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Assessment of degree of risk from sources of microbial contamination in cleanrooms; 1: Airborne

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The degree of risk from microbial contamination of manufactured products by sources of contamination in healthcare cleanrooms has been assessed in a series of three articles. This first article considers airborne sources, and a second article will consider surface contact and liquid sources. A final article will consider all sources and the application of the risk method to a variety of cleanroom designs and manufacturing methods.

The assessment of the degree of risk from airborne sources of microbial contamination has been carried out by calculating the number of microbes deposited from the air (NMD_A) onto, or into, a product from various sources. A fundamental equation was used that utilises the following variables (risk factors): concentration of source microbes; surface area of product exposed to microbial deposition; ease of microbial dispersion, transmission and deposition from source to product; and time available for deposition. This approach gives an accurate risk assessment, although it is dependent on the quality of the input data. It is a particularly useful method as it calculates the likely rate of product microbial contamination from the various sources of airborne contamination.

Key words: Risk assessment, degree of risk, source, airborne contamination, micro-organisms, microbe carrying particles, MCP.

Introduction

The requirements for minimising microbial contamination in pharmaceutical cleanrooms are in regulatory documents published by authorities that include the European Commission[1] and the Food and Drug Administration in the USA[2]. These authorities also suggest the use of risk management and assessment techniques to identify and control sources of microbial contamination[3,4]. The authors of this article have described risk management methods for products manufactured in cleanrooms[5–7], and risk assessment techniques to determine the relative importance of sources of microbial contamination[8,9]. An overview and discussion of other approaches is provided by Mollah *et al*. (2013)[10].

Risk is defined[11] as 'the combination of the severity of harm and the probability of occurrence of that harm', and can be calculated from Equation 1.

Equation 1

$$\textit{Degree of risk} = \textit{severity of harm} \times \textit{probability of harm}$$

The word 'criticality' is often used as a synonym for 'severity of harm'. 'Severity of harm' from microbial contaminants, when applied to products manufactured in cleanrooms, can be determined by the following risk factors.

a. The concentration of source microbes.
b. The area of the product exposed to airborne deposition or surface contact.
c. The ease by which source microbes are dispersed, transmitted and deposited into, or onto, a product.

The 'probability of harm' can be assessed by the frequency of deposition, which is either the number of surface contacts, or the time available for airborne deposition.

Actual values of these risk factors are often not available and surrogate descriptors, such as 'high', 'medium' and 'low', etc. are utilised. Scores are then assigned to these descriptors, and the scores combined in the best way to give an assessment of the degree of risk of a source[6,7].

The assignment of descriptors and scores to risk factors is subjective, and assigned values are often difficult to align with actual values. Also, the method of combining risk scores to obtain the degree of risk from a source may not accurately model the actual mechanisms of dispersion, transmission and deposition of microbial contamination. In addition, the mechanisms through air, surface contact and liquid are different, and the associated risks are, therefore, not readily comparable. Owing to these problems, inaccurate risk assessments are often

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completed, and it would be beneficial if a technique was available to overcome such drawbacks. This would be especially welcome if the risk assessment also calculated the product's contamination rate from various cleanroom sources, and would be a useful advance in the management of microbial contamination in cleanrooms.

Whyte and Eaton[8] have provided equations to calculate the exact amount of microbial contamination of a product and demonstrated their use in risk assessment; this approach is expanded in this article. This article deals with airborne sources and the next article will consider sources of surface contact and liquid contamination.

Calculation of airborne microbial contamination of a product

Equation 2 has been derived by Whyte and Eaton[8] to calculate the number of microbe-carrying particles (MCPs) deposited from air onto or into a product.

Equation 2

$$NMD_A = c*a*s_v*t$$

where, NMD_A = number of MCPs deposited from air onto a single product, c = concentration of microbes in the air next to the product, a = area of product exposed to microbial deposition, s_v = settling velocity through air of MCPs , t = time of airborne deposition.

It is important to ensure that the units of measurement are consistent in the risk equations, and those used in Equation 2 in this article are centimetres and seconds. Airborne concentrations are usually given as number per m^3, but to align the concentrations with other risk factors, number per cm^3 is used.

The above NMD_A is calculated from knowledge of the MCP concentration next to the product. However, some sources of airborne risk will be a distance away from the product, and in these situations it is necessary to know the proportion of MCPs transmitted to the area next to the product. This proportion is known as the transfer coefficient, which is the ratio of the concentration of MCPs at the product to the concentration at the source. This proportion is included in Equation 3.

Equation 3

$$NMD_A = c*p*a*s_v*t$$

where, p = proportion of MCPs that are transmitted from a source to the area next to the product (transfer coefficient).

The NMD_A onto one product unit is calculated, and gives the expected contamination rate of a product from a given source. Its numerical value is usually well below 1 but, if required, can be converted to a more conventional contamination rate. For example, if the NMD_A is 1×10^{-6}, the contamination rate of the product is 1 in 10^6, or 1 in a million units.

Most of the values of risk factors, i.e. the variables required to solve Equation 3, are known by cleanroom users, or can be determined. However, the settling velocity of MCPs falling through cleanroom air is not well known. MCPs rarely occur in cleanroom air in a unicellular form, but are found on skin or clothing detritus dispersed from personnel. The MCPs have an average equivalent particle diameter of about 12 μm[12,13], and settle under the influence of gravity at a velocity of about 0.46 cm/s[14]. It is assumed that the area of the product exposed to airborne contamination is the surface in the horizontal orientation. However, should the exposed surface be at an angle, the 'effective' area for MCPs that deposit under the influence of gravity will be reduced. It can be calculated by multiplying the area by Cos σ, where σ is the angle that the surface is to the horizontal.

Equation 3 uses the concentration of MCPs in a volume of air, as determined by a microbial air sampler. However, settle plates can be used to accurately and directly measure the deposition rate of MCPs. If a settle plate is used to sample air adjacent to product, its count can be used to calculate the NMD_A by proportioning and use of Equation 4.

Equation 4

$$NMD_A = \text{settle plate count} \times \frac{\text{area of exposed product}}{\text{area of settle plate}} \times \frac{\text{time product exposed}}{\text{time settle plate exposed}}$$

However, because of the greater popularity of air samplers in evaluating airborne microbial contamination, and greater availability of counts, air sampler concentrations are used in this article.

Description of cleanroom studied

A pharmaceutical cleanroom, where aseptic filling of vials is carried out, is used to demonstrate the NMD_A method. The cleanroom is fictitious but typical of those cleanrooms where aseptic filling of small batches of pharmaceutical products is carried out in a unidirectional airflow workstation, rather than in a restricted access barrier system (RABS) or isolator. Increasing regulatory expectations are leading to manufacturing facilities being designed with such separative devices, but to illustrate the wider application of the risk assessment method to a variety of healthcare facilities, the following cleanroom and manufacturing process is used as an example.

Vials with an internal neck area of 2 cm^2 are aseptically filled with 2 ml of an aqueous product solution and sealed with sterile closures. This is carried out in batches of 4000, which take about 4 hours to process.

1. The vials are heat sterilised in a depyrogenation tunnel and conveyed into a vertical unidirectional airflow (UDAF) workstation (EU Guideline to Good Manufacturing Practice (GGMP) grade A), which is known as the 'filling workstation', where they are automatically filled and sealed by inserting a stopper.

The average time the vial is open to airborne contamination, i.e. between exiting from the depyrogenation tunnel and being sealed, is 10 minutes (600 s).

2. Vial closures (rubber stoppers) are held in a hopper within the filling workstation, which has a capacity of 1000 closures, and is replenished every hour.
3. The air supply and extract system and the particle removal efficiency of the supply air filters are fully described in the relevant section of this article. However, all terminal filtration of the supply air is by H14 high-efficiency particulate air (HEPA) filters, as rated according to EN 1822: 2012[15].
4. The filling workstation is situated in a non-unidirectional airflow cleanroom (EU GGMP grade B) which is known as the 'filling cleanroom'. It is 10 m x 10 m x 3 m, i.e. 300 m^3 in volume, and supplied with 3.33 m^3/s of HEPA-filtered air, which is equivalent to 40 room air changes per hour.
5. Two people work in the filling cleanroom, with one of these attending to the filling machine within the filling workstation. Access into the filling workstation is through plastic-strip curtains that hang down to just above the floor.
6. Personnel wear cleanroom clothing consisting of a woven one-piece polyester coverall with hood, overboots, mask and goggles. Sterilised, latex, double gloves are worn over disinfected hands. There are no areas of exposed skin.
7. Hard surfaces, which do not come into contact with product, vials or closures, are disinfected. Hard surfaces, such as pipework that contact product, or product-contacting surfaces, such as sterile closures, closure's hopper and track-ways, are sterilised.
8. Eight litres of aqueous solution of product is prepared in an adjacent cleanroom (EU GGMP grade C) and piped from the preparation vessel through a sterilised, sterilising-grade filter, into the filling workstation. An aseptic connection is made in the filling workstation with the product filling equipment.

Sources of airborne contamination

Figure 1 shows the airflow in the cleanroom under consideration, and **Figure 2** gives a risk diagram that shows the various sources of airborne contamination, their control measures, and routes of transfer to product. Personnel are considered the prime source of microbial contamination in a cleanroom and disperse MCPs into the air of both the filling workstation and filling cleanroom. Airborne contamination may also enter the filling workstation and cleanroom through the HEPA supply filters, especially if they are damaged.

Calculation of degree of risk to product in cleanroom

The degree of risk from the sources shown in **Figure 2** can be determined by calculating the NMD_A into, or onto, one product vial. The NMD_A is calculated by use of Equation 3, using centimetres and seconds as the units of measurement. Each variable in the equation, i.e. the risk factors, is assigned a value that the authors consider 'typical' of the cleanroom described. For simplicity, and because it is peripheral and has a very small risk, the risk associated with 'air within adjacent cleanrooms', although included in **Figure 2**, is not calculated.

When sampling air in an EU GGMP grade A zone, only an occasional MCP is found, and most samples have zero counts. Average concentrations are, therefore, used, and calculated as the number of MCPs isolated from the total volume of air sampled over numerous consecutive operational periods. The average concentration of airborne microbes is required for the whole period when the product or closures are exposed

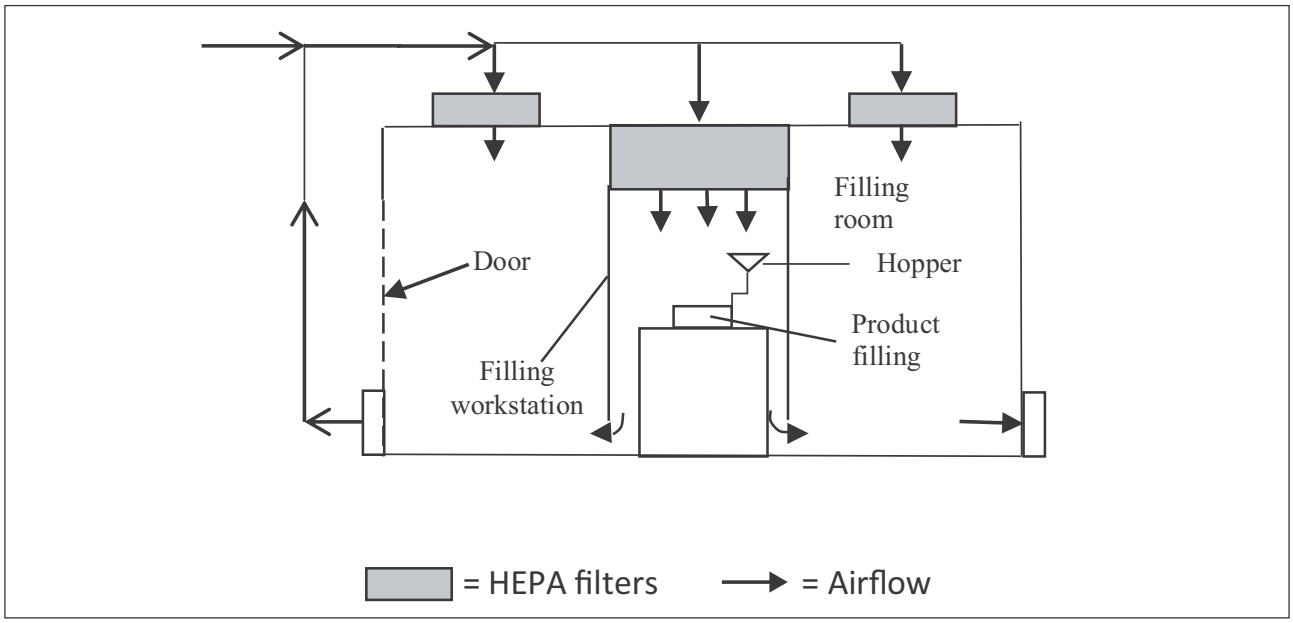

Figure 1. Airflow in a cleanroom.

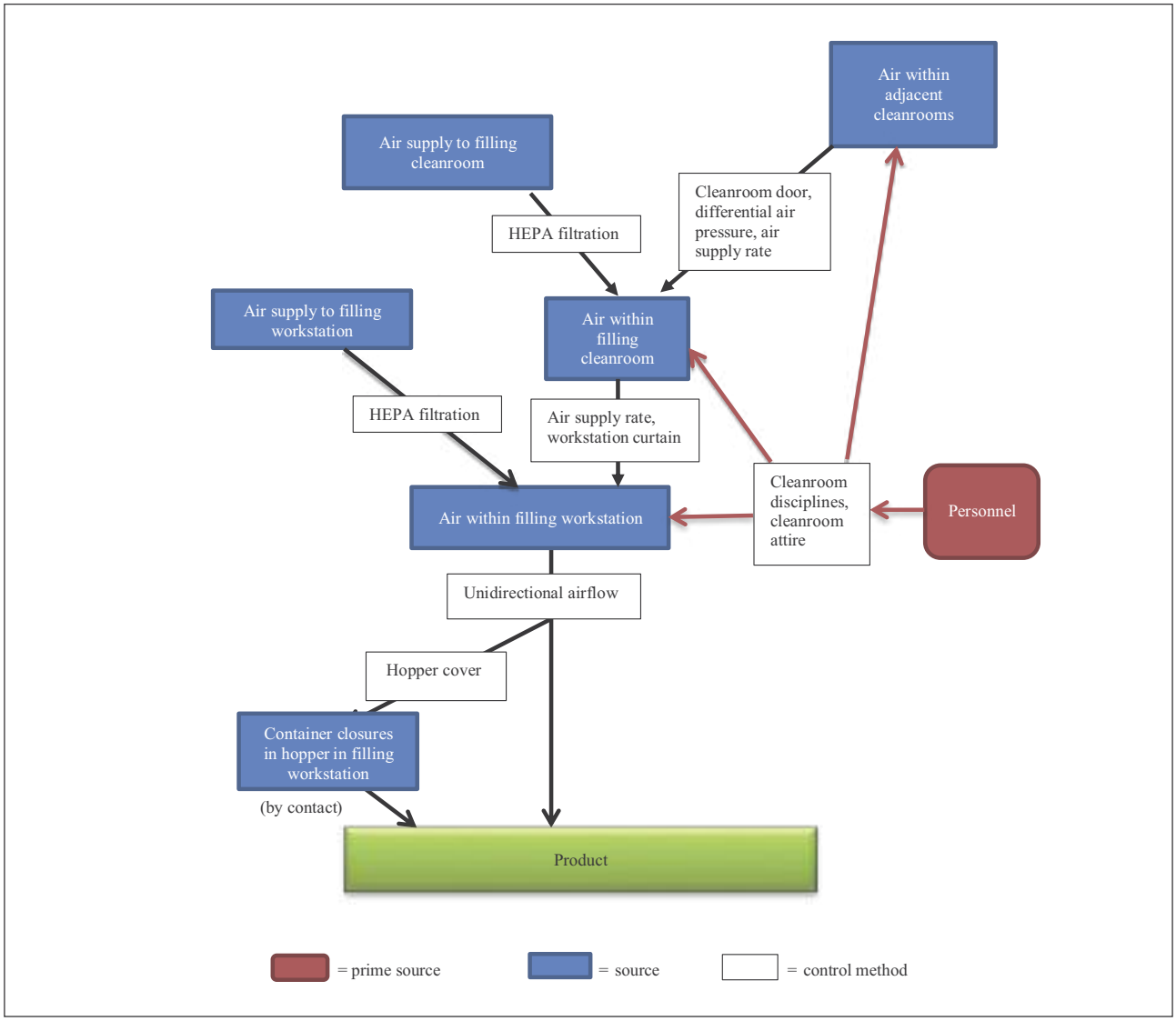

Figure 2. Risk diagram showing airborne sources of microbial contamination, control methods, and routes of transfer to product.

to microbial deposition. Microbial air sampling is often carried out for one single period during manufacture but, for the purposes of risk assessment, several samples should be taken to provide an average of the whole period of exposure, including periods when personnel are not in attendance.

Degree of risk from airborne MCPs dispersed by personnel within filling workstation

The average concentration of MCPs in the air of an EU GGMP grade A filling workstation during manufacturing depends on whether personnel work inside or from outside the workstation, the number of personnel involved, their time within the workstation, their activity, and type of cleanroom clothing worn. An average value should be ascertained over the whole of the manufacturing time. The lowest average value at the filling point is likely to be about 1 x 10^{-4}/m^3 (1 x 10^{-10}/cm^3) but the average concentration in our example is taken as 0.01/m^3 (1 x 10^{-8}/cm^3). To calculate the degree of risk to product from air in the filling workstation, the values of the variables (risk factors), and the solution of Equation 3, is considered as follows.

1. **Concentration of airborne MCPs (number/cm^3)**: An average concentration close to where vials are exposed during filling and over the whole of the manufacturing period is 0.01/m^3 (1 x 10^{-8}/cm^3).
2. **Transfer coefficient**: The air is sampled adjacent to the exposed vials, and, therefore, a transfer coefficient is not necessary, and taken as 1.
3. **Area of product exposed (cm^2)**: The inner neck area of the vial is 2 cm^2.
4. **Time of deposition (s)**: The time the vial is exposed is 600 s.
5. **Settling velocity of MCPs through air (cm/s)**: As discussed in the 'Calculation of airborne microbial contamination of a product' section, the average setting velocity of MCPs through the air and into the vial is assumed to be 0.46 cm/s.

Using Equation 3, the NMD_A can be calculated;
$NMD_A = c*p*a*t*s = 1 \times 10^{-8}*1*2*600*0.46 =$ **5.5×10^{-6}**

Degree of risk from airborne MCPs dispersed by personnel within filling cleanroom

The concentration of airborne MCPs in an EU GGMP grade B filling cleanroom during manufacturing is dependent on the effectiveness of the cleanroom ventilation system, the number and activity of personnel, and type of cleanroom clothing worn. Depending on these variables, the lowest average value is usually about $1/m^3$ ($1 \times 10^{-6}/cm^3$) but the average concentration in our example is taken as $5/m^3$ ($5 \times 10^{-6}/cm^3$).

It is necessary to know what proportion of MCPs in the filling cleanroom is transferred to product, i.e. the transfer coefficient. The filling workstation has a plastic curtain round its perimeter to minimise this transfer. However, personnel who move between the filling room and the filling workstation, or pass their arms through the curtains, will cause filling cleanroom air to be transferred into the filling workstation. Also, by working round the product and disturbing the unidirectional airflow, filling cleanroom air may be transferred to product.

Ljungqvist and Reinmuller[16] have measured the proportion of airborne particles that are transmitted from outside a unidirectional airflow workstation to product when personnel work through the curtain and around the workstation. Using this information, it is assumed that the proportion transferred (transfer coefficient) from the filling cleanroom is 1×10^{-4}. However, the time personnel spend in attending to machinery in the filling workstation is about 10% of the total time spent in the cleanroom. The time of airborne deposition of MCPs sourced in the filling cleanroom is, therefore, taken as 60s. The degree of risk to product from air in the filling cleanroom is now determined as follows.

1. **Concentration of airborne MCPs (number/cm^3)**: The average concentration in the filling cleanroom is taken as $5/m^3$ ($5 \times 10^{-6}/cm^3$).
2. **Transfer coefficient**: The proportion of MCPs in the filling cleanroom transmitted to product is assumed to be 1×10^{-4}.
3. **Area of product exposed (cm^2)**: The inner neck area of the vial is 2 cm^2.
4. **Time of airborne deposition (s)**: The time a vial is exposed to contamination originating in the filling cleanroom is 60 s
5. **Settling velocity of MCPs through air (cm/s)**: As discussed in the 'Calculation of airborne microbial contamination of a product' section, the average velocity of MCPs settling through the air and into the vials is 0.46 cm/s.

Using Equation 3, the NMD_A can be calculated;
$NMD_A = c*p*a*t*s = 5 \times 10^{-6}*1 \times 10^{-4}*2*60*0.46 =$ **2.8×10^{-8}**

Degree of risk from the filtered air supply

The previous two sections of this article have calculated the NMD_A of MCPs in the filling workstation and filling cleanroom. These calculations considered the risk from airborne MCPs dispersed by personnel working in these areas. However, there is also a degree of risk from the filtered air supply, and this is now considered.

Calculating the penetration of MCPs through air filters

The concentration of MCPs after an air filter is calculated by Equation 5.

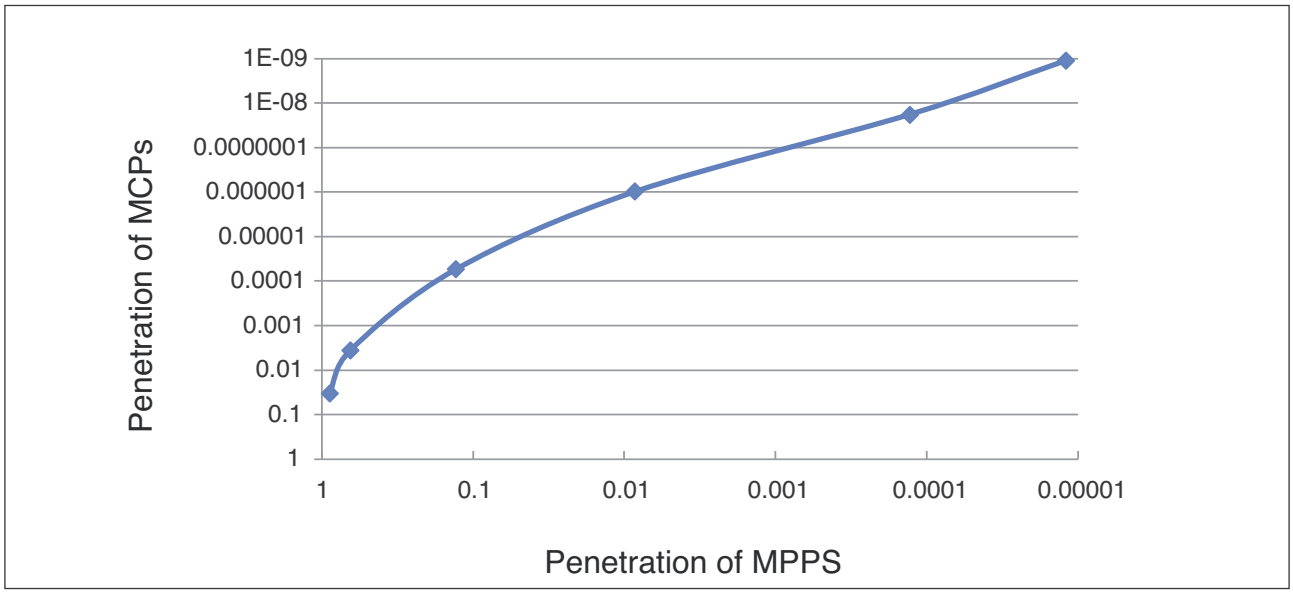

Figure 3. Relationship between penetration of most penetrating particle size (MPPS) and MCPs through high efficiency filters.

Equation 5

Concentration after filter = concentration before filter × penetration through filter

Air filters are rated according to their removal efficiency, which is usually given as a percentage, or to the proportion of airborne contamination that penetrates the filters. These two quantities are related in Equation 6.

Equation 6

Penetration = 1 – (removal efficiency/100)

The high efficiency filters used in cleanrooms are classified by EN 1822: 2009[15], which uses the removal efficiency of the filter's most penetrating particle size (MPPS) of about 0.3 μm. Because of their much larger size, fewer MCPs penetrate HEPA filters, and this relationship has been investigated[17] and shown in **Figure 3**.

Calculating the MCP concentration in supply air

The design of a typical air ventilation system used in a cleanroom is shown in **Figure 4**. Air is extracted from the cleanroom, mixed with some fresh air, passed through an air conditioning plant, and returned to the filling cleanroom and workstation. Fresh air is added for the health of the personnel, and to make up the total air supply so that the cleanroom is continually pressurised. This proportion of fresh air is about 0.1 of the total air supplied to the cleanroom.

Typically, fresh air is mixed with recirculated air and filtered by a primary filter before being passed through the air conditioning plant. The conditioned air is then filtered by a secondary filter to extend the life of the terminal filter, and reduce the contamination risk to the product, should a terminal supply filter in the ceiling be damaged.

The concentration of MCPs in the air supplied to both the filling cleanroom and workstation can be calculated by Equation 7. If necessary, this equation can be modified for other designs of ventilation systems.

Equation 7

$$C_S = (p_1 * C_R + p_2 * C_{FA}) * \eta_P * \eta_S * \eta_T$$

where;

- Cs is the MCP concentration supplied to the filling cleanroom and workstation,
- C_R is the MCP concentration in the filling cleanroom and recirculated air,
- C_{FA} is the MCP concentration in fresh air,
- p_1 is the proportion of recirculated air in the total air supply volume,
- p_2 is the proportion of fresh air in the total air supply volume,
- η_P is the proportion of MCPs removed by the primary filters
- η_S is the proportion of MCPs removed by the secondary filters,
- η_T is the proportion of MCPs removed by the terminal filters.

Fresh air has an MCP concentration of about 50/m^3, and the concentration in the filling cleanroom is assumed to be 5/m^3. The proportion of fresh air is 0.1 and, therefore, the proportion of recirculated air is 0.9. The mixture of fresh and recirculated air is filtered by primary filters, which are E10 bag filters with a removal efficiency against the MPPS of about 85% (penetration = 0.15) and, as given in **Figure 3**, they have an MCP penetration of about 1 x 10^{-4}. The secondary filters are H13, with a removal efficiency against the MPPS of 99.95%, and an MCP penetration of about 1 x 10^{-7}. The terminal H14 filters have a removal

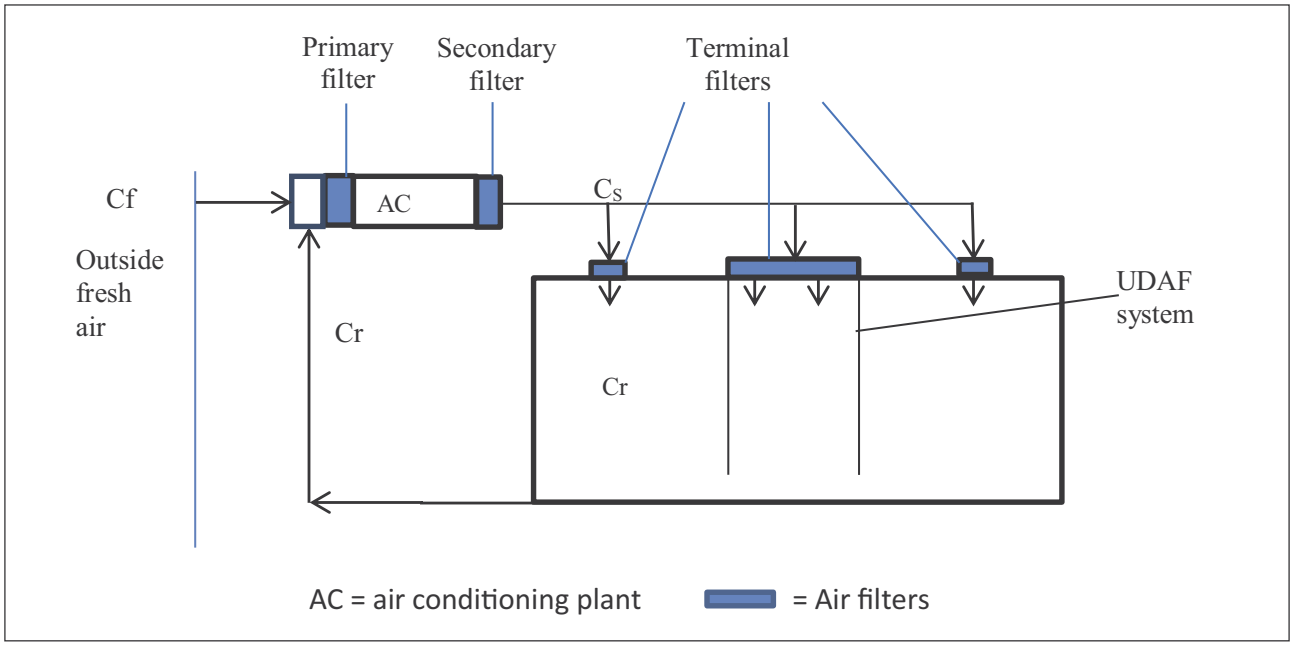

Figure 4. Typical cleanroom ventilation system.

efficiency against the MPPS of 99.995%, and an MCP penetration of about 1 x 10^{-8}.

The airborne concentration of MCPs supplied to the filling cleanroom and workstation is therefore:

$$C_S = \{(0.9*5 \times 10^{-6}) + (0.1*5 \times 10^{-5})\}*1 \times 10^{-4}*1 \times 10^{-7}*1 \times 10^{-8}$$
$$= \{(4.5 \times 10^{-6}) + (5 \times 10^{-6})\}*1 \times 10^{-19}$$
$$= 9.5 \times 10^{-25}$$

The concentration of airborne MCPs approaching and passing through a leak in the terminal HEPA filters will be required in later calculations. The air passing through the leak is assumed to be unfiltered by the terminal filter and, therefore, has the same concentration as the air supplied to the terminal filters. This can be calculated in the manner given in the previous paragraph, and is 9.5 x $10^{-17}/m^3$.

Degree of risk from air supplied into filling workstation by HEPA filters without leaks

If the filter system that supplies air into the filling workstation has no leaks, the NMD_A from the supply air can be calculated as follows.

1. **Concentration of MCPs in air supply (number/cm³)**: The filling workstation is supplied by air from the air conditioning plant that uses primary, secondary and terminal filters of the type described in the previous section. The average concentration of MCPs in the air supplied from the terminal HEPA filters, without leaks, has been calculated to be 9.5 x 10^{-25}.
2. **Transfer coefficient**: Air from the terminal HEPA filter in the filling workstation flows in a unidirectional manner to product and the airborne concentration at product is assumed to be the same as at the filter face, and the transfer coefficient is 1.
3. **Area of product exposed to microbial deposition (cm²)**: The inner neck area of the vial is 2 cm^2.
4. **Time of deposition (s)**: The time the vial is exposed is 600 s.
5. **Settling velocity of MCPs through air (cm/s)**: As discussed in the 'Calculation of airborne microbial contamination of a product' section, the average velocity of MCPs settling through air to vials is 0.46 cm/s.

Using Equation 3, the NMD_A can be calculated;
$NMD_A = c*p*a*t*s = 9.5 \times 10^{-25}*1*2*600*0.46 =$ **5.2×10^{-22}**

In some filling workstations, the air supply is not from the air conditioning plant but drawn from the filling cleanroom where the airborne concentrations of MCPs is 5/m^3 (5 x $10^{-6}/cm^3$). This air may be only filtered by the H14 terminal filters, with an overall removal efficiency of 99.995% (penetration = 0.00005) against the MPPS, and, therefore, a penetration of MCPs of about 1 x 10^{-8}. Thus, the airborne concentration of MCPs supplied by the terminal H14 filters in the filling workstation, as determined by Equation 5, is as follows:

$$C_S = 5 \times 10^{-6}*1 \times 10^{-8} = 5 \times 10^{-14}$$

Using the same approach as in the box above, the NMD_A is,

$$NMD_A = c*p*a*t*s = 5 \times 10^{-14}*1*2*600*0.46 = \mathbf{2.8 \times 10^{-11}}$$

Degree of risk from air supplied into filling workstation by HEPA filters with a leak

HEPA filters are routinely tested for leaks by generating sub-micrometre particles before the filter and scanning the filter's supply face with a probe, so as to obtain the particle penetration through the filter. Leaks are considered to occur if the penetration of the particle challenge is greater than 0.01%.

The area of a leak is much smaller than the filter's supply face area, and as the airflow in the filling workstation is unidirectional, leaking air may pass through the filling workstation at sufficient distance away from the product vials that no contamination occurs. However, the leak may be directly above the vials, and this worst case situation is considered. Such a filter leak is considered when (a) air is supplied by the air conditioning plant or (b) air is drawn into the filling workstation from the filling cleanroom. A maximum leak of 100%, and a minimum of 0.01% are investigated in each of these situations.

(a) Air supplied by the air conditioning plant

A maximum leak of 100% in a terminal HEPA filter will only occur after an exceptional amount of filter damage, and it is assumed that the large hole made will allow the unfiltered supply air to pass through it. The NMD_A can be calculated as follows.

1. **Concentration of airborne MCPs (number/cm³)**: The average MCP concentration passing through a leak in the HEPA filter and into the cleanroom has been calculated in the 'Calculating the MCP concentration in supply air' section to be 9.5 x $10^{-17}/cm^3$.
2. **Transfer coefficient**: Air from the HEPA filter face flows in a unidirectional manner, and the concentration of MCPs at product is assumed to be the same as at the filter face, and the transfer coefficient is therefore 1.
3. **Area of product exposed (cm²)**: The inner neck area of the vial is 2 cm^2
4. **Time of airborne deposition (s)**: Although the time the vial is exposed is 600 s, vials are on a conveyor, and the time directly below a filter leak is considered to be 10 s.
5. **Settling velocity of MCPs through air (cm/s)**: As discussed in the 'Calculation of airborne microbial contamination of a product' section, the average settling velocity of MCPs through the air and into the vials is 0.46 cm/s.

Using Equation 3, the NMD_A can be calculated;
$NMD_A = c*p*a*t*s = 9.5 \times 10^{-17}*1*2*10*0.46 =$ **8.7×10^{-16}**

A minimum leak is taken as 0.01%, and these leaks are not usually an actual hole but broken fibres in the filter media, or a thinning of the depth of fibres. MCPs are, therefore, less likely to penetrate than the MPPS particles. However, the actual penetration of MCPs in this situation is unknown, and thus the worst condition is assumed, where the penetration of MCPs is the same as MPPS particles. It is also assumed that the filter leak is directly above the vials.

Therefore,

$$NMD_A = c*p*a*t*s = (9.5 \times 10^{-17}*0.0001)*1*2*10*0.46 = \mathbf{8.7 \times 10^{-20}}$$

(b) Air drawn from the filling cleanroom

If the filling workstation draws its air from the EU GGMP grade B filling cleanroom, the MCP concentration in the air approaching the filter can be assumed to be the same as in the filling cleanroom, which is 5/m^3 (5 x 10^{-6}/cm^3). For a maximum leak of 100% in the terminal HEPA filters, the NMD_A is,

$$NMD_A = c*p*a*t*s = 5 \times 10^{-6}*1*2*10*0.46 = \mathbf{4.6 \times 10^{-5}}$$

If the leak has a minimum penetration of 0.01%, the NMD_A is,

$$NMD_A = c*p*a*t*s = (5 \times 10^{-6}*0.0001)*1*2*10*0.46 = \mathbf{4.6 \times 10^{-9}}$$

Degree of risk from air supply to filling cleanroom

The risk to product from the air within the filling cleanroom has been considered in the 'Degree of risk from airborne MCPs dispersed by personnel within filling cleanroom' section. That section considers the airborne MCPs dispersed by personnel, but there may also be a contribution from the air supplied from the terminal HEPA filters. This may occur in a filter system with full integrity, or with a leak in the system.

(a) Full-integrity filtration system

The degree of risk from fully filtered air supplied to the filling cleanroom is calculated as follows.

1. **Concentration of MCPs in airborne source (number/cm^3)**: The average concentration of MCPs in the filling cleanroom attributed to the air supply is the same concentration as coming from the terminal air filters. Other MCPs in the air of the filling cleanroom that are dispersed by personnel are considered in the 'Degree of risk from airborne MCPs dispersed by personnel within filling cleanroom' section. The concentration from terminal filters has been calculated in the 'Calculating the MCP concentration in supply air' section and is 9.5 x 10^{-25}/cm^3.
2. **Transfer coefficient**: The MCPs in the cleanroom air must pass across the unidirectional airflow in the filling workstation, to reach the product. The proportion that does so has been discussed in the 'Degree of risk from airborne MCPs dispersed by personnel within filling cleanroom' section, and considered to be 1 x 10^{-4}.
3. **Area of product exposed (cm^2)**: The inner neck area of the vial is 2 cm^2.
4. **Time of deposition (s)**: The time the vial is exposed to MCPs from filters is 600 s.
5. **Settling velocity of MCPs through air (cm/s)**: As discussed in the the 'Degree of risk from the filtered air supply' section, the velocity of MCPs settling through the air and into vials can be assumed to be 0.46 cm/s.

Using Equation 3, the NMD_A can be calculated;
$NMD_A = c*p*a*t*s = 9.5 \times 10^{-25}*1 \times 10^{-4}*2*600*0.46 = \mathbf{5.2 \times 10^{-26}}$

(b) Leak in terminal filter system

The risk to product from a 100% penetration leak in a HEPA filter that supplies the filling cleanroom, is now considered.

The volume of air that passes through a hole in the filter system can be calculated by Bernoulli's equation. This requires knowledge of the area of the hole, the pressure difference across the hole, and the density of the air. The effect of the type of hole on the airflow volume is accounted for by a coefficient of discharge.

$$Q = C_D.A.\left[\frac{2\Delta p}{\rho}\right]^{0.5}$$

where, Q = flow rate (m^3/s), C_D = discharge coefficient, A = area (m^2), Δp = pressure difference (Pa), and ρ = air density (kg/m^3).

Using a pressure difference across a HEPA filter of 250 Pa, the area of a large hole in the filter media of 0.5 cm^2, a discharge coefficient of 0.7, and an air density of 1.225 kg/m^3, the air volume passing through the hole can be calculated to be 0.0007 m^3/s.

The air leaking through the hole in the filter will enter the filling cleanroom where it will mix with the rest of the air supply that has been correctly filtered. The total amount of air supplied to the filling cleanroom room is 3.33 m^3/s, and the volume of air from the leak is 0.0007 m^3/s. Therefore, 3.329 m^3/s of correctly filtered air will pass into the filling cleanroom.

The concentration of MCPs in leaking air has previously been shown to be 9.5 x 10^{-17}/cm^3 and in the filtered air it is 9.5 x 10^{-25}/cm^3. The air from the leak will mix in the filling cleanroom with the correctly-filtered air supply, and exit the room. The average concentration in the mixed air is obtained by proportioning the appropriate concentration of MCPs with the volumes of filtered and leaking air, is as follows:

$$\text{Average MCP concentration} = (9.5\times10^{-25}*3.329) + (9.5 \times 10^{-17}*0.0007) = 3.2 \times 10^{-24} + 6.7 \times 10^{-20} = 6.7 \times 10^{-20}/\text{cm}^2$$

Using this MCP concentration, the NMD_A can be calculated as previously shown.

$$NMD_A = c*p*a*t*s = 6.7 \times 10^{-20}*1 \times 10^{-4}*2*600*0.46 = \mathbf{3.7 \times 10^{-20}}$$

Degree of risk from airborne contamination of closures in the hopper

The hopper that contains closures is located within the filling workstation. If it is open to workstation air, MCPs may deposit from air onto closures and could subsequently be introduced into product.

If the hopper is cone shaped, the surface area of stoppers exposed to deposition of MCPs will diminish as stoppers are used. To give an approximation of the average area of closures exposed, the area is taken as half the surface of the top of the closures in the full hopper. The degree of risk to product of vial closures is now calculated as follows.

1. **Concentration of MCPs in the air (number/cm^3)**: The average MCP concentration over the period that the closures are exposed to airborne deposition is assumed to be the same as that suggested in the 'Degree of risk from airborne MCPs dispersed by personnel within filling workstation' section for the concentration at the filling location, and is 1 x 10^{-8}/cm^3.
2. **Transfer coefficient**: The concentration of MCPs is measured adjacent to the hopper, and a transfer coefficient is not necessary, or taken as 1.
3. **Area of product exposed to deposition (cm^2)**: The diameter of the hopper opening is 50 cm, with an associated surface area of 1964 cm^2. The average surface area exposed to airborne deposition is therefore assumed to be half of this surface area, i.e. 982 cm^2.
4. **Time of airborne deposition(s)**: The closures are replenished 4 times throughout the 4-hour filling operation. The time the closures are exposed to airborne deposition is therefore 3600 s.
5. **Settling velocity of MCPs through air (cm/s)**: As discussed in the 'Calculation of airborne microbial contamination of a product' section, the average settling velocity of MCPs in the air and into vials is 0.46 cm/s.

Using Equation 3, the NMD_A onto all closures in the hopper is;

$NMD_A = c*p*a*t*p = 1 \times 10^{-8}*1*982*3600*0.46 = 1.6 \times 10^{-2}$

This contamination will be deposited onto some of the 1000 closures in the hopper. Also, when a closure is inserted into a container, only about half of its area is in the container, and half the MCPs are introduced. Therefore, for one stopper, the NMD_A can be calculated;

$NMD_A = 1.6 \times 10^{-2}*1 \times 10^{-3}*0.5 = \mathbf{8.1 \times 10^{-6}}$

Hoppers can also be used with air-tight lids to minimise airborne contamination. However, when the lid is lifted and closures added to the near-empty hopper, the general air turbulence will cause most of the hopper air to be exchanged for filling workstation air. Also, because of higher activity of personnel during replenishment, and greater exposure to air transmitted across the curtains, the concentration of airborne MCPs round the hopper during the period of replenishment will be higher than the average taken over the whole of the manufacturing time, and assumed to be 1 x 10^{-7}.

The hopper has a height of 15 cm and radius of 25 cm, and hence its volume ($\pi . r^2 . h/3$) is 9818 cm^3. After the closures have been added, and the hopper lid shut, the number of MCPs sealed in the hopper are,

Number of MCPs sealed in hopper
= volume of air in hopper x concentration MCPs in air
= $9818*1 \times 10^{-7} = 9.8 \times 10^{-4}$

As MCPs have an average deposition velocity of 0.46 cm/s and 3600 s to deposit, it is reasonable to assume that most of the MCPs sealed in the hopper will deposit onto some of the 1000 closures in the hopper. Also, when a closure is inserted into a vial, only about half of its area is in the container, and thus only half the MCPs are introduced. Therefore, for one stopper, the NMD_A can be calculated to be,

$$NMD_A = 9.8 \times 10^{-4}*1 \times 10^{-3}*0.5 = 4.9 \times 10^{-7}$$

Relative importance of sources of airborne contamination

Shown in **Table 1** are the NMD_A of sources of airborne contamination found in the cleanroom used as an example. The NMD_A values are given in order of importance.

Discussion and conclusions

The risk to a product from sources of airborne microbial contamination in healthcare cleanrooms has been assessed. This was carried out by calculating the number of microbes deposited from air (NMD_A) into, or onto, a product. The NMD_A was calculated by use of Equation 3, which uses the following risk factors as variables: concentration of source microbes; area of product exposed to airborne deposition; the ease of microbial dispersion; transmission and deposition from a source to a product; time available for deposition to occur. Equation 3 is a fundamental equation and if the values of the risk factors are correct then the result will be exact. There are other advantages to this method, as the calculation of the degree of risk of sources is more accurate than typical methods in use, and it also gives the actual contamination rate of the product.

Many of the risk factors required to solve Equation 3 are available, or can be obtained. Even if this is not possible, an informed assessment will lead to a more accurate risk value than methods used at present. Much of

Table 1. Importance of sources of airborne microbial contamination in a pharmaceutical cleanroom.

Risk importance	Source of airborne microbial contamination	NMD_A
1	**Filling workstation (EU GGMP grade A) filters** – air drawn from filling cleanroom, 100% leak in filter directly above vials	4.6×10^{-5}
2	**Closures hopper** – closures in open hopper	8.1×10^{-6}
3	**Filling workstation (EU GGMP grade A)** – MCPs generated by personnel working in workstation	5.5×10^{-6}
4	**Closures hopper** – closures in lidded hopper	4.9×10^{-7}
5	**Filling cleanroom (EU GGMP grade B)** – MCPs generated by personnel in room	2.8×10^{-8}
6	**Filling workstation (EU GGMP grade A) filters** – air supply drawn from filling cleanroom, 0.01% leak in filter directly above vials	4.6×10^{-9}
7	**Filling workstation (EU GGMP grade A) filters** – air drawn from filling cleanroom, no leaks in filter	2.8×10^{-11}
8	**Filling workstation (EU GGMP grade A) filters** – air supply from air conditioning plant, 100% leak in filter directly above vials	8.7×10^{-16}
9	**Filling workstation (EU GGMP grade A) filters** – air drawn from air conditioning plant, 0.01% leak in filter directly above vials	8.7×10^{-20}
10	**Filling cleanroom (EU GGMP grade B) filters** – air supply from air conditioning plant, 100% leak in filter	3.7×10^{-20}
11	**Filling workstation (EU GGMP grade A) filters** – air supply from air conditioning plant, no leak in filter	5.2×10^{-22}
12	**Filling cleanroom (EU GGMP grade B) filters** – air supply from air conditioning plant, no leak in filter	5.2×10^{-26}

the required information is not difficult to obtain, e.g. average airborne concentration of MCPs, area of product exposed to airborne deposition, deposition velocity, and time product is exposed to contamination. However, the ease of transfer of MCPs from source to product, as given by transfer coefficients, may be missing. If this is so, then values can be obtained by the method advocated by Ljungqvist and Reinmuller[16].

To demonstrate how the NMD_A method can be used in a wide variety of cleanroom situations, a pharmaceutical cleanroom that uses a UDAF workstation and small-batch aseptic filling is given as an example, and the results summarised in **Table 1**. In this example, the higher risks are associated with personnel activities within the filling workstation, and the highest of these is caused by airborne contamination of vial closures within an open hopper, and subsequent transfer to product (8.1×10^{-6}). Use of a lidded hopper reduces this risk by approximately 16-fold to 4.9×10^{-7}. The risk from deposition of MCPs dispersed by personnel in the workstation is also high (5.5×10^{-6}). If a reduction in the levels of risk from personnel is considered necessary, a review of the associated risk factors will indicate where reductions can be best achieved. In this case, it may be appropriate to reduce the airborne concentration of MCPs by means of the use of a separative device, such as a RABS or isolator. Methods of managing risk in this situation, and in situations with different ventilation and manufacturing methods, will be considered more fully in the final article of the series.

The degree of risk from air supplied by the terminal HEPA filters in both the filling workstation and filling cleanroom was assessed. When the terminal air filters have no leaks, and the air conditioning plant supplies the air, the contribution from the supply air presents the lowest risk of product microbial contamination ($<1 \times 10^{-21}$) and the risk can be ignored. When there are no leaks in the workstation's supply filter system but the supply air is drawn from the filling cleanroom, there is an increase in the NMD_A to 2.8×10^{-11}.

Leaking filters were also assessed. The worst of these scenarios occurs when there is a leak in the HEPA filter directly above the product vials in the filling workstation, and when the supply air is drawn directly from the filling cleanroom and not the air conditioning plant. When a 100% leak occurs in these conditions, this gives the highest risk of product contamination (4.6×10^{-5}). However, when air is supplied from the air conditioning plant, the risk is substantially reduced by a factor of approximately 10^{11} to an NMD_A of 8.7×10^{-16}, which is caused by additional filtration in the air conditioning system prior to the filter with a 100% leak. A similar risk reduction for a HEPA filter with a 0.01% leak can also be achieved if the air is sourced from the air conditioning system. The risk of product contamination from a leaking HEPA filter within the filling workstation can, therefore, be effectively managed by using an

additional filter in the supply air.

This article only reports on the assessment of the degree of risk from airborne sources, and risks from surfaces and liquids will be considered in a second article. A final paper will consider the risks from all microbial sources in various types of healthcare cleanrooms, and methods of managing these risks.

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