Systematic Review and Meta-Analysis of the Persistence and Disinfection of Human Coronaviruses and Their Viral Surrogates in Water and Wastewater

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ABSTRACT: A systematic review and meta-analysis was conducted to identify decay rate constants (k) of human coronaviruses and their viral surrogates (i.e., animal coronaviruses and the enveloped bacteriophage Phi6) in water and wastewater and disinfection rates with exposure to free chlorine and germicidal ultraviolet light (UV254). Here, 73 k were identified, with only 12 for human coronaviruses, as opposed to animal coronaviruses or Phi6. In the absence of disinfectants, k increased with temperature. Between 22 and 25 °C, mean k for coronaviruses ranged from 0.19 ± 0.06 d−1 in laboratory buffer (n = 4) to 2.9 ± 0.03 d−1 in sterilized wastewater (n = 3), which are within the ranges observed for Phi6 and nonenveloped viruses. No free chlorine or UV254 disinfection studies for coronaviruses were identified that met the systematic review inclusion criteria, although evidence from the literature suggests that coronaviruses would be inactivated if disinfectant doses recommended for nonenveloped viruses were applied. Three disinfection experiments were identified for Phi6. However, given different genome compositions and virion structures between coronaviruses and Phi6, it is not clear whether Phi6 should be used as a surrogate for evaluating free chlorine or UV254 k. Therefore, there is a critical need for additional studies that specifically evaluate disinfection kinetics of coronaviruses in the aqueous environment.

INTRODUCTION

Over the last two decades, novel coronaviruses (CoV) have captured the world’s attention by causing local outbreaks and global pandemics.1,2 The severe acute respiratory syndrome coronavirus (SARS-CoV-1) outbreak of 2003 and Middle Eastern respiratory syndrome coronavirus (MERS-CoV) outbreak of 2012, for example, caused thousands of infections with mortality rates of 10% and 40%, respectively. The emergence of SARS-CoV-2, the causative agent of COVID-19, in December 2019 has caused a global pandemic with unprecedented and ongoing global health and economic impacts. At the time of submission of this article (April 17, 2020), there have been an estimated 2.2 million clinically diagnosed illnesses and 149,000 deaths across the globe,3 more than one-third of the world’s population is under stay-at-home orders to reduce transmission rates,4 and the incidence of illness is continuing to grow.

While primary transmission of coronaviruses is through respiratory droplets, viral RNA can be excreted fecally.5–12 The RNA of SARS-CoV-1,13 SARS-CoV-2,7–10 MERS-CoV11,12 and human coronavirus HUK12, for example, have been detected in the stool of some infected patients. Furthermore, researchers in Australia13 and The Netherlands14 observed SARS-CoV-2 RNA in raw sewage samples collected during the first few weeks of their respective outbreaks. It has been suggested that coronaviruses can replicate in the gastrointestinal tract,15 however, there is limited evidence as to whether infectious virions can be excreted in feces. One study to date has reported observing infectious SARS-CoV-2 virions in feces,16 whereas two others did not find evidence of this, despite detection of high virus RNA concentrations in the stool samples,13,17 and data from Zang et al.17 suggest that SARS-CoV-2 is inactivated in fluids of the gastrointestinal tract.

While it is not believed that water and wastewater will play an important role in coronavirus transmission,19 there is still a need to understand the fate of these viruses outside the human host, including their persistence in water and wastewater, and inactivation with exposure to commonly used disinfectants. A greater knowledge of coronavirus decay rates in wastewater can also inform wastewater epidemiology, which has been
Coronaviruses are 120–160 nm diameter, enveloped viruses with a single-stranded, nonsegmented RNA genome between 26.4 and 31.7 kilobases long. The presence of a lipid-containing envelope and relatively large ssRNA genome are attributes that set coronaviruses apart from the nonenveloped waterborne viruses that are more commonly evaluated for persistence and disinfection in water and wastewater (e.g., adenoviruses, enteroviruses, coliphage). Properties of the viral genome and envelope can have an outsized influence on virus decay rates in aqueous environments; therefore, coronaviruses are hypothesized to have persistence and disinfection behaviors that differ from smaller, nonenveloped viruses.

The disinfection of nonenveloped waterborne viruses and fecal indicator bacteria has traditionally been used to determine required disinfection doses for water and wastewater unit processes, to monitor disinfection unit process performance, and to evaluate pathogen persistence in natural waters. Therefore, it is of interest to understand the disinfection and persistence of coronaviruses relative to these organisms. Thus, the goal of this work was to conduct a systematic review and meta-analysis of the peer-reviewed literature to collate rates of decay of coronaviruses in aqueous environments—including water and wastewaters—and with exposure to free chlorine and germicidal ultraviolet light (UV254).

Given safety challenges in conducting laboratory work with highly infectious human coronaviruses, many researchers have instead evaluated closely related viral surrogates, including animal coronaviruses [e.g., murine hepatitis virus (MHV), transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV)] and the enveloped bacteriophage Phi6. These surrogates were included in the systematic review. MHV, TGEV, and FIPV are viruses within the Coronaviridae family that infect mice, pigs, and cats, respectively, and have been used as surrogates for human coronaviruses given similar size, composition, and morphology. Phi6 (family Cystoviridae) is an 85 nm diameter, enveloped bacteriophage that infects Pseudomonas spp. bacteria. Phi6 has a double capsid structure and a segmented, dsRNA genome, with a total length of approximately 13.4 kilobases. There are therefore key differences in the capsid and genome structure between coronaviruses and Phi6 (see the abstract graphic) that may affect their relative persistence and disinfection in water.

**Methods**

The systematic review and meta-analysis followed PRISMA guidelines. The goal of the review was to compile quantitative information from the peer-reviewed literature on the decay rates of human coronaviruses and their viral surrogates in water and wastewater, with and without exposure to common water and wastewater disinfectants (i.e., chlorine, chloramines, and germicidal ultraviolet light (UV254)). Viruses included in the review were coronaviruses (family Coronaviridae) and the enveloped bacteriophage Phi6 (family Cystoviridae), which has been suggested as a surrogate for mammalian enveloped viruses.

Web of Science core collection (search field = topic), Scopus (search field = article title, abstract, keyword), and PubMed (search field = all fields) were searched on March 18, 2020 (Table 1). Two searches were conducted: one targeting virus
persistence in water and wastewater and the other targeting virus inactivation with exposure to disinfectants. For the persistence search, the search terms were “(X) AND (water OR seawater OR stormwater OR groundwater OR wastewater) AND (die-off OR persistence OR survival OR inactivat* OR decay)” where X is the target-specific text (“search term” in Table 1). For the disinfection search, the search terms were “(X) AND (water OR seawater OR stormwater OR groundwater OR wastewater) AND (chlorine OR chloramine OR UV OR UV254 OR ultraviolet OR UVC)”. Identified articles were assembled, and duplicates were removed. Details of the review process, which involved two independent full-text reviews of papers, are provided in Boehm et al.24 The inclusion criteria were that the paper: (1) properties of the experimental solution (i.e., the type of constituents), (2) experimental conditions (i.e., temperature, experimental reactor volume and sample size, disinfectant concentration, starting concentration of the target virus, whether experiments were conducted with light exposure or in the dark), (3) virus enumeration method, and (4) the model used to calculate virus decay rates.

Fifteen percent of the papers from which data were extracted by a single reviewer were randomly chosen for a second round of data extraction by a different reviewer. Data extracted by the two reviewers were compared to ensure consistency. A single reviewer conducted detailed review of all data sets to identify missing data, data outliers, and data entry mistakes.

When necessary, k values were log10-transformed (log10 k) to facilitate comparison and visualization of decay rate constants over multiple orders of magnitude. Data compiled from this review are available in the Supporting Information.

### RESULTS AND DISCUSSION

#### Systematic Review.
The systematic review identified 46 decay rate constants (k) for coronaviruses (from seven publications)28–31,37–39 and 27 decay rate constants (from five publications)30,31–34 for bacteriophage Phi6, which has been suggested as a surrogate for coronaviruses (Table 1). Each k is from a different “experiment”, where experiment is defined as one viral target exposed to a unique set of conditions (e.g., type of experimental matrix, temperature, disinfectant exposure). All inactivation data from the identified experiments were determined using culture-based assays [i.e., cell culture (TCID50 MPN, and plaque assays) for coronaviruses and double agar layer plaque assays for Phi6], thereby indicating loss of virus viability.

Seventy of the identified experiments were conducted without exposing the viruses to disinfectants; these experiments are designated as persistence experiments. One study was identified that evaluated chlorine disinfection of SARS-CoV-1 in wastewater,40 and one evaluated UV254 disinfection of SARS-CoV-1 in cell culture supernatant.39 However, neither study provided enough information on the disinfectant dose

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**Table 2. Number of Decay Rate Constants Identified for Persistence of Each Virus in Each Experimental Matrix (i.e., in the absence of disinfectant exposure) for All Temperatures between 4 and 56 °C**

<table>
<thead>
<tr>
<th>Laboratory Water/Buffer</th>
<th>Cell Culture Supernatant</th>
<th>Fresh Water</th>
<th>Tap Water</th>
<th>Raw Wastewater</th>
<th>Sterilized Wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse hepatitis virus (MHV)</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Feline infectious peritonitis virus (FIPV)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Transmissible gastroenteritis virus (TGEV)</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Human coronavirus (HCoV)229E</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>HCoV Oc43</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SARS-CoV-1</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Coronaviruses (total)</td>
<td>18</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Bacteriophage Phi6</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

“*” denotes that no k values were identified.

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In this formulation, C is the virus concentration at time t, and C0 is the concentration at the start of the experiment (i.e., t = 0). k and its associated error, as well as model fit parameters, were recorded.

In addition to extracting information on the decay of the viral target, a record of each experiment was kept, including (1) properties of the experimental solution (i.e., the type of water or wastewater used, whether it was filtered or disininfected, salinity, pH, concentrations of organic and inorganic constituents), (2) experimental conditions (i.e., temperature, experimental reactor volume and sample size, disinfectant concentration, starting concentration of the target virus, whether experiments were conducted with light exposure or in the dark), (3) virus enumeration method, and (4) the model used to calculate virus decay rates.

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Seventy of the identified experiments were conducted without exposing the viruses to disinfectants; these experiments are designated as persistence experiments. One study was identified that evaluated chlorine disinfection of SARS-CoV-1 in wastewater, and one evaluated UV254 disinfection of SARS-CoV-1 in cell culture supernatant. However, neither study provided enough information on the disinfectant dose
applied to meet inclusion criteria and allow for extraction of \( k \) values; therefore, these papers were not included in the total experiment count. Only three experiments were identified that evaluated Phi6 disinfection: two with exposure to chlorine\(^{20,32} \) and one with exposure to UV\(^{24} \).

Of the 46 persistence experiments identified for coronaviruses, 10 were conducted with murine hepatitis virus (MHV) as a target, six with feline infectious peritonitis virus (FIPV), 18 with transmissible gastroenteritis virus (TGEV), eight with human coronavirus (HCoV) 229E, two with HCoV OC43, and two with SARS-CoV-1 (Table 2). No experiments were identified that examined the persistence of SARS-CoV-2 or MERS in aqueous solution.

Of the 70 coronavirus and Phi6 persistence experiments, 28 were conducted in distilled water or laboratory buffer (i.e., phosphate or HEPES buffer), eight in cell culture supernatants, six in environmental fresh waters (i.e., river or lake water), seven in tap water, 12 in raw wastewater, and nine in sterilized (i.e., filtered or pasteurized) wastewater (Table 2). Experiments were conducted at temperatures between 4 and 56°C. No experiment was indicated as being conducted with exposure to light; we therefore assumed that all experiments were conducted in the absence of sunlight, which could have impacted decay rates (see discussion below).

For all experiments except for one, the studies either provided figures illustrating that there was a log-linear (i.e., first-order) decay curve or noted within the text that virus inactivation followed log-linear decay. The one experiment that deviated from log-linear decay was for Phi6 inactivation in pasteurized wastewater at 22°C, which had a biphasic decay curve.\(^{34} \) Data for this experiment were extracted and fit with a log-linear decay curve, which resulted in \( k = 1.5 \text{ d}^{-1} (\log_{10} k = 0.18) \) and \( R^2 = 0.49; \) for consistency, we used this calculated first-order rate constant in the meta-analysis. Two of the identified experiments (i.e., MHV decay at 4°C in laboratory water and in lake water) had negligible decay rates over the course of the experimental time frame (49 and 14 d, respectively). These experiments were assigned \( k = 0.01 \text{ d}^{-1} \), given that a value of zero cannot be log\(_{10}\)-transformed, and the lowest identified \( k \) at this temperature was 0.012 d\(^{-1} \). The two experiments with these assigned \( k \) values are designated in Figures 1 and 2.

**Persistence in Water and Wastewater.** In the absence of exposure to disinfectants, the decay rate constants (\( k \)) of coronaviruses and bacteriophage Phi6 were observed to increase with temperature regardless of the experimental matrix (Figure 1). This trend of increased \( k \) with increased temperature was also previously observed for nonenveloped viruses.\(^{24} \) The slope of the best-fit linear regression line for the coronavirus \( \log_{10} k \) data versus temperature was 0.065 ± 0.006 (y-intercept = −1.66; \( R^2 = 0.75 \)), meaning that \( \log_{10} k \) (where original units of \( k \) are d\(^{-1} \)) increased by 0.065 for every 1°C increase in temperature. This slope is within the range previously reported for nonenveloped viruses (i.e., 0.03–0.07).\(^{25} \) The best-fit linear regression line for Phi6 \( \log_{10} k \) data versus temperature was not found to be significantly different from that of the coronaviruses (\( p = 0.6 \)).

Within the range of environmentally relevant temperatures (i.e., 4–30°C), the identified coronavirus experiments were conducted at 4, 10°C, and room temperature (defined as 22–25°C); therefore, mean \( \log_{10} k \) values were calculated for each of these ranges and for each experimental water type (Table 3). Within each temperature range, mean and median \( \log_{10} k \) were greatest in wastewater and lowest in laboratory water and buffer (Figure 2). It has been suggested that increased \( k \) of the enveloped viruses in wastewater could be attributed to a variety of factors, including enzymatic activity, predation, and the presence of solvents, detergents, and organic matter in wastewater,\(^{28,29,31} \) although there has yet to be specific evidence to definitively attribute these factors to virus decay.

To put coronavirus and Phi6 persistence data into context, these data were compared to \( \log_{10} k \) of nonenveloped waterborne viruses (i.e., enteroviruses, rotaviruses, noroviruses, hepatitis A virus, adenoviruses, astroviruses, and F+ and somatic coliphages; Figure 2), which were compiled in a previously conducted systematic review by Boehm et al.\(^{24} \) The \( \log_{10} k \) values for the nonenveloped viruses presented herein were determined in unaltered environmental surface waters (i.e., fresh, estuarine, and marine waters) in the dark (i.e., without sunlight exposure) and quantified using infectivity assays (Table 4). There was more variability in the temperatures used for the nonenveloped virus decay experiments; therefore, \( \log_{10} k \) values from experiments conducted at a range of temperatures ±2°C around each target temperature were included for the nonenveloped viruses.

A similar range of \( \log_{10} k \) values were identified for coronaviruses, Phi6, and the nonenveloped viruses. It was hypothesized that the lipid-containing envelope that surrounds the coronavirus nucleocapsid and Phi6 capsids would make these viruses more susceptible to degradation than the nonenveloped viruses. However, the virus decay data identified in this review suggests that enveloped and nonenveloped viruses have similar rates of persistence in the aqueous environment in the dark, despite different structures.

All identified coronavirus and Phi6 persistence experiments were conducted under dark conditions. Sunlight exposure was previously found to result in greater average \( k \) for the nonenveloped viruses,\(^{24} \) as compared to in the dark, likely owing to the ability of absorbed photons in the UVB region of

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**Figure 1.** \( \log_{10}\)-transformed first-order decay rate constants (\( \log_{10} k \)) of coronaviruses (orange circles) and bacteriophage Phi6 (blue circles) as a function of temperature. Each marker represents one \( \log_{10} k \) value extracted from the literature. These data include all experiments identified in the review that were not conducted in the presence of a disinfectant (\( n = 70 \)). Solid line is the best-fit linear regression line for the coronavirus data (slope = 0.065 ± 0.006; y-intercept = −1.66; \( R^2 = 0.75 \)). Dashed line is the best-fit linear regression line for the Phi6 data (slope = 0.070 ± 0.011; y-intercept = −1.72; \( R^2 = 0.65 \)). The coronavirus markers with a cross (\( n = 2 \)) designate experiments with minimal virus decay that were assigned \( k = 0.01 \text{ d}^{-1} \). \( k_{90} \) is the time (d) required for 99% inactivation.

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the solar spectrum to damage genomic DNA and RNA. It is therefore expected that coronavirus and Phi6 would be greater than those presented herein if measured in sunlit waters. While not proven, there is a possibility that sunlight exposure would have a larger impact on increasing coronavirus than it would on the waterborne viruses, given that coronaviruses have a ssRNA genome that is much larger than the genomes of the nonenveloped ssRNA viruses (e.g., enteroviruses ~7–8.5 kb). The large coronavirus genome presents more targets for direct sunlight-induced RNA damage, which could result in faster rates of inactivation. This would not necessarily be the case for Phi6, given that dsRNA genomes have been found to be more resistant to UV damage. A further discussion of this is provided in the UV254 disinfection section below.

Disinfection with UV254. Only one experiment was identified for Phi6 disinfection with UV254 (conducted in laboratory buffer at 23 °C; k = 0.067 cm²/mJ; log10 k = −1.17; table 3. mean log10-transformed first-order decay rate constants (log10 k) for coronaviruses and bacteriophage phi6 in each experimental matrix and each temperature

Figure 2. Log$_{10}$-transformed first-order decay rate constants (log$_{10}$k) of viruses for persistence experiments (without exposure to disinfectants) at (a) 22–25 °C, (b) 9–12 °C, and (c) 4–6 °C. Each coronavirus (orange circles) and Phi6 (blue circles) marker represents one log$_{10}$k value extracted from the literature; the horizontal bar represents the median value. Coronavirus markers with a cross (n = 2) designate experiments with minimal virus decay that were assigned k = 0.01 d$^{-1}$. The log$_{10}$k values for the nonenveloped viruses (i.e., enteroviruses, rotaviruses, noroviruses, hepatitis A virus, adenoviruses, astrovirus, and F+ and somatic coliphages; green circles) were collected from the systematic review by Boehm et al. and are decay rate constants determined in unaltered environmental surface waters (i.e., fresh, estuarine, and marine waters) in the dark and quantified using culture-based methods (n for each is presented in Table 4). t$_{99}$ is the time (d) required for 99% inactivation.
UV254 dose required for a 37% reduction in virus infectivity (J/cm²) and the genome length to predict UV254 inactivation are correlated with nucleic acid type), and rates. Brieﬂy, eq 1 was used to calculate values as a function of fluence; these values were calculated using log₁₀ inactivation data. We calculated true k values measured with monochromatic UV254 light were included. Gray blocks for the human viruses and Phi6 are the range of log₁₀ time) required for 99.99% microorganism inactivation. The gray y-axis represents the UV254 dose (i.e., UV254 irradiance × time) required for 99.99% microorganism inactivation. The gray horizontal line represents the USEPA recommended UV254 dose for 99.99% virus inactivation.

Figure 3. Log₁₀-transformed first-order decay rate constants (log₁₀ k) with exposure to germicidal ultraviolet light (UV254). The Phi6 log₁₀ k value (blue circle) is from Ye et al. The remaining log₁₀ k values were extracted from the UV254 disinfection review paper by Hijnen et al. (bacteria represented by gray circles and nonenveloped viruses represented by green circles). Only k determined with low pressure UV254 light (as opposed to medium pressure UV) were included. Gray blocks for the human viruses and Phi6 are the range of log₁₀ k values predicted by Lytle and Sagripanti (see main text for details). The secondary y-axis represents the UV254 dose (i.e., UV254 irradiance × time) required for 99.99% microorganism inactivation. The gray horizontal line represents the USEPA recommended UV254 dose for 99.99% virus inactivation.22

Table 4. Mean Log₁₀-Transformed First-Order Decay Rate Constants (log₁₀ k) for Nonenveloped Waterborne Viruses

<table>
<thead>
<tr>
<th>Nonenveloped Viruses</th>
<th>Mean log₁₀ k ± sdev (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteroviruses</td>
<td>-0.51 ± 0.41 (35)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>-1.05 ± 0.45 (5)</td>
</tr>
<tr>
<td>Noroviruses</td>
<td>-1.70 (1)</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>-1.26 ± 0.14 (2)</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>-1.29 ± 0.13 (3)</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>-1.19 (1)</td>
</tr>
<tr>
<td>F+ coliphages</td>
<td>-1.15 ± 1.01 (21)</td>
</tr>
<tr>
<td>Somatic coliphages</td>
<td>-1.87 ± 1.47 (7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonenveloped Viruses</th>
<th>Mean log₁₀ k ± sdev (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.29 ± 0.55 (5)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>0.10 (2)</td>
</tr>
<tr>
<td>Poliovirus Type 1</td>
<td>0.44 (2)</td>
</tr>
<tr>
<td>Poliovirus Type 2</td>
<td>1.47 (7)</td>
</tr>
<tr>
<td>Reovirus 3A</td>
<td>0.63 (6)</td>
</tr>
<tr>
<td>Reovirus 3B</td>
<td>1.70 (1)</td>
</tr>
<tr>
<td>Reovirus 3C</td>
<td>0.99 (2)</td>
</tr>
<tr>
<td>Reovirus 3D</td>
<td>0.69 (2)</td>
</tr>
<tr>
<td>Reovirus 3E</td>
<td>0.59 (2)</td>
</tr>
<tr>
<td>Reovirus 3F</td>
<td>0.55 (2)</td>
</tr>
<tr>
<td>Reovirus 3G</td>
<td>0.50 (2)</td>
</tr>
<tr>
<td>Reovirus 3H</td>
<td>0.45 (2)</td>
</tr>
<tr>
<td>Reovirus 3I</td>
<td>0.40 (2)</td>
</tr>
<tr>
<td>Reovirus 3J</td>
<td>0.35 (2)</td>
</tr>
<tr>
<td>Reovirus 3K</td>
<td>0.30 (2)</td>
</tr>
<tr>
<td>Reovirus 3L</td>
<td>0.25 (2)</td>
</tr>
</tbody>
</table>

Figure 3. Log₁₀-transformed first-order decay rate constants (log₁₀ k) with exposure to germicidal ultraviolet light (UV254). The Phi6 log₁₀ k value (blue circle) is from Ye et al. The remaining log₁₀ k values were extracted from the UV254 disinfection review paper by Hijnen et al. (bacteria represented by gray circles and nonenveloped viruses represented by green circles). Only k determined with low pressure UV254 light (as opposed to medium pressure UV) were included. Gray blocks for the human viruses and Phi6 are the range of log₁₀ k values predicted by Lytle and Sagripanti (see main text for details). The secondary y-axis represents the UV254 dose (i.e., UV254 irradiance × time) required for 99.99% microorganism inactivation. The gray horizontal line represents the USEPA recommended UV254 dose for 99.99% virus inactivation.

\[
\frac{1}{k} = D_{37} = \frac{SnS}{GS}
\] (1)

LS predicted coronavirus UV254 k to be between 2.6 and 4.0 cm²/mJ (log₁₀ k = 0.41±0.60; based on a genome length of between 20 and 31 kilobases; Figure 3) and validated these values through comparison with a previously measured inactivation rate constant of a torovirus (i.e., Berne virus). Toroviruses are a genus of enveloped ssRNA viruses with a virion size, genome length, and morphology similar to coronaviruses. In fact, toroviruses were classified as a genus within the Coronaviridae family until 2018, when the genus was reclassified under the Toroviridae family. The Berne virus used for validation of modeled UV254 k had a measured k of 1.9 cm²/mJ (log₁₀ k = 0.29; data extracted from Figure 2 in Weiss and Horzinek), which was close to, but slightly lower than, the range of k predicted by LS.

Lytle and Sagripanti focused their effort on viruses infecting vertebrates. As such, the authors did not include predicted UV254 disinfection rates for bacteriophage, such as Phi6. Therefore, we calculated a range of estimated k for Phi6 using eq 1, with GS equal to 13.4 kilobases and two values of SnS representing median SnS values for the two dsRNA viruses included in the analysis by LS (i.e., Birnaviridae and Reoviridae; median SnS = 1400 and 3800 J/m²-kilobase, respectively). The resulting predicted Phi6 k ranged between 0.035 and 0.096 cm²/mJ (log₁₀ k = -1.45 to -1.02), which overlaps with measured Phi6 UV254 k.

To compare Phi6 and predicted coronavirus UV254 k with those of fecal indicator bacteria and nonenveloped viruses, k for the latter were extracted from the UV254 disinfection review paper by Hijnen et al. In that review, the researchers compiled UV254 disinfection data from studies that (i) used culture-based assays to quantify microorganism viability, (ii) provided data on the UV254 fluence applied, and (iii) corrected the fluence for light attenuation in the experimental solution. Hijnen et al. used the compiled data to calculate k values as a function of fluence; these values were calculated using log₁₀ inactivation data. We calculated true first-order k by converting these values to a ln scale (i.e., the values reported by Hijnen et al. were multiplied by ln(10)); only k values measured with monochromatic UV254 light were included.

The k values from Hijnen et al. were as follows (error is presented as the 95% conﬁdence interval; n = number of studies included by Hijnen et al. in calculating k for each microorganism; Figure 3): E. coli = 1.2 ± 0.11 cm²/mJ (not including O157:H7, n = 6), Enterococcus faecalis = 0.72 ± 0.074 cm²/mJ (not including O157:H7, n = 6), Enterococcus faecalis = 0.72 ± 0.074 cm²/mJ (not including O157:H7, n = 6).
coronaviruses have larger genomes than most other ssRNA and smaller than those of the other included bacteria and viruses, chlorine dose for 99.99% virus removal at pH between 6 and 9 and temperatures between 20 and 25 °C. Diamonds represent experiments where the temperature was not indicated. The secondary y-axis represents the free chlorine dose (i.e., free chlorine concentration × time) required for 99.99% microorganism inactivation. The gray horizontal band represents the USEPA recommended chlorine dose for 99.99% virus removal at pH between 6 and 9 and temperatures between 20 and 25 °C.

**Coronavirus k with Exposure to Free Chlorine**. No experiments of coronavirus k with exposure to free chlorine were identified that met the systematic review inclusion criteria. Two experiments were identified that evaluated Phl6 disinfection with exposure to chlorine. Both experiments were conducted in laboratory buffers: one was conducted at pH 7 and 5 °C and the other at pH 7.4 and 23 °C. A greater free chlorine k value was observed for Phl6 at 23 °C than at 5 °C, which is expected, given that increased temperatures typically result in greater observed chlorine disinfection rates.

Disinfection with Free Chlorine. No experiments of coronavirus k with exposure to free chlorine were identified that met the systematic review inclusion criteria. Two experiments were identified that evaluated Phl6 disinfection with exposure to chlorine. Both experiments were conducted in laboratory buffers: one was conducted at pH 7 and 5 °C and the other at pH 7.4 and 23 °C. A greater free chlorine k value was observed for Phl6 at 23 °C than at 5 °C, which is expected, given that increased temperatures typically result in greater observed chlorine disinfection rates.

Free chlorine decay rate constants for indicator bacteria and nonenveloped viruses (all quantified using culture-based viability assays) were identified from the literature to place Phl6 free chlorine k in perspective. While the review of the literature describing free chlorine disinfection of these additional microorganisms was not systematic, they at least give an indication of relative free chlorine susceptibility. The Phl6 free chlorine k at 5 °C was within the range of k measured for the nonenveloped viruses at the same temperature: Phl6 k was lower than those of the adenoviruses but greater than those of MS2 and the picornaviruses (i.e., echoviruses, coxsackieviruses). Interestingly, Phl6 free chlorine k at 23 °C was greater than those of *E. coli* and *Enterococcus faecalis* measured around the same temperature, suggesting greater free chlorine susceptibility of the enveloped bacteriophage compared to the indicator bacteria.

One paper was identified that described a UV254 inactivation experiment conducted with SARS-CoV-1 in cell culture supernatant. This experiment was not included in the analysis because there was no description of how the reported UV254 irradiance used in experiments was measured or if it was corrected for light attenuation in the experimental solution. Nonetheless, the study suggested a lower coronavirus UV254 k than the range predicted by LS. This discrepancy, combined with limited UV254 disinfection data available for coronaviruses, highlight a critical need for more research to generate additional data on UV254 inactivation of actual coronaviruses. Future experiments should be conducted with careful measurement of the UV254 fluence applied. Additionally, given different nucleic acid types and genome length, it is unclear whether Phl6 is a good surrogate for coronaviruses when evaluating or monitoring UV254 disinfection.
Ye et al.\textsuperscript{20} evaluated the targets of oxidative damage for bacteriophage Phi6 exposed to free chlorine. While some oxidation of the genome and membrane-bound lipids and proteins was observed, the main driver of free chlorine inactivation of Phi6 was found to be oxidation of proteins in the capsid (which is located within the lipid envelope). It is unclear whether this disinfection mechanism, and subsequent decay rate constants, can be extrapolated to coronaviruses.

**Implications.** The United States Environmental Protection Agency (USEPA) provides tables outlining the disinfectant doses [i.e., \( \text{UV}_{254} \) irradiance \( \times \) time (mJ/cm\(^2\)) or chlorine concentration \( \times \) time (mg-min/L)] required for 4-log (i.e., 99.99\%) inactivation of viruses with exposure to \( \text{UV}_{254} \) and free chlorine.\textsuperscript{23} We converted \( k \) reported above to disinfectant doses for 4-log inactivation using the following equation

\[
\text{disinfectant dose}_{99.99\%} = \frac{-\ln(0.0001)}{k}
\]  

(2)

The coronavirus \( \text{UV}_{254} \) dose required for 4-log virus disinfection (based on \( \log_{10} k \) predicted by LS\textsuperscript{11}) was lower than the USEPA recommended value (disinfectant dose\(_{99.99\%} = 186 \text{ mJ/cm}^2 \); Figure 3), suggesting that coronaviruses would be efficiently inactivated if following the USEPA recommended \( \text{UV}_{254} \) dose for virus disinfection. The free chlorine dose required for 4-log disinfection of Phi6 was lower than the USEPA recommended dose (e.g., disinfectant dose\(_{99.99\%} = 3 \text{ mg-min/L} \) at 20 °C and pH between 6 and 9; Figure 4); however, there is no direct evidence of what this would mean for coronavirus disinfection by free chlorine.

Overall, there is very little data available in the literature on coronavirus inactivation in aqueous solution with exposure to disinfectants. There is therefore a critical need for additional studies that specifically evaluate the disinfection kinetics of human coronaviruses and their surrogates that infect animals (e.g., MHV, TGEV, and FIPV). Given different genome compositions and virion structures between coronaviruses and bacteriophage Phi6, it is not clear whether Phi6 should be used as a surrogate for evaluating coronavirus free chlorine or \( \text{UV}_{254} \) for gastrointestinal infection of SARS-CoV-2.

Alternatively, the available data on coronavirus survival in the absence of disinfectants suggest that these viruses are as persistent as nonenveloped viruses and bacteriophage Phi6 in the aqueous environment, including in natural waters and wastewaters. Additional research on the persistence of coronaviruses in water, as well as in the presence of sunlight, will further our understanding of their fate outside of the human body.

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**Notes**

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