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ORIGINAL ARTICLE

Kinetics of UV_{254} inactivation of selected viral pathogens in a static system

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Abstract

Aims: The objective of this study was to estimate UV_{254} inactivation constants for four viral pathogens: influenza virus type A, porcine respiratory and reproductive syndrome virus (PRRSV), bovine viral diarrhoea virus (BVDV) and reovirus.

Methods and Results: Viruses in culture medium were exposed to one of nine doses of UV_{254} and then titrated for infectious virus. Analysis showed that viral inactivation by UV_{254} was more accurately described by a two-stage inactivation model *vs* a standard one-stage inactivation model.

Conclusions: The results provided evidence for the existence of two heterogeneous viral subpopulations among the viruses tested, one highly susceptible to UV_{254} inactivation and the other more resistant. Importantly, inactivation constants based on the one-stage inactivation model would have underestimated the UV_{254} dose required for the inactivation of these viruses under the conditions of the experiment.

Significance and Impact of the Study: To improve the accuracy of estimates, it is recommended that research involving the inactivation of micro-organisms evaluates inactivation kinetics using both one-stage and two-stage models. These results will be of interest to persons responsible for microbial agents under laboratory or field conditions.

Introduction

Ultraviolet (UV) radiation is the part of the electromagnetic spectrum that lays between the high-energy X-rays (≤100 nm) and the visible spectrum (>400 nm). UV_{254} inactivation has been a proven method of disinfection since 1930s (Brickner *et al.* 2003). In 1960s, it was shown that inactivation of microbes occurred through dimer formation by nucleic acids, with 260 nm determined to be the UV wavelength most efficiently absorbed by genetic material (Kowalski *et al.* 2001).

The efficacy of UV inactivation is dependent upon several factors, including the microbe's susceptibility to UV_{254}. Microbial susceptibility to UV_{254}, generally termed the inactivation constant (k), is frequently modelled using the following Eqn (1):

$$k = \frac{\log_{10}\left(\frac{N_t}{N_0}\right)}{\text{Dose}} \quad (1)$$

where

k = the inactivation constant
N_0 = quantity of microbes at time zero
N_t = quantity of microbes at UV_{254} exposure time 't'
Dose_t = depth (centimetres) of the solution irradiated by UV_{254}

Thus, the one-stage inactivation model (Eqn 1) describes the one-hit model which, when $\log_{10}$-transformed, is described by a linear relationship between the survival

fraction of the microbial population and the UV_{254} exposure dose (Goldberg *et al.* 1958). This model long served as the primary method for describing microbial susceptibility to UV_{254}. In 1964, Hiatt described the limitations of the model and proposed several alternate computational models to describe the inactivation of microbes (Hiatt 1964). More recently, Kowalski *et al.* (2001) described a two-stage inactivation model for UV_{254} inactivation of viruses. The objectives of this study were to compare the one-stage and two-stage inactivation models and report the inactivation constants for two enveloped $^{(+)}$ssRNA viruses [porcine respiratory and reproductive syndrome virus (PRRSV) and bovine viral diarrhoea virus (BVDV)], an enveloped $^{(-)}$ssRNA virus (influenza virus type A) and a nonenveloped dsRNA virus (reovirus) in a static environment. The viruses chosen include economically significant animal pathogens for which estimates of UV_{254} inactivation are not available.

Materials and methods

Experimental design

Viruses were suspended in culture medium and exposed to specific doses of UV_{254} by placing aliquots into wells of a modified 8-well plate (VWR, West Chester, PA, USA). Each plate included a positive control virus (BVDV, PRRSV, reovirus or influenza) that was protected from UV_{254} exposure by covering the well with aluminium foil. To reduce bias, the placement of each virus within the plate, the unexposed viral control for each plate and the UV_{254} dose for each plate were randomized. Following UV_{254} exposure, the samples were harvested and stored at −80°C until titrated for infectious virus. Viral inactivation data were analysed using both the one-stage inactivation and two-stage inactivation models (Goldberg *et al.* 1958; Kowalski *et al.* 2001).

Viruses

Influenza virus

Influenza A/Swine/Iowa/73 (H1N1) (USDA: National Veterinary Service Laboratories, Ames, IA, USA) was propagated on Madin–Darby canine kidney (MDCK) cells (American Type Culture Collection (ATCC), Manassas, VA, USA). Cells were grown in 162-cm^2 flasks (Corning, Corning, NY, USA) using Dulbecco's modified Eagle's medium (DMEM) (Mediatech Inc., Manassas, VA, USA) supplemented with 0·25 μg ml^{-1} amphotericin B (Sigma Chemical Co., St Louis, MO, USA), 50 μg ml^{-1} gentamycin (Sigma Chemical Co.), 0·5 mol l^{-1} L-glutamine (Gibco, Carlsbad, CA, USA), 300 international units (IU) ml^{-1} penicillin (Sigma), 300 μg ml^{-1} streptomycin (Sigma), 1·0% nonessential amino acids (HyClone, Logan, UT, USA), 25 mmol l^{-1} HEPES buffer (Sigma) and 10·0% heat-inactivated foetal bovine serum (FBS) (Sigma, verified BVDV free). When cells were confluent, the media were discarded and the flasks rinsed with 15 ml of complete infecting medium (CIM): 500 ml of DMEM growth medium, 7·8% bovine serum albumin (BSA) (Sigma) in place of 10% FBS, and 0·83 ml of trypsin working stock. Trypsin working stock was formulated at 100 mg of L-(tosylamido-2-phenyl) ethyl chloromethylketone (TPCK-treated) trypsin (Worthington Biochemical, Lakewood, NJ, USA) diluted into 100 ml of CIM. Flasks were inoculated with 5 ml of CIM containing SIV at a titre of $1{\cdot}26 \times 10^6$ $TCID_{50}$ ml^{-1} and placed on a rocking platform in a 37°C humidified 5% CO_2 incubator for 90 min, after which the inoculum was discarded and replaced with 50 ml of maintenance medium (MM): DMEM growth medium with 4% heat-inactivated FBS in place of 10% FBS. Flasks were incubated at 37°C in a humidified 5% CO_2 incubator and cells examined daily for cytopathic effect (CPE). When ≥75% CPE was observed, flasks were freeze-thawed and the cell lysate was harvested. The harvested lysate was centrifuged at 10 000 ***g*** for 20 min at 4°C and decanted, and the supernatant was stored at −80°C.

Porcine reproductive and respiratory syndrome virus

A type 2 PRRSV isolate MN-184 (provided by Dr Scott Dee, University of Minnesota) was propagated in MARC-145 cells (Iowa State University, Veterinary Diagnostic Laboratory, Ames, IA, USA), and confluent cells were inoculated with 5 ml of MN-184 virus at a titre of $1 \times 10^{3{\cdot}5}$ $TCID_{50}$ ml^{-1}. The flasks were placed on a rocking platform in a 37°C humidified 5% CO_2 incubator for 90 min, then 40 ml of DMEM growth medium with 4% heat-inactivated FBS in place of 10% FBS was added, and the flasks were returned to the incubator for 36 h. Cell culture supernatant was harvested by flask freeze/thaw and centrifugation (10 000 ***g*** for 20 min at 4°C). Viral stock was stored in 25-ml aliquots at −80°C.

Bovine viral diarrhoea virus

Singer strain BVDV type 2 (provided by Dr J. Ridpath, USDA: NADC) was propagated on bovine turbinate cell line T7 (provided by Dr J. Ridpath, USDA: NADC). The cells were grown in 162-cm^2 flasks using EME growth medium: EME medium (Sigma) containing 0·25 μg ml^{-1} amphotericin B (Sigma), 50 μg ml^{-1} gentamycin (Sigma), 300 IU ml^{-1} penicillin (Sigma), 300 μg ml^{-1} streptomycin (Sigma) and 10% FBS (Sigma, verified BVDV free). When cells were 75% confluent, the media were discarded and the flasks inoculated with 5 ml of EME growth medium containing BVDV at a titre of $1{\cdot}26 \times 10^6$ $TCID_{50}$ ml^{-1} and placed on a rocking platform in a 37°C humidified 5% CO_2 incubator for 90 min. Thereafter, 40 ml of EME growth

medium was added and the cells were examined daily for CPE. When *c.* 75% CPE was observed, cell culture supernatant was harvested by flask freeze/thaw and centrifugation (10 000 ***g*** for 20 min at 4°C). Viral stock was stored in 25-ml aliquots at −80°C.

Reovirus

Reovirus strain $T3D^C$ (provided by Dr Cathy Miller, Iowa State University) was propagated in spinner culture-adapted murine fibroblasts (L929 cells) as described by Qin *et al.* (2009). Confluent L929 cells were incubated with a multiplicity of infection of ten plaque-forming units per cell in Joklik c-MEM (Sigma) and stirred for 3 days at 35°C. Thereafter, the cells were pelleted, the supernatant was discarded, and the pellet was resuspended in an equal volume of fresh media. The resuspended cells were sonicated, as described by Nibert and Fields (1992), and then centrifuged at 3000 ***g*** for 5 min. The supernatant was adjusted to a viral concentration of $1{\cdot}7 \times 10^9$ PFU ml^{-1} with Joklik c-MEM media and stored at 4°C until used.

Viral titration

Samples were assayed for infectious virus following the procedures described in the following paragraph. To avoid bias, sample aliquots were assayed blind and in random order. Viral titres were calculated using the Spearman–Kärber method and expressed as the median tissue culture infective dose ($TCID_{50}$) ml^{-1} (Hubert 1992).

Influenza virus microinfectivity and hemagglutination assays

Swine testicle (ST) cells (ATCC CRL-1746) were propagated in 96-well tissue culture plates (Costar, Corning, NY, USA) using MEM growth medium: (MEM) minimum essential Eagle's medium (Sigma) containing 0·25 μg ml^{-1} amphotericin B (Sigma), 50 μg ml^{-1} gentamycin (Sigma), 2 mmol l^{-1} L-glutamine (Sigma), 100 IU ml^{-1} penicillin (Sigma), 100 μg ml^{-1} streptomycin (Sigma) and 10% heat-inactivated FBS (Atlas, Fort Collins, CO, USA). Duplicate samples were serially tenfold diluted (10^{-1}–10^{-6}) in MEM growth medium with 2% heat-inactivated FBS in place of 10% FBS. Test and control sample serial dilutions were plated at 100 μl per well onto confluent cells. Plates were incubated in a 37°C humidified 5% CO_2 incubator and observed daily, when ≥50% CPE was observed, or at the end of the 7-day incubation, the plates were frozen at −80°C.

All test wells were HA tested for confirmation using a hemagglutination (HA) assay. In brief, 50 μl of positive control [Influenza A/Swine/Iowa/73 (H1N1)], negative control (1× PBS, pH = 7·2) and fluid from freeze/thawed CPE-positive wells was added to an untreated 96-well round bottom microtitration plate (Evergreen Scientific, Los Angeles, CA, USA) and serially twofold diluted (1 : 2 to 1 : 256) in 1× PBS. Thereafter, 50 μl of 0·55% turkey red blood cells (USDA: NVSL) was added to each well, and the plates were incubated for 30 min at 25°C. A positive reaction produced red blood cell agglutination, i.e. a 'mat' at the bottom of the well. Samples demonstrating CPE on the microinfectivity assay and an HA viral titre ≥1 : 4 were considered positive for infectious SIV. Viral titres were calculated on the basis of the number of wells in the microinfectivity assay showing CPE. Each sample was run in duplicate with the titres averaged.

Porcine reproductive and respiratory syndrome virus microinfectivity assay

MARC-145 cells propagated in 96-well tissue culture plates (Corning) using DMEM growth medium were incubated at 37°C in a humidified 5% CO_2 incubator for 72 h. Samples to be assayed were serially tenfold diluted (10^{-1}–10^{-5}) in DMEM growth medium without FBS. The growth medium was discarded, and five wells were inoculated with 100 μl of sample at each dilution. The plates were placed in a 37°C humidified 5% CO_2 incubator for 90 min, the inoculum was discarded, and 100 μl per well of growth medium containing 4% FBS, rather than 10% FBS, was added to each well. Plates were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. Following incubation, cells were fixed with cold 80% acetone/water solution and stained for 30 min at 37°C with SDOW-17F, fluorescein isothiocyanate–conjugated monoclonal antibody specific for PRRSV diluted, according to manufacturer's instructions (Rural Technologies Inc., Brookings, SD, USA). Viral titres were calculated on the basis of the number of wells showing a PRRSV-specific fluorescence reaction at each dilution. Each sample was run in triplicate and the titres were averaged.

Bovine viral diarrhoea virus microinfectivity assay

Madin–Darby bovine kidney cells (MDBK), (ATCC) were propagated in 96-well plates (Costar) using complete Ex-Cell™ MDBK-MM Medium (Sigma) containing 0·25 μg ml^{-1} amphotericin B (Sigma), 50 μg ml^{-1} gentamycin (Sigma), 2 mmol l^{-1} L-glutamine (Sigma), 100 IU ml^{-1} penicillin (Sigma), 100 μg ml^{-1} streptomycin (Sigma) and 5% heat-inactivated equine serum (HyClone). Plates were incubated at 37°C in a humidified 5% CO_2 incubator until the cell monolayer was confluent. Samples were serially tenfold diluted (10^{-1}–10^{-6}) in complete Ex-Cell™ MDBK-MM Medium, and 100 μl per well of each sample dilution was applied over the cell suspension. Plates were

then incubated at 37°C in a humidified 5% CO_2 incubator for 24 h, and then, the growth medium was replaced with 200 μl per well of complete medium. The plates were returned to the incubator for an additional 24 h and viewed for CPE. The plates were then frozen at −80°C until confirmatory testing to be carried out. Confirmatory testing and genotyping of BVDV results were carried out using RT-PCR with visualization on a 2% agarose gel (Ameresco, Solon, OH, USA). The viral RNA was extracted as per the manufacturer's specifications, using the Ambion MagMAX™-96 Viral RNA Isolation kit (Ambion, Austin, TX, USA), reverse-transcribed and amplified using the Qiagen® One-Step RT-PCR kit (Qiagen, Valencia, CA, USA). Thermal cycling conditions were as follows: 50°C for 30 min, melt at 95°C for 15 min, 35 cycles of amplification at 94°C for 30 s, 50°C for 30 s, 72°C for 1 min and extension for 10 min at 72°C. Primers used were 5′-TCGACGCCTTRRCATGAAGGT-3′ and 5′-TCGACGCTTTGGAGGACAAGC-3′. The 180-basepair product was run on a 2% agarose gel and visualized under UV illumination.

Reovirus plaque assay

L929 cells in Joklik's minimum essential medium (Sigma) containing 10% FBS (Atlanta Biological, Lawrenceville, GA, USA) were added to 6-well plates (Corning) at a concentration of 2×10^6 cells per well and incubated at 37°C overnight. Thereafter, the medium was removed and the cells were washed once with sterile 1× PBS. To conduct the assay, the sample was serially diluted tenfold, and 100 μl per well was added and allowed to adsorb for 1 h at 25°C. The wells were then overlaid with 2 ml of complete 1× Medium 199 at 37°C. Overlay was formulated as 2× Medium 199 (Sigma) containing 4 mmol l^{-1} ml^{-1} L-glutamine (Mediatech), 200 IU ml^{-1} penicillin, 200 μg ml^{-1} streptomycin (Mediatech) and 20 μg ml^{-1} trypsin (Mediatech) to which was added an equal volume of 58°C 2% agar (BD Bacto™, Franklin Lakes, NJ, USA). The plates were incubated for 48 h in a 37°C humidified 5% CO_2 incubator prior to counting plaques.

Exposure of viruses to ultraviolet (UV_{254})

Source of ultraviolet

The UV_{254}-emitting apparatus consisted of an 18-inch (46 cm), 2-lamp, surface-mounted, 110-volt, Ultraviolet fixture (American UV Company, Lebanon, IN, USA) fitted with two standard output, quasi-collimated beam, low-pressure, mercury vapour germicidal lamps emitting monochromatic UV radiation at 253·7 nm (American UV Company). For uniform output, the lamps were 'burned in' for 8 h prior to use. The UV_{254} source was mounted within an environmental chamber capable of maintaining a constant temperature of 4°C (Percival Scientific, Perry, IA, USA). The inside of the environmental chamber was draped with black burlap to eliminate the reflection of the UV_{254}. To allow lamps to reach full operating pressure within the 4°C environmental chamber, UV-emitting lamps were turned on 2 h prior to commencing the irradiation experiment and 20 min before each exposure treatment, thereafter.

Viral exposure to ultraviolet

Prior to UV_{254} exposure, the clarified viral solutions were thawed and mixed thoroughly to disperse viral aggregates. Initial concentrations of $1 \times 10^{8{\cdot}4}$ (BVDV), $1 \times 10^{6{\cdot}0}$ (PRRSV), $1 \times 10^{6{\cdot}0}$ (influenza virus) and $1 \times 10^{9{\cdot}5}$ (reovirus) $TCID_{50}$ ml^{-1} were exposed to specific UV_{254} doses (25, 50, 75, 100, 125, 150, 200, 250 and 300 mJ cm^{-2}) by placing aliquots (2 mm depth) of each virus into four separate wells. One positive control virus (either influenza virus type A, PRRSV, BVDV or reovirus) was placed in a covered fifth well. The UV_{254} exposure dose (intensity × time) was determined using the integration function of two commercial radiometers equipped with UV_{254} sensors (Technika, Scottsdale, AZ, USA). The UV_{254} doses ($n = 9$) were replicated five times, which corresponded to 45 plates.

To achieve the predetermined sample exposure dose, the two centre wells in one row of each plate were removed to accommodate a UV_{254} sensor. The viruses were exposed to UV_{254} by the use of an electronically controlled shutter system. After lamp warm-up (20 min), the shutters were opened and the plate was exposed to the target dose. When the dose was reached, the UV_{254} emitter was shut off, the shutter system reset, the next plate placed in the environmental chamber, and the UV_{254} emitters warmed up for 20 min prior to exposure. Following UV_{254} exposure, each viral suspension was harvested using a pipette, placed in a 2-ml storage vial labelled with the viral species and a random number and frozen at −80°C until titrated for infectious virus.

In accordance with the Lambert–Beer's Law, the absorbance of any UV_{254} by the liquid medium was determined by measurements taken at the top of the 2 mm depth of the sample and beneath a quartz cuvette containing an equal (2 mm) depth of medium. The measured intensity was corrected for absorbance by the viral medium using Eqn 2 (Thurston-Enriquez *et al.* 2003):

$$I_{\text{average}} = \frac{I_0(1-e^{-a_e L})}{a_e L} \quad (2)$$

where

I_{average} = average UV_{254} intensity (mW cm^{-2})

a_e = absorbance of the viral suspension to the base e

I_0 = UV_{254} intensity after passing through solution
L = depth (centimetres) of the solution irradiated by the UV_{254} energy.

Statistical analysis

Two statistical models were used for analysing the UV_{254} inactivation of each virus.

Model 1. The one-stage inactivation model (Eqn 1) assumed uniform susceptibility of the viral population and utilized simple linear regression (Eqn 3), with the log-transformed viral concentration as the response and the dose of UV_{254} as the explanatory variable:

$$\log_{10} N_t = \log_{10} N_0 - k \cdot \text{Dose}_t \quad (3)$$

where
N_t = quantity of virus in the test sample after treatment with Dose_t
N_0 = quantity of virus in the unexposed control sample
k = the inactivation rate
Dose_t = average UV_{254} intensity × time
In the one-stage inactivation model, the inactivation constant (k) is the quotient of the survival fraction $\left(\frac{N_t}{N_0}\right)$ plotted against the dose. This linear regression model encompasses all of the data points and forces the points into one curve using the classic regression formula, $\left(-b = \frac{mx}{y}\right)$.

Model 2. The two-stage inactivation model (Eqn 4) assumed two heterogeneous subpopulations: one relatively susceptible and the other relatively resistant to UV_{254} treatment (Nibert and Fields 1992):

$$\log_{10} N_t = \log_{10} N_0 + \log_{10}[(1-f) \cdot 10^{-k_1 \cdot \text{Dose}_t} + f \cdot 10^{-k_2 \cdot \text{Dose}_t}] \quad (4)$$

where
N_t = quantity of virus in the test sample after treatment with Dose_t
N_0 = quantity of virus in the unexposed control sample
f = the resistant fraction of the total initial viral population with inactivation rate k_2
$(1 - f)$ = the susceptible viral population fraction with inactivation rate k_1
k_1 = the inactivation rate of the inactivation curve for the 'fast decay population'
k_2 = the inactivation rate of the inactivation curve for the 'resistant population'
Dose_t = average UV_{254} intensity × time
The two-stage inactivation model provides two inactivation constants based on the quotient of the survival fractions of two separate microbial populations: the UV-susceptible population and the resistant population. The susceptible population $(1 - f)$ has an inactivation constant of (k_1); the resistant population (f) has an inactivation constant of (k_2).

The analysis of the data was conducted using SAS® ver. 9.2 (SAS® Institute Inc., Cary, NC, USA). Simple linear regression analysis was performed for Model 1 using SAS® procedure GLM, and nonlinear regression analysis was performed for Model 2 using SAS® procedure NLIN. Lack-of-fit F tests were performed to assess whether the two models fit the data adequately well, by comparing with a full ANOVA model. A P-value ≤0·05 was considered significant.

Results

Analysis of the data showed that the one-stage inactivation model did not fit the data (Table 1). Specifically, the lack-of-fit tests for influenza virus (P = 0·0009), PRRSV (P = 0·0002), BVDV (P < 0·0001) and reovirus (P = 0·0410) showed a statistically significant difference between the observed variation in the data and the variation explained by the single curve model for each virus. Because of the lack of fit, the inactivation constants (k) derived from the one-stage inactivation analysis cannot accurately describe the amount of UV_{254} needed for the inactivation of these viruses under the conditions of the study.

Further analysis of the data showed a good fit between the observed variation in data and the variation explained by the two-stage inactivation model with lack-of-fit-test P-values >0·05 for all viruses (Table 1). The two-stage model described inactivation curves composed of two slopes separated by an inflection point denoting the inactivation kinetics of the two viral populations (Figs 1–4). The two-stage inactivation analysis estimated the susceptible population $(1 - f)$ as 98·8% (influenza virus), 94·1% (PRRSV), 99·9% (BVDV) and 87·3% (reovirus) of the total viral population. Inactivation rates (k_1) in the susceptible population were calculated as 0·0651 (influenza virus), 0·4995 (PRRSV), 0·0663 (BVDV) and 0·0142 (reovirus). The resistant population (f) was a low proportion of the total population of influenza virus (1·2%), PRRSV (5·9%) and BVDV (0·07%), but a larger proportion of the reoviral population (12·7%). Compared with the UV_{254}-susceptible population, the resistant populations showed lower inactivation constants (k_2), i.e. 0·0055 (influenza virus), 0·0143 (PRRSV), 0·0095 (BVDV) and 0·0035 (reovirus).

Discussion

Chick's law describes the rate of microbial inactivation as the ratio of the viable population (N_t) to the original microbial population (N_0) in relation to exposure time (t) to an inactivator (Rubin and Elmaraghy 1977). The resulting one-stage inactivation constant (slope) is an estimate of

Table 1 Parameter estimates and lack-of-fit-test results for two inactivation models

	Influenza virus*	PRRSV†	BVDV‡	Reovirus§
One-stage inactivation model				
Intercept	4·9474	4·4367	7·1911	9·1991
Inactivation rate (*K*)	−0·0124	−0·0258	−0·0188	−0·0066
Lack-of-fit-test *P*-value	0·0009	0·0002	<0·0001	0·0410
Two-stage inactivation model¶				
Intercept	5·7970	4·7979	8·4495	9·3996
Susceptible population proportion (1 − *f*)	0·988	0·941	0·999	0·873
(K_1) Inactivation rate (1 − *f*)	−0·0651	−0·4995	−0·0663	−0·0142
Resistant population proportion (*f*)	0·012	0·059	0·001	0·127
(K_2) Inactivation rate (*f*)	−0·00552	−0·0143	−0·00948	−0·00347
Lack-of-fit-test *P*-value	0·7146	0·0734	0·2227	0·2807

BVDV, bovine viral diarrhoea virus; PRRSV, porcine respiratory and reproductive syndrome virus.
*Influenza A/Swine/Iowa/73 (H1N1) provided by USDA: National Veterinary Service Laboratories, Ames, IA.
†PRRSV type 2 (isolate MN-184) provided by Dr Scott Dee, University of Minnesota, St Paul, MN.
‡BVDV type 2 (Singer strain) provided by Dr J. Ridpath, USDA: National Animal Disease Center, Ames, IA.
§Reovirus strain $T3D^C$ provided by Dr Cathy Miller, Iowa State University, Ames, IA.
¶Kowalski *et al.*, 2001.

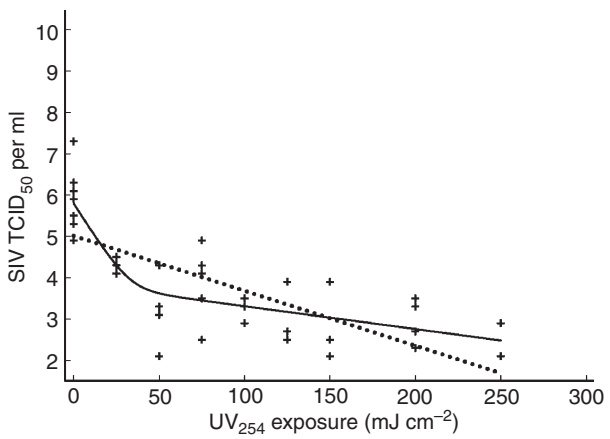

Figure 1 Influenza virus: Linear regression (lack-of-fit $P = 0{\cdot}0009$, $k = -0{\cdot}0124$), two-stage inactivation (lack-of-fit $P = 0{\cdot}7146$, $k_1 = -0{\cdot}0651$, $k_2 = -0{\cdot}00552$). (·····) One-stage inactivation curve and (——) Two-stage inactivation curve.

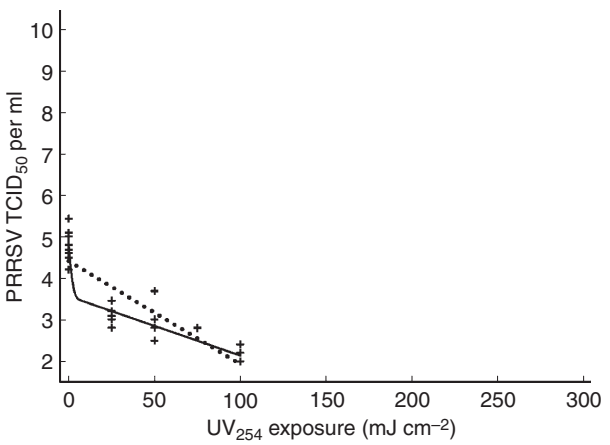

Figure 2 Porcine respiratory and reproductive syndrome virus: Linear regression (lack-of-fit $P = 0{\cdot}0002$, $k = -0{\cdot}0258$), two-stage inactivation (lack-of-fit $P = 0{\cdot}0734$, $k_1 = -0{\cdot}4995$, $k_2 = -0{\cdot}0143$). (·····) One-stage inactivation curve and (——) Two-stage inactivation curve.

the rate of microbial inactivation based on the assumption of a linear relationship between microbial inactivation and contact time. This relationship has been used to describe the kinetics of microbial populations under a variety of inactivating conditions, including exposure to UV_{254} irradiation (Chang *et al.* 1985; Kowalski *et al.* 2001; Thurston-Enriquez *et al.* 2003). One-stage inactivation estimates may be accurate for describing UV_{254} inactivation in homogenous microbial populations (Hiatt 1964). However, one-stage estimates do not address the problem of 'tailing off' of UV inactivation curves and may overestimate the rate of inactivation (Hiatt 1964; Hijnen *et al.* 2006). Jagger (1967) hypothesized that microbial populations were actually composed of two subpopulations: a larger UV_{254}-susceptible population and a smaller UV_{254}-resistant population. Others have hypothesized that the UV-resistant population reflects differences in viral clumping, sample preparation, viral coat structure or viral genomics (Thurston-Enriquez *et al.* 2003). Regardless of the mechanism, Riley and Kaufman (1972) proposed an equation that accurately described the UV_{254} inactivation of *Serratia marcescens* as a two-stage process. Perhaps because deriving estimates for the two-stage inactivation model were computationally demanding prior to the availability of desktop computers, the two-stage approach has not been widely used to describe the kinetics of microbial inactivation. For example, there are no estimates of UV_{254} inactivation of BVDV,

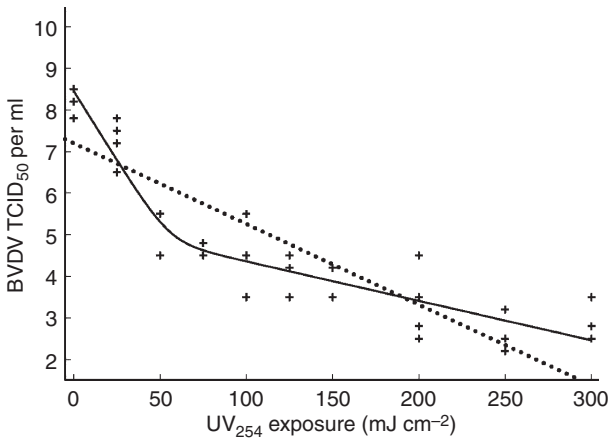

Figure 3 Bovine viral diarrhoea virus: Linear regression (lack-of-fit $P \leq 0{\cdot}0001$, $k = -0{\cdot}0188$), two-stage inactivation (lack-of-fit $P = 0{\cdot}2227$, $k_1 = -0{\cdot}0663$, $k_2 = -0{\cdot}00948$). (·····) One-stage inactivation curve and (——) Two-stage inactivation curve.

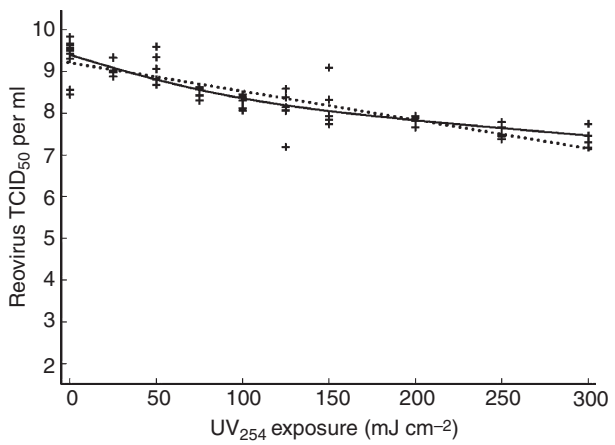

Figure 4 Reovirus: Linear regression (lack-of-fit $P \leq 0{\cdot}0410$, $k = -0{\cdot}0066$), two-stage inactivation (lack-of-fit $P = 0{\cdot}2807$, $k_1 = -0{\cdot}0142$, $k_2 = -0{\cdot}0035$). (·····) One-stage inactivation curve and (——) Two-stage inactivation curve.

PRRSV, reovirus strain $T3D^C$ and influenza virus using the two-stage analysis with which to make comparisons to the results reported herein. However, our population proportions are in agreement with those hypothesized by Hiatt (1964) and demonstrated by Riley and Kaufman (1972).

Overall, this study supported the validity of the two-stage approach as a method for evaluating UV_{254} inactivation kinetics. In addition, these results provide guidelines to persons responsible for the prevention and control of these agents in the environment. Importantly, a comparison of one-stage and two-stage inactivation models using experimental data showed that inactivation of the viruses in the study was a two-stage process, as confirmed by a statistical lack-of-fit test.

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