Biosafety performances of biotechnology equipment: Consideration of Performance Criteria and of Equipment Categorisation.

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SUMMARY

This paper identifies leak tightness, cleanability and sterilisability as the characteristics of biotechnology equipment which are of primary importance in biosafety. Performance
criteria which assign equipment to one of three classes in each of these respects are defined. Leaktightness classes have a uniform definition over the range of biotechnology processes; cleanability classification is recognised to depend on the nature of the soil and of the cleaning protocol, and thus will vary with the intended use. A distinction is drawn between equipment which can be sterilised and that which cannot, but in which target organisms can be destroyed. It is proposed that these criteria could form the basis of biosafety classification of units of equipment and of whole plant.

1. INTRODUCTION

Biotechnology equipment plays a vital role in determining process integrity, product quality and in preventing harm to people and the environment. Technical performance criteria for equipment are well established and described in standards and guides to Good Manufacturing Practice. Criteria for biological safety are not well established and an approach to developing them is discussed here. The paper is not concerned with other aspects of equipment safety, which are well regulated by existing Directives, regulations, standards and guidelines, nor primarily with the many aspects of product quality where equipment performance is important. However, the criteria defined below impinge on product quality because they affect control over the manufacturing process, for example by affecting the penetration of contaminant organisms into production equipment.

Performance criteria standards for biotechnology equipment are being developed by the European Standardisation Organisation, CEN, as part of the work of its Technical Committee 233 on biotechnology (10). This paper presents the views of the authors, building on the discussions of Working Group 4 of this committee, which is responsible for drafting the standards for equipment.

Biosafety criteria affect both the new and the traditional biotechnology industries and care is needed to avoid harming the traditional activities such as brewing, wine production, waste water treatment, etc., by producing criteria which are too stringent or unrelated to the risks presented by these industries.

2. SIGNIFICANT FACTORS.

Hazardous or potentially hazardous living organisms and/or their products may cause harm when they are released during:

- primary production;
- downstream processing;
- cleaning and maintenance operations.

The important properties of equipment which affect release are:
- leak tightness during primary and downstream processing;
- cleanability, which affects whether undesirable organisms or products are present during servicing/maintenance and
- sterilisability, which determines whether relevant organisms can be destroyed when necessary.

These properties interact. Thus residues present after unsatisfactory cleaning can reduce the effectiveness of a sterilising procedure and unclean equipment may have an increased tendency to leak.

Because of their importance, criteria for leak tightness, sterilisability and cleanability need to be defined. Accordingly, the Commission of the European Union has mandated the European Standards Organisation, CEN, to do this for 21 units of equipment. This decision underlines the significance of actual performance, rather than design. Undue insistence on existing design criteria may affect biotechnology negatively in the longer term.

The questions which arise are:

a) how can performance be defined and
b) how can equipment which performs in different ways be categorised.

3. QUANTIFICATION

We believe that performance should be defined quantitatively where possible. Operators and regulators will then know what is required, avoid subjective decisions and will be able to audit processes from a biosafety viewpoint. If such quantitative criteria cannot be developed, semi-quantitative or widely accepted qualitative criteria should be adopted.

The boundary conditions are that a unit of equipment should be leaktight, sterilisable and cleanable either to a very high degree or to a very low degree. Thus a fermenter may be categorised as completely leak tight or as failing to provide biological containment. It may be cleanable so that soil is undetectable or it may be impossible to clean. It may be sterilisable so that all living organisms are destroyed or be impossible to sterilise.

Some of the wide range of biotechnology processes require the highest possible performance and others lower performance. Thus complete leaktightness may be essential for fermenters used with a hazardous organism but for many traditional biotechnology processes - brewing, pickling, etc - containment is unimportant from the biosafety viewpoint.
A high degree of cleanability is essential for the manufacture of some injectable products; the requirements can usually be much less stringent for plant in which industrial or agricultural chemicals are to be manufactured.

High sterilisability is often needed, especially for processes of long duration using slow growing organisms in rich media and also when the organism used must be destroyed before the plant is opened for routine maintenance. Sterilisation may not be important (or may be undesirable) in traditional processes and may be impossible for example in domestic waste water treatment or when wooden vessels are used.

Intermediate situations between the extreme boundary conditions also exist. A fermenter may not be sterilisable but may be treatable to remove important organisms. For example, non-sporing bacteria or yeasts may be the target organism but complete sterilisation is not required to destroy them. When organisms rapidly generate inhibitory media, high sterilisability may not be required; many yeast fermentations are of this type. In some circumstances in the traditional biotechnologies, such as waste treatment, the persistence of significant production organisms from batch to batch is essential. Many processes need plant which is clean but not to the maximum extent; a degree of leak tightness less than the maximum is commonly sufficient and acceptable from a biosafety point of view.

It is concluded that it is necessary to define the extreme boundary conditions and at least one intermediate condition of leaktightness, cleanability and sterilisability.

A principal difficulty is that of quantifying the boundary conditions. That this is difficult is instanced by the use of terms such as "minimise" or "strongly minimise" by OECD (1) and NIH (3), and in national regulations (eg 2) designed to implement the Contained Use (4) and Biological Agents (5) European Directives, in relation to the release of potentially hazardous organisms. The possible levels are discussed below for the three significant parameters.

Leaktightness

Both the European Commission and the National Institutes of Health have laid down broad requirements for biotechnology plants, in relation to the degree of perceived hazard.

Thus in Directive 90/219/EEC (4) and the associated guidelines (5) there is a requirement to "minimise release", to "strongly minimise release" or to prevent release for different groups of organisms. In the revised NIH guidelines (3), similar terminology is used. These terms present difficulties to those who need to use them, for there is no objective definition of their meaning. The availability of quantitative criteria would be of great value to plant operators, machinery manufactures, designers and regulators.
The regulatory intent is that, in some circumstances, there shall be no release of organisms from biotechnology plant. This represents one boundary condition. While this objective is generally regarded as unattainable in practice (7, 11, 12), it is reasonable to restate it as "organisms originating in the biotechnology plant should not be detectable in the workplace and/or the environment". This is the criterion for the highest category of leaktightness. The opposite boundary is that of no containment. One or more intermediate conditions may be defined, when there is some detectable leakage, combined with appreciable containment. This means that organisms will be detectable in the workplace/environment surrounding biotechnology equipment but that the number is substantially reduced compared with the situation of no containment. This situation may be acceptable when the risk (being hazard x exposure) is acceptably low. Such an approach is taken in assessing the performance of safety cabinets (6,8). The number of such intermediate categories could be large but in practice it is advantageous to define only one. The numerical evaluation of this is discussed below.

**Sterilisability**

Formally, to sterilise means to destroy all organisms present in the equipment. In practice, because different processes have different objectives, the term is used not only in this sense but also with the meaning that unwanted organisms have been destroyed. Thus it is required that some plants are completely sterilised with any living organisms present being destroyed; this is one boundary condition and will be required when release or survival of any organism present is considered to be a major hazard to safety or to the product. At the other extreme, some traditional processes can operate without procedures to destroy organisms, because there is no organism present which is believed to be a hazard to people, the environment or the product. This is the other boundary condition.

An intermediate situation is where plant can be treated to remove unwanted organisms, though absolute sterilisation cannot be achieved. Equipment which can be pasteurised but not sterilised is in this class. The key criterion here is that a defined target organism can be destroyed.

Again, the interests of practicability indicate that only one intermediate class should be created.

**Cleanability**

Absolute cleanliness, defined as the absence of any contaminating material on the surfaces of the equipment, cannot be achieved. There are thus parallels with the leaktightness situation, where absolute containment cannot be expected. As in this latter case, it seems that one boundary condition for cleanability must be the inability to detect contaminating material, following the BATNEEEK principle (Best Available Technology Not Entailing
Excessive Cost).

Contaminating soil is always a mixture of substances, which vary in their ease of removal; cleanability must therefore always be assessed by measurement of a named substance. This must be specified with care and again with the concept of BATNEEC applying. It also seems appropriate to include visual observation as one factor when assessing cleanability, because of its sensitivity in some circumstances and because it may be poorly matched by complex and expensive technology. In addition, the cleaning methodology must be defined, because this has a major influence on the extent to which contamination can be removed.

As a result of these considerations, the cleanability of equipment cannot be regarded as an absolute characteristic, in that it may be very high for some contaminants and low for others. Thus equipment made with poor surface finish may be highly cleanable with one soil and poorly cleanable with another.

QUANTITATIVE EXPRESSION OF LEAKTIGHTNESS

While no detectable leakage may sound a rigorous condition, its significance is determined by sensitivity of the assay method. Factors such as the low recovery rate of small particles, the loss of viability of organisms in air streams, the difficulty of ensuring growth of stressed organisms on agar media, especially when they have been genetically constructed to be disadvantaged in the environment, mean that in some circumstances recovery may be very low, especially for small bacteria. Recovery of only 1% of released organisms is not uncommon. In other circumstances (eg 9) more than half of the organisms released have been recovered.

The significance of these considerations is that the failure to detect organisms in the workplace may still reflect appreciable release. Many biotechnology plants have 1000 cubic metres of air in the workplace. When 1 cubic metre is sampled and the efficiency of recovery is 1%, at least 100 organisms must be present in the sampled air to allow detection. This means that the air in the plant as a whole must contain at least 100,000 target organisms. When air changes result in a fivefold dilution per hour, then 500,000 must escape per hour to allow detection. This is leakage per hour of 0.5 ml of medium containing $10^6$ organisms per ml. or $10^12$ per cubic metre. In this case, failure to detect organisms in the environment means only that the leakage is less than 25 ml in a 50 hour fermentation period.

For a fermentation plant containing 25 m$^3$ of microbial suspension, this represents less than 0.0001% of the volume over the whole period, or leakage of <0.00005% per day or <0.000002% per hour. This may be expressed as a leak factor or, by analogy with the approach taken in safety cabinet studies, in terms of a protection factor, defined as the
ratio:

\[
\text{Organism concentration in the fermentation medium} \\
\frac{\text{Organism concentration in the air}}{}
\]

This has a value (per metre cubed) of $10^{12}/10^2 = 10^{10}$ for the situation described above.

This clearly represents very substantial containment, even though release is not completely prevented. There are many factors which determine the value of the true protection factor in the context of no detectable release. These include the fact that leaked organisms will not be evenly distributed and that the volume of air in the plant will not bear a constant ratio to the volume of microbial suspension in the contained vessel.

As detection technology improves, for example via PCR technology, the quantitative meaning of "no detectable leakage" could change, though it must be emphasized that practical criteria must relate to methodology which can readily be applied and is not prohibitively expensive or too complex for routine use.

The intermediate condition of some leakage coupled with appreciable containment needs to be considered. The use of a protection factor value seems to be valuable here. The concept is already used in relation to safety cabinets, where containment which gives a protection factor of $10^5$ is regarded as a desirable value. If higher protection is needed, the use of glove boxes, which provide higher containment, is recommended (6). For biotechnology plant the concentration of organisms in the fermenter may be high and their absolute concentration in air, rather than the fraction escaping, may be significant for worker's health. A higher protection factor than $10^5$ thus seems appropriate for the equipment in the intermediate class; it is suggested that to be classified in the intermediate class, the protection factor should be at least $10^8$. In the conditions described above, this would mean that leakage of 0.002% of the fermenter contents could be tolerated per hour from equipment.

**CATEGORIES AND CRITERIA**

The degrees of leak tightness, cleanability and sterilisability have been expressed as three categories, in each case. These are expressed in Tables below, in two ways. For the boundary conditions for each parameter, there is no difficulty in defining the criteria: the ones quoted represent those currently being considered by Working Group 4 of CEN Technical Committee 233. The intermediate classes present some difficulty in the case of leak tightness and sterilisability and for these we quote both the qualitative approach currently being followed by the above Working Group (Column (a)) and the quantitative...
values suggested by the present authors Column (b). We recognised that both may require modification on the basis of experience but at the time of writing they appear not only to be reasonable but also to of value as the first step in a wider discussion of the topic.

**TABLE 1. PERFORMANCE CATEGORIES FOR LEAKTIGHTNESS**

<table>
<thead>
<tr>
<th>Performance category</th>
<th>Performance criterion (a)</th>
<th>Performance criterion (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-A</td>
<td>Open equipment or release level not specified</td>
<td></td>
</tr>
<tr>
<td>L-B</td>
<td>Release tested under defined conditions</td>
<td>Release restricted to give a protection factor of at least $10^8$ (1)</td>
</tr>
<tr>
<td>L-C</td>
<td>Release not detectable</td>
<td>Release not detectable (2)</td>
</tr>
</tbody>
</table>

1) Based on comparison of microbial concentrations inside the fermenter and in the workplace air.
2) Based on tests following the principles of BATNEEC (best available techniques not entailing excessive costs)

**Table 2: PERFORMANCE CRITERIA RELATED TO CLEANABILITY**

<table>
<thead>
<tr>
<th>Performance category</th>
<th>Performance criterion (a)</th>
<th>Performance criterion (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-A</td>
<td>Visible soil or cleanability level not specified</td>
<td></td>
</tr>
<tr>
<td>C-B</td>
<td>Cleanability level specified in terms of defined soil and cleaning protocol</td>
<td>95% of defined soil removed by defined cleaning protocol</td>
</tr>
<tr>
<td>C-C</td>
<td>Defined soil not detectable (1) after application of a defined cleaning protocol</td>
<td></td>
</tr>
</tbody>
</table>

(1) Based on tests following the principles of BATNEEC (best available techniques not entailing excessive costs)
(1) assessed by BATNEEC

Table 3. PERFORMANCE CRITERIA RELATED TO STERILIZABILITY

<table>
<thead>
<tr>
<th>Performance category</th>
<th>Performance criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI-A</td>
<td>Sterilizability of equipment not specified</td>
</tr>
<tr>
<td>SI-B</td>
<td>Equipment cannot be sterilised but defined target organisms can be destroyed</td>
</tr>
<tr>
<td>SI-C</td>
<td>Equipment can be sterilised</td>
</tr>
</tbody>
</table>

DISCUSSION

In biotechnology, as in other industries, some processes are held to be hazardous to a greater or lesser degree. Where there is risk of harm to people or to the environment, measures are taken to minimise the risk by the selection of suitable operating conditions and appropriate equipment. Regulatory authorities have the responsibility of defining the degree of risk; plant designers and managers have the responsibility of constructing and operating the plant appropriately. Equipment characteristics dominate plant performance but, until recently, little attention has been paid to defining the biosafety performance criteria which make equipment suitable for particular operations. In this paper, criteria have been defined for leaktightness, cleanability and sterilisability of equipment. Three classes have been specified in each case. They are differentiated by possessing the property to a high degree, to an intermediate extent or not at all. It is hoped that the classification of equipment in this way will allow suppliers to define their products more precisely with regard to biosafety. Equally, users of equipment will be able to select for the intended use on the basis of performance and monitor performance in practice against agreed criteria.

The application of the classification scheme requires appropriate test methods and, while these exist to some extent, it is clear that more research is need before there is a fully adequate methodology. The insensitivity of existing testing methods is discussed in relation to leak tightness; here it is clear that failure to detect escaped organisms can coexist with appreciable lack of containment.
ACKNOWLEDGEMENT

We have valued the opportunity to discuss performance criteria for equipment with colleagues in Working group 4 of Technical Committee 233 of the European Standardisation Organisation, CEN.

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