Environmental Science & Technology Letters

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Letter



# A Statistical Framework for Assessing Heterogeneous Sensitivity of Viruses to Ultraviolet, Ozone, and Free Chlorine

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Cite This: *Environ. Sci. Technol. Lett.* 2025, 12, 904−910

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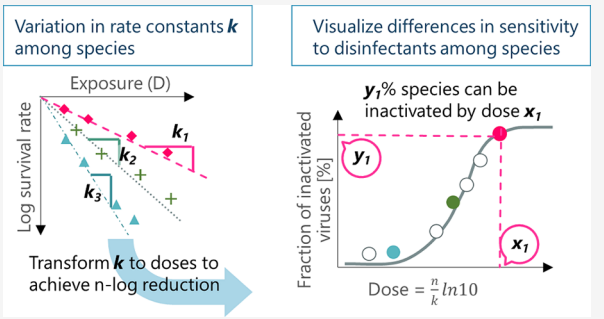
**ABSTRACT:** Disinfection is key to controlling the infection risk caused by viral contamination. Current disinfection guidelines often refer to a single virus resistant to the disinfectant of interest, despite the large variation in sensitivity to disinfectants among viruses or even among strains within the same species. Here, we demonstrate a statistical framework that integrates multiple experimental data sets and model the variation in sensitivity to disinfectants across different virus species using a parametric distribution termed the disinfectant sensitivity distribution. To illustrate this framework, we used 37, 9, and 28 species-dependent inactivation rate constants for ultraviolet (UV), ozone, and free chlorine, respectively, from systematic reviews. We estimated the sensitivity distributions of these disinfectants by incorporating the uncertainty in the individual inactivation rate constants using a Bayesian framework. The estimated sensitivity distributions suggested that it should be possible to achieve 4-log inactivation of 93.0% (95% credible interval (CrI): 84.2%−97.5%), 99.4% (95% CrI: 86.7%−100%), and 95.0% (95% CrI: 85.5%−98.8%) of the examined virus species using UV, ozone, and free chlorine, respectively, if the disinfectant dose complies with the values recommended by the US EPA. The proposed approach provides a reasonable extrapolation of observed inactivation kinetics to untested viruses and tools for more transparent risk assessments.




**KEYWORDS:** *Disinfection, Free chlorine, Inactivation kinetics, Ozone, Species sensitivity distribution, Ultraviolet irradiation, Virus*



## INTRODUCTION

Disinfection is a key component in controlling microbial risks in water, food, air, and other environments. Pathogenic viruses can be inactivated by exposure to disinfectants such as free chlorine, ozone, and ultraviolet (UV) radiation, and these disinfection technologies have been widely applied in various industries, including pharmaceutical engineering and the food industry, as well as in (waste)water treatment and healthcare facilities.[1] Each disinfectant has a different mode of action,[2] and thus does not always achieve high inactivation efficacy against all viruses, which possess diverse genome and protein structures. To compare the disinfection efficacy across different types of viruses, the inactivation rate constant (often denoted as $k$)—or its reciprocal, the required disinfectant dose (e.g., UV fluence or CT value) for a given level of inactivation—has been used as a common indicator. This metric is typically obtained by fitting the Chick−Watson model to disinfection experiment data, assuming a dilution coefficient of one.[3,4]

Recent disinfection studies have revealed substantial heterogeneity in sensitivity to disinfectants across different types of viruses, including among species, genotypes, and even strains, under identical experimental conditions.[5−14] Despite this, most regulatory guidelines for water treatment evaluate virucidal efficacies of disinfection processes, referring to a single virus that is known to be resistant to disinfectants and clinically important. For example, the United States Environmental Protection Agency (US EPA) Guidance Manual refers to adenovirus for UV, hepatitis A virus for free chlorine, and poliovirus for ozone.[15] However, recent studies have suggested the existence of more resistant viruses (e.g., coxsackievirus B5 (CVB5) for free chlorine), and the research community is debating whether such findings should be incorporated into guidelines as worst-case scenarios for the treatment of drinking water and wastewater.[16−18]

Despite its practicality, the "single reference" approach has two inherent limitations. First, it is infeasible to test all newly identified viruses, given their virtually infinite diversity. As demonstrated by the example of CVB5, previously untested viruses may exhibit unexpectedly high resistance to disinfection. As more experimental data accumulate, the inactivation kinetics of known viruses are becoming increas-

Received: May 8, 2025
Revised: June 5, 2025
Accepted: June 5, 2025
Published: July 1, 2025

ingly well understood, sometimes revealing resistance greater than that previously recognized. This leads to continuous revisions of which virus is considered the most resistant and can be used as a reference pathogen, and this process is likely to continue indefinitely. Second, even if recommended thresholds (e.g., the CT values for 4-log reduction) for the reference virus are met, the actual proportion of virus species in an environmental sample—including untested ones—that are effectively inactivated remains uncertain.

The same motivation—how to extrapolate empirical data on tested organisms to untested ones—has been investigated in the fields of ecotoxicology and ecological risk assessment. This approach, known as species sensitivity distribution (SSD),[19−22] assesses the ecological risk of chemicals by estimating the proportion of species affected at a given concentration. Typically, a parametric distribution is fitted to acute or chronic toxicity data for multiple species (e.g., using EC*x* values, which represent the effective concentration at which *x*% of the population exhibits the (toxic) outcome of interest). This yields a distribution of sensitivities to the chemical of interest across an assemblage of species present in an environmental sample. Subsequently, the fifth percentile value is typically used to derive benchmarks, such as the predicted no-effect concentration. In a similar manner, we argue that viral disinfection experimental data can be summarized to infer a distribution of disinfectant sensitivity across an assemblage of virus species, thereby enabling extrapolation from individual virus-level observations to disinfection outcomes under broader treatment scenarios.

In this study, we propose a statistical framework that produces distributions that describe various viruses' sensitivities to disinfectants by integrating available data sets on inactivation rate constants. To demonstrate this framework, we used experimental data on disinfection using UV, ozone, and free chlorine from existing systematic reviews.[3,23,24] We also discuss how the inferred sensitivity distributions can be used to translate required log-reduction values into the proportions of potentially inactivated viruses by referring to current disinfection guidelines.

## METHODS AND MATERIALS

**Data Collection.** We used 220, 31, and 82 reported values of inactivation rate constants for UV ($mJ^{-1}$ $cm^2$), ozone ($mg^{-1}$ $min^{-1}$ L), and free chlorine ($mg^{-1}$ $min^{-1}$ L), respectively, from systematic reviews.[3,23,24] These reviews employed screening criteria to assess whether the experimental conditions had been explicitly provided in the collected articles required to determine the disinfectant dose (e.g., disinfectant concentration and decay during the experiment). As detailed in the Supporting Information, the collated data sets were either standardized under specific pH and temperature conditions or collected under similar experimental conditions to account for the variation in the inactivation rate constant due to biological differences. The collated data were then aggregated by viral species, which is the lowest rank in the formal taxonomic hierarchy recognized by the International Committee on the Taxonomy of Viruses. A virus species is defined as a single monophyletic group of viruses that is distinguishable from other groups by a set of shared properties.[25]

The inactivation rate constants and the guideline disinfection values (i.e., the values recommended by the current US EPA manuals for water disinfection[15]) for the three disinfectants were transformed into doses (with units of mJ $cm^{-2}$ for UV and mg min $L^{-1}$ for ozone and free chlorine) for achieving a given *n*-log reduction value ($n$ = 2, 4, or 6). The guidelines for water disinfection served as examples for illustrating the relationship between the single reference value and the framework proposed in this study. A more detailed description of the processing of these data is given in the Supporting Information.

**Estimation of Disinfectant Sensitivity Distributions.** The disinfectant sensitivity distributions for UV, ozone, and free chlorine were estimated by fitting three parametric distributions to the collated inactivation rate constants using a Bayesian approach.[26,27] For the main analysis, we chose to use a log-normal distribution as this is widely used in estimating SSDs for ecological risk assessment;[28,29] the Weibull and gamma distributions were selected as alternative distributions.

A Bayesian framework was employed to estimate the parameters of the selected distributions (see the Supporting Information for details). This method considers the uncertainty in the observed inactivation rate constants as interval data by assuming that an individual observation is uniformly distributed over the interval defined by the mean ± 2SD and by incorporating interval censoring directly into the likelihood. As the inactivation rate constants and guideline values were defined for a given *n*-log reduction, we estimated the disinfectant sensitivity distributions for each combination of the three disinfectants and *n*-log reduction values.

The goodness-of-fit of each model was assessed using the Widely Applicable Information Criterion (WAIC) and Leave-One-Out Information Criterion (LOOIC). 95% credible intervals (CrIs) were obtained from posterior samples generated by Markov Chain Monte Carlo (MCMC). More details of the computational settings are given in the Supporting Information.

**Proportions of Potentially Inactivated Virus Species.** The disinfectant sensitivity distributions provided the proportions of potentially inactivated species for a given disinfectant dose and a given *target of n*-log reduction. We compared the proportion of species expected to be inactivated by the disinfectant doses given in the US EPA guidelines. The guideline values are based on the required doses for the inactivation of adenovirus by UV with an 80% CrI,[30] poliovirus inactivation by ozone at a pH of 6−9 and a temperature of 20 °C with a 3-fold safety factor,[23] and hepatitis A virus inactivation by chlorine at a pH of 6−9 and a temperature of 20 °C with a 3-fold safety factor[15] (see the Supporting Information for details). In addition, the doses required for 4-log reduction of certain proportions of virus species using the three disinfectants (i.e., the doses at 95th, 99th, and 99.9th percentiles) were estimated.

## RESULTS

**Data Description.** The collated data contained the reported inactivation rate constants for UV, ozone, and free chlorine for 37, 9, and 28 species, respectively. The histograms of transformed mean inactivation rate constants (transformed dose) are shown in Figure S1 in the Supporting Information. The mean and SD values obtained for each virus species were employed in the subsequent analysis.

**Estimated Disinfectant Sensitivity Distributions and Proportion of Viruses Inactivated.** Disinfectant sensitivity distributions for UV, ozone, and free chlorine were first estimated using three parametric distributions: log-normal,


https://doi.org/10.1021/acs.estlett.5c00467
Environ. Sci. Technol. Lett. 2025, 12, 904−910

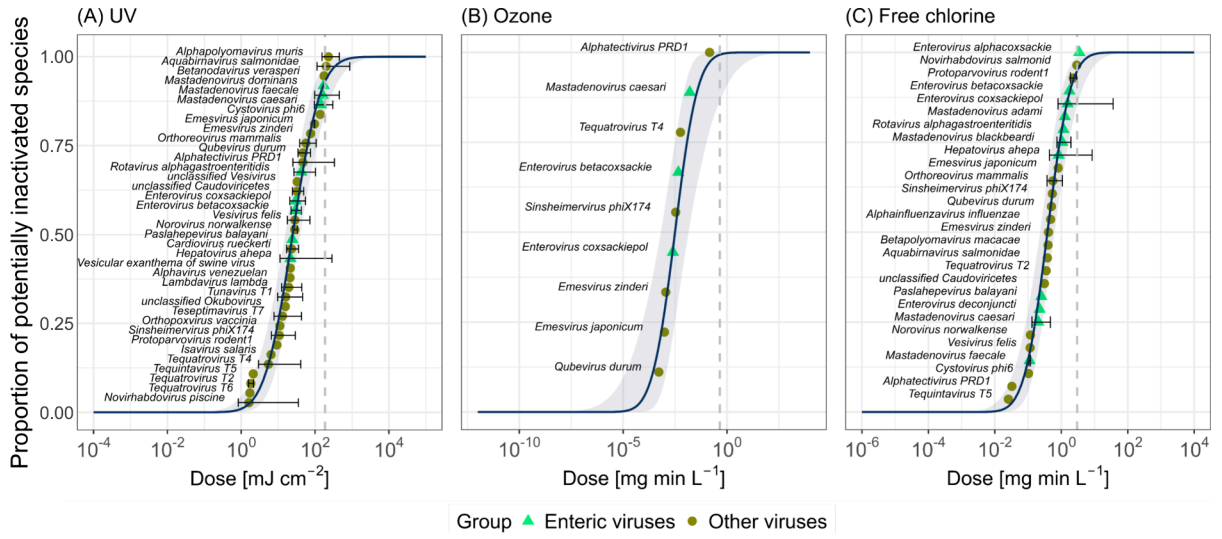



**Figure 1.** Disinfectant sensitivity distributions of viruses for UV (A), ozone (B), and free chlorine (C) for a 4-log reduction target: The points represent the transformed mean inactivation rate constants; the error bars mark ±2SD from the mean (where computable). The green triangles represent enteric viruses, and the dark circles represent other viruses. The species names are shown next to each point. The solid lines are the estimated disinfectant sensitivity distributions, and the shaded areas indicate the 95% credible intervals computed using posterior samples. The dashed lines represent the recommended doses given in the US EPA Guidance Manual for each disinfectant.

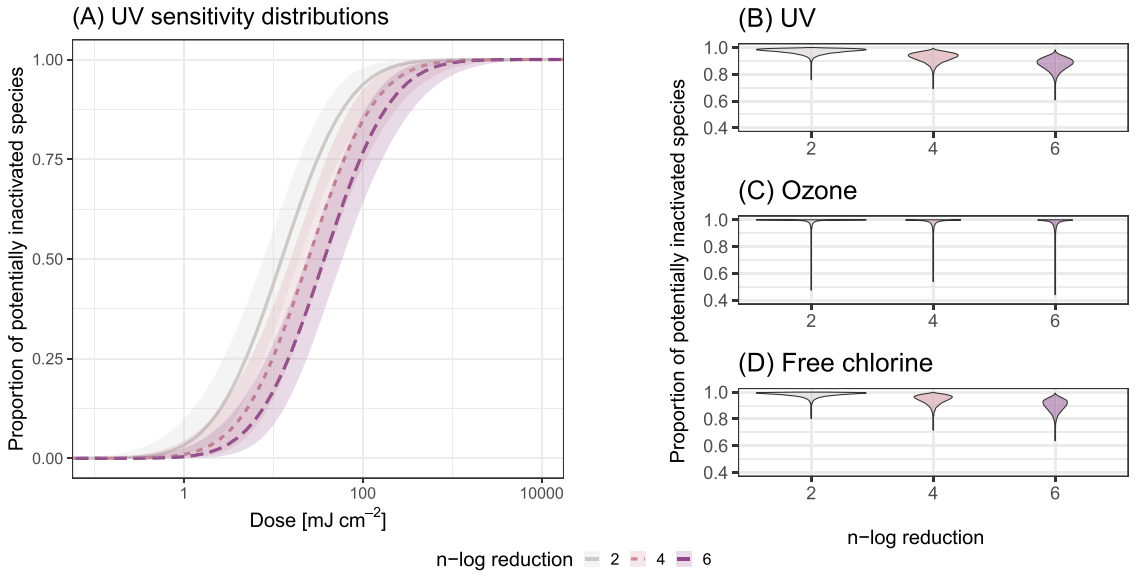



**Figure 2.** Comparison of the estimated UV sensitivity distributions for different target log reduction values (A). The light gray, light red, and purple lines represent 2-, 4-, and 6-log reduction, respectively. The posterior distributions of the proportion of viruses expected to be inactivated by the doses recommended by the US EPA guidance for UV (B), ozone (C), and free chlorine (D), when 4-log reduction is targeted.

Weibull, and gamma. The estimated parameters and goodness-of-fit for each distribution for 4-log reduction are summarized in Table S1. Figure 1 and Figures S2−S4 illustrate the relationships between the dose of each disinfectant required to achieve *n*-log reduction and the proportion of potentially inactivated species using a log-normal distribution. The three estimated parametric distributions for each disinfectant and the *n*-log reduction mostly overlapped each other, but there were differences in the estimated uncertainty ranges in the upper and lower tails of the distributions because of the different functional forms. The computed WAIC and LOOIC for each disinfectant sensitivity distribution showed small differences, suggesting that all three models described the observed data nearly equally well.[31] Based on this, the following discussion focuses on the results of log-normal sensitivity distributions across different virus species.

The recommended doses for 4-log reduction given in the US EPA Guidance Manual are 186 mJ cm$^{-2}$ for UV, 0.532 mg min L$^{-1}$ at a temperature of 20 °C for ozone, and 3.0 mg min L$^{-1}$ at a pH of 6−9 and a temperature of 20 °C for free chlorine. Our analysis showed that 93.0% (95% CrI: 84.2%−97.5%), 99.4% (95% CrI: 86.7%−100%), and 95.0% (95% CrI: 85.5%−98.8%) of virus species were expected to be inactivated by 4-log by UV, ozone, and free chlorine, respectively. Notably, we found that *Enterovirus alphacoxsackie* could not be inactivated by free chlorine at this dose level. Figure 1 also provides the coverage of the viruses expected to be inactivated when the required dose levels are determined based on single reference species. For example, disinfection at the dose required to


https://doi.org/10.1021/acs.estlett.5c00467
Environ. Sci. Technol. Lett. 2025, 12, 904−910

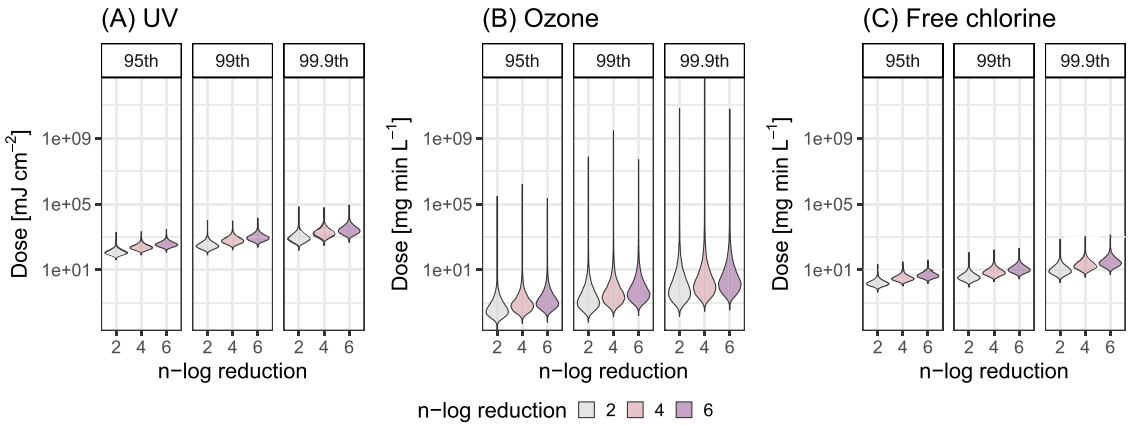



**Figure 3.** Doses of UV (A), ozone (B), and free chlorine (C) required to achieve particular target combinations of the log reduction value and proportion of inactivated viruses. The light gray, light red, and purple plots represent 2-, 4-, and 6-log reduction, respectively. The uncertainty in each estimate is based on the posterior distributions for each disinfectant; the summary statistics for each posterior distribution are provided in Table S2.

achieve 4-log reduction of *Emesvirus zinderi* (which includes bacteriophage MS2) would result in inactivation of only 33%−78% of virus species.

**Comparison of Different Guidelines and Reference Values.** The disinfectant sensitivity distributions were estimated by varying the target *n*-log reduction value ($n$ = 2, 4, or 6). Figure 2A illustrates the estimated log-normal sensitivity distributions for UV, and shows that, due to the model's assumed functional form, the distribution curves shifted in parallel to the higher range of the dose while the curves maintained their shapes (i.e., linear scaling of the $x$-axis) as the log reduction values increased (Figures S2−S4 show the results for all combinations of the disinfectants, log reduction values, and distributions.) We also compared the distributions of the proportion of species potentially inactivated by the disinfectant doses recommended in the US EPA guidance for 4-log reduction (Figure 2B−D).

Figure 3 shows the posterior distributions of the dose of the three disinfectants required to inactivate 95%, 99%, and 99.9% of virus species (the estimated values are summarized in Table S2). For instance, to inactivate 95% of virus species at 4-log reduction, the required dose of disinfectants would be $2.34 \times 10^2$ mJ cm$^{-2}$ (95% CrI: $1.28 \times 10^2$ to $5.48 \times 10^2$ mJ cm$^{-2}$) for UV, 0.083 mg min L$^{-1}$ (95% CrI: 0.016−3.02 mg min L$^{-1}$) for ozone at a pH of 6.5−8.5 and a temperature of 15−25 °C, and 3.02 mg min L$^{-1}$ (95% CrI: 1.60−7.80 mg min L$^{-1}$) for free chlorine at a pH of 7.53 and a temperature of 20 °C. This suggests that, in the case of ozone, the doses recommended by the US EPA are higher than those necessary for more than 95% of virus species, whereas this is not the case for UV and free chlorine. As Figure 3 depicts, the required dose of disinfectant increases exponentially as the target log reduction value or desired proportion of inactivated species increases. Because the amount of data for ozone was more limited than that for the other two disinfectants, the uncertainty in the sensitivity of viruses to ozone was greater (Figure S3), and this produced the wider range of estimated values shown in Figure 2C and Figure 3B.

## DISCUSSION

In this study, we have proposed a statistical framework for quantifying the variation in sensitivity to standard disinfectants across different virus species by summarizing the available experimental data on inactivation kinetics. This framework can be used to estimate disinfectant sensitivity distributions across various virus assemblages and translate a single reference value (e.g., a log reduction value recommended by guidelines) into the proportion of virus species expected to be inactivated.

Our proposed framework complements a single reference approach by characterizing the disinfectant sensitivity of viruses as a distribution rather than a fixed value. This allows for the quantitative evaluation of the proportion of virus species that may not achieve the target log reduction. While recent modeling efforts focus on predicting individual-level (single-virus and condition-specific) inactivation kinetics,[24] our approach summarizes such individual estimates as an overall disinfectant sensitivity distribution for a particular assemblage of virus species. To illustrate its adaptability to different virus assemblages, we performed additional analyses and derived genus-based and enterovirus-specific sensitivity distributions (see Tables S3 and S4 and Figures S5 and S6). Another strength of our Bayesian approach is that it can incorporate the uncertainty in observed experimental data by treating reported values from different experiments as intervals rather than exact points in the form of interval data. This allows for coherent synthesis of available data sets and quantification of uncertainty in required dose levels as a posterior distribution (e.g., Figure 3).

The large data sets utilized for this analysis allowed for more robust extrapolation across species. The analyzed data set also included nonpathogenic viruses, which were tested as surrogates for pathogenic viruses. This inclusion enables a more robust extrapolation by utilizing their shared virological characteristics. Similar challenges in species selection have long been addressed in the field of ecotoxicology,[19,22,32] and various methodological approaches and data-driven criteria are now used to form the current guidelines for ecological risk assessment. Although inclusion criteria for viral species should be carefully considered (e.g., including nonpathogenic viruses may shift the disinfectant sensitivity distribution due to differences in biological or physicochemical properties), the existing methodologies in ecotoxicology could help guide future research in identifying key factors influencing disinfectant sensitivity distributions.

This study aimed to support rational, empirical extrapolation of disinfection kinetics from tested to untested viruses using



https://doi.org/10.1021/acs.estlett.5c00467
Environ. Sci. Technol. Lett. 2025, 12, 904−910

the best available data at the time of analysis. Our framework infers disinfectant sensitivity distributions based on viruses historically tested for virological or operational reasons,[3,23,24] and assumes that future testing will follow similar patterns. To examine this assumption, we reconstructed UV sensitivity distributions using data that were available in 1990, 2000, 2010, and 2020 (Figure S7). Although slight shifts in the distributions were observed, the estimated parameters and the corresponding doses required for 4-log inactivation of 95% of virus species were consistent for the different years (Tables S6 and S7). These results support the framework's applicability to future data sets. Nevertheless, the extrapolation of results to untested viruses remains inherently conditional on the currently available data; we therefore highlight that, while the framework may capture some untested viruses, it cannot predict those that emerge outside these assumptions.

Several limitations to our study remain. Limited data coverage may influence the stability of the fitted distributions and, in turn, the estimated doses required to inactivate a large proportion of viral species (e.g., 95% or more). Although our analysis included a relatively large number of virus species compared to benchmarks in ecotoxicology,[22] further accumulation of disinfection data, in particular for ozone, would enhance the robustness of the estimation of distributions. This study did not fully account for uncertainty and potential bias in the source data, as point estimates from systematic reviews were used without propagating the uncertainty in the original disinfection experiments. Future work could address this through multilevel modeling or statistical weighting methods. Potential biases may arise because the reported inactivation rate constants were derived from linear portions of inactivation curves, often excluding higher doses where efficacy may decline due to virus aggregation, adsorption to particles, or biological variability within the target population (i.e., tailing effects).[13,33] Finally, required log reduction values in practice are determined not only by inactivation rate constants but also by the health significance and environmental occurrence of specific viruses. Further research is needed to quantify the impact of tailing effects on dose estimates[34] and to translate these findings more effectively into risk assessment frameworks.

In conclusion, our proposed framework reveals the variation in sensitivity to UV, ozone, and free chlorine across different types of viruses and quantifies the proportion of viruses expected to be inactivated by a given disinfection dose. This approach is simple and interpretable—for instance, if the goal is to inactivate 95% of virus species, the required dose can be estimated from the disinfectant sensitivity distributions. This makes the framework easy to communicate to potential users such as risk assessors and environmental engineers. The use of disinfectant sensitivity distributions complements current practices in probabilistic risk assessment and serves as a supportive tool to promote more transparent risk communication.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

All codes and analyzed data are available via the authors' GitHub link (https://github.com/miinay/disinfection_sensitivity_distribution).

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.5c00467.

Details of the data collection, transformation of guideline values, and the Bayesian framework used to estimate parameter sets for the different distributions; histograms of reported inactivation rate constants (**Figure S1**); comparison of three fitted sensitivity distributions for the disinfectants (**Figures S2−S4**); genus-level disinfectant sensitivity distributions (**Figure S5**); enterovirus-specific disinfectant sensitivity distributions (**Figure S6**); comparison of UV sensitivity distributions by year of data publication (**Figure S7**); summary statistics for the three estimated parametric distributions and computed goodness-of-fit (**Table S1**); estimated dose required to achieve the inactivation of 95%, 99%, and 99.9% of viruses (**Table S2**); summary statistics for genus-level disinfectant sensitivity distributions (**Table S3**); summary statistics for enterovirus-specific disinfectant sensitivity distributions (**Table S4**); estimated dose levels required to achieve the inactivation of 95%, 99%, and 99.9% of viruses (enterovirus-specific disinfectant sensitivity distributions) (**Table S5**); summary statistics for UV sensitivity distributions by year of data publication (**Table S6**); estimated dose levels required to achieve the inactivation of 95%, 99%, and 99.9% of viruses (comparison of UV sensitivity distributions by year of data publication) (**Table S7**) (PDF)

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### Author Contributions

[▽]F.M. and S.T. contributed equally to this study.


### Funding

M.Y. acknowledges funding from the Japan Society for the Promotion of Science (JSPS) KAKENHI, grant number 21J00885. M.Y. and F.M. were supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT), to a project on Joint Usage/Research Center−Leading Academia in Marine and Environmental Pollution Research (LaMer). F.M. acknowledges funding provided by JSPS KAKENHI, grant number 20J00793, and by JST (JPMJPR23RA). S.T. was funded by the JSPS through

Grant-in-Aid for Young Scientists (grant number JP24K17379) and the Kurita Water and Environmental Foundation.

### Notes

An earlier version of this manuscript was deposited as a preprint: Yanagihara, M.; Smeets, P.; Miura, F.; Torii, S. Statistical framework for assessing heterogeneous sensitivity of viruses to ultraviolet, ozone, and free chlorine. 2025, 10.1101/2025.01.20.633964, *bioRxiv*, https://www.biorxiv.org/content/10.1101/2025.01.20.633964v1.

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors thank Dr. Leo Posthuma of the Dutch National Institute for Public Health and the Environment and Radboud University for his invaluable input in improving the manuscript. We also thank the three anonymous reviewers for their constructive comments, which helped improve the clarity and quality of the manuscript.

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https://doi.org/10.1021/acs.estlett.5c00467
*Environ. Sci. Technol. Lett.* 2025, 12, 904−910