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Microbiological contamination models for use in risk assessment during pharmaceutical production

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This paper describes the fundamental mechanisms of microbial contamination during manufacture of pharmaceutical products. Models are derived that describe air and surface contact contamination. These models can be used to develop and improve methods of microbial risk assessment. The use of the FMEA (FMECA) method of risk assessment is discussed and, when used with the correct risk factors, its use endorsed.

Introduction

The risk to a pharmaceutical product from microbial contamination must be controlled. Control methods, such as the use of aseptic manipulation techniques, cleanroom clothing and appropriate ventilation, are well-established and described in documents issued by the Regulatory Authorities[1]. However, there has been a recent interest in risk management systems. ISO 14698-1[2] requires the use of a formal risk management system to control microbial contamination. The FDA has recently issued a document advocating a greater use of risk assessment systems[3].

A number of systems exist for managing risk during manufacturing. These include Fault Tree Analysis (FTA)[4], Failure Mode and Effect Analysis (FMEA)[5], Hazard and Operational Studies (HAZOP)[6], and Hazard Analysis and Critical Control Point (HACCP)[7,8]. The first three systems were produced for the electrical and mechanical industries, but can be used in aspects of pharmaceutical production such as safety, reliability, and validation. However, they have been little used in assessing microbial risk to the product, although a FMEA method has been suggested[9], and its use is gaining popularity[10].

The HACCP system was developed for use in the food industry and is more easily applied in the pharmaceutical industry, as it uses principles familiar to those working there, and also considers control and monitoring methods as important parts of its system. If adapted for use in the pharmaceutical industry, the HACCP system offers a systematic way of assessing, controlling, and monitoring microbial risk[11].

All risk systems manage risk in a similar way, an important component being risk assessment, which calculates or assigns a degree of risk to a hazard. For a risk management system to work well, the risk assessment method must accurately estimate the degree of risk. To ensure this occurs, accurate models are required that define the actual variables governing the process and show how these variables must combine to correctly predict risk. If such models are available, microbial risk can be assessed by combining the best choice of risk factors, in the correct way. This paper derives such models.

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Fundamental microbial transfer model

The chance of microorganisms being transferred from a source to a product is dependent on the likelihood of them being dispersed, transmitted and deposited onto a product. To calculate the number of microorganisms that would be deposited during pharmaceutical production, as shown on **Figure 1**, onto a given area of product in a given time, **Equation 1** has been derived. This equation is universally applicable to all sources and routes.

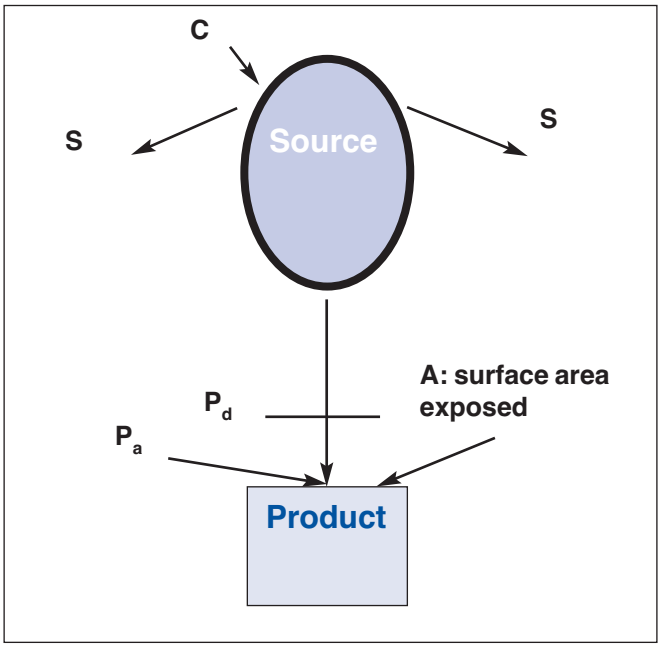

Figure 1. Transfer of microorganisms from source to product.

Equation 1

$$\text{No. of microbes deposited on a product} = C \times S \times P_d \times P_a \times A \times T$$

where:

- C = concentration of microbial contamination on, or in, a source (number/cm^2 for a surface, or number/cm^3 for air);
- S = the quantity of surface material, or air, that is dispersed, from a source in a given time (cm^2 /s for surfaces, and cm^3 /s for air dispersion); this can also be expressed as the quantity dispersed per frequency of occurrence;
- P_d = proportion of microorganisms dispersed from a source that are transferred to the area adjacent to the product;
- P_a = proportion of microorganisms in the adjacent area that are deposited per unit area of the product (/cm^2);
- A = area of surface onto which microbes are deposit (cm^2);
- T = time, during which transfers occur (s); this can also be expressed as frequency of occurrence.

A practical example of the mechanisms described in **Equation 1** would be the airborne transfer of skin microorganisms from personnel to a product. Here, the number of microorganisms that would deposit on a product would depend on:

(a) the concentration of microorganisms on personnel's skin surface;
(b) the surface area of the skin that is dispersed in a given time;
(c) the proportion of microorganisms dispersed that are able to pass through cleanroom clothing and transverse the air space to the area adjacent to the product (this is dependent on control measures);
(d) the proportion of microorganisms adjacent to product that would be deposited from the air in a given time onto a given area of exposed product; and
(e) the time over which this deposition occurs.

Derived deposition models

Equation 1 is a fundamental equation that governs deposition of microbes onto, or into, a product from all sources, and by all routes. However, in a cleanroom, the two main routes of microbial contamination are air deposition and surface contact, and it is important both from a theoretical and practical point of view to keep these two routes separate. Contamination by the liquid route is another possibility not considered here, but, if required, an analogous equation to that derived for airborne deposition can be used. Equations are now developed for the two main routes of contamination i.e. contact routes and air deposition.

Deposition of microorganisms by surface contact

An equation that will calculate the number of microorganisms deposited by surface contact can be derived from the fundamental **Equation 1** by combining the dispersion, transfer and deposition variables into one overall term i.e. 'transfer coefficient'. Thus, **Equation 1** can be reformatted to the following **Equation 2** that calculates the number of microbes deposited on a given area of product in a given time.

Equation 2

Number of microorganisms deposited by surface contact (no.) = microbes on contacting surface (no./cm^2) × transfer coefficient × area of product that is contacted (cm^2) × frequency of contact over the given time

where:

the 'transfer coefficient' is the proportion of microorganisms on a contaminating surface that is transferred to the product.

A practical example, shown in **Figure 2**, would be the contact of a contaminated gloved hand with a product, where the number of microbes transferred could be calculated from:

(a) the concentration of microorganisms on the glove surface;
(b) the proportion of microorganisms on the glove surface that are transferred to the product i.e. transfer coefficient;
(c) the area of the product that is touched and;
(d) the frequency of touching the product surface in the given time.

Unfortunately, all of the information required to solve this equation is seldom available, especially values for the 'transfer coefficient' and the 'frequency of contact' with the product. However, the main object of deriving this equation is to provide the correct contamination model on which a risk assessment method can be based.

Deposition of microorganisms by airborne route

Most of the microbe carrying particles in the cleanroom air come from the skin of personnel. People shed approximately 10^9 skin cells per day[12], these skin cells being approximately 33μm × 44 μm[12], and are found in the cleanroom either as whole cells or fragments. A proportion of these skin cells contains microorganisms, and cleanroom personnel can disperse through cleanroom clothing several hundred microbe carrying particles per minute[13]. For these reasons, microorganisms are normally found in cleanrooms attached to skin particles (or very occasionally a clothing fibre). There is a spectrum of sizes to be found in the cleanroom air, but the average size, expressed as an equivalent particle diameter, is similar to

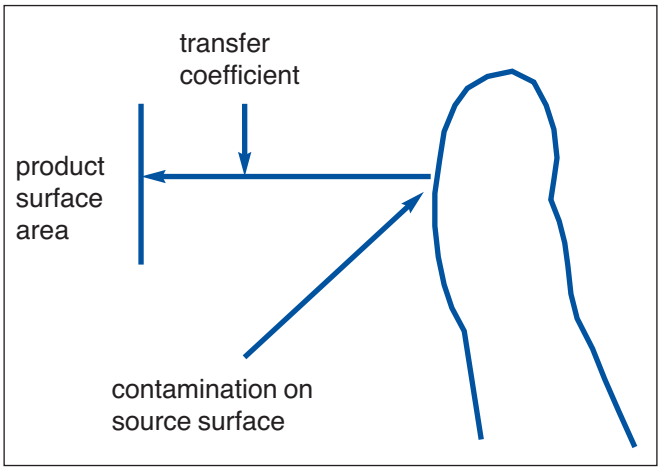

Figure 2. Mechanism of surface contact transfer.

skin cells. Owing to skin cell fragmentation, and the varying effect of the filtering action of cleanroom clothing and ventilation, the average size of microbe carrying particles will vary between about 8μm and 20μm[14, 15, 16], this size often increasing as the rate of ventilation increases. These sizes of particles have quite high deposition velocities of between 0.2cm/s and 3cm/s, the deposition velocity being the velocity that microbe carrying particles in the air will deposit into, or onto, the product.

The mechanism of airborne deposition is illustrated in **Figure 3** and expressed mathematically in **Equation 3**, where the number of airborne microbes deposited on a given area over a given time can be calculated. **Equation 3** is derived by combining the first three components of **Equation 1** to give the concentration of microorganisms found adjacent to the product. This is a useful approach, as this is a value measured in the cleanroom by volumetric air sampling. The proportion deposited can then be calculated from knowledge of the deposition velocity, using a time component expressed as the time the product is exposed to airborne contamination.

Equation 3

Number of airborne microorganisms deposited onto the product (no.) = airborne microbial count (number/cm^3) × deposition velocity of microbes from air (cm/s) x area of product exposed (cm^2) × time of exposure (s)

To solve **Equation 3** it is necessary to know the deposition velocity of the microbe carrying particles in the air surrounding the product. This is generally unknown, although it can be calculated from a knowledge of the volumetric and settle plate counts[16]. However, by combining the first two variables, **Equation 3** can be simplified to the following:

Equation 4

Number of airborne microorganisms deposited onto the product in a given time (no.) = Deposition rate (no./(cm^2.s)) × area of product exposed (cm^2) × time of exposure (s)

Settle plates exposed adjacent to the product give the number of microbe carrying particles that deposit onto a known area of settle plate exposed for a given time. This information can be easily recalculated as a deposition rate of no./(cm^2.s). The likely number of airborne microorganisms that will deposit onto a product can then be calculated.

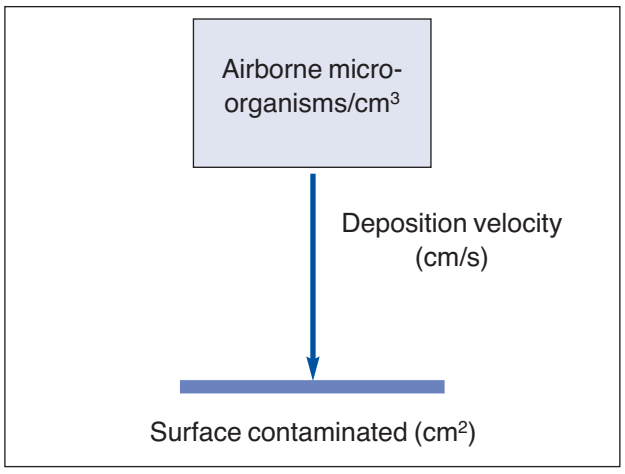

Figure 3. Mechanism of airborne deposition

Equations 2 and **4** are the best equations to use to calculate the amount of surface contact or airborne deposition onto a pharmaceutical product. If numerical values e.g. microbial concentrations, or the number of times a product is touched, are available to substitute into the equations, then the most accurate solution can be obtained. However, numerical values required to mathematically solve **Equation 2** for surface contact are generally unavailable, although those required for the airborne deposition equation should be available. If they are not, then a solution can be found by substituting risk descriptors. These risk descriptors are surrogates for the actual numerical values and are often verbal descriptors of risk e.g. 'high', 'medium' and 'low'. Both numerical values and risk descriptors should be chosen from the information available to best reflect the required equation variables and their relative importance to each other. How this might be done is described in a further article in this journal[17].

Correlation of the contamination models with criticality risk assessment

The Failure Mode and Effect Analysis (FMEA) method has been in use since the late 1940s, but in 1985 the International Electronic Commission published a report on a procedure for Failure Mode and Effect Analysis (FMEA)[5]. This report, which has the status of an ISO standard, is considered to describe the definitive method of FMEA. The report also includes a description of Failure Mode and Effect and Criticality Analysis (FMECA) that incorporates a method for assessing the 'criticality' of risk. In this, risk is defined by two components, namely, its 'criticality' and 'frequency' of occurrence. Criticality may be considered to be the degree of risk from an undesirable event i.e. its undesirability or importance. Frequency can be considered as the number of times an event occurs, or the length of time over which it occurs. This concept is shown graphically in **Figure 4**, where it can be seen that as the 'criticality' and 'frequency' of occurrence increases, either separately or in combination, the risk will increase.

Risk may be defined mathematically in **Equation 5**.

Equation 5

Risk = criticality of the occurrence × frequency of occurrence

To use **Equation 5** to assess risk from microbial contamination requires a definition of the words 'frequency' and 'criticality', as interpretation of the meaning of these words varies between manufacturing industries and applications. In the previous sections of this paper, the fundamental equations that predict the microbial contamination of a product were derived. These

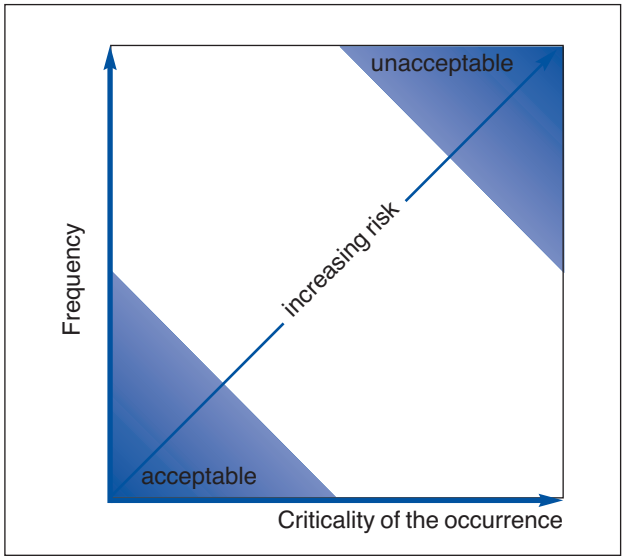

Figure 4. Increase in risk caused by an increase in the frequency and the criticality of an occurrence.

equations show that the amount of microbial contamination is dependent on:

(a) the concentration of microorganisms on a contaminating surface, or within air;
(b) how much of this contamination is dispersed and transferred to the area next to the product;
(c) how much is deposited onto the product and;
(d) a variable of time.

A combination of the variables 'a', 'b' and 'c', should be considered to express 'criticality' in terms of microbial contamination, i.e. the likelihood that microbes will be dispersed, transferred and deposited onto the product. If the time is taken as the time available for contamination to occur, or the frequency of an occurrence of contamination, then it may be seen that **Equation 5** is, in essence, the same as the fundamental equations of microbial contamination derived in this paper. The correctness of the use of the FMECA criticality risk assessment method when working with microbial contamination is thus clarified and, when used with the correct risk factors, its use endorsed.

Choosing the correct microbial risk assessment model

The Failure Mode and Effect Analysis (FMEA) method of risk assessment, as outlined by Keiffer[9], has been gaining popularity[10], and is used in safety, reliability, and validation, as well as in microbiological contamination. Although this method is called FMEA in the Keiffer paper it would be considered a Failure Mode and Effect and Criticality Analysis (FMECA) method in the IEC document. It is considered by Keiffer that:

Equation 6

Risk (priority number) = probability × severity × likelihood of detection

Equation 6 is similar to **Equation 5** in that 'probability' can be considered to be 'frequency' and 'severity' to be 'criticality', but there is an additional third variable of 'detection'. A risk assessment based on **Equation 6**, and using a third additional variable of 'detection', works well in aspects of engineering manufacturing such as reliability of electronic goods, where 'detection' of a faulty circuit is clearly relevant to the likelihood of identifying the breakdown of the product. It is also useful in controlling non microbiological aspects of pharmaceutical production, and by measuring pH, optical properties, weight etc. faults in the process can be detected and the risk reduced. However, it is not possible to 'detect' microbial contamination of a product during manufacture. Even if this was possible, its detection could not be used to modify the actual degree of risk, or likelihood of contamination. It is also possible to interpret 'detection' as the frequency of monitoring of risks. This interpretation is incorrect, as frequency of monitoring will not affect degree of risk; it will only measure the concentration of microorganisms with more surety. For the reasons outlined in this paragraph, we consider that the factor designated 'likelihood of detection' should not be used to assess the 'risk' of microbial contamination and **Equation 5**, an equation without the 'detection' variable, used. Also, if the detection variable is left out, the risk assessment model will be in agreement with the fundamental contamination models derived in this paper.

Practical application of models

The authors of this paper have used the models derived in this paper to assess microbial risk in cleanrooms. Several cleanrooms have been assessed, and this experience suggests that risk assessment is best carried out in two stages. The first stage should be carried out using an overall risk assessment that allows all of the risks within the manufacturing suite to be assessed. This can best be done if a risk assessment, using suitable numerical values and risk descriptors, is combined with the HACCP system to give a structured method by which all the microbial risks are established, assessed, action and alert levels set, the correct type of monitoring and frequency established, and the effectiveness of the system verified[11].

Use of the overall risk assessment method will normally demonstrate that the highest risk is at the critical area, when the product is open and exposed to microbial contamination. Therefore, it is best to carry out an additional risk assessment of this critical area. Knowledge of the fundamental mechanisms of microbial contamination given in this paper, as well as practical experience, shows that this must be done by separating airborne from surface contact contamination. The most accurate solution is to solve **Equations 2** and **4** using numerical values from every source of contamination in the cleanroom using each method of transfer. This is possible with airborne contamination, as the combined sources of airborne contamination can be measured adjacent to the product and their deposition calculated, but it is not possible with surface contact. Information on the concentration of microbes on surfaces will be available,

although almost certainly incomplete, but practically no information will be available on microbial dispersion, transfer and deposition, and how frequently it occurs. A numerical solution is therefore not possible and risk descriptors that act as surrogates for the required numerical values should be used. In practical terms this means that for each stage of production, the concentration of microbes on the sources, the likelihood of microbial transfer, the area open to contamination, and the frequency of occurrence is assessed by the use of suitable risk factors that may be either numerical values or risk descriptors. These factors should be combined in a way that best reflects the contamination models so as to give an accurate risk rating. Examples of these methods will be reported in a further article in this journal[17].

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