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Comparative Inactivation of Enteroviruses and Adenovirus 2 by UV Light

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The doses of UV irradiation necessary to inactivate selected enteric viruses on the U.S. Environmental Protection Agency Contaminant Candidate List were determined. Three-log reductions of echovirus 1, echovirus 11, coxsackievirus B3, coxsackievirus B5, poliovirus 1, and human adenovirus type 2 were effected by doses of 25, 20.5, 24.5, 27, 23, and 119 mW/cm^2, respectively. Human adenovirus type 2 is the most UV light-resistant enteric virus reported to date.

Under, the 1996 amendments to the Safe Drinking Water Act, the U. S. Environmental Protection Agency is required to publish a list of unregulated contaminants that are known or expected to occur in public water systems and whose presence in drinking water may pose a health risk. The first of these lists, published in 1998, identified 10 microbiological contaminants, including echoviruses, coxsackieviruses, and adenoviruses. All of these viruses have been associated with outbreaks involving drinking water (1, 10, 12). UV light disinfection is being increasingly used in the treatment of both wastewater and potable drinking water since such treatment does not produce disinfectant by-products and is effective against *Cryptosporidium* oocysts (2). The goal of this study was to compare the ways inactivation by UV light varies among the enteroviruses and a nonenteric adenovirus.

Human adenovirus type 2 Adenoid 6 (ATCC VR-846) was propagated and assayed in the PLC/PRF/5 cell line (6). Coxsackievirus B5 HA (ATCC VR-688), coxsackievirus B5 Faulkner (ATCC VR-185), echovirus 1 Farouk (ATCC VR-31), echovirus 11 Gregory (ATCC VR-41), and poliovirus 1 LSC-2ab were propagated in the BGM cell line. The viruses were grown in serum-free medium, and after the production of cytopathogenic effects in the cell monolayer, the medium was frozen and thawed once at −20°C to release the virus. This was followed by centrifugation at 2,800 × *g* for 12 min, addition of polyethylene glycol (9%) and sodium chloride (5.8%), and stirring overnight at 4°C. The virus suspension was then subjected to high-speed centrifugation (10,000 × *g* for 30 min). The resulting virus pellet was resuspended in 0.01 M phosphate-buffered saline to 18% of the original volume. Resuspension was followed by chloroform extraction in which equal parts of virus suspension and chloroform were mixed and shaken for 10 min. The virus-chloroform suspension was then centrifuged at low speed (2,500 × *g* for 5 min), and the upper aqueous layer was collected, the titers were determined, and the material was stored at −80°C.

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Viruses were assayed in 24-well plastic cell culture plates by the most-probable-number method. Six wells were used for each dilution, and the titers of the viruses were determined by using a general-purpose program adapted from the method described by Hurley and Roscoe (9). The production of cytopathogenic effects was used as an indication of a viable virus. All experiments were performed at least four times, with the exception of that for poliovirus 1 (performed twice), which was included as a control.

A collimated beam incorporating an 8-W low-pressure mercury UV lamp (Sankyo Denki model G8T5.2N), as previously described, was used (14). An IL-2000 radiometer (International Light, Newburyport, Mass.) set with a model 254 photodetector (catalog no. SED 240/NS254/W) was utilized to measure the UV light intensity. A viral suspension was added to a glass petri dish, which was placed on a magnetic stir plate, and continuously stirred. For ease of calculation, it was determined that the petri dish must contain 14 ml of solution in order to achieve a 1-cm distance from the bottom of the petri dish to the surface of the viral suspension. This experimental design allows for an accurate irradiance measurement when low-pressure UV lamps are used (2). A different petri dish was used for each exposure period. Five to six different exposure times were used for each virus. In addition, a control petri dish containing virus was held under the same conditions to assess whether any viral decay occurred during the experiment. UV intensity was calculated as described by Meng and Gerba (14).

Statistical analysis of the inactivation kinetics for each of the viruses was performed by using SYSTAT (version 9) and EXCEL (Microsoft Office 97). The *P* values were computed and compared at the confidence level of 95%, or 0.05.

Figure 1 shows the dose versus log survival data for the viruses studied, and Table 1 indicates the UV light doses needed to inactivate different amounts of the studied viruses. The doses in Table 1 were interpolated from the linear-regression lines for each of the respective viruses. An analysis of variance indicated that there was a significant difference between the dose required to inactivate the enteroviruses and that needed to inactivate adenovirus 2 ($P = 3.3 \times 10^{-6}$). Poliovirus 1 was used as a control in this study, and the results

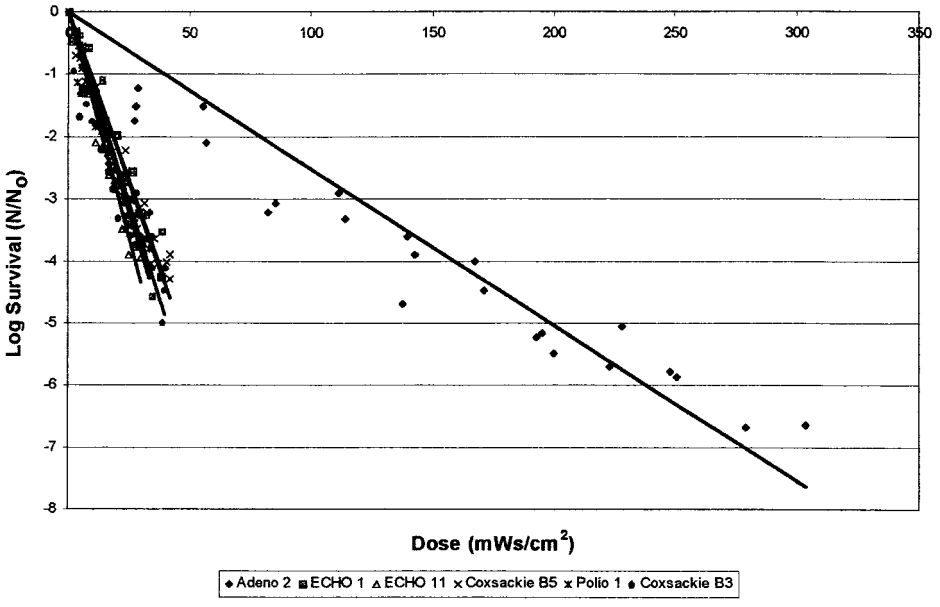

FIG. 1. Comparison of virus survival with UV dose. N, concentration of virus after exposure to the indicated dose; N_0, initial concentration of virus.

indicate that the dose required to achieve a 99.9% inactivation was similar to that previously reported in the literature (16).

The guidance manual published by the U.S. Environmental Protection Agency (7) recommends a UV light dose of 21 mW/cm^2 for a 2-log$_{10}$ inactivation and a dose of 36 mW/cm^2 for a 3-log$_{10}$ inactivation of enteric viruses for drinking water treatment. The results of this study indicate that this would be adequate for enteroviruses but not for adenovirus type 2. The enteroviruses showed little variability in resistance to UV light, and some were slightly more sensitive than previously reported (15). In the present study, adenovirus type 2 demonstrated more resistance than adenovirus types 40 and 41 did in a previous study (14). The greater resistance of adenovirus type 2 may have been due to genetic differences, its structure, or the way in which the virus was prepared. Meng and Gerba (14), in an effort to release as many viral particles as possible from the host cells, performed the cell culture freeze-thaw procedure five times, compared to only once in the present study. It is possible that multiple freezing-thawing procedures damage the viral particle, making it more susceptible to disinfection. The greater resistance of adenovirus type 2 may also have been due to the fact that it has only one fiber projection at each penton capsomere whereas adenovirus type 40 has two fibers; this fiber projection may cause a shadowing effect or a disruption in the adsorbance by the viral nucleic acids. However, it is more likely that adenoviruses display greater resistance than either enteric viruses or spore-forming bacteria (15) because they contain double-stranded DNA and are able to use the host cell enzymes to repair damages in the DNA caused by UV irradiation. Adenoviruses have been used to measure the occurrence of repair enzymes in normal cells and cancer cells (4, 5). The presence and availability of these enzymes affect the ability of the virus DNA to be repaired. Thus, it is possible that the assay of adenovirus in different cell lines after UV light exposure may provide different results, i.e., the greater availability of repair enzymes may result in a greater number of viral particles surviving UV treatment.

TABLE 1. Dose requirements for inactivation of viruses by UV light

Virus	UV exposure (mW/cm^2) needed for virus inactivation of[a]:			
	90.0%	99.0%	99.9%	99.99%
Echovirus 1	8	16.5	25	33
Echovirus 2	7	14	20.5	28
Coxsackievirus B5	9.5	18	27	36
Coxsackievirus B3	8	16	24.5	32.5
Poliovirus 1	8	15.5	23	31
Adenovirus type 2	40	78	119	160

[a] The starting concentration of the viruses ranged from 2×10^7 to 1×10^6 per ml.

The results of this study suggest that double-stranded DNA viruses are likely the most resistant viruses to UV light disinfection. These viruses have been responsible for both drinking water and recreational waterborne disease outbreaks (9, 12). The enteric adenoviruses, types 40 and 41, cause mortalities as great as 50% in immunocompromised individuals (8). Adenoviruses are believed to occur in greater concentrations than other enteric viruses (11) and have been detected in treated drinking water (13). The use of UV light in the disinfection of drinking water and wastewater has increased rapidly in recent years (3). Consideration should be given to the resistance of adenoviruses to UV light disinfection when appropriate doses for the control of waterborne viruses are being determined.

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