Dispersion of microbes from floors when walking in ventilated rooms

W Whyte, WM Whyte, S Blake and G Green
School of Engineering
University of Glasgow
Glasgow G12 8QQ

Abstract

The redispersion factor of microbe-carrying particles, which is the ratio of the concentration of floor-derived microbes in room air to those on a floor surface, was determined, as was the percentage of floor-derived microbes in room air. These relationships were shown to vary according to conditions in the room. Equations were derived that allow these relationships to be calculated for a variety of room conditions, including air supply rates, levels of personnel activity, and the effect of gravitational deposition on microbe-carrying particles.

The redispersion factor in ventilated rooms, such as cleanrooms and operating rooms, when the floor surface concentration was measured by nutrient agar contact dishes, was found to vary from about 1.5×10^{-4} to 7.4×10^{-6}, and the percentage of floor-derived microbes in room air from about 0.004% to 10.5%. In a typical cleanroom, the redispersion factor is likely to be about 1.0×10^{-4}, and the percentage of floor-derived microbes, 0.7. In a typical operating room, the redispersion factor is likely to be about 5.2×10^{-6} and the percentage of floor-derived microbes, 2.

Key words: floors; walking; dispersion; redispersion; microbes; micro-organisms

1 Introduction

The floors of cleanrooms, operating rooms, and rooms contaminated with dangerous microbes, are potential sources of microbial contamination. When people walk about, microbes are dispersed into the air and these may be transferred and deposited onto a manufactured product, a surgical wound, or breathed by occupants.

Lidwell (1967) reviewed the limited information available at that time on the dispersion of microbes and radioactive particles from floors, and discussed values of a redispersion factor, which was defined as follows:

$$\text{Redispersion factor} = \frac{\text{airborne concentration of floor-derived contamination/m}^3}{\text{surface concentration of contamination/m}^2} \quad (1)$$

Hambraeus et al (1978) and Karlsson et al (1999) determined the redispersion factor of microbe-carrying particles (MCPs) in occupied rooms. Sehmel (1980) reviewed the values of redispersion factors obtained from studies of non-viable particles, and more recent work on non-viable particles has been carried out by researchers, such as Thatcher and Layton (1995), Gomes et al (2007) and Qian and Ferro (2008). The combined results of all these studies show that the redispersion factor varies from about 1×10^{-3} to 1×10^{-8}. To determine the importance of the floor as a source of contamination, or calculate the airborne concentration of floor-derived microbes from a given concentration of floor microbes, the correct values of the aforementioned relationship should be known, and the reasons for any variability understood. The reasons for variability have been discussed in the scientific articles quote above, but there are variables that need further consideration, such as (a) collection efficiency of the air and surface sampling methods (b) walking time to reach the steady-state airborne concentration (c) failure to distinguish between particles dispersed from the floor and from the

skin and clothing of people (d) walking activity (e) air supply rate. These have been investigated in this paper, along with the use of the redispersion fraction in models used to predict the redispersion factor, the airborne concentration of floor-derived contaminants, and the percentage of floor-generated contaminants in room air.

2 Material and Methods

2.1 Generation of a known size of microbe-carrying particles (MCPs)

Hambraeus and Laurell (1980) found in a hospital operating room that there was little difference between the microbial concentrations on the upper surface of an operating lamp that derived its contamination from the air, and those on the floor, and concluded that most of the microbes on the floors of operating rooms came from the air. Microbes grow on skin, and the main source of airborne MCPs in occupied rooms are skin particles dispersed by people (Davis and Noble, 1962). Skin cells are about 44µm x 33µm in surface area, and about 3-5 µm thick, although they will fragment (McIntosh et al, 1978). Experiments have shown that the average equivalent diameter of MCPs in room air is about 12µm (Noble et al, 1963, Whyte, 1986 and Whyte and Hejab, 2007). The floor of the experimental room described in this article was therefore seeded with MCPs with an equivalent diameter of 12µm, these particles being generated by a Spinning Top Aerosol Generator (STAG).

The STAG was the type described by May (1966). A 2 cm diameter cone spins at high speed, and liquid is fed onto the cone's uppermost surface at a rate of 1ml per minute and spun out as liquid droplets, the droplet diameter depending on the speed of the cone. After being spun out, the droplets quickly dry to a diameter that is dependent on the solid material contained within the wet droplet. In these experiments, an aqueous solution of Sodium Chloride (10% w/v), containing 2 x 10^{7}/ml of bacterial spores, was used. The spores used to tag the droplets were *Bacillus atrophaeus* (ATCC 9372, equivalent to NCIB 8058), these bacteria being previously known as *Bacillus subtilis var. globigii*.

The dry microbe-carrying particles were sampled 40 cm from the STAG by means of a 0.45µm membrane filter (Millipore). After sampling, the membrane was viewed through microscope oil by means of a light microscope, and the average diameter of 50 particles determined by a calibrated New Porton eyepiece graticule (Graticules Ltd). With a knowledge of the density of Sodium Chloride, the average equivalent particle diameter was obtained. This dry sizing method was tedious, as drying produced particle shapes that were variable and awkward to size. A wet particle sizing method (May, 1950) was a faster method, and used to check that the conditions of the experiment were correct.

2.2 Determining the dispersion of MCPs from the floor

Experiments were carried out in a small room with a floor area of 2.68m x 2.23m and a height of 2.70 m. An enclosed bench, with cupboards, stretched along the longer length of the floor and was built against the wall. The volume of the room, with the bench volume deducted, was 14.83 m^3. The floor area, without the bench, was 4.02m^2, and the combined horizontal surface area of the floor and bench, was 6 m^2. The room did not have a filtered air supply but a wall-mounted extract fan.

Five settle plates (90 mm diameter), containing tryptone soya agar (Oxoid), were placed around the floor to measure the concentration of test MCPs on the floor. The settle plates were opened and the STAG run for one or two minutes. The generated MCPs were mixed with room air for a few minutes using a small table fan. The MCPs were then given 30 minutes to settle onto the floor and settle plates, after which time the room extract fan was switched on, and left on overnight so as to remove any remaining test MCPs in the air. The settle plates were closed in the morning.

The spore-bearing MCPs on the floor were dispersed into the air by a person walking. The person was instructed to briskly walk for 100 steps on all parts of the floor. This was done to the beat of a metronome that was set to produce steps at a rate of 3 every 2 seconds i.e. 1.5/s, It was considered that his body volume and speed of movement, in such a small room, were sufficient to ensure good mixing of the air and an even

airborne microbial contamination. Flat-soled shoes were worn, and covered with a thin-plastic blue shoe of the type used in cleanrooms. The area of the sole and heel of the shoe that contacted the floor was determined by applying the shoe to graph paper, and found to be 109 cm^2. To ensure that no contamination came from the previous experiment, a fresh 100μm-thick sheet of disinfected PVC was used to cover the floor for each experiment. PVC was chosen, as it is the plastic commonly used in flooring.

To avoid dispersing microbes from the floor before the walking experiment started, the air sampler was sited and activated in the room adjacent to the experimental room, and connected through the wall by a short, large diameter tube. The air sampler was a high volume (700 l/min) slit sampler (Casella Ltd), which collected the MCPs by impaction onto Petri dishes containing tryptone soya agar (Oxoid) nutrient agar. Air samples were taken before the walking experiment started to check the background concentration. Immediately after walking stopped, an air sample was taken for two minutes. All Petri dishes were incubated for 36 hours at 35°C. After incubation, both the colonies of the test MCPs, and MCPs generated from the skin and clothing of the person walking, were counted.

3 Experimental results

Shown in Table 1 are the results of the walking experiments. The first experiment had higher air and surface concentrations because the aerosol dispersion time was greater than the two subsequent experiments. However, the redispersal factors calculated from the individual experiments were similar and an average of the results was used.

The average surface concentration of the spore-bearing test MCPs on the floor, as determined by the settle plates, was 14.3/cm^2 (1.4 x 10^5/m^2). The average airborne concentration of test MCPs, immediately after walking, was 10.73/m^3. The concentration of airborne microbes before walking was 0.23/m^3 and this concentration was deducted from the count after walking to give the airborne concentration of floor-derived MCPs of 10.5/m^3. The average redispersal factor was therefore 7.3 x 10^{-5}.

Table 1 Surface and air concentrations, and redispersion factors

Experiment No.	Surface concentration of MCPs /cm^2 (/m^2)	Air concentration of MCPs /m^3				Redispersal factor: $\frac{no./m^3}{no./m^2}$
		Before walking		After walking		
		Marker microbes	Other microbes	Marker microbes	Other microbes	
1	27.6 (276 000)	0.7	12.1	23.6	102.1	8.3 x 10^{-5}
2	8.3 (83 000)	0	14.3	3.6	53.6	4.3 x 10^{-5}
3	7.0 (70 000)	0	42.1	5.0	102.1	7.1 x 10^{-5}
Average	14.3 (143 000)	0.23	22.83	10.73	257.8	7.3 x 10^{-5}

MCPs, other than the spore-bearing test particles, were also measured. They could only have come from outside the experimental room, or from the skin and clothing of the person who walked about the room. Their concentration was measured immediately after walking (257.8/m^3), and the background contamination before walking (22.83/m^3), which was assumed to be from outside the experimental room, was deducted. The percentage of floor-derived MCPs in the room air was then calculated by dividing the average concentration of MCPs generated from the floor (10.5/m^3) by the average concentration of MCPs from the skin and clothing of personnel (235/m^3); this gave 4.5%.

4 Number of microbes dispersed by one step

It was shown in the previous section that when a person takes 100 steps over a floor which has an average surface concentration of 14.3/cm^3, at a rate of 1.5 steps per second, then at the end of walking the concentration in room air was 10.5/m ^{3}. The volume of the experimental room was 14.83 m^3 and, therefore, the total number of marker MCPs in the room air was 158.7. This number was produced by 100 steps, and hence the number of MCPs produced per step appears to be 1.6. However, this result does not take account of losses caused by gravitational sedimentation of the airborne MCPs onto the floor and bench top, during both the sampling and walking periods.

4.1 Losses during airborne sampling

Microbial air samples were taken for two minutes immediately after walking stopped. It is not possible to instantly measure a microbial airborne concentration, as microbial sampling methods only give an average concentration over the time of sampling. It has been shown that the average equivalent particle diameter of MCPs in the air of an occupied room is about 12 µm (Noble et al. 1963; Whyte, 1986 and Whyte and Hejab, 2007) and that the main mechanism of deposition is by gravitational settling, with a deposition velocity of 0.0046 m/s (Whyte, 1981; Whyte, 1986). Therefore, during the two minutes of sampling, the concentration of the MCPs in the room air would decay owing to surface deposition, and the air sample give a lower count than the concentration immediately after walking.

To determine the actual concentration in the experimental room, immediately after walking, it is necessary to use the average concentration i.e. the sample count, and the following Equation A12 that is derived in the Annex.

$$C_W = \frac{C_A . N_D . t}{1 - e^{-N_D . t}}$$

Where, C_W is the airborne concentration immediately after walking, C_A is the average concentration obtained by air sampling, N_D is the decay rate caused by gravitational deposition, and t is the sampling (decay) time.

The marker microbes used in the experiments were spores, and it was assumed that there was no decay in their viability during sampling, as the concentration of the spore suspension had dropped by an insignificant amount in over 40 years.

As there was no air supply to the experimental room, the decay of the airborne concentration was only caused by gravitational settling onto surfaces and, as discussed in the Annex, the following Equation A8 should be used to determine the decay rate.

$$N_D = \frac{V_D . A}{V}$$

Where, N_D is the decay rate /s owing to surface deposition, V_D is the average deposition velocity of airborne particles onto surfaces (m/s), A is the horizontal deposition area (m^2), and V is the volume of the room (m^3).

As the volume of the experimental room, excluding the bench, is 14.83m^3, and the horizontal surface area is 6 m^2, the decay rate of test MCPs owing to surface deposition is equal to 0.00186/s.

The average concentration of test MCPs (C_A) obtained from an air sample taken for two minutes immediately after walking stopped, was 10.5/m^3. Therefore, using Equation A12, the concentration immediately after walking (C_W) was found to be 11.7m^3.

4.2 Losses during walking

The airborne concentration of marker MCPs in the experimental room, immediately after walking stopped, was calculated in the previous section to be 11.7/m^3. However, this concentration does not include test MCPs that are lost during the walking period by gravitational settling from room air. To ascertain the total dispersion

rate of MCPs from the floor during walking, it is necessary to assume that there was no removal by gravitational settling, and use the following Equation A13 that is derived in the Annex,

$$D_F = \frac{N_D.V.C}{1 - e^{-N_D.t}}$$

Where, D_F = microbes dispersed from the floor/s by walking, N_D = decay rate of MCPs owing to surface deposition (0.00186/s), V = volume of room (m^3), C = airborne concentration/m^3 after an interval of t seconds.

It is known that the average airborne concentration of test MCPs immediately after 100 steps i.e. 67 seconds of walking was 11.7/m^3, the room volume was 14.83 m^2, and the decay rate of MCPs owing to gravitational settling was 0.00186/s. Therefore, the dispersion rate (D_F) was 2.75 /s. As the walking rate was 1.5 steps per second, the total number of microbes dispersed into the air by one step, including those lost by gravitational settling, was 1.83.

The number of MCPs dispersed per step during the experiments, when gravitational sedimentation is not included, was calculated in the opening paragraph of this section to be 1.6. Therefore, the loss of floor MCPs caused by deposition from the air during walking and sampling, was 13%.

5 Calculation of the redispersion fraction

The 'resuspension fraction' has been defined by Karlsson et al (1999) as 'the fraction of particles emitted from the contact area e.g. the area of sole and heel of one shoe, during one cycle of activity e.g. one foot step.' The experiments described in this article had an average concentration of marker microbes on the floor of 14.3/cm^2, and the area of the shoe in contact with the floor was 109 cm^2. The number of marker MCPs on the floor that were walked on per step was therefore 1558.7. The calculations in the previous section showed that the average number of airborne microbes dispersed from the floor by a single step, including those redeposited onto the floor by gravity, was 1.83. The redispersion fraction is therefore 0.0012.

The redispersion fraction was also calculated for each of the three experiments. In the first experiment, with a floor surface concentration of MCPs of 27.6/cm^2, it was 0.0013. In the second experiment, with a surface concentration of 8.3/cm^2, it was 0.0007, and for the third experiment with a concentration of 7.0/m^2, it was 0.0011. Results from microbial testing of surfaces can be quite variable, and hence reduce the confidence in the accuracy of individual results, but it was noted that the surface concentration did not appear to affect the redispersion fraction.

6 The effect of room conditions on the redispersion factor and airborne concentration of floor-derived microbes

The average value of the redispersion factor found in the experimental room was 7.3 x 10^{-5}. However, this value will vary in rooms with different conditions, as will the airborne concentration of floor-derived microbes. The variables involved are determined here, as are equations for calculating the redispersion factor, and airborne concentration of floor-derived microbes, in different room conditions.

The total walking activity (W_T), which is the total number of steps per second taken by all the people in a room is calculated to be as follows:

$$W_T = N \text{ x } W \text{ x } P \qquad (2)$$

Where, N = number of people in room, W = walking rate (number of steps/s), and P = proportion of time spent walking.

The total number of microbes dispersed from the floor per second by all personnel when walking (D_F) is as follows.

$$D_F = C_F \text{ x } A_S \text{ x } R_F \text{ x } W_T = C_F \text{ x } A_S \text{ x } R_F \text{ x } N \text{ x } W \text{ x } P \qquad (3)$$

Where, C_F = concentration of MCPs (no./m^2) on floor surface, A_S = area of shoe in contact with floor (m^2), R_F = redispersion fraction.

At the start of work, people enter an empty cleanroom room or operating room, where the supply of filtered air, and the room's positive pressure with respect to adjacent areas, ensure that the airborne microbial concentration is practically zero. As work starts, the airborne microbial concentration rises until it reaches a steady-state, where the MCPs dispersed by personnel are equal to those removed through the exhaust grilles and deposited on surfaces.

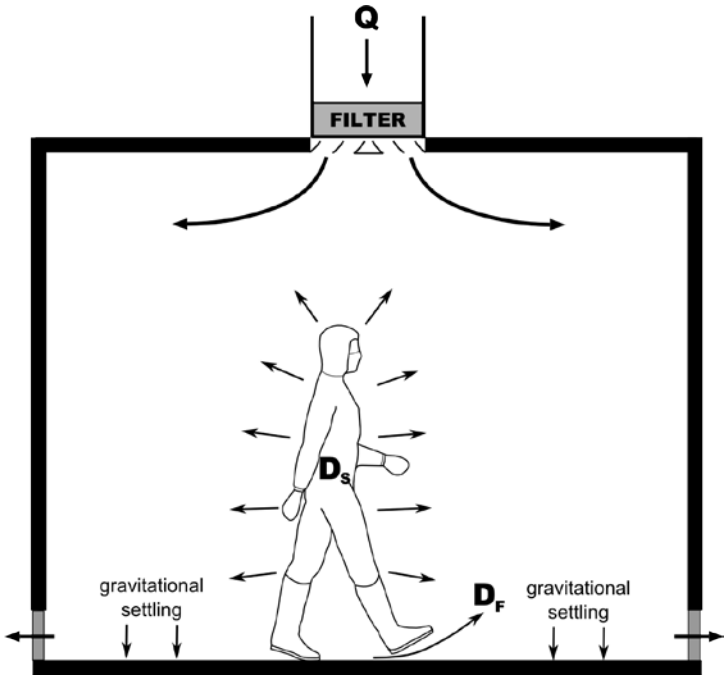

Figure 1 Dispersion and removal of MCPs in a non-unidirectional airflow cleanroom

The airborne concentration (C) of floor-derived MCPs in a ventilated room where the room air is well mixed and in a steady-state condition can be calculated by the following Equation A6, which is discussed in the Annex.

$$C = (D_F/(Q + V_D.A)$$

Where, D_F is the total number of MCPs dispersed from the floor into the air/s, Q is the air supply volume (m^3/s), V_D is the deposition velocity (0.0046 m/s) of MCPs, and A is the deposition area (m^2) in the room (normally equal to the floor area).

When the value of D_F in Equation 3 is substituted into Equation A6, the following equation is obtained:

$$\text{Airborne concentration of floor-derived MCPs /m}^3 = \frac{C_F \times A_S \times R_F \times N \times W \times P}{Q + V_D.A} \quad (4)$$

By substituting into Equation 1, which defines the redispersion factor, the airborne concentration of floor-derived MCPs/m^3 given in Equation 4, the following equation is obtained:

$$\text{Redispersion factor} = \frac{C_F \times A_S \times R_F \times N \times W \times P}{(Q + V_D.A) \times C_{FS}} = \frac{A_S \times R_F \times N \times W \times P}{Q + V_D.A} \quad (5)$$

Equations 4 and 5 show the constants and variables that affect the airborne concentration and the redispersion factor. The equations also show that if it can be assumed that the redispersion fraction does not vary with concentration, the microbial concentration on the floor has no effect on the redispersion factor, although it affects the room's airborne concentration. To demonstrate the practical effect of these constants and variables,

the redispersion factor and air concentration were calculated for a non-unidirectional cleanroom with the following characteristics.

1. Floor area of 10m x 10m, and 3m high.
2. Personnel walked at a rate of 1.5 steps per second. The surface area of the sole and heel of the shoe was 109 cm^2, and the redispersion fraction was 0.0012.
3. Low and high volumes of air supply i.e. 20 air changes/ hour (air supply rate, 1.67 m^3/s), and 80 air changes per hour (air supply rate, 6.68 m^3/s).
4. A 'low' and 'high' level of total walking activity. For low-level activity, two people were assumed to walk for a proportion of 0.2 of the time i.e. the total walking activity was 0.6/s. For 'high' level, it was assumed that 10 people walked for 0.8 of the time, i.e. the total walking activity was 12/s.
5. The concentration of MCPs on the floor was $0.1/m^2$.

The airborne concentrations and redispersion factors were calculated for combinations of the above variables, and given in Table 2. The second-last column gives the redispersion factors calculated by assuming that the microbial floor concentration was accurately measured by the settle plate method used in the experiments. However, the common methods of ascertaining microbial surface contamination are by swabbing or by use of nutrient agar contact dishes, the most common method being the use of Replicate Organism Detection And Counting (RODAC) contact dishes. The redispersion factor was therefore recalculated by assuming that the surface concentration was measured by an agar contact method, such as the RODAC method with a collection efficiency of 50% (Whyte, Carson and Hambraeus, 1989), and the results are given in the last column.

Table 2 Variability of the concentration of airborne floor-derived microbes and redispersion factors in a cleanroom

Concentration on floor surface /cm^2 (C_F)	Total walking activity/s (W_T)	Microbial dispersion from floor/s (D_F)	Air change rate/hour (air supply-m^3/s)	Concentration in room air/m^3 (C_A)	Redispersion factor	Redispersion factor, when RODAC dishes used
0.1	Low – 0.6	0.0078	20 (1.67)	0.0037	3.7×10^{-6}	7.4×10^{-6}
0.1	Low – 0.6	0.0078	80 (6.68)	0.0011	1.1×10^{-6}	2.2×10^{-6}
0.1	High – 12	0.157	20 (1.67)	0.074	7.4×10^{-5}	1.5×10^{-4}
0.1	High - 12	0.157	80 (6.68)	0.022	2.2×10^{-5}	4.4×10^{-5}

The redispersion factor was also calculated for a 'typical' operating room, with a floor area of $35m^2$, ceiling height of 3.2m, air change rate of 20 per hour (air supply rate of $0.62m^3/s$), 4 people walking continuously, a microbial surface concentration of $1/cm^2$ when measured by nutrient agar contact plates (Suzuki et al, 1984). Using Equation 5, along with a shoe area of 109 cm^2 and a redispersion fraction of 0.0012, gives a redispersion factor of 1.0×10^{-4}.

A redispersion factor was also calculated for a 'typical' cleanroom with a floor area of 10m x 10m, an air change rate of 40 per hour (air supply rate of $3.34m^3/s$), two people walking about for half the time, a shoe area of 109 cm^2, a redispersion fraction of 0.0012, and a floor surface concentration of $0.1/cm^2$ when measured by nutrient agar contact plates. The redispersion factor was 5.2×10^{-6}.

7 The effect of room conditions on the percentage of floor-derived MCPs in room air

The percentage of floor-derived MCPs in room air is defined as follows:

$$\text{Floor-derived microbes in room air (\%)} = \frac{\text{concentration of floor-derived MCPs in room air}}{\text{concentration of MCPs dispersed from people in room}} \times 100 \qquad (6)$$

The percentage determined in our experiments was 4.5, but it will vary according to the room conditions. The variables that affect the percentage are determined in this section, along with an equation to calculate the percentage in different conditions.

As discussed in the Annex, the concentration of MCPs can be calculated in the steady-state condition by means of Equation A6, which can be applied to either floor-derived microbes, or those dispersed from the skin and clothing of people in the room. If the concentrations obtained from these equations are substituted into Equation 6, the following equation is obtained:

$$\text{Floor-derived microbes in room air (\%)} = \frac{D_F}{Q + V_D.A} \div \frac{D_S.N}{Q + V_D.A} \text{ x } 100 = \frac{D_F}{D_S.N} \text{ x } 100 \qquad (7)$$

Where, D_F is the total number of MCPs dispersed/s from the floor by people in the room, D_S is the number dispersed/s from the skin and clothing of one person, and N is the number of people in the room.

Substituting the value of D_F obtained from Equation 3 into Equation 7, and cancelling,

$$\text{Floor-derived microbes in room air (\%)} = \frac{C_F \times A_S \times R_F \times W \times P}{D_S} \text{ x } 100 \qquad (8)$$

Equation 8 shows the variables and constants that affect the percentage of floor-derived microbes in room air. It also shows that the number of people in the room, the air supply rate, and floor area, have no influence.

To demonstrate the practical effect of the variables given in Equation 8, percentages were calculated for a non-unidirectional airflow cleanroom with the following characteristics.

1. Floor area of 10m x 10m, and 3m high;
2. Personnel walked at a rate of 1.5 steps per second. The surface area of the sole and heel of the shoes was 109 cm^2, and the redispersion fraction was 0.0012.
3. A ‘low’ and ‘high’ level of total walking activity. In the low-level activity, people were assumed to walk for a proportion of 0.2 of the time, and in the high-level a proportion of 0.8 was assumed.
4. Personnel wore either (a) efficient cleanroom clothing i.e. hood, mask, coverall, and knee-high boots made from tightly-woven fabric or (b) a cleanroom or operating room type of gown over their normal indoor working clothing. Whyte and Hejab (2007) investigated the dispersion rate of 30 females and 25 males and found that when wearing their ordinary indoor clothing they dispersed an average of 40 MCPs per second into the air, and when wearing efficient, occlusive, cleanroom clothing they dispersed 3 MCPs/ second. The effect of a cleanroom or operating room-type gowns has also been investigated, and Whyte et al, 1976 found that they gave a reduction in the dispersion of MCPs of about 50%. A dispersion rate of 20 MCPs/s was therefore assumed when a gown is worn.
5. The concentration of floor microbes was assumed to be either ‘low’ (0.01/cm^2) or ‘high’ (1/cm^2) when measured by RODAC contact plates. The collection efficiency of these plates is known to be 50% (Whyte et al, 1989) and the actual surface concentrations were therefore 0.02/cm^2 and 2/cm^2.

The lowest percentage of floor-derived microbes in the room air was found to be 0.004, and this occurred when the dispersion from the skin was high and both the floor contamination and walking activity were low. When the magnitudes of these variables were reversed, the maximum percentage was 10.5.

The percentage was also calculated of floor-derived MCPs in the air of a typical cleanroom, which had the same properties and conditions as the cleanroom described at the end of Section 6; the percentage was 0.7.

The percentage was also calculated for a typical operating room with the same properties as the cleanroom described at the end of Section 6; the percentage was 2.0%.

8 Discussion and conclusions

Experiments were carried out to establish in occupied and ventilated rooms the relationship between the concentration of microbe-carrying particles (MCPs) on the floor, and the air. The relationship can be expressed by a redispersion factor, which is the ratio of the floor-derived microbes/m^3 in room air, to those on the floor/m^2. There have been few investigations into the dispersion of MCPs from the floor but Hambraeus et al (1978) found that the redispersion factor when 4 people walked continuously in an unventilated operating room was 3.5×10^{-3}. Karlsson et al (1999) also studied MCPs and, found the redispersion factor for one person continuously walking to be about 1.8×10^{-4}, and for four people it was about 7×10^{-4}. Other researchers, working in a variety of experimental conditions, have reported redispersion factors for non-microbial particles of between 1×10^{-3} to 1×10^{-8} (Sehmel, 1980; Thatcher and Layton, 1995; Karlsson et al, 1999; Gomes et al, 2007; Qian and Ferro, 2008). The reasons for such a wide range of results were investigated in this article, and models derived to calculate in a variety of room conditions, the redispersion factor, the airborne concentration of floor-derived microbes, and the percentage of floor-derived microbes in the room air.

It is clear from this investigation that the value of the redispersion factor will only apply to the conditions in the room where it was measured. There are several reasons for this. Firstly, the collection efficiencies of the sampling methods will change the value of the redispersion factor. For example, we used a method that accurately measured the microbial concentration on the floor but Hambraeus et al (1978) used RODAC nutrient agar contact plates, which have a sampling efficiency of about 50%, and will therefore double the calculated redispersion factor. Similarly, the collection efficiency of microbial air sampling methods can vary by up to ten-fold (Ljungvist and Reinmuller, 1998). In addition, it is not possible for a microbial air sampler to give instant measurements of concentration, and an average measurement is obtained from the several minutes of sampling. During that time MCPs can sediment from the room's air. Also, during walking, some of the MCPs dispersed into the air will be re-deposited by gravitational sedimentation. The sampling and walking losses in these experiments were found to reduce redispersion by 13%. This reduction is small but will be greater in other experimental situations where the walking and sampling times are greater than the one or two minutes used in these experiments.

The second reason for the variation in the value of the redispersion factor is the time spent walking. Our walking was concluded in a minute, but it was calculated in the Annex that it would require at least 30 minutes before the airborne concentration in our experiments came close to the maximum concentration found in the steady-state condition. In one minute, the airborne concentration of floor-derived microbes would only have reached 11% of the steady-state concentration. Any experiments where the steady-state condition has not been reached, will give low redispersion factors.

A third reason is a failure to distinguish between particles dispersed from the floor and those dispersed from the skin and clothing of people. Some researchers e.g. Thatcher and Layton (1995) have assumed that all the particles found in the room air during walking were dispersed from the floor. However, this paper shows the floor-derived MCPs are likely to be between 0.004% and 10.5% of the MCPs dispersed from the skin and clothing. Failing to remove the skin and clothing dispersion from the calculation will overestimate the value of the redispersion factor.

The fourth reason is walking activity, as the more people walking, and the greater their walking rate, the greater the redispersion factor. Fifthly, the redispersion factor is dependent on the dilution of floor-dispersed microbes by the filtered air supply, and to a lesser extent by the surface area onto which the airborne microbes will redeposit; the greater the supply rate and surface area, the lower the redispersion factor. The sixth reason

is the variation of the value of the redispersion fraction, as the greater the fraction of floor microbes that are dispersed into the air by a step, the greater the redispersion factor.

All of the reasons discussed above explain why redispersion factors differ between scientific articles, and suggest that a mathematical model should be used to calculate the redispersion factor or airborne concentration of the floor-derived contamination, for different room conditions. The value of the redispersion fraction is important in these calculations, and is likely to vary according to a person's weight, speed of walking, gait, type of floor surface, type of footwear, and particle size. Some of these variables have been investigated by Thatcher and Layton (1995), Butter et al (2002), Ferro et al (2004), Gomes et al (2007) and Qian and Ferro, (2008). The particle size, floor surface and shoe type in the present experiments were chosen to mimic those found in cleanrooms, and it was considered that the value of the redispersion fraction found in these experiments i.e. 0.0012 was reasonably accurate when applied to cleanrooms, operating rooms, and similar rooms. However, it was also assumed that the redispersion fraction was independent of surface concentration. This was a reasonable assumption, as the distance between the MCPs particles was very large compared to their size, and therefore the MCPs were unlikely to influence each other in the normal distances found in normal rooms. This was confirmed by a limited amount of experimental evidence. However, information on the values of the redispersion fraction requires further investigation.

The mathematical models derived in this article show that the variables that influence the redispersion factor, or airborne concentration in a room, are the total walking activity, redispersion fraction, shoe area, air supply rate, and deposition on surfaces. The concentration of microbes on the floor has no effect on the redispersion factor but is required in order to calculate the airborne microbial concentration of floor microbes.

Redispersion factors were calculated for MCPs in a range of conditions found in a variety of cleanrooms and operating rooms. Assuming the surface concentration was measured by RODAC contact plates, the redispersion factor was found to vary from 1.5×10^{-4} to 7.4×10^{-6}. For a typical operating room, the redispersion factor was calculated to be 1.0×10^{-4} and for a typical pharmaceutical cleanroom, 5.2×10^{-6}.

The percentage of floor-derived airborne microbes in room air was found to be dependent on the microbial floor concentration, walking activity, redispersion fraction, shoe area, and the rate of microbial dispersion from a person's skin and clothing. It was not influenced by the number of people in the room, or by the air supply rate and deposition on surfaces, and was determined experimentally to be 4.5. Over the range of conditions found in cleanrooms and operating rooms, when the surface concentration was measured by RODAC plates, the percentage ranged from 10.5% to 0.004%. In a typical operating room, the percentage of floor-derived microbes in the room air was found to be 2, and in a typical pharmaceutical cleanroom, it was 0.7.

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Annex: The build-up, steady state, and decay of airborne contamination in rooms

A1 The ventilation equations

A set of equations, often known as the 'ventilation equations', are used in ventilated rooms to determine the build-up, steady-state, and decay of airborne contamination such as undesirable gases, in the manner shown in Figure A1. A derivation of these equations is given in textbooks, such as those written by Jones (2002) and Eastop and Watson (1992). The general equation that applies to all three conditions is as follows:

$$C = [D/Q + C_B].[1 - e^{-[Qt/V]}] + C_I.\ e^{-[Qt/V]} \qquad \text{Equation A1}$$

Where, C = concentration of contaminants /m^3 in room at a given time; D = release rate of airborne contaminant/s; Q = air volume supply rate (m^3/s); C_B = background concentration of contamination /m^3 entering the room in the air supply; t = elapsed time (s); V = room volume (m^3), and C_I = initial concentration of contaminants /m^3 in a room.

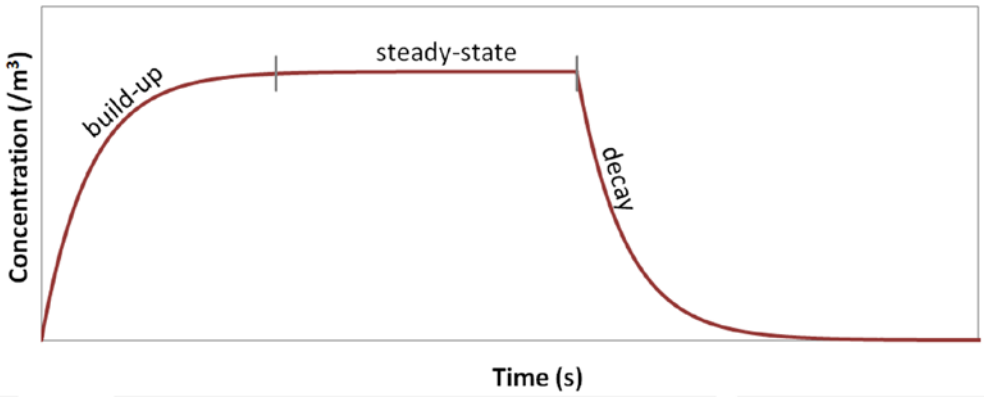

Figure 1: Build-up, steady state, and decay of airborne contamination in a ventilated room

The application of the ventilation equations to the build-up, steady-state and decay of airborne contamination in cleanrooms and operating rooms has been discussed by Whyte et al (2012). The overall ventilation equation (A1) allows the inclusion of background contamination of gases such as CO_2 that enter the room from outside, as well as the contamination generated within the room. In cleanrooms and operating rooms, where particles and MCPs are the problem contaminants, the high efficiency filtration of the supply air ensures that there is no background contamination, and this component can be left out of the equation. In addition, 'Q/V' can be replaced in Equation 1 by the air change supply rate to the room, as these two components are the same. If the good air mixing is obtained, the following equations apply.

Build-up: $C = \left(\frac{D}{Q}\right)(1 - e^{-N_V t})$ (A2)

Steady-state: $C = D/Q$ (A3)

Decay: $C = C_{\mathrm{I}} . e^{-N_V t}$ (A4)

In an empty ventilated room, such as a cleanroom or operating room, the concentration of airborne particles and MCPs will be practically zero. As people enter the room and move about, the airborne contaminants will build-up in a manner that can be calculated by Equation A2. A 'steady state' concentration, will be reached where the dispersion of contaminants is balanced by the dilution and removal by the room's ventilation and, to a lesser extent, by surface deposition. This concentration will vary with activity but an average value can be calculated by Equation A3. If the machines are switched off and everyone leaves the cleanroom, the airborne contamination will decay to a practically zero concentration, and the decay can be calculated by Equation A4.

The dispersion rate '*D*' given in equations A2, A3 and A4 can be a dispersion rate from the floor when walking (D_W), a dispersion rate coming only from the skin and clothing of a person (D_S), or the total dispersion of MCPs from both sources.

The ventilation equations A2, A3 and A4 assume that the removal of airborne contamination through the deposition of particles onto surfaces does not occur. However, these equations can be modified to take account of surface deposition, as occurs with MCPs. This modification has been discussed by Whyte (2012) and results in the following equations.

Build-up: $C = \left(\frac{D}{Q+V_D.A}\right)\left(1 - e^{-(N_D+N_V)t}\right)$ (A5)

Steady-state: $C = D/(Q + V_D.A)$ (A6)

Decay: $C = C_{\mathrm{I}} . e^{-(N_D+N_V)t}$ (A7)

V_D is the deposition velocity of the airborne particles onto surfaces, and N_V is the air change rate/s produced by the air supply to the room. The value 'N_D' given in Equations A5 and A7 allows the effect of the surface

deposition of particles to be represented by an equivalent air change rate which produces the same decay rate as produced by the air change rate. Thus, if the decay rate by surface deposition (N_D) is 6 per hour, this would give the same decay as a room supplied with 6 changes of filtered air per hour. The equivalent air change rate caused by surface deposition can be added to the air change rate of the room by ventilation.

The equivalent air change rate owing to surface deposition has been shown (Whyte, 2012) to be the same as the decay rate owing to surface deposition, and calculated as follows:

$$N_D = \frac{V_D.A}{V} \quad \text{(A8)}$$

Where, N_D is the decay rate /s owing to surface deposition, V_D is the average deposition velocity of airborne particles onto surfaces (m/s), A is the horizontal deposition area (m^2), which is normally the floor area, and V is the volume of the room (m^3).

Equations A5 to A7 will accurately predict the airborne concentration of contamination in rooms as long as there is good mixing of the room air and the surface deposition velocity is known. A knowledge of the particle diameter and the mechanism of deposition, along with experimental measurements, will give a good estimate of deposition velocity.

MCPs are major contaminants in cleanrooms and operating rooms. The main source of MCPs in the air of occupied rooms is microbe-carrying skin particles dispersed by people (Davis and Noble, 1962). Microbes grow on skin, and a person sheds one layer of skin per day, which amounts to about 10^9 skin cells. Skin cells are about 44µm x 33µm in surface area, and 3-5 µm thick, although they will fragment (McIntosh et al, 1978). Experiments have shown that the average equivalent particle diameter of MCPs in the air of an occupied room is about 12 µm (Noble et al, 1963, Whyte, 1986; Whyte and Hejab, 2007) with a deposition velocity owing to gravitational settling of 0.0046 m/s (Whyte, 1981; Whyte, 1986). This deposition has the same effect on the concentration of airborne contamination as dilution by an air supply of between 5 to 7 air changes per hour of filtered air (Whyte et al, 2012). A deposition velocity of 0.0046m/s can therefore be used when dealing with airborne MCPs in rooms.

Equations A5 to A7 can be further modified for use in an unventilated room, such as the experimental room used in this investigation, where the decay of airborne contamination is caused by only surface deposition. If the air change rate (N_V) and air supply rate (Q) are assumed to be zero, and there is good air mixing, then the following equations apply.

Build-up: $C = (\mathrm{D}/(V_D.A))(1 - e^{-N_D.t})$ (A9)

Steady-state: $C = D/V_D.A$ (A10)

Decay: $C = C_I.e^{-N_D.t}$ (A11)

A2 Derivation of equation to calculate the microbial airborne concentration immediately after walking

In the experiments described in this paper, the airborne concentration of MCPs was sampled for two minutes immediately after walking. Microbial air samplers do not give instant measurements but an average over the sampling time. During the sampling, the concentration of MCPs in the air being sampled will decay owing to the gravitational sedimentation of MCPs onto surfaces in the room, and the measured concentration will be less than the actual concentration immediately after walking. An equation that calculates the concentration immediately after walking, in terms of the average measured concentration, is therefore desirable.

The experimental room had no supply of filtered air, and decay of MCPs was only by gravitational settling, and therefore Equation A11 is applicable.

$C = C_I.e^{-N_D t}$

The average concentration (C_A) obtained from an air sample can be calculated by dividing the total concentration (C_T) by the sampling time (t),

$C_A = C_T / t$

The total concentration (C_T) is the integral of C taken over the sampling time, 0 to t.

Therefore,

$$C_A = \frac{1}{t}\int_0^t C.dt$$

Substituting Equation A11 into the above equation, when $C_I = C_W$, which is the concentration immediately after walking stops, gives the following equation,

$$C_A = \frac{1}{t}\int_0^t C_W.e^{-N_D.t}.dt$$

If the equation is integrated and rearranged, the following equation is obtained that allows the concentration immediately after walking stops to be calculated.

$$C_W = \frac{C_A.N_D.t}{1-e^{-N_D.t}} \qquad \text{(A12)}$$

A3 Equation to calculate the actual dispersion rate of MCPs during walking

When a person walks over a floor, microbes are dispersed into the air. However, some will return to the floor owing to the effect of gravitational settling. To determine the actual number of MCPs dispersed by walking, the gravitational loss should be included.

Equation A9 gives the build-up of microbial contamination in an unventilated room (such as occurred in the experimental room), when the dispersion of MCPs is only from walking, i.e.

$$C = (D_F / V_D.A)(1 - e^{-N_D t})$$

Where, D_F is the number of MCPs dispersed from the floor/s, V_D is the average deposition velocity (0.0046 m/s) of MCPs, A is the deposition area (m^2) in the room, N_D is the decay rate owing to surface deposition, and t is the elapsed time.

It is known from a rearrangement of Equation A8, that

$V_D.A = N_D.V$

By substitution, and rearrangement of Equation A9,

$$D_F = \frac{N_D.V.C}{1-e^{N_D.t}} \qquad \text{(A13)}$$

The concentration immediately after walking can be calculated by Equation A12, which was derived in the previous section and, knowing that concentration, the actual dispersal rate by walking, including those MCPs lost by gravitational settling, can be calculated by use of Equation A13.

A4 Time to reach the steady-state concentration in experimental room

To obtain the correct rate of airborne dispersion of MCPs from floors during walking, the airborne concentration in a room must have reached the maximum concentration to be found in the steady-state condition. However, walking in the experimental room was carried out over a short period of 67 seconds, and it is useful to calculate how close the airborne concentration after 67s was to the steady-state concentration.

The experimental room had no air supply, and therefore the build-up of MCPs in the room during walking can be calculated by Equation A9, as can the steady-state condition from Equation A10. Using these

equations, it can be calculated that it would take 30 minutes to reach 95% of the steady-state concentration, and after 67 seconds of walking, the airborne concentration would have reached only 11% of the steady-state concentration.