals of the parameter estimates of equation 8 based on the Bonferroni method are:

| | |
|---|---|
| R: | ( 0.0384, 0.4096) |
| a: | (-0.2321, -0.3031) |
| 1 + b: | ( 0.0792, 0.2977) |
| c: | ( 1.9756, 3.1117) |
| d: | (-0.2192, -0.0724) |

## Regulatory Application

Many of the uncertainties about the various data sets might be used to calculate C·t values. The random variable analysis shows the statistical incompatibility among most of these data sets. More work must be done to define the impact of strain variation and *in vivo* verses *in vitro* techniques on C·t values. In order to provide conservative estimates for C·t values when using the best available methodology the authors suggest the approach illustrated in Figure 1.

In Figure 1, the 99% confidence interval at the 4 log inactivation level is calculated. First order kinetics are then assumed so that the inactivation "line" passes through 1 at C·t = 0 and the upper 99% confidence interval of the C·t value at 4 logs of inactivation. As can be seen the inactivation line consists of higher C·t values than do all of the mean predicted C·t values (mean) from equation 17, and most of the data from data set 1 and most of data set 2 data points. Conservative C·t values for a specified level of inactivation can be obtained from the inactivation line prescribed by the disinfection conditions. For the example indicated in Figure 1, the appropriate C·t for achieving 99.9% inactivation would be 105. This approach (assumption of first order kinetics) also provides the basis for establishing credits for sequential disin-fection steps.

Table 5 compares the C·t values calculated, with the use of the modified approach, to the C·t values from the SWTR.

## Summary and Conclusions

Amendments to the SDWA clearly require that all surface water suppliers in the United States filter and/or disinfect to protect the health of their customers. *G. lamblia* has been identified as one of the leading causes of waterborne dise outbreaks in the United States. *G. lam* cysts are also one of the most resis organisms to disinfection by f chlorine. EPA's Office of Drinking W has adopted the C·t concept to qua the inactivation of *G. lamblia* cysts disinfection. If a utility can assure th large enough C·t can be maintaine ensure adequate disinfection th depending upon site specific factor may not be required to install filtra Similarly, the C·t concept can be app to filtered systems for determir appropriate levels of protection.

In this paper, an equation has b developed that can be used to predic values for the inactivation of *G. lam* by free chlorine based on the interac of disinfectant concentration, t perature, pH, and inactivation level. parameters for this equation have b derived from a set of animal infect data and excystation data. The equa can be used to predict C·t values achieving 0.5 to 4 logs of inactiva within temperature ranges of 0.5 to 5 chlorine concentration ranges up t mg/L, and pH levels of 6 to 8. Altho the equation was not based on pH va above 8, the model is still conside applicable to pH levels of 9 for rea discussed elsewhere. The equa shows the effect of disproportio increases of C·t versus inactivation le With the use of 99% confidence inter at the 4 log inactivation levels and applying first order kinetics to these points a conservative regulatory stra for defining C·t at various level inactivation has been proposed. approach represents an alternative to regulatory strategy previously propos

***Table 4.*** *Parameter Estimates for Equation 12*

| Variable | DF | Parameter Estimate | Standard Error | T for HO: Parameter = 0 | PROB > \| t \| | Variance Inflation |
|---|---|---|---|---|---|---|
| INTERCEP | 1 | -0.902 | 0.200 | -4.518 | 0.0001 | 0.000 |
| LOGI | 1 | -0.268 | 0.014 | -19.420 | 0.0001 | 1.183 |
| LOGCHLOR | 1 | -0.812 | 0.042 | -19.136 | 0.0001 | 1.033 |
| LOGPH | 1 | 2.544 | 0.221 | 11.535 | 0.0001 | 1.032 |
| LOGTEMP | 1 | -0.146 | 0.028 | -5.117 | 0.0001 | 1.179 |

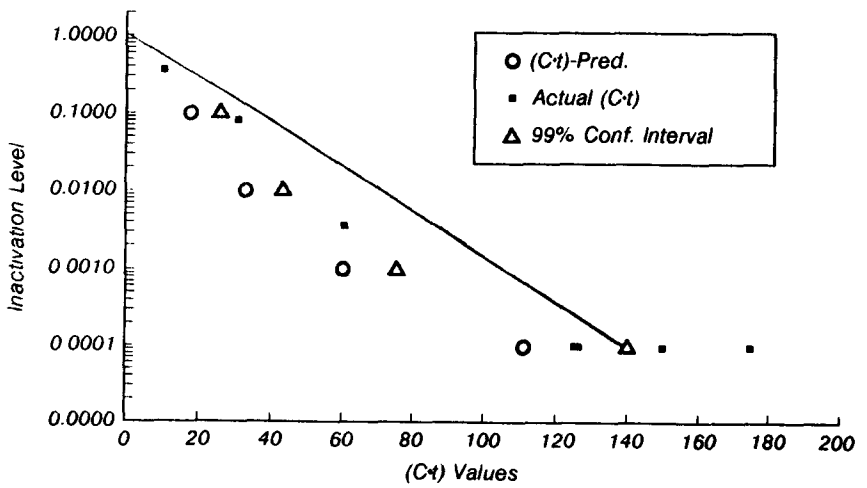


***Figure 1.*** *99% Confidence levels using Hibler-Jarroll Equation for chlorine = 1 mg/l; pH = 6; temperature = 5°C.*

***Table 5.*** *Comparison Between Modified Approach (Means) and Rule C·ts at 99.9% Inactivation and 5°C*

| Concentration mg/L | pH | | | | | |
|---|---|---|---|---|---|---|
| | 6 | | 7 | | 8 | |
| | Eq. 22 | Rule | Eq. 22 | Rule | Eq. 22 | Rule |
| 1 | 105 | 108 | 149 | 165 | 216 | 238 |
| 2 | 116 | 122 | 165 | 186 | 243 | 269 |

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*The complete report, entitled "Predicting the Inactivation of Giardia lamblia: A Mathematical and Statistical Model," (Order No. PB 89-233 472/AS; Cost: $15.95, subject to change) will be available only from:*

*National Technical Information Service*
*5285 Port Royal Road*
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