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# UV inactivation and model of UV inactivation of foot-and-mouth disease viruses in suspension

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ABSTRACT

Foot-and-mouth disease virus (FMDV) causes one of the most contagious diseases affecting cloven-hoofed animals (e.g., cattle and swine) and causes severe economic loss for many countries. The resistance to UV irradiation of FMDV strains isolated from outbreaks in Thailand was investigated in phosphate-buffered saline at 25 °C. Since the regression coefficients of linear regression were large and root mean square errors were small, UV inactivation could be appropriately summarized and fitted well by a linear equation. The first-order kinetics then could describe UV inactivation, which was experimentally and mathematically shown in this study to be an effective means to inactivate FMDV in suspension. The decimal inactivation dose (DID) was modified from D value in traditional thermal-inactivation kinetics. The DID was the amount of UV irradiation required to reduce the number of microorganisms by a factor of 10, or by 90% DIDs of FMDV serotypes O189, A132, A-Sakol, and AS1 ranged from 19.66 to 31.31 mWs/cm$^2$. FMDV serotype AS1 was the most UV-resistant, and FMDV serotype A132 was the least UV-resistant. UV resistance of FMDV did not vary significantly among strains and serotypes ($P$ value$N$0.05). DID raw data were used to determine the fitted probability distributions by simulation software @Risk. The fitted distributions suggested were Exponential, Logistic, Normal, and LogNormal. Exponential distribution was the best fit by Chi-square test, Kolmogorov–Smirnov test, and Anderson–Darling test ($P$ value$N$0.10). The parameter $\beta$ of the Exponential distribution, equivalent to the mean DID, was 24.173 mWs/cm$^2$ as a first-order model. Poisson probability distribution described the uncertainty of parameter $\beta$ for the second-order model. After running simulations of both first-order and second-order models, the curves of both first-order and second-order models were overlaid on the same graph, which indicated that both models were only slightly different. This study concluded that FMDV in suspension was effectively inactivated by UV irradiation, the fitted probability distribution for UV inactivation was Exponential, and source of total uncertainty of this UV-inactivation model was not the uncertainty component.



## 1. Introduction

Foot-and-mouth disease virus (FMDV) is a virus of the family Picornaviridae and classified within genus Aphthovirus. There are seven immunologically distinct serotypes: A, O, C, SAT1, SAT2, SAT3, Asia 1; however only 3 serotypes, which are O, A, and Asia 1, have been circulating in Southeast-Asian countries, including Thailand. FMDV is a non-enveloped, icosahedrally symmetric, and single-stranded RNA virus; therefore it is somewhat resistant to harsh conditions (e.g., thermal treatment, radiation, low water activities, ultraviolet radiation, and disinfection; 4). FMDV causes foot-and-mouth disease (FMD), which is one of the most contagious diseases among cloven-hoofed animals (e.g., cattle and swine). The common mode of transmission of FMDV is direct contact, yet oral-route transmission as indirect contact has frequently caused epidemics in swine. FMD is the first disease on the OIE List A and also was the first disease for which the OIE established an official list of free countries and zones. FMD has a great potential for causing severe economic loss for many countries (Alexandersen et al., 2003). In order to prevent or control the spread of FMD, the vaccination of susceptible animals is a method of choice. Viral inactivation methods for vaccines, together with cell and tissue culture, include chemical treatments (e.g., formaldehyde (Bartley et al., 2002), beta-propiolactone (Culbertson et al., 1956), etc.) and physical treatments (e.g., heat (Raettig, 1981), high hydrostatic pressure (Ishimaru et al., 2004), and ultraviolet (UV) irradiation (Bogaerts and Durville-van der, 1972), etc.. In the slaughter, where there may be subclinical cases of FMD, the wastewater, which is primarily treated to eliminate organic matter, is supposed to be free of FMDV. The contaminated discharges of raw sewage, in surface water that is fed to cattle and swine, could lead to an epidemic (Schijven et al., 2005). Additionally, the cross contamination of FMDV from FMD animals to FMD-free meat, food-contact surfaces, and environment

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within the slaughterhouse should be deemed a prime risk factor for FMD outbreaks domestically and internationally (Schijven et al., 2005). Not only can UV irradiation be used to inactivate FMDV during vaccine preparation (Bogaerts and Durville-van der, 1972), UV irradiation is applicable to controlling FMDV spread by treating FMDV-contaminated effluent from the slaughterhouse and FMDV-contaminated surfaces of meat and other processing-plant equipment and environment.

UV irradiation is a physical means of inactivation and has been used to disinfect pathogens that contaminate water (Drescher et al., 2001), air (Jensen, 1964), food (Altic et al., 2007), and food-contact surfaces (Gardner and Shama, 2000). The mechanism of UV inactivation of RNA viruses is the formation of pyrimidine dimers in the same strand of nucleic acid, thus preventing RNA transcription and replication (Hodges et al., 1980). In contrast to most (chemical) disinfectants, UV radiation effectively and rapidly inactivates pathogens by transfer of the electromagnetic energy from a mercury-arc lamp, through photochemical reaction with their nucleic acids (Hodges et al., 1980). This makes it unnecessary to generate, handle, transport, or store corrosive and hazardous chemical reagents on the premises. Furthermore, UV disinfection equipment requires less space than that for other disinfection methods. For these reasons, UV radiation has been widely used to disinfect (ground) water and wastewater (Drescher et al., 2001).

Like thermal inactivation, UV irradiation's inactivation is a function of dose, in this case specified by UV intensity and time. The UV dose is defined and usually measured as incident energy (not absorbed energy), which is the product of constant UV intensity or dose rate in unit of $mW/cm^2$ and time in unit of seconds. The inactivation curve of UV irradiation is characterized by a survival curve for microorganisms like bacteria or a dose–response curve. In general, the inactivation curve of UV irradiation generally follows first-order kinetics. Analogous to the decimal reduction time (D value or DRT or $D_T$) of thermal-inactivation kinetics, the decimal inactivation dose (DID) is the amount of UV irradiation required to reduce the number of microorganisms by a factor of 10, or by 90%(Nuanualsuwan et al., 2002). Very limited studies have demonstrated UV inactivation of FMDV using irradiation (Dekker, 1998). However these studies employed gamma radiation and did not cover strains which were epidemic in Southeast-Asian countries or Thailand.

An application of inactivation data is to assess the risk of FMDV or other microbial contamination of commodities or biological products (Heng and Wilson, 1993). One crucial component of risk is the likelihood of having a hazard (e.g., microorganism) in the commodity of interest. In the case of FMDV, this likelihood is strongly associated with the titer of FMDV in the commodity. Therefore, the UV resistance of various serotypes and strains of FMDV is a necessary variable to quantify the FMDV residual titer when UV inactivation is applied. In general, the aim of doing experiments is often to infer the application of the experimental result to a population of interest that could not possibly be tested directly (Daniel, 1995). In this case, FMDV strains used are assumed to be representative of FMDV strains that might be epidemic in the field. In conjunction with the nature of experimental design, UV resistance of FMDV always includes serotype or strain variability and uncertainty as a result of small sample size (Vose, 2008). The model of UV inactivation in the form of probability distribution is designated to take into account any possible range of values, and the probability distribution also specifies a probability that corresponds to each value of the variable; thus this model inherently includes both variability and uncertainty (collectively called total uncertainty) of UV inactivation of FMDV (Vose, 2008).

Therefore, the objectives of this study were to determine the UV inactivation kinetics in phosphate-buffered saline and the DID of FMDV serotypes and strains isolated from outbreaks in Thailand, to fit a probability distribution for UV inactivation, and to determine the source of total uncertainty of the UV-inactivation model.

## 2. Materials and methods

### 2.1. Virus and cell culture

FMDV serotypes O (strains O189), A (strains A132 and A-Sakol) and Asia 1 (strain AS1) were obtained from the Regional Reference Laboratory (RRL) for FMD in Southeast Asia, Pakchong, Thailand. All of these strains have been isolated from epidemics in Thailand and then been used as the vaccine strains produced and employed domestically.

The maintenance of the baby hamster kidney (BHK-21) cell line was modified from a previous publication (Clarke and Spier, 1980). Briefly, BHK-21 was grown in a medium composed of Eagle's minimum essential medium "Nissui" No.1, supplemented with 5% normal bovine serum, 0.07% sodium bicarbonate, 0.25 mg/ml of amphotericin (Fungizone® Bristol-Myers Squibb Ltd., Thailand), 0.1 mg/ml of kanamycin, 0.117 mg/ml of penicillin, and 0.1 mg/ml of streptomycin. The maintenance medium was like the growth medium, but contained only 2% fetal bovine serum and 0.21% sodium bicarbonate. Cells for virus propagation and assay were grown in 96-well tissue culture plates (Corning, NY, U.S.A.).

### 2.2. Virus isolation

Several serotypes of FMDV were isolated from FMD epidemics in Thailand. The epithelial tissue in the oral cavity of animals infected with FMD was prepared in 10%(w/v) suspension. Monolayer primary cultures of lamb kidney cells were inoculated with epithelial suspension and held at 37 °C for 60 min (International Office of Epizootics, 2004). The maintenance medium (Eagle's minimum essential medium "Nissui" No.1) was added on the monolayer and held at 37 °C. The cultures were observed for cytopathic effect (CPE), and the harvested fluids were centrifuged at 2000 ×g for 10 min to separate the cell sediment from the fluid medium (International Office of Epizootics, 2004). The serotyping of FMDV was done by ELISA as previously described (Blacksell et al., 1994). If no CPE was observed in the first passage within 48 h, the cells were frozen, thawed, used to inoculate fresh culture and examined for CPE for another 48 h. The second and third blind passages employed the same procedure as the first blind passage, except that the monolayer culture was BHK-21 cells (International Office of Epizootics, 2004). The other application of virus isolation was to determine any residual virus after UV inactivation.

### 2.3. Virus preparation

The viruses were prepared as previously described (Nuanualsuwan and Cliver, 2002). Briefly, the FMDV was propagated in BHK-21 cells. After CPE was observed, harvested fluids were centrifuged to separate the cell sediment from the fluid medium. The sediments were mixed with the cell monolayer, which had been treated with sodium dodecyl sulfate. The supernatant and treated cell monolayer were pooled, filtered (0.2 μm), and kept at −70 °C until used.

### 2.4. Virus assay

Ten-fold virus dilutions were inoculated in equal volumes (50 μl) with BHK-21 cell suspension in MEM growth medium into 96-well plates. There are four wells per dilution. The control wells were inoculated with 50 μl viral diluent. The viral diluent was phosphate-buffered saline (PBS) having 137 mmol NaCl, 2.7 mmol KCl, and 10 mmol phosphate buffer. Plates were incubated at 37 °C for 48 h to observe CPE. Virus titer was recorded as $TCID_{50}$ value according to the method of Reed and Muench (1938).

### 2.5. UV system

A low-pressure mercury-vapor discharge lamp (germicidal lamp: Phillips TUV 36W/G36 T8) was used for this study. The germicidal

lamp with tubular glass envelope emits short-wavelength UV radiation with the peak (monochromatic) at 253.7 nm, with only about 1% of other wavelengths. The intensity of the ultraviolet radiation was measured by a digital radiometer model VLX-3W (Vilber Lourmat, France). This radiometer has an CX-254 sensor designed particularly to measure monochromatic bandwidth at the 254 nm UV wavelength from a low-pressure mercury lamp. The sensor was calibrated prior to use.

### 2.6. UV treatments

The UV treatments have been slightly modified from those previously described (Nuanualsuwan et al., 2002). A summary of the treatment follows. The relative dose of ultraviolet radiation was measured from a source of constant UV radiation within a period of time depending on the specific conditions of the experimental setup. The UV dose is defined and usually measured as incident energy (not absorbed energy), which is the product of constant UV intensity or dose rate in unit of $mW/cm^2$ and time in unit of seconds. The range of UV doses used in the experiments was within 150 $mWs/cm^2$. In order to achieve reproducible results, the UV lamp was warmed up for ca. 20 min before starting the experiments. A 36-W UV lamp 91 cm long was positioned above the sample petri dish whereby the UV irradiation from the UV source to the sample surface displayed a UV intensity of approximately 1.0 $mW/cm^2$. This vertical-beam irradiation makes an infinite plane source of UV, thus making the irradiation independent of distance from UV lamp to virus sample suspension. The continuous ventilation in the biosafety cabinet, and the glass of the lamp tube filtering out 185 nm wavelength radiation, prevented ozone formation in the air between UV source and sample. The UV irradiation effect could have been biased by ozone, which could dissolve in the sample virus suspension and be harmful to the virus. The digital UVX radiometer was also warmed up ca. 10–15 min before measuring the UV intensity and calibrated to 0 $mW/cm^2$ with the sensor face down. Intensities of UV radiation were monitored concomitantly with UV inactivation throughout the experiment and average intensities calculated. The average intensity was about 1.0 $mW/cm^2$. The stock virus suspensions were diluted 10-fold with phosphate-buffered saline to prepare the working virus suspension, essentially to eliminate UV absorption by any proteins left over from the cell culture maintenance medium. Phosphate buffer solution has only slight absorption of wavelengths more than 220 nm, even with solutions 1 cm deep. Virus suspensions, ca.1–3 ml, were dispensed to form a layer of fluid less than 2 mm deep in a round, flat petri dish of 4 cm diameter. The relationship between the volume of virus suspension and the diameter of the container was such that a meniscus did not form at the edges of the fluid or there was no capillary effect where the virus suspension contacted the petri dish wall, so the focusing effect was negligible and uniform irradiation was achieved. The samples were not stirred because the thin layer of virus suspension made this unnecessary; however, vibration from the fan in the biological safety cabinet also agitated the virus suspensions at all times. The UV exposure was initiated and terminated by moving a piece of aluminum foil so as to uncover and cover the virus suspension, respectively. The desired UV doses determined how long the virus suspension was irradiated. Immediately after UV exposures of the virus suspensions were complete, the samples were serially diluted and quantitated by the virus assay to determine the residual titers of UV-irradiated virus.

### 2.7. Rate of UV inactivation

Since the main objective was to determine the number of virus units that were activated at various inactivation times, it was understood that some virus suspensions still had infectious virus left to be assayed. The UV inactivation of a microorganism is dependent on intensity of irradiation and time exposed. At a constant intensity of irradiation and temperature, the decrease in the number of microorganisms generally follows first-order kinetics and can be expressed as:

$$\mathrm{Log}(N_d/N_0) = -t/\mathrm{DID} \quad (1)$$

where $N_d$ and $N_0$ are the concentrations of FMDV at dose d and zero, respectively. The decimal inactivation dose (DID) is the amount of UV irradiation at specified temperature required to reduce the number of microorganisms by a factor of 10, or by 90%(Nuanualsuwan et al., 2002). Therefore, DID represents the UV resistance or rate of microbial inactivation and can be calculated by the negative reciprocal of the slope of the inactivation curve where log $[N_d/N_0 N_0]$ against inactivation dose d is linear on a semi-logarithmic plot.

### 2.8. Statistical analyses

For inactivation curve, the regression analysis was employed to determine if regression coefficient (in the form of negative reciprocal of slope or DID) is significantly different from zero (i.e., UV irradiation is capable of inactivating FMDV). The correlation coefficient ($r^2$) was calculated to determine the correlation of inactivation dose and virus concentration. Root mean square error (RMSE) was employed to determine goodness-of-fit of the regression equations to the observed data. The analysis of variance (ANOVA) was employed to determine the statistical significance of difference in UV resistance across FMDV strains. When ANOVA indicated a statistically significant difference, a multiple comparison was employed to determine specific differences between pairs of FMDV strains (Kleinbaum et al., 1988).

### 2.9. UV inactivation model

For the purpose of quantitative microbial risk assessment, it is more conventional and useful to express the result of a certain variable in the form of probability distribution (Vose, 1997, 1998). A total of 20 results of DID raw data from tested FMDV strains was analyzed to determine the fitted probability distributions by Monte Carlo Simulation software @Risk® Version 4.5 (Palisade, corporation), using a feature called "Fitting distribution to data." The tests for goodness-of-fit were Chi-square (Garcia-Perez and Nunez-Anton, 2004), Kolmogorov–Smirnov (K–S) (Haas et al., 1999), and Anderson–Darling (A–D).

In order to determine the source of total uncertainty, variability and uncertainty were analyzed separately by setting up a first-order model and second-order model (Vose, 2008). The first-order model contributed the variability per se, whereas the second-order model contributed total uncertainty. On one hand, the first-order model for a probability distribution was set up in such a way that the parameter of the model was fixed and simulated to an expected value using either Monte Carlo or Latin Hypercube re-sampling techniques (Murray, 2004; Vose, 2008).

$$\mathrm{DID} = \mathrm{Exponential}(\beta) \quad (2)$$

For example in Eq. (2), the parameter of "Exponential" probability distribution or β is fixed at a certain value. On the other hand, the parameter of first-order model is uncertain and replaced by a probability distribution as a "mixed model." Then the variability and uncertainty were simulated altogether using either Monte Carlo or Latin Hypercube re-sampling techniques (Murray, 2004; Vose, 2008). The choice of re-sampling technique is dependent on the number of iterations used in the simulation. If the number of iterations is small, the Latin Hypercube is chosen; otherwise the Monte Carlo re-sampling technique is an option (Murray, 2004; Vose, 2008).

$$\mathrm{DID} = \mathrm{Exponential}(\mathrm{Poisson}(\lambda)) \quad (3)$$

For example in Eq. (3), the parameter of Exponential probability distribution β is replaced by an uncertainty variable which has

Table 1
DIDs (mWs/cm$^2$) of FMDV strains in PBS

| FMDV | DID[a] | $r^2$ [b] | RMSE[c] | P value[d] |
|---|---|---|---|---|
| O189 | 25.23 ±2.88$^A$ | 0.96 | 0.35 | 0.021 |
| A132 | 19.66 ±1.66$^A$ | 0.99 | 0.28 | 0.019 |
| A-Sakol | 22.10 ±2.07$^A$ | 0.98 | 0.33 | 0.026 |
| AS1 | 31.31 ±4.78$^A$ | 0.92 | 0.56 | 0.004 |

[a] Values are mean of five replications. Values followed by the same letters are not statistically different (Pb0.05).
[b] Correlation coefficients.
[c] Root mean square error.
[d] $H_0$: regression coefficient =0.

"Poisson" probability distribution. Eventually, the discrepancy of these two models indicated the effect of uncertainty (Murray, 2004). The number of iterations for simulation is 10,000 by @Risk simulation software.

## 3. Results

### 3.1. Decimal inactivation dose (DID)

In order to demonstrate whether UV irradiation was effective against FMDV serotypes in suspension form, the biological hypotheses were tested (Table 1). The statistical results indicated that UV irradiation was significantly effective against tested FMDV serotypes which are strains O189, A132, A-Sakol and AS-1 (P valueb0.05). Therefore the biological explanation is that in this experiment the decreasing number of FMDV is a result of UV irradiation.

DIDs of FMDV serotypes O189, A132, A-Sakol, and AS1 ranged from 19.66 to 31.31 mWs/cm$^2$ (Table 1 and Fig. 1). FMDV serotype AS1 was the most UV-resistant, with the largest DID; whereas FMDV serotype A132 was the least UV-resistant, with the smallest DID. Different strains of the same FMDV serotype (e.g., serotype A) have a comparable DID; the observed discrepancy was also not statistically significant (PN0.05). Furthermore UV resistances of tested FMDV serotypes were not significant different (PN0.05). The regression coefficients were in the range of 0.92 and 0.99 (Table 1). The large regression coefficients indicated that the UV inactivation could be appropriately explained by linear equation. The root mean square errors (RMSEs) were in the range of 0.28 to 0.56 (Table 1). The small root mean square error indicated that the raw data from the experiments were well fitted with the linear equations that explained reducing FMDV concentrations as a function of UV dose.

### 3.2. UV inactivation model

A total of 20 results of DID raw data from tested FMDV strains, to determine the fitted probability distributions by Monte Carlo Simulation software @Risk®Version 4.5 (Palisade, corporation) using feature so-called "Fitting distribution to data," is shown in Table 2. The distribution of DID of FMDV serotypes was shown in Fig. 2. The histogram clearly shows that most of DID were in less than 20 mWs/cm$^2$ and the rest gradually decreased in the range of 20 and 35 mWs/cm$^2$. The fitted probability distributions suggested by simulation software were Exponential, Logistic, Normal, and LogNormal as shown in Table 3. In order to test for fitted probability distribution, the null hypothesis was that candidate probability distribution is fit to DID raw data. One will accept the null hypothesis when the test value of goodness-of-fit test is less than the critical value (Daniel, 1995). The smaller the test value, the fitter the probability distribution. In other words, the larger P value of goodness-of-fit test indicated that the probability distribution was fitter to the raw data (Daniel, 1995). Exponential probability distribution was the best fit (Fig. 2) among all candidate probability distributions by Chi-square test (P valueN0.10), Kolmogorov–Smirnov test (P valueN0.10), and Anderson–Darling test (P valueN0.10) because all test values of Exponential probability distribution were much smaller than those of other candidate probability distributions (Table 3). For the Chi-square test, the other

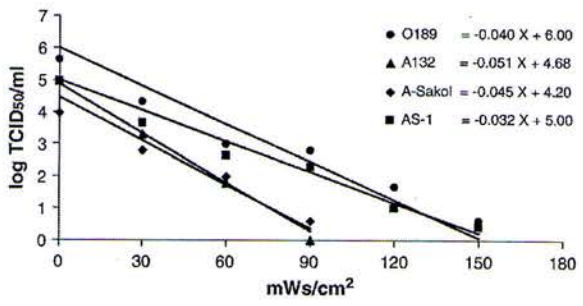


Fig. 1. UV inactivation of foot-and-mouth disease virus serotypes O, A, and Asia1 in PBS. The log-linear inactivation equations of FMDV serotypes are shown in the legend where FMDV serotypes symbolize the numbers of FMDV and X is the inactivation dose. DID values are the reciprocal of the slope of the inactivation curve.

Table 2
DIDs (mWs/cm$^2$) of FMDV strains used to fit probability distribution

| FMDV | Replication | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| O189 | 20.93 | 31.56 | 34.41 | 22.72 | 16.52 |
| A132 | 18.75 | 19.48 | 24.19 | 17.57 | 18.28 |
| A-Sakol | 16.98 | 22.11 | 29.56 | 17.08 | 24.77 |
| AS1 | 34.91 | 31.21 | 47.04 | 24.72 | 18.65 |

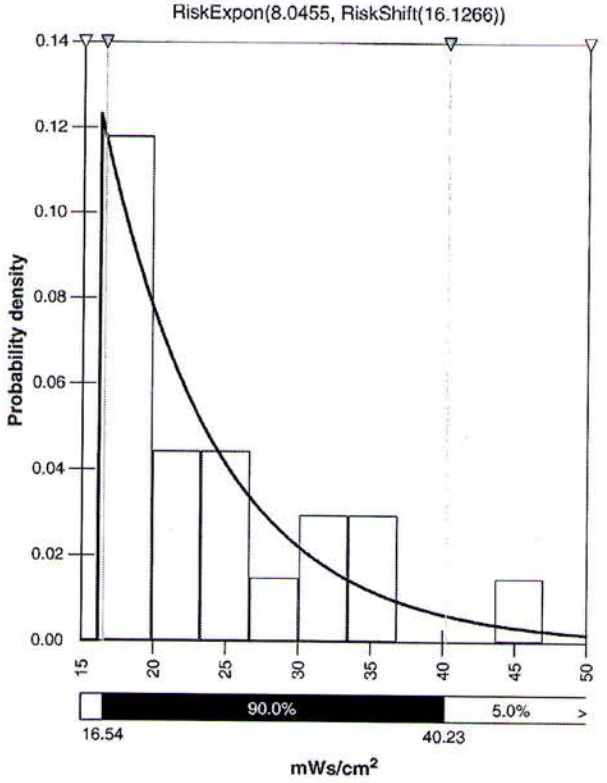


Fig. 2. Exponential probability distribution (line) that fitted to the histogram of DIDs of FMDV strains (bar). The parameter of Exponential probability distribution is the mean, which is equal to 8.045 with the shift of the distribution 16.1266 mWs/cm$^2$ to the right.

Table 3
Goodness-of-fit test[a] of probability distributions of DID

| Candidate distributions | Goodness-of-fit tests | | | | | |
|---|---|---|---|---|---|---|
| | Chi-square | | K–S[b] | | A–D[c] | |
| | CV[d] | TV[e] | CV | TV | CV | TV |
| Exponential | 7.814 | 0.400 | 0.231 | 0.111 | 0.818 | 0.219 |
| LogNormal | 7.814 | 1.600 | N/A | 0.189 | N/A | 0.889 |
| Normal | 7.814 | 1.600 | 0.192 | 0.190 | 0.720 | 0.878 |
| Logistic | 7.814 | 2.000 | 0.168 | 0.162 | 0.759 | 0.747 |

[a] $H_0$: candidate distribution was fitted to raw data.
[b] K–S: Kolmogorov–Smirnov significance test.
[c] A–D: Anderson–Darling significance test.
[d] CV: critical value of the fit statistics at the 5%level of significance.
[e] TV: test value of the fit statistics at the 5%level of significance. $H_0$ was accepted if the test value was less than the critical value.

three probability distributions were considered to fit the raw data with much smaller degree of goodness-of-fit, since the test values were much higher than that of Exponential probability distribution. Unlike the Chi-square test, the Kolmogorov–Smirnov test indicated that Logistic and Normal probability distributions similarly fit to the DID raw data in comparison with Exponential probability distribution, since test values of all these three probability distributions were not far from the corresponding critical values. For Anderson–Darling test, only Exponential and Logistic probability distributions were fit. However test value of Logistic probability distribution was close to the corresponding critical value than that of Exponential probability distribution.

According to the goodness-of-fit test, the best fit probability distribution is Exponential. Exponential probability distribution as shown in Eq. (2) is generally used to describe interval measured in either amount (liter, kilometer, etc.) or time between successive occurrences of the event e.g. dose to inactivate FMDV, etc. (Vose, 2008). The parameter β is the mean amount or time to the occurrence which is equal to 8.046 +16.127 mWs/cm$^2$ (Murray, 2004; Vose, 2008). Therefore the first-order model as shown in Eq. (4) will have the mean of DID =24.173 mWs/cm$^2$ as fixed expected value.

Mean DID ¼ Exponential(8:046) + 16:127 (4)

The Exponential and Poisson probability distributions are among the exponential family of distributions. Therefore the Poisson probability distribution appropriately describes the uncertainty of the parameter of Exponential probability distribution (β) (Murray, 2004; Vose, 2008). For the second-order model as shown in Eq. (5), the Poisson probability distribution as uncertainty parameter had its parameter (λ) equal to 8.049 after simulation (Fig. 3).

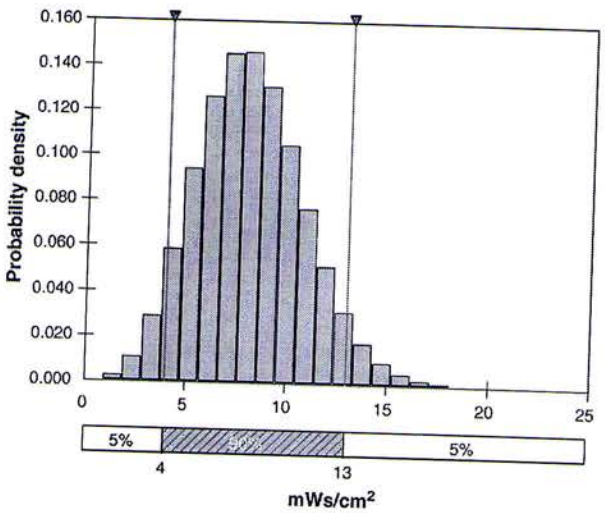


Fig. 3. Poisson probability distribution [RiskPoisson (8.046)] as an uncertain parameter of UV inactivation model.

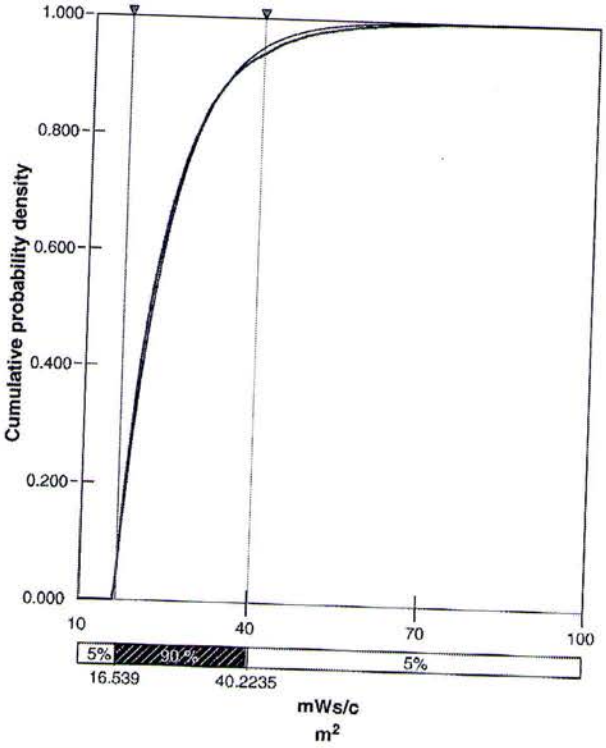


Fig. 4. A comparison of first-order model, which represented the uncertainty parameter (thin line), and second-order (mixed) model, which represented variability and uncertainty (bold line).

Mean DID ¼ Exponential(Poisson(8:046)) + 16:127 (5)

After simulation of both first-order and second-order models, the probability distributions of both models from the simulation were only slightly different, as shown in Fig. 4.

## 4. Discussion

The UV irradiation applied to FMDV suspensions produced decreases in the FMDV numbers as a function of UV doses. Additionally, since the regression coefficients were large and root mean square errors were small, the UV inactivation could be appropriately summarized and fitted well by a linear equation. Thus first-order kinetics can predict the UV inactivation, which is experimentally and mathematically shown in this study to be an effective means to inactivate FMDV in suspension. UV inactivation of viruses has been studied; however, most earlier studies did not determine the inactivation kinetics and the viruses were not in fluid suspension (Den Boon et al., 1995). There are many more UV-inactivation studies with bacteria, and some of these quantitatively determined the bacterial reductions (Basaran et al., 2004). The inactivation of bacteria indicated that UV inactivation could be linear or non-linear (e.g., logistic regression) (Forney et al., 2004). The difference might be related to the media used in the study (e.g., water, juices, etc.), since food particles could shield the UV effect.

In this study, the theory of thermal-inactivation kinetics was applied to describe the UV-inactivation kinetics of FMDV. The

decimal-reduction time is the time at a specified temperature required to reduce the number of microorganisms by a factor of 10 (Singh and Heldman, 2001); then the efficiency of thermal inactivation is a function of time and temperature. For UV inactivation, the parameters of inactivation are exposure time and UV intensity from a UV lamp, analogous to inactivation temperature. Because of variability of UV intensity from UV lamps (Duffy et al., 2000), UV doses must be expressed to include both time and intensity parameters simultaneously. The decimal-inactivation dose (DID) was modified from decimal-reduction time or D value (Singh and Heldman, 2001). When effective-inactivation UV dose and the D value concept were combined, the decimal inactivation dose or DID was introduced to describe the UV inactivation of various microorganisms. Unlike thermal inactivation, where D value is designated by temperature, UV inactivation is then independent of the UV intensity. Therefore DID is more practical than D value, as DIDs can be readily compared without specifying UV intensity.

UV resistance of FMDV did not vary significantly among strains and serotypes, so strains of FMDV serotypes, particularly serotype A, have a similar UV resistance. In addition, the UV resistance of tested FMDV serotypes essentially did not differ. The strain and serotype similarity in terms of UV inactivation of FMDV was also observed for thermal inactivation (Kamolsiripichaiporn et al., 2007). It seems possible that any chosen serotype or strain of FMDV isolated in Thailand can be a good representative for further in-depth studies (e.g., mechanisms of UV inactivation, vaccine development, etc.).

Inactivation kinetics of physical methods, especially heating and UV irradiation, against FMDV has a tendency to be analogous. FMDV serotype AS-1 was more resistant than FMDV serotype A to both UV irradiation and thermal inactivation at all ranges of inactivation temperatures (Kamolsiripichaiporn et al., 2007); whereas FMDV strains A132 and A-Sakol were less resistant than FMDV serotypes O to both UV irradiation and thermal inactivation (Kamolsiripichaiporn et al., 2007). These results indicate that pattern of resistance of FMDV serotypes and strains is independent of the physical method of inactivation. Additionally, this inactivation pattern also corresponded well to the result of UV-inactivation kinetics which was fitted by a first-order kinetics equation. Even though the inactivation kinetics of both UV and heat are similar, the mechanisms of inactivation are different. The UV energy focuses on the nucleic acid and usually causes the pyrimidine dimer formation (Betina et al., 1993), whereas the thermal energy primarily changes the conformation of viral capsid protein (Nuanualsuwan and Cliver, 2003).

According to the histogram of DID (Fig. 2), the peak of DID was less than 20 mWs/cm$^2$, with less frequent values down to 35 mWs/cm$^2$. This pattern of distribution indicated the strain or serotype similarity, which corresponded to the statistical analysis of UV resistance across FMDV strain and serotypes. Among the probability distributions suggested by @Risk software, the probability distributions that peak at the left side of the distribution earned good rankings (e.g., Exponential (Fig. 2), Logistic, and LogNormal). When comparing the test and critical values of probability distribution candidates, Chi-square and A–D goodness-of-fit tests indicated that the Exponential probability distribution was appropriate to represent the DID raw data. Chi-square test did not clearly define number and location of the bins however this analysis used the equal-probability bin. Unlike Chi-square test, K–S and A–D tests do not need a bin, so they are less arbitrary. A–D test differ from the K–S test in that A–D also emphasize the differences between the tails of fitted-distribution and raw data. In this study, even though most DID aggregated on the left side, few DID were on the right tail of histogram. This explained why the best candidate, as judged by A–D, was not Exponential probability distribution.

In this study, the main source of total uncertainty was the variability; therefore, the sample size to determine the UV inactivation model is adequate. Traditionally, a fitted-probability distribution is used to model variability; whereas the parameter of that probability distribution, which describes the model, remains uncertain. The total uncertainty of the model is in fact derived from both uncertainty and variability (Murray, 2004; Vose, 2008). The first-order and second-order models were simulated and shown in Fig. 4. The ascending cumulative curves of both first-order and second-order models were overlaid on the same graph, indicating that the models were only slightly different and that the main source of total uncertainty was not due to uncertainty (Murray, 2004; Vose, 2008). Therefore reduction of uncertainty by collecting further information or doing more experimental replications to improve the DID estimate in the future is not necessary. In other words, reduction of variability is not possible either by increasing replications or measurements, so the quantitative microbial risk assessment model, particularly in this case the DID model obtained, was mathematically correct since the variability component and uncertainty component were separately analyzed and compared in both first-order and second-order models for UV inactivation (Vose, 2008).

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