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Methodology for determining the susceptibility of airborne microorganisms to irradiation by an upper-room UVGI system

C.B. Beggs[1], C.J. Noakes[2], P.A. Sleigh[2*], L.A. Fletcher[2], K.G. Kerr[3]

[1] Medical Engineering Group, School of Engineering, Design and Technology, University of Bradford, Bradford BD7 1DP, UK

[2]Aerobiological Research Group, School of Civil Engineering, University of Leeds, Leeds LS2 9JT, UK

[3] Dept of Microbiology, Harrogate Health Care Trust, Harrogate District Hospital, Lancaster Park Road, Harrogate HG2 7SX, UK

*Corresponding author: Dr P Andrew Sleigh, email P.A.Sleigh@leeds.ac.uk phone +44(0)113 343 2398, fax +44(0)113 343 2265

Abstract

Whilst a number of researchers have demonstrated the disinfection effectiveness of upper-room UV irradiation devices against a range of airborne microorganisms, it is technically difficult to determine the performance of such systems because the biological and physical processes involved can be complex. In particular, most of the quantitative data on the susceptibility of airborne microorganisms to UV irradiation is obtained from single-pass experiments which are not representative of the fragmented UV exposure experienced by airborne microorganisms in real rooms. This paper presents complete and partial mixing models for predicting an effective UV susceptibility constant, Z_{eff}, that is appropriate for quantifying the behaviour of airborne microorganisms when irradiated using an upper-room system. The use of both decay and continuous contamination experimental techniques are discussed and related to the models presented. Experimental results are presented which indicate that Z_{eff} for *Serratia marcescens* is up to an order of magnitude lower than the susceptibility constants derived from single-pass experiments, suggesting that using these data to design upper-room UV systems may lead to a lower than expected performance.

Keywords
Upper-room UV, ultraviolet, UVGI, air disinfection, experimental method, UV susceptibility, airborne microorganisms, ventilation model.

1. Introduction

Airborne microorganisms are particularly vulnerable to damage from ultraviolet (UV) light at 254 nm. At this wavelength, photons of light are absorbed by deoxyribonucleic acid (DNA) to form pyrimidine dimers and other lethal photoproducts (Beggs, 2002). UV light can therefore be used to inactivate airborne pathogens such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB). However, UV-C irradiation is hazardous to humans, causing skin and eye irritation under conditions of direct exposure, and therefore must be used with caution. One effective way to disinfect air is to use wall or pendant mounted fittings to create an open UV field in an upper-room zone (see Figure 1). By shielding the fittings and mounting them above head height, it is possible to irradiate a large area of the room whilst protecting any occupants from exposure. Such systems rely on the convection currents which occur naturally in a room, rather than fans, to transport airborne microorganisms through the UV field. By using the convection currents in rooms it is possible to disinfect very large volumes of air relatively quickly (Miller *et al*., 1999).

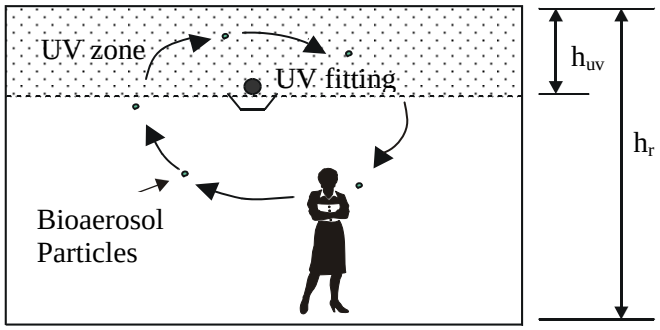


Figure 1. An upper-room UV installation

While a number of researchers (Miller *et al*., 1999, Riley *et al*., 1976, Miller and Macher, 2000) have demonstrated that upper-room UV fields might be effective in controlling the spread of TB and other airborne infections in enclosed spaces, no firm guidelines exist for the design of such systems. The absence of guidelines is largely due to the complexity of the biological and physical processes involved in the air disinfection process, and a lack of good quality microbiological data. It is thus difficult to predict how upper-room UV systems will perform, let alone determine how they should be optimised. Indeed, such is the shortage of good microbiological data that designers have little idea of how medically important microorganisms behave when exposed to UV light at 254 nm. In particular, little is known about the effect of sub-lethal doses of UV irradiation on many significant microorganisms (Beggs, 2002).

The susceptibility of airborne microorganisms to UV light is generally determined using a single pass airflow apparatus (Ko e*t al*., 2000, Fletcher *et al*., 2003), in which microorganisms suspended in an air stream pass once through an accurately calibrated UV field. However, in a real room the airborne microorganisms may pass in and out of the upper-room UV field several times and may therefore experience DNA repair processes such as photoreactivation (Beggs and Sleigh, 2002, Peccia and Hernandez, 2001) as well as multiple exposures to the UV field. As a result the microorganism susceptibility characteristics measured in a single pass apparatus may not be representative of the behaviour in a real situation. This paper addresses this issue by presenting several analytical models, of varying degrees of complexity, which may be used in conjunction with experimental data to determine the susceptibility of airborne microorganisms irradiated by upper-room UV systems. The study demonstrates how the models can be used to calculate the value of an *effective susceptibility constant*, Z_{eff}. This value is comparable to the microorganism susceptibility data obtained from single pass apparatus, but it also allows for the additional decay and repair mechanisms that may occur in a real situation. The relative merits of the models are examined using both theoretical analysis and actual experimental data from an aerobiological test chamber.

2. UV Susceptibility

The susceptibility of airborne microorganisms to damage from UV light depends on both the microorganism species and the climatic conditions. If a microbial population is exposed to UV light at 254 nm, the DNA will suffer damage: the greater the UV dose received, the greater the damage and ultimately the greater the number of microorganisms killed. While UV light at 254 nm damages most microorganisms, the injury caused varies between microbial genera and species. The UV susceptibility of any given microorganism to UV damage is quantified by a UV susceptibility constant, Z (m^2/J), and calculated from experimental measurements using equation (1):

$$Z = \frac{-\ln(C_t / C_0)}{H_{eff}} \qquad (1)$$

where: C_t = Bioaerosol concentration at time t (cfu/m^3)
C_o = Initial bioaerosol concentration at (i.e. at time t = 0) (cfu/m^3)
H_{eff} = Effective UV dose received by microorganisms (J/m^2)

UV susceptibility constants have been determined experimentally by a number of researchers over many years using a single pass airflow apparatus, similar to those described by Ko e*t al*., 2000 or Fletcher *et al*., 2003. Aerosolised microorganisms are passed once through known UV fields under controlled climatic conditions and sampled using a suitable aerobiological sampler. The surviving microorganisms are incubated and enumerated, and the subsequent reduction in microorganism concentration is determined for each UV field. By plotting the natural log of the reduction against UV dose and fitting a linear regression line to the data, the value of Z can be found by calculating the gradient of the line, as shown by equation (1). This experimental procedure is, in practice, quite difficult to undertake and achieve a good consistency of measurements (Fletcher *et al*., 2003). This is indicated by the data in Table 1, which presents published Z values for a range of microorganisms in the aerosolised state.

Table 1 UV susceptibility constants for various aerosolised microorganisms calculated from single-pass experiments

Microorganism	***Z*** **(m^2/J)**	**RH range**	**Source**
Bacillus subtilis (mixed spores)	0.0190	-	Rentschler (1941)
Bacillus subtilis (vegetative cells)	0.063-0.066	20-40%	Peccia *et al* (2001)
Bacillus anthracis	0.0510	-	(Sharp 1938)
Pseudomonas aeruginosa	0.5721	-	Sharp (1940)
Serratia marcescens	0.57±0.42[a]-0.58±0.21[b]	[a]49-62%, [b]22-33%	Ko *et al* (2000)
Serratia marcescens	0.095[c]-0.94[d]	[c] 78%, [d] 48%	Fletcher et al (2003)
Serratia marcescens	0.35-0.45	40-50%	Peccia *et al* (2001)
Serratia marcescens	1.83-2.45	50%	Riley *et al*. (1976)
Serratia marcescens	0.4449	-	Sharp (1940)
Mycobacterium bovis BCG	0.37-0.39, 0.23-0.28[*]	50%	Riley *et al*. (1976)
Mycobacterium bovis BCG	0.17±0.13[a]-0.27±0.20[b]	[a]49-62%, [b]22-33%	Ko *et al* (2000)
Mycobacterium parafortuitum	0.12-0.15[e],0.2-0.22[f]	[e]50-90%,[f]20-40%	Peccia *et al* (2001)
Mycobacterium tuberculosis	0.23-0.42, 0.44-0.55[*]	50%	Riley *et al*. (1976)
Staphylococcus aureus	0.3476	-	Sharp (1940)
Staphylococcus aureus	0.9602	-	Luckiesh (1946)
Escherichia coli	0.3759	-	Sharp (1940)
Adenovirus	0.0546	-	Jensen (1964)
Influenza A virus	0.1187	-	Jensen (1964)
Aspergillus amstelodami	0.00344	-	Luckiesh (1946)
Penicillium chrysogenum	0.00434	-	Luckiesh (1946)

[*]Two different cultures used

The data in Table 1 reveal a wide range of *Z* values for the various microorganisms. *Escherichia coli* for example, is approximately a hundred times more susceptible to UV irradiation than *Aspergillus amstelodami*. In addition, the data show considerable variation between the values obtained by different researchers for the same microorganism; for example, for *Serratia marcescens* values range from 0.095 – 2.14 m^2/J. While this variance may, in part, be attributable to the use of different strains by various researchers, it may also be attributable to variations in experimental technique. In particular differences in relative humidity between different experiments is likely to result in significant variations in the measured susceptibility constant. Several authors have noted that there is a species dependent relationship between UV susceptibility and relative humidity with most reporting a decrease in susceptibility to UV irradiation at high relative humidities (Riley and Kaufman 1972, Peccia et al 2001, Fletcher et al 2003). However it is only the more recent studies (Ko et al 2000, Peccia et al 2001, Fletcher et al 2003) that fully report both the relative humidity data and the respective UV susceptibility constants.

3. Complete air mixing models

The complete air mixing model is developed by considering a ventilated room in which the air is assumed to be fully mixed. If there is no recirculation of ventilation air and the ventilation supply air is clean the only source of microorganisms is from within the room, for example disseminated into the air by one of the room occupants. Assuming that ventilation is the only contaminant removal mechanism the theoretical rate of change of microbial contamination is given by

$$V\frac{dC}{dt} = q - CNV \tag{2}$$

where:

C	=	Bioaerosol contamination level at time t (cfu/m^3)
V	=	Room volume (m^3)
N	=	Steady state air change rate (s^{-1}) = Number of air changes per hour/3600
q	=	Constant bioaerosol contamination rate in the room space (cfu/s)

With an initial concentration in the room of C_0, the general solution to equation (2) yields the contamination in the room at time t, as given by equation (3).

$$C = \frac{q}{NV} + \left(C_0 - \frac{q}{NV}\right)e^{-Nt} \tag{3}$$

Although this equation describes the general build up or decay of the contaminant in the room, it is not particularly useful for analysing experimental data in its current form. Therefore two specific cases are considered which are relevant to experiments characterising contamination removal by ventilation and upper room UV devices

The first case considers the calculation of how long it takes for the contamination level to fall in enclosed spaces. Should the contamination source cease (or leave the room) then with $q = 0$ equation (3) reduces to the *decay model*.

$$C = C_0 e^{-Nt} \tag{4}$$

The second case assumes *continuous contamination* of the room space and examines the contaminant level in the room under steady-state conditions. Here the left hand side of equation (2) is zero and the

microbial contamination level can be predicted by using equation (5). This indicates that the steady-state concentration is inversely proportional to the air change rate *N*.

$$C = \frac{q}{NV} \tag{5}$$

The above equations only consider the removal by the ventilation system and do not take into account bioaerosol 'removal' by upper room UV systems. To include this, a further constant inactivation rate term, ϕ_{uv}, is required. This is added to *N*, to give the total inactivation rate $N + \phi_{uv}$. Beggs and Sleigh (2002) have shown that under conditions of complete air mixing ϕ_{uv} is given by:

$$\phi_{uv} = Z_{eff} E \frac{h_{uv}}{h_r} \tag{6}$$

where;

h_{uv}	=	Height of UV zone (m) (see Figure 1)
h_r	=	Height of room (m) (see Figure 1)
Z_{eff}	=	Effective UV susceptibility constant (m^2/J)
E	=	Average UV irradiance (W/m^2)

Including this inactivation term in equation (2) yields the following expression for the rate of change of contaminant concentration in the presence of both UV irradiation and ventilation removal, C_{uv}

$$V \frac{dC_{uv}}{dt} = q - C_{uv} V (N + \phi_{uv}) \tag{7}$$

Integrating this results in the following general solution for the contamination in the room:

$$C_{uv} = \frac{q}{(N + \phi_{uv})V} + \left(C_0 \frac{q}{(N + \phi_{uv})V} \right) e^{-(N+\phi_{uv})t} \tag{8}$$

As previously this expression can be simplified for the particular case where the contamination ceases at time zero to give a new *decay model* as

$$C_{uv} = C_0 e^{-(N+\phi_{uv})t} \tag{9}$$

Similarly the following *continuous contamination model* now represents the contaminant concentration in the steady state case

$$C_{uv} = \frac{q}{(N + \phi_{uv})V} \tag{10}$$

Xu et al's (2003) experimental evaluations of upper room UV systems inactivating Mycobacterium parafortuitum showed that equivalent UV inactivation rates of between 1.2 and 17 AC/h could be achieved with a room ventilation rate of 3 AC/h, depending on the UV lamp power. This indicates that ϕ_{uv} is usually of a similar order of magnitude as *N* and consequently it is very important to quantify both rates as accurately as possible.

In reality the rate at which airborne microorganisms are inactivated or removed from a room space depends on more than the physical actions of the ventilation system or UV lamps. Viable bioaerosol particles are also 'removed' from the air by natural biological and physical processes including inactivation of microorganisms resulting from environmental stress due to factors such as oxygen toxicity, nutrient starvation and desiccation (Cox 1995) and by physical deposition on room surfaces. It is possible to include these effects in the above models by replacing N with

$$\phi_n = N + k_d \tag{11}$$

where

k_d = Bioaerosol inactivation rate arising from natural decay processes and deposition or impaction on room surfaces (s^{-1})

The term ϕ_n combines the actions of the ventilation system, natural biological decay and deposition of particles on room surfaces. The value of ϕ_n is generally greater than N, however, as shown below, it is only possible to evaluate the magnitude of k_d in some of the experiments described in this paper.

4. Application of Theory to the Experimental Determination of Z_{eff}

Although the value of Z_{eff} is a characteristic of the microbial behaviour, it also has some dependence on the room characteristics and UV field, as this determines how many times the microorganisms are passed through the field and hence the level of DNA repair that is experienced. In theory Z_{eff} should be evaluated for the actual room where the upper room UV system is implemented. However in practice it is not possible to perform experiments in every location, therefore typical values of Z_{eff} for different organisms and room characteristics can be determined from experiments. These may be conducted in a ventilated test chamber with an upper-room UV field using either the *decay model* (equations (4) and (9)) or the *continuous contamination model* (equations (5) and (10)). A typical test chamber used for such experiments may be similar to the bioaerosol test chamber described in section 6.

4.1 Calculating Z_{eff} using the Decay Model

The experimental procedure needed to evaluate Z_{eff} depends on the model selected for analysis. If the *decay model* is used, it is necessary to nebulise microorganisms into a ventilated chamber until a high steady-state contamination level is reached with the UV system switched off. Once this level has been reached, the nebulisation should cease and the airborne microbial burden in the chamber sampled at regular time intervals to determine the concentration of colony forming units (cfu/m^3) and hence provide data to enable an exponential decay curve to be plotted, such as that shown in Figure 2. The same experiment should then be repeated with the UV lamps in operation to obtain a second decay curve, also shown in Figure 2.

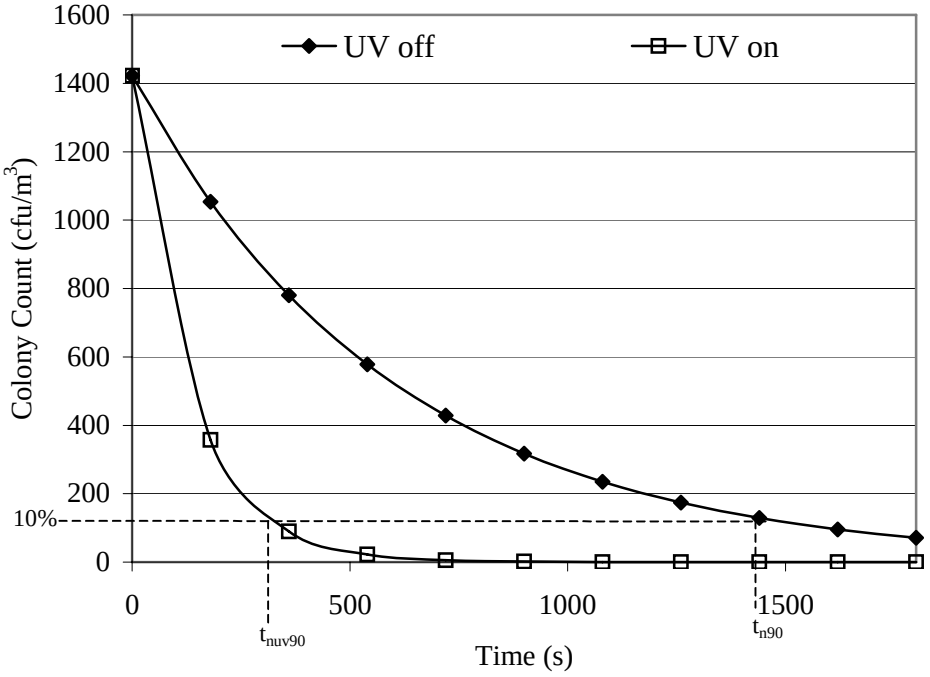


Figure 2. Decay curves typical of an experiment with and without UV irradiation, with nebulization ceasing at time $t = 0$ s

From simple rearrangement of equation (4) and (11) the value of ϕ_n may be determined by choosing a suitably high value of biological 'inactivation' and reading the time taken to achieve this from the decay curve. Choosing a 90% inactivation results in $C/C_0 = 0.1$ in equation (4) and ϕ_n is given by equation (12), where the time taken to achieve 90% bioaerosol 'inactivation' with the UV lights off is t_{n90}.

$$\phi_n = -\frac{1}{t_{n90}} \ln 0.1 \tag{12}$$

A similar equation to give ϕ_{uv} may be obtained by rearranging equation (9). With a 90% inactivation ϕ_{uv} is given by equation (13). In this equation t_{nuv90} is the time taken to achieve 90% bioaerosol 'inactivation' with both the ventilation system running and the UV lamps on.

$$\phi_{uv} = -\frac{1}{t_{nuv90}} \ln 0.1 - \phi_n \tag{13}$$

Having determined the value of ϕ_{uv}, it is then possible determine the value of Z_{eff} by rearranging equation (6) as follows:

$$Z_{eff} = \frac{\phi_{uv} h_r}{E\ h_{uv}} \tag{14}$$

In addition to calculating the UV susceptibility, it is also possible to use this decay method to evaluate the contribution of the additional decay processes, k_d. If the ventilation rate has been measured by another method, such as using a capture hood or tracer gas, then the value of N will be known. Therefore equation (11) can be rearranged to easily calculate the value of k_d. This calculation also does not depend on the measurement of the microorganism decay in the presence of the UV lamps and the method could therefore be used to carry out general experiments to evaluate the natural decay of airborne microorganisms in ventilated rooms.

4.2 Calculating Z_{eff} using the Continuous Contamination Model

An alternative to the *decay model* is to use the *continuous contamination model*. As with the *decay model*, when the *continuous contamination model* is used to evaluate experimental data, it is necessary to nebulise microorganisms into a ventilated chamber until a relatively high steady-state condition is reached. Once steady-state has been reached and sampled over a period of time, the UV lamps should then be switched on, while still maintaining a constant bioaerosol production. The air should then be sampled again at regular intervals over a period of time to record the contamination levels when the new steady-state condition is achieved. Theoretical example data for such a procedure is shown in Figure 3.

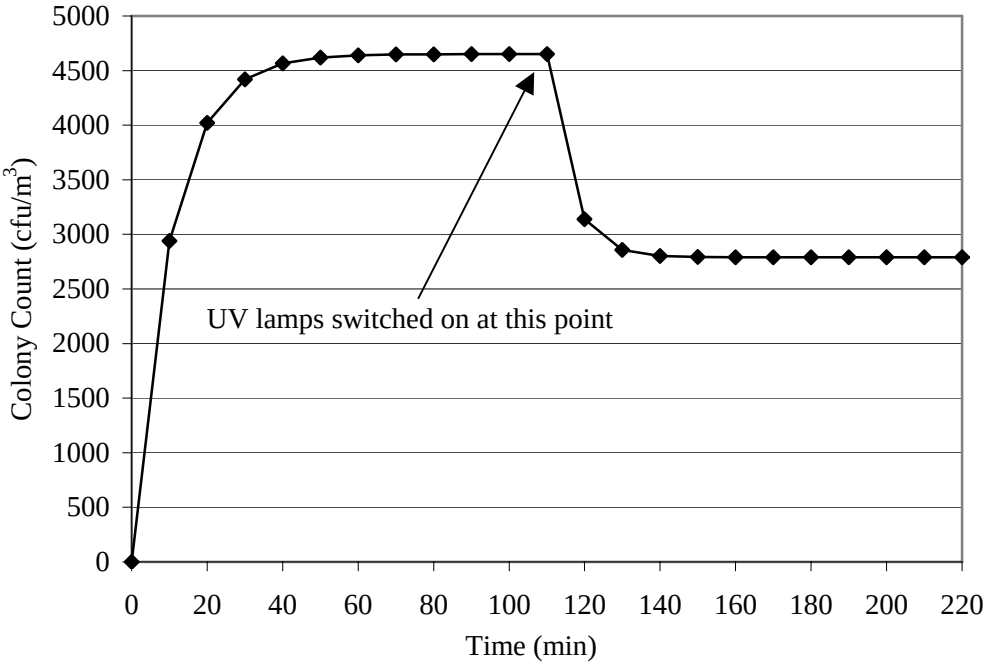


Figure 3. Typical bioaerosol concentration curve, before and after UV irradiation, with continuous nebulization.

When using the *continuous contamination model* the measured data can be evaluated using equations (5) and (10). As the contamination rate is constant throughout the experiment, the term q in equations (5) and (10) can be equated to yield

$$\phi_{uv} = N\left(\frac{C}{C_{uv}} - 1\right) \tag{15}$$

As with the *decay model*, equation (14) can then be used to determine the value of Z_{eff}. In this case it is always necessary to find the ventilation rate N by additional measurements to be able to use the above equation. To be able to evaluate the natural decay mechanisms in this case it is also necessary to know the value of q, whereupon equations (5) and (11) can be rearranged to evaluate ϕ_n and hence k_d. In theory it is possible to find q by evaluating the bacteria concentration in the nebulizer broth and the rate at which this is transferred into the room during the experiment. However in practice this tends to give unrealistic results as the difference between the sampled value and the input rate also includes additional decay due to the stress in the nebulization process.

Because both the *decay* and *continuous contamination* methods are based on models which assume complete air mixing, experimental procedures ideally should ensure good air mixing at all times.

4.3 Experimental Pros and Cons

By using either the *decay method* or the *continuous contamination method*, it is theoretically possible to determine realistic values for Z_{eff}, which reflect both the irradiation damage and DNA repair characteristics of microorganisms subjected to real upper-room UV systems. However, each experimental method has its drawbacks and advantages. Because the *continuous contamination method* only uses steady-state values to calculate Z_{eff}, it is not necessary to collect data during the non-steady-state 'build-up' and 'decay' stages. Consequently, the amount of air sampling and microbiological culturing required can be greatly reduced. This, of course, pre-supposes that the duration of the 'build-up' and 'decay' stages is known; a 'scoping' test run may therefore be necessary to discover the duration of these periods. One drawback of the *continuous contamination method* is that it can be difficult to accurately determine steady-state levels; variability in the air sampling and culturing process, and incomplete room air mixing can cause colony counts to fluctuate, particularly at higher contamination levels. It is therefore recommended that several samples be taken during each steady-state period to obtain the mean airborne microorganism concentrations and the standard deviation.

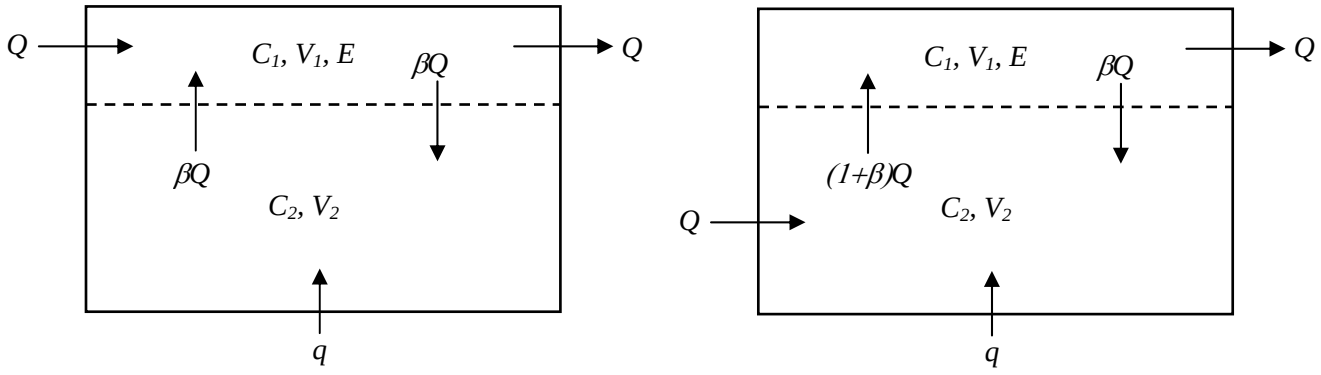
This problem of variability is not shared by the *decay method*, as in our experience it generally produces curves in which the fluctuations are smoothed out. However, the *decay method* has two major disadvantages. Firstly, to accurately measure a decay curve, it is often necessary to take a large number samples in quick succession. For example, if a multi-stage Anderson impactor is used it may be difficult to dissemble and reassemble the sampler in the time available, particularly under high ventilation rate conditions. Using several samplers would overcome this issue, however in many cases this is prohibitively expensive. The second and possibly more important difficulty is due to the lamp warm up time. Because UV lamps take several minutes to reach full power when first switched on, recorded values for UV decay curves may well be inaccurate, especially if the decay is very rapid. In theory this problem can be overcome by constructing some form of shield, which can be removed once the lamps have warmed up. However, in practice this is a difficult and complex procedure as it is not possible to enter the chamber during experiments. The *continuous contamination method* does not suffer from the 'warming up' problem, because the UV lamps warm-up before a steady-state condition is reached. For this reason (more than any other) the *continuous contamination method* appears to be the superior of the two methods.

From equation (14) it is evident that the value of Z_{eff} is strongly influenced by the average irradiance E, of the UV field. Accurate prediction of E is therefore essential when determining the value of Z_{eff}. The value of E can either be determined experimentally (Miller and Macher, 2000) or by calculation. Because light travels in straight lines it is possible to accurately calculate E, provided the photometric characteristics of the UV fittings are known. The value of E can be calculated by a variety of techniques, either by fitting mathematical equations to experimentally determined photometric data (Dumyahn and First, 1999, Rudnick, 2001), or by using line source models (Beggs *et al.*, 2000, Beggs and Sleigh, 2002), or view factor models (Kowalski, 2000).

5. Incomplete air mixing models

While the complete mixing models described in section 3 may yield a good approximation of Z_{eff} for rooms in which the air is well mixed, they may be of limited value when applied to rooms in which the air is poorly mixed. Given that many spaces are far from fully mixed, it is important to consider how upper-room UV systems might behave under such conditions. Theoretical models to evaluate the effectiveness of upper-room UV under conditions of incomplete mixing are generally based on zone-mixing models (Skåret, 1986, Brouns and Waters, 1991). These models enable contaminant concentrations to be estimated in different regions of a room by dividing the space into two or more zones. The air is assumed to be fully mixed within each zone, but not between zones, leading to 'short-circuiting' of the air and different contaminant concentrations in each zone. When applied to a UVGI installation, one or more upper-room zones are specified which contain the UV field. Such zonal models have previously been developed by several authors to analyse specific cases. Riley and Permutt (1971) developed a two-zone decay model which they used to evaluate their experimental data. Nicas and Miller (1999) considered a three-zone model with upper and lower room zones and a near field zone around an infectious patient. This was also formulated as a decay model and applied to experimental data.

A continuous contamination (steady-state) model for a room with incomplete air mixing, can be developed by modifying a two-zone ventilation model (Brouns and Waters, 1991) to incorporate the UV inactivation of airborne microflora (Riley and Permutt, 1971). In addition to estimating the concentration differences between zones due to UV inactivation, zonal models also include the type of ventilation system in a room. This allows the effectiveness of both the UV lamps and the ventilation system at removing airborne microorganisms to be assessed. A general steady-state model, suitable for assessing UV systems for a wide range of ventilation regimes was presented in Noakes *et al.* (2004a). Two specific cases from this model are used below to illustrate how such models may also be used to find the effective inactivation rate, Z_{eff} of a microorganism from experimental data. Schematics of these cases are shown in Figure 4.



(a) Ventilation in high-out high

(b) Ventilation in low-out high

Figure 4. Schematics of two-zone ventilation model with an upper room UV field for two ventilation regimes

In each case the room is divided into upper and lower zones, volume V_1 and V_2 (m^3), and the room ventilation flow rate is designated as Q (m^3/s) which is given by $Q = \phi_n(V_1 + V_2)$. Case (a) has a ventilation system where the supply and extract points are both in the upper zone, while case (b) is a piston ventilation system with the supply air in the lower zone and the extract in the upper zone. It is assumed that the supply air is uncontaminated, a UV field with average irradiance E is present in the upper

zone only, and bioaerosol contaminants are injected uniformly and continuously into the lower zone only, at a rate of q colony forming units per second (cfu/s).

In case (a) the inter-zonal airflow rate is represented as βQ, where β is a dimensionless mixing factor, which describes the inter-zonal air change rate relative to the room ventilation rate. The higher the value of β, the more times the air is exchanged between the zones and the better the room mixing. In the piston type regime of case (b), the zone transfer rate in the overall ventilation direction is $(1+\beta)Q$, to reflect both the main ventilation flow and the effect of short circuiting. Beggs and Sleigh (2002) suggested that the value of β may be estimated for a particular room from the equation:

$$\beta Q = \frac{A v_{\text{int}}}{2} \tag{16}$$

where A (m^2) is the area of the interface between the two zones and v_{int} (m/s) is the average velocity of air crossing this interface.

These inputs and outputs to and from the room result in overall steady-state microorganism concentrations C_{1UV} and C_{2UV} (cfu/m^3) in the upper and lower zones respectively, given by the expressions in Table 2. Details of the derivation of these expressions are given in Noakes *et al.* (2004a). With no UV irradiation ($E = 0$) the upper and lower zone concentrations C_1 and C_2 in Table 2 are those given by Brouns and Waters (1991).

Table 2 Upper and lower zone steady-state contaminant concentrations for two ventilation regimes

Case	With Upper room UV	No Upper room UV (E = 0)
(a)	$C_{1UV} = \frac{q}{Q + Z_{eff} E V_1}$ (17a)	$C_1 = \frac{q}{Q}$ (17b)
	$C_{2UV} = \frac{q(Q + \beta Q + Z_{eff} E V_1)}{\beta Q (Q + Z_{eff} E V_1)}$ (18a)	$C_2 = \frac{q(1+\beta)}{Q\beta}$ (18b)
(b)	$C_{1UV} = \frac{q}{Q + Z_{eff} E V_1}$ (19a)	$C_1 = \frac{q}{Q}$ (19b)
	$C_{2UV} = \frac{q(Q + \beta Q + Z_{eff} E V_1)}{Q(Q + Z_{eff} E V_1)(1+\beta)}$ (20a)	$C_2 = \frac{q}{Q}$ (20b)

If a continuous contamination experiment is carried out to evaluate either the effectiveness of a UV lamp or to determine Z_{eff} for a particular microorganism, the rate at with microorganisms enter the chamber (q) should remain constant throughout the entire experiment (i.e. without UV and with UV), to allow the two cases to be successfully compared. As in the fully mixed case this fact allows the equations in Table 2 to be manipulated to find the value of Z_{eff} in terms of either the upper zone or lower zone reduction. By equating q in each pair of equations, Z_{eff} can be expressed as

$$Z_{eff} = \frac{Q}{E V_1}\left(\frac{C_1}{C_{1UV}} - 1\right) \tag{21}$$

or

$$Z_{eff} = \frac{Q}{EV_1} \frac{(1+\beta)\left(\frac{C_2}{C_{2UV}} - 1\right)}{\left((1+\beta) - \frac{C_2}{C_{2UV}}\right)} \tag{22}$$

Despite the zone concentration equations being different for cases (a) and (b) in Table 2, the expressions for Z_{eff} turn out to be the same for both cases. The usage of equations (21) and (22) depends on the experimental data that is collected. If air samples are taken in the upper zone of the room, then C_1 and C_{1UV} will be known and equation (20) is appropriate. Likewise, samples taken in the lower part of the room require equation (21) to analyse the data, although in this case the analysis is complicated by the need to determine a suitable mixing factor, β. This can be calculated using Equation (16), with the average interfacial velocity, v_{int} determined by carrying out measurements of the vertical velocity at a number of points in the room at the height of the specified zone interface. As the value of β is used as an indicator of room mixing, measurements made with a hand held anemometer of sufficient sensitivity should be adequate.

The generic relationship for both zones C_i/C_{iUV} that appears in equations (21) and (22)is the reciprocal of the fraction of microorganisms that survive following UV irradiation in zone i (i = 1 or 2). Figure 5 shows graphically the relationship between this survival fraction for the upper zone and the effective susceptibility constant Z_{eff}. It should be noted that Figure 5 does not include the reduction achieved by the ventilation system and displays only the survival fractions achieved through the action of the UV lamps. It can be seen, as expected that a survival fraction of 1 (no reduction due to UV) results in a zero value of Z_{eff}, while Z_{eff} tends to infinity as the survival fraction approaches 0 (total 'kill'). The figure also demonstrates that the relative impact of the UV irradiation reduces as the ventilation rate is increased. This is because UV irradiation time decreases as the room ventilation rate increases. For a microorganism with a characteristic Z_{eff}, the upper zone survival fraction is greater at higher ventilation rates.

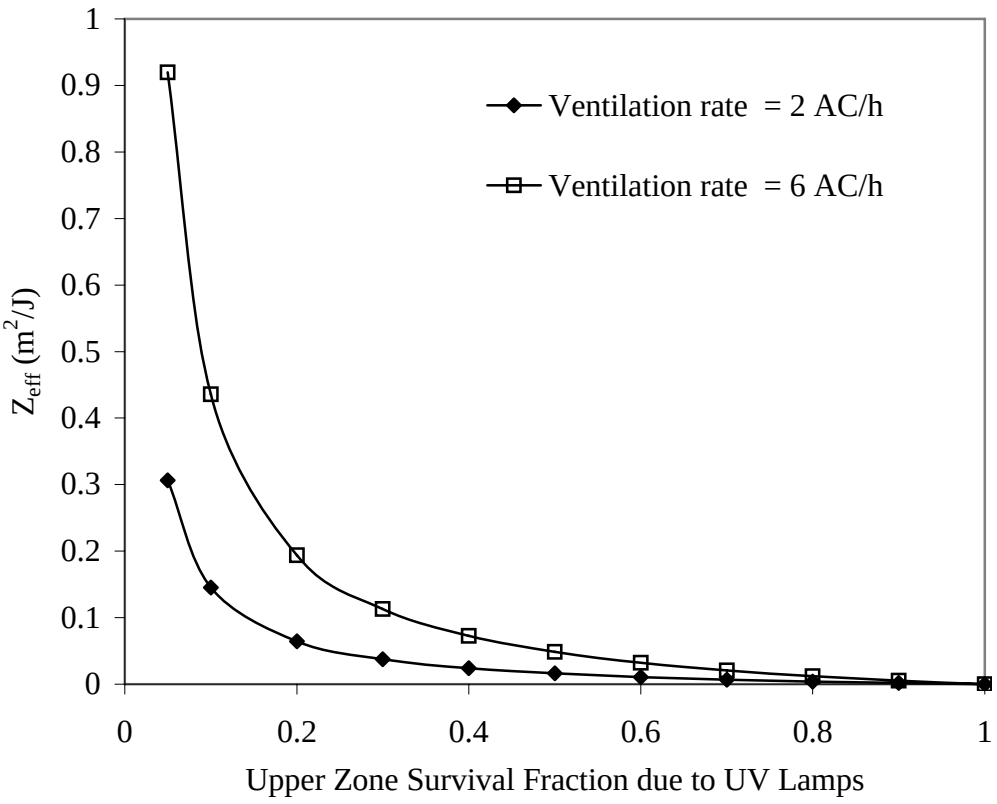


Figure 5. Variation in Z_{eff} with upper zone survival fraction at two ventilation rates

6. Experimental Results

In order to test the validity of steady-state models described in sections 3 and 5 a continuous contamination UV irradiation experiment was carried out in the bioaerosol test chamber shown in Figure 6. This facility is a climatically controlled 32.25 m^3 chamber with room ventilation that can be varied between 1 and 12 AC/h and may be supplied and extracted through either ceiling or wall mounted vents. Bioaerosols generated by a nebulizer can be introduced to the centre of the room via a tube and diffuser, to simulate continuous or variable contamination.

The experiment involved nebulizing a liquid culture of *Serratia marcescens* (NCTC 1377) into the centre of the chamber at a constant rate over a period of 6.5 hours – 3 hours without UV irradiation followed by 3.5 hours with UV irradiation. The culture was grown in nutrient broth (Oxoid) in a shaker at 37°C after which it was centrifuged and resuspended in sterile phosphate buffered saline. Appropriate dilutions of the concentrated microbial suspension was then prepared in sterile distilled water to give a concentration of approximately 10^6 cfu/ml. The bioaerosols were generated using a six-jet Collision nebulizer (CN 25, BGI Inc, USA) which was operated at a constant pressure of 20psi and flow rate of 12l/m, monitored by a flow meter.

The UV field was supplied using two wall mounted UV lamps (Lumalier Inc. USA, WM136 & WM236) which were both switched on to produce a stronger field. The two UV fittings were located on adjacent walls, 0.5 m below the 2.26m high ceiling. The average UV field over the upper 0.5m of the room was determined as 0.12 W/m^2 using a 3D computer model based upon irradiance data obtained from the manufacturer. The accuracy of these data was confirmed by measuring the UV irradiance at a number of points within the test chamber using a radiometer.

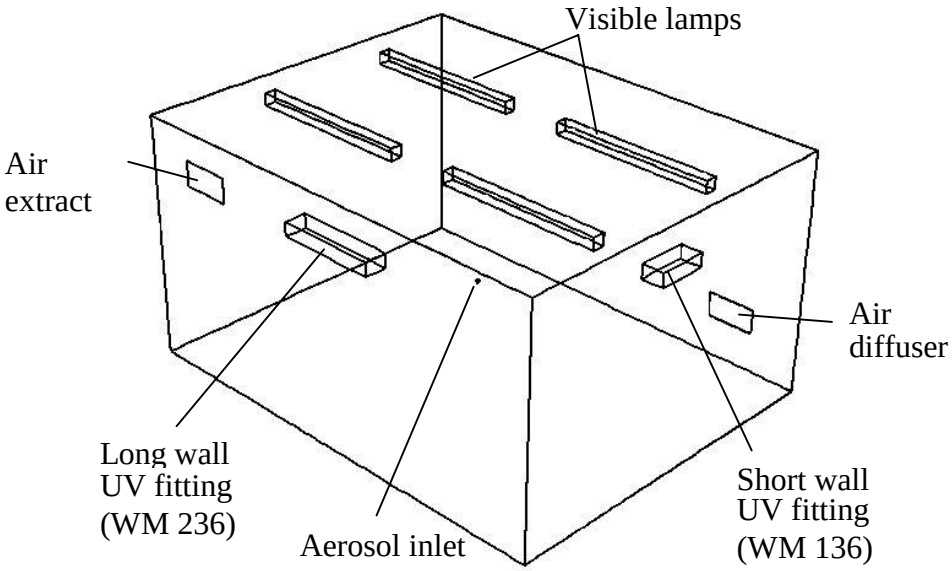


Figure 6. Aerobiology test room geometry

During the experiment clean, HEPA filtered air entered the chamber at the low level wall diffuser and exited via the high level vent on the opposite wall (see Figure 2 and case (b) in Figure 4). The ventilation rates in the chamber were determined by measuring the air flow into and out of the inlet and exhaust grills using a capture hood and thermal anemometers (Air Flow, ProHood) and was adjusted such that a slight negative pressure was maintained (0.12 in.wg) in the chamber. In the experiments carried out in this study the ventilation rate was set at 6 or 9 AC/h and the temperature and relative humidity were maintained close to ambient, as indicated in table 3. Throughout both steady-state periods of the experiment, the microbial concentration in the test chamber air was sampled at regular intervals via a sampling tube located directly in front of the exhaust grill. This was connected to the inlet of a six-stage Andersen sampler (Andersen Instruments, USA), loaded with two sterile dry nutrient agar plates on stages 5 and 6 only, operated at a flow rate of 28.3 l/min. The decision to use only stages 5 and 6 was based on initial investigations that revealed that the 95 % of the particles in the test chamber were collected on these plates. Following sampling the agar plates were incubated in the dark at 37°C for 24 hours, and the number of colonies on each of the plates was counted. These values were corrected to account for multiple impaction (Macher 1989) and then used to calculate the microbial concentration in the room.

During the first three hour period the UV lamps were switched off and nine samples were taken at regular intervals to determine the mean steady state concentration with no irradiation. The UV lamps were then switched on and left for 30 minutes to reach their operating intensity before 10 further samples were taken over the next three hour period. From these samples the mean concentration of airborne microorganisms in the presence of UV irradiance was calculated. In all cases the experiments were carried out in the absence of visible light. The results from the two experiments are given in Table 3.

Table 3 Results of the two steady-state UV irradiation experiments, showing values as means and standard deviations in each case

Ventilation Rate AC/h	Temperature (C)	Relative Humidity (%)	Survival Fraction following UV irradiation (C_{UV}/C)
6	21.52 (SD 0.77)	48.70 (SD 2.34)	0.5724 (SD 0.20)
9	20.99 (SD 0.34)	48.16 (SD 1.63)	0.6851 (SD 0.06)

The value of Z_{eff} was calculated using both the complete mixing and two zone steady-state continuous contamination models. In the case of the complete mixing model Z_{eff} was calculated using equations (14) and (15), with the height of the UV zone taken as 0.5m and the survival fractions calculated from the experiments taken as the whole room averages. The calculation using the two-zone model assumed the survival fractions calculated from the experiments to be the upper zone survival, C_{1UV}/C_1, as the experimental samples were taken close to the extract vent, in the upper zone of the room. Therefore equation (21) was used to evaluate Z_{eff} in terms of the two-zone model. As only the upper-zone was considered it was not necessary to define the mixing factor, β, in this calculation. The results from both models are presented in Table 4, together with experimental values from the literature for single pass systems (as shown in Table 1).

Table 4 Comparison of Z_{eff} calculated from test room experiments and values from single pass experiments

Model	**Value of Z_{eff}** (m^2/J)	
	6 AC/h	**9 AC/h**
Test room (complete mixing model)	0.0469 ([*] 0.018-0.106)	0.0433 ([*] 0.032-0.057)
Test room (two zone model)	0.0469 ([*] 0.018-0.106)	0.0433 ([*] 0.032-0.057)
Single pass experiment (Riley 1976)	1.83-2.45[a]	
Single pass experiment (Peccia et al 2001)	0.35-0.45[b]	
Single pass experiment (Ko et al 2000)	0.57[c]-0.58[d]	
Single pass experiment (Fletcher et al 2003)	0.095[e]-0.94[f]	

[a] 50% RH, [b] 40-50% RH, [c] 49-62% RH, [d] 22-33% RH, [e] 78% RH, [f] 48% RH

[*] Calculated from experimental survival fraction +/- 1 standard deviation

From the results presented in Table 4 it is clear that both the models yield identical values of Z_{eff}, which initially seems to be a surprising result. However examination of equation (14), (15) and (21) shows that both models are written in the same form with C_1/C_{1UV} replaced by the room average values C_e/C_{nuv} for the fully mixed models. The reason that both models yield the same result is due to how the available experimental data is used. In the fully mixed model, the experimentally determined survival fraction is treated as an average value for the whole room, whereas in the two-zone model this is assumed to be for the upper-zone only. Providing that the sampled value is representative of the upper zone concentration, this shows that either model can be used to determine a suitable value for Z_{eff}. However, it does indicate that the fully mixed model may be overestimating the effectiveness of the UV irradiation. If equation (22) is rearranged, the ratio of the lower zone concentration without and with UV irradiation is given by

$$\frac{C_2}{C_{2UV}} = \frac{(1+\beta)\left(\frac{EV_1Z_{eff}}{Q}+1\right)}{\left((1+\beta)+\frac{EV_1Z_{eff}}{Q}\right)} \qquad (23)$$

Using the calculated values of Z_{eff} in Table 4 and a mixing factor $\beta = 3.6$ the lower zone survival fractions, C_{2uv}/C_2, can be estimated as 0.665 at 6 AC/h and 0.754 at 9 AC/h. These values are clearly dependent on the mixing factor, but as previous studies (Noakes *et al.*, 2004b) have shown that typically $3<\beta<10$, it is likely to be a reasonable estimate and indicates the underestimation of the fully mixed model.

It is also notable that the calculated values of Z_{eff} shown in Table 4 are lower than the susceptibility constants presented in the literature for *Serratia marcescens* determined from single pass experiments, including those determined in our own study under similar climatic conditions. This suggests that in the test room experiments *Serratia marcescens* is harder to inactivate than in the single pass experiments under similar conditions. This may be due to the potential for repair between passes and the effects of repeated exposure upon the bacterium.

7. Discussion

The models presented here represent an alternative method to the single pass irradiation technique for calculating the UV susceptibility of airborne microorganisms. However, the value determined is an effective value which represents the summation of all the various DNA damage and repair mechanisms which occur during the room residence time of bioaerosol particles. Given that with upper-room UV systems, for any given aerosol particle there may be many minutes between successive irradiation doses, it would appear that it is the Z_{eff} value and not the single-pass Z value which most accurately reflects the behaviour of the air disinfection process.

The discussion in section 6 highlights the importance of ensuring that the appropriate zone mixing equation is applied to the data collected. For example, in Table 4 the complete mixing and two-zone models give the same value for Z_{eff}, only because the air sampling point is located in the upper-room zone, with the result equations (14,15) and (21) are essentially the same. If the sampling point had been located in the lower zone, then unless the room air is very well mixed, it is likely that the two models would have yielded different values. This raises important questions about the nature and validity of sampling points used during experimentation. If the sampling point is located in the lower-zone, it becomes necessary to use equation (22) and know the value β in order to accurately determine the value of Z_{eff}. If however, the sampling point is in the upper-zone, then both equations (14,15) and (21) can be used, and if air is sampled from several points in the upper and lower zones simultaneously, then equations (14,15) should be used with the average sample value.

The complete mixing model has an inherent weakness that, with some experimental data, it predicts the survival fraction in the upper-room zone, and thus overestimates the microbial reduction achieved in the lower-zone. However, despite being inferior to the two-zone model it is much easier to use since it does not require the value of β to be known. Indeed, if the value β is greater than 20 (Noakes *et al*., 2004a), the room air is likely to be well mixed and so equations (14,15) will yield a good approximation of the survival fraction in the lower-room zone.

The results from experiments carried out in an aerobiology test chamber indicate that the calculated value of Z_{eff} is similar at different ventilation rates, but is up to an order of magnitude lower than values obtained in single pass experiments. This would suggest that the repeated exposure and subsequent potential for DNA repair seen in real room situations may result in a need for increased UV irradiation intensity to achieve the levels of inactivation suggested by the experiments carried out in the single pass test apparatus. Although in some situations expressing the impact of the UV irradiation as an equivalent air change rate (i.e. ϕ_{uv}) this is only useful once a UV system is in place. In terms of designing a system in a ventilated room to achieve an equivalent air change rate against a specific pathogen, it is necessary to use UV susceptibility data in the calculations. Work is currently ongoing to examine the relationship between Z_{eff} calculated from room studies and the single pass data for other microorganism species and climatic conditions. If similar results are found, this may suggest that using UV susceptibility constants derived

from single pass experiments to predict the performance of upper room devices in real situations may result in a significant under prediction of the efficacy of such systems.

8. Conclusions

The following conclusions can be drawn from this study:

- It is possible to determine an effective UV susceptibility constant Z_{eff}, of an airborne microorganism by using an upper-room UV field in a ventilated test chamber.

- The *continuous contamination method* appears to be superior to the *decay method* as an experimental method for evaluating the air disinfection performance of UV lamps.

- While the complete mixing model accurately predicts the microbial survival fraction in the upper-room zone, if the room air is not well mixed it will significantly underestimate the survival fraction in the lower room. Therefore, where possible the two-zone model should be used.

- The lower susceptibility constants for *Serratia marcescens* calculated from experiments in a test chamber compared to single pass experiments suggest that using single pass data to size upper-room UV installations may underestimate the required UV power.

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