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Categories of Large-Scale Containment for Manufacturing Processes with Recombinant Organisms

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Introduction

Why is it necessary to review the measures needed for the large-scale containment of genetically engineered organisms? The guidelines issued should be clear enough and a review ought not to undermine them. Not that I would wish to do that, particularly to documents that I have had a hand in drafting. A general review is also unhelpful in applying the guidelines because that must, at a large scale, depend on an analysis of the case in question. 'Horses for courses' is the old saw that applies. Moreover, the guidelines themselves are in a state of flux; this year's review, even if it were up-to-date with all the recent changes, would be next year's history. The question remains: 'Why bother with a review?'

There is perhaps one reason. The guidelines will describe a set of containment measures structured into a hierarchy of increasing stringency. Anyone who compares one set of guidelines with another will find subtle differences between them. Occasionally the impact on their application is out of all proportion to the differences themselves. A review would be useful if it could provide an approach to containment which highlights these differences, and which creates a logical framework for a hierarchy of containment measures. My recent experience in helping to plan a contained plant for the

Abbreviations: ACDP, Advisory Committee on Dangerous Pathogens (United Kingdom); ACGM, Advisory Committee on Genetic Manipulation (United Kingdom); COSHH, Control of Sustances Hazardous to Health; GILSP, Good Industrial Large Scale Practice; GLSP, Good Large Scale Practice; GMAG, Genetic Manipulation Advisory Group (United Kingdom); GManP, Good Manufacturing Practice; GMicP, Good Microbiological Practice; HASAW Act, Health and Safety at Work, etc. Act; HAZOP, Hazard and Operability (study); HEPA, high efficiency particulate air (filter); HSE, Health and Safety Executive (United Kingdom); NIH, National Institutes of Health (United States); OECD, Organisation for Economic Cooperation and Development; SMicT, Safe Microbiological Technique.

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recovery of microbial products convinces me that such a framework is essential if any set of guidelines is to be applied in a consistent fashion.

A word of caution is necessary. Throughout this article I have reproduced in a shortened form parts of the various guidelines for handling recombinant organisms. This was necessary to illustrate particular points but the extracts are not a substitute for the guidelines themselves and the comments must not be taken as a summary of them.

Lord Robens and the Gordon Conference

Our current attempt to regulate the use of genetically engineered organisms has two strands, both of which begin in the early 1970s. One is social, and was clearly expressed in the United Kingdom through the 'Robens' Report' which set out a new approach to safety at work (Robens' Report, 1972). The other is scientific and arose out of the Gordon Research Conference on Nucleic Acids held in the USA in 1973 (Singer and Soll, 1973). They continue to influence the discussion not least because they both insisted on a prospective rather than a retrospective analysis of the hazards which underlie new technology.

Until that time, as the Robens' Report (1972) pointed out, it had been 'a melancholy fact that much of our preventative legislation was introduced following events that were both unforseen and disastrous' (para. 19). To be fair, it was an approach which had served well.

> This practical, empirical approach has been associated with the development of high standards of safety and health protection, and the attainment of a degree of systematic official supervision which is probably unsurpassed anywhere in the world. Paradoxically, however, this source of strength is also a source of weakness. It results in a body of detailed law which must be constantly extended to deal with new problems. In an age of rapid change in industrial structures and technologies as well as in social attitudes and expectations this traditional empirical approach cannot keep pace. (para. 23)

The new approach which the Robens' Report (1972) advocated embodied an explicit wish to identify the hazards in advance; '. . . the nature of the problems must be methodically assessed and the assessments translated into practical objectives and courses of action' (para. 45). New legislation followed the Report. The Health and Safety at Work etc. Act 1974 (HASAW, 1974), placed a duty on the manufacturer '. . . to carry out or arrange for the carrying out of any necessary research with a view to the discovery and, so far as is reasonably practicable, the elimination or minimisation of any risks to health and safety to which the design or article may give rise' [para. 6(2)]. This duty remains following the amendments that were later incorporated in the Consumer Protection Act (1987).

This emphasis of the new Act (HASAW, 1974) on the need to explore the dangers of technological advances, was closely allied to the attitudes expressed

by the group who had publicly launched a revolution in molecular genetics at the previous year's Gordon Research Conference on Nucleic Acids (Singer and Soll, 1973).

The Conference gave a hearing to several papers that showed that DNA molecules from separate sources could be covalently joined and transferred between living organisms. This technique opened up the prospect, with which we are now so familiar, of expressing the information contained within hybrid molecules of DNA whose synthesis and location, would, from then on, be under experimental control. A majority of the delegates to the conference recognized that the technique was fraught with unpredictable hazards. Their concern was expressed in letters (Singer and Soll, 1973) to the US National Academy of Sciences and the US National Institute of Medicine. They wrote '. . . that these experiments offer exciting and interesting potential both for advancing knowledge of fundamental biological processes and for the alleviation of human health problems. Certain such hybrid molecules may prove hazardous to laboratory workers and to the public. Although no hazard has yet been established, prudence suggests that the potential hazard be seriously considered. . .'. The Academies were asked to '. . . establish a study committee to consider the problem and to recommend specific actions or guidelines. . .'. The parallel with the HASAW Act (1974) is obvious.

The US National Academy of Sciences responded and set up their Committee on Recombinant DNA Molecules. Its immediate impact was the 'voluntary embargo' on some of the possible manipulations of DNA (*Table 1*; Berg *et al.*, 1974). While this was important psychologically, of more lasting importance was the underlying attempt to classify experiments with recombinant DNA.

The embargo forbade the introduction of antibiotic resistance into plasmids or strains of bacteria not known to carry that particular trait. Similar experiments were permitted with strains where the resistance was known to occur naturally. The embargo also extended to some experiments with DNA

Table 1. The classification of experiments in genetic engineering—the Voluntary Embargo (Berg *et al.*, 1974)

Type 1 Experiments—Construction of new plasmids			
FORBIDDEN			
Plasmids carrying	antibiotic resistance or bacterial toxin synthesis	into	bacterial strains not known to carry these traits
ALLOWED			
Plasmids carrying	antibiotic resistance	into	bacterial strains known to carry these traits
Type 2 Experiments—Synthesis of self-replicating DNA			
FORBIDDEN			
Introduction of segments of	oncogenes or animal viruses	into	bacterial plasmids or other viral DNA
ALLOWED (WITH CAUTION)			
Introduction of segments of	animal DNA	into	bacterial plasmids

coding for bacterial toxin synthesis, oncogenes or animal viruses. No outright ban on the handling of DNA from animals was suggested, although the Committee advised that all such experiments should be done with caution (*Table 1*).

The latter group were the subject of considerable debate and some biologists did argue for their separate classification (as Type 3 experiments) and for an outright ban (Wade, 1974). The Committee seems to have recognized that their powers of enforcement were limited to moral pressure, and that their embargo would stand only through the general scientific acceptance of the experimental classification that it had created. A voluntary embargo, which would be too readily ignored because of disagreement over the hazards involved, is of little use; better to accept one based on a general agreement that some experiments could have a dangerous outcome, particularly as the dangers were not proven. Moreover, the product of some Type 3 experiments could be a protein of therapeutic and commercial value whose exploitation would be highly desirable.

The dilemma is an inevitable consequence of prospective safety legislation based on possible hazards rather than on an analysis of the effect of known ones. It is hardly surprising that the Committee should have wanted it resolved. It asked (Berg *et al.*, 1974) the US National Institutes of Health (NIH) to oversee an experimental programme designed to evaluate:

1. The biological and ecological hazards of recombinant DNA molecules; and
2. The procedures designed to minimize their spread within human and other populations.

Again the parallel with the approach of the HASAW Act (1974) is clear. These should be important questions in any debate about the safety of recombinant DNA. They have a direct bearing on the final recommendation from the Committee, that the NIH should set out guidelines for investigators handling the molecules.

The Asilomar Conference

The first attempt to create such guidelines was made at the Asilomar Conference in the following year (Berg *et al.*, 1975). The delegates took the view that containment should become an essential feature of experimental design, and that the effectiveness of the containment should match, as closely as possible, the estimated risk. The publication which followed (Berg *et al.*, 1975) formulated a hierarchy of increasing containment to match the estimated risks of the technology (*Table 2*). It also described a hierarchy of experimental techniques likely to create those risks (*Table 3*), which were seen to derive as much from the recombinant DNA itself, as from the recombinant organisms. The need for the containment, and particularly for inactivation of the biological agent, therefore applied to the DNA as well as to its host.

The containment was of two kinds, one physical, the other biological. The physical containment ranged from Good Microbiological Practice (GMicP) to

Table 2. The Asilomar containment levels (Berg *et al.*, 1975)

Containment level	1	2	3	4
Level of risk	Minimal	Low	Moderate	High
	Accurately assessed and minimal	Novel biotypes but no evidence of pathogenicity or environmental effect	Significant potential of pathogenicity or environmental damage	High potential of pathogenicity or environmental damage
Physical containment	Disinfection of waste			Inactivate waste
	Plugged or mechanical pipettes			
		No mouth pipetting		
		Biological safety cabinets where aerosols likely		
			Biological safety cabinets for all transfers	
			Protect vacuum lines with filters	
			Negative pressure in laboratories	
				Filter exhaust air
				Isolation with airlocks
Method of work		No eating, drinking or smoking in laboratories		
	Wear laboratory coats		Wear gloves	Change clothes
		Access limited to laboratory personnel		
				Use protective clothes
				Shower on exit
Biological containment		Safe vectors where available	Disabled vectors only	Vectors whose growth is limited to the laboratory

Table 3. The classification of experiments in genetic engineering—the Asilomar Classification (Berg *et al.*, 1975)

Level of risk	Minimal	Low	Moderate	High
Transfer of prokaryotic DNA to prokaryotes	Exchange of DNA between strains which exchange it naturally	Exchange of DNA between strains where this does not occur naturally (novel biotypes)	Novel biotypes where the transferred DNA affects pathenogenicity, therapeutic treatment, antibiotic resistance or relationship with the environment	
Transfer of animal DNA to prokaryotes		Purified animal DNA where the DNA performs a known function	Purified animal DNA where the DNA codes for a hazardous product Random cloning of animal DNA	Uncharacterized DNA from warm-blooded animals and particularly primates
Transfer of DNA into animal cells			DNA from viral or low-risk agents with low-risk animal DNA as a vector (e.g. viral or mitochondrial)	

the more stringent measures thought necessary for handling uncharacterized primate DNA. It is too often forgotten that the Conference took the view that 'Stringent physical containment and rigorous laboratory procedures can reduce but not eliminate the possibility of spreading potentially hazardous agents.' The alternative was to provide biological barriers to contain the recombinant organism itself or to limit the spread of the recombinant vector (*Table 4*). Indeed at the less stringent end, where there is only minimal risk, some release is almost inevitable and the only realistic containment is provided by the biological barriers, most of which are the natural defences of one organism against another.

Table 4. Biological barriers limiting the spread of recombinant organisms (Berg *et al.*, 1975)

Fastidious hosts unable to survive outside of the laboratory
Fastidious vectors which are non-transmissable, except in specified hosts

Where these biological barriers are crucial their efficiency needs to be checked, but it is not difficult to see in this the seeds of a paradox about containment that needs to be resolved. If the physical containment is unreliable, it must always be complemented with biological barriers; but if the biological barriers are effective, why do we need the physical containment; and if they are not effective, what containment is left?

Table 5. Experiments to be deferred under the Asilomer classification (Berg *et al.*, 1975)

The cloning of DNA	1. from highly pathogenic organisms 2. which codes for toxins
The manufacture of products harmful to man, animals or plants at greater than a 10 l scale	

Concerns of this kind, while not explicitly stated, found their expression in a continuing, if more restricted, embargo on some work (*Table 5*). Its main effect was to establish the concept of an upper limit of 10 l on the scale of laboratory experiments. Here was the explicit assumption that large-scale processes were 'riskier than equivalent experiments done on a small scale and therefore require more stringent containment procedures'.

The OECD Guidelines

The Asilomer and Gordon Conferences and the Robens' Report are the roots from which our current guidelines have grown. Over the following 10 years, as research experience accumulated, so the initial concerns abated, but as the manufacture of proteins with recombinant organisms grew in scale it became necessary to control the larger-scale processes. This did not take place in a vacuum, uninfluenced by other developments. Those in microbiology have been well reviewed (Collins, 1984), but the evolution of the code of Good Manufacturing Practice (GManP) for the pharmaceutical industry (Sharp, 1983) is also relevant to the large-scale work because of the nature of the

products that the recombinant organisms are designed to make. The result of this activity is several sets of containment hierarchies of which those recommended in the Organisation for Economic Cooperation and Development (OECD) Report (1986) are typical.

Table 6. The principles of Good Industrial Large Scale Practice (OECD Report, 1986)

1. To keep the workplace and environmental exposure to any physical, chemical or biological agent to the lowest practicable level
2. To exercise engineering control measures at source and to supplement these with appropriate personal clothing and equipment when necessary
3. To test adequately and maintain control measures and equipment
4. To test when necessary for the presence of viable process organisms outside the primary physical containment
5. To provide training of personnel
6. To establish biological safety committees or subcommittees as required
7. To formulate and implement local codes of practice for the safety of personnel

The containment measures which the OECD have suggested range from Good Industrial Large Scale Practice (GILSP, see *Table 6*), through a series of three increasingly stringent levels of containment (*Table 7*). In proposing GILSP the OECD Report recognized that the

> . . . vast majority of organisms now used in traditional manufacturing industry can be regarded as safe, because in the long periods of industrial use, sometimes extending to centuries, they have rarely given rise to safety problems. In the same way, modified organisms prepared by inserting segments of DNA that are well characterized and free from known harmful sequences into such organisms to improve their performance, are also unlikely to pose any risk.

The OECD Report then suggests criteria for defining these organisms (*Table 8*). The detailed characteristics of the GILSP used to handle them, in the pharmaceutical industry at least, would be those of GManP (Sharp, 1983), and not of GMicP unless they arose as a natural consequence of the manufacture.

This now faces the manufacturing industry with at least an inconsistency, if not a second paradox. GILSP is clearly not a containment category in the strict sense; it does not necessarily contain the micro-organism. Nor indeed does OECD Containment Category 1, in which, for example, the operator is asked to minimize the release of viable organisms in the exhaust gases (*Table 7*). The reason why these are sensible measures is that the organisms which may be handled under them will have 'built-in environmental limitations' (*Table 8*). This biological containment is deemed to be sufficient and complete physical containment is unnecessary.

The difficulty of this approach, realistic though it is, lies in the relationship these categories bear to a deliberate release of a recombinant organism, for which other criteria of safety are used. The recent report of the Royal Commission on Environmental Pollution (Report, 1989) explained (para. 5.17) why organisms with simple engineered gene deletions could not

Table 7. OECD Containment Levels 1 to 3 (from the OECD Report, 1986, courtesy of OECD, Paris)

Specifications	Containment categories		
	1	2	3
1. Viable organisms should be handled in a system which physically separates the process from the environment (closed system)	Yes	Yes	Yes
2. Exhaust gases from the closed system should be treated so as to:	Minimize release	Prevent release	Prevent release
3. Sample collection, addition of materials to a closed system and transfer of viable organisms to another closed system, should be performed so as to:	Minimize release	Prevent release	Prevent release
4. Bulk culture fluids should not be removed from the closed system unless the viable organisms have been:	Inactived by validated means	Inactived by validated chemical or physical means	Inactived by validated chemical or physical means
5. Seals should be designed so as to:	Minimize release	Prevent release	Prevent release
6. Closed systems should be located within a controlled area	Optional	Optional	Yes, and purpose-built
(*a*) Biohazard signs should be posted	Optional	Yes	Yes
(*b*) Access should be restricted to nominated personnel only	Optional	Yes	Yes, via an airlock
(*c*) Personnel should wear protective clothing	Yes work clothing	Yes	A complete change
(*d*) Decontamination and washing facilities should be provided for personnel	Yes	Yes	Yes
(*e*) Personnel should shower before leaving the controlled area	No	Optional	Yes
(*f*) Effluent from sinks and showers should be collected and inactived before release	No	Optional	Yes
(*g*) The controlled area should be adequately ventilated to minimize air contamination	Optional	Optional	Yes
(*h*) The controlled area should be maintained at an air pressure negative to atmosphere	No	Optional	Yes
(*i*) Input air and extract air to the controlled area should be HEPA filtered	No	Optional	Yes
(*j*) The controlled area should be designed to contain spillage of the entire contents of the closed system	No	Optional	Yes
(*k*) The controlled area should be sealable to permit fumigation	No	Optional	Yes
7. Effluent treatment before final discharge	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactived by validated chemical or physical means

Table 8. Suggested criteria for rDNA GILSP organisms (from OECD Report, 1986, courtesy of OECD, Paris)

Host organism	rDNA engineered organism	Vector/insert
Non-pathogenic	Non-Pathogenic	Well characterized and free from known harmful sequences
No adventitious agents	As safe in industrial setting as host organism, but with limited survival without adverse consequences in environment	Limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the construct in the environment (unless that is a requirement of the intended function)
Extended history of safe industrial use OR		Should be poorly mobilizable
Built-in environmental limitations permitting optimal growth in industrial setting but limited survival without adverse consequences in environment		Should not transfer any resistance markers to micro-organisms not known to acquire them naturally (if such acquisition could compromise use of drug to control disease agents)

necessarily be considered safe in the environment, and have argued (para. 6.7) that a 'case by case assessment of every proposal to release a genetically engineered organism to the environment is essential'. The Commission does accept that manufacture with recombinant organisms will produce a biomass whose accidental release (paras 10.5–10.8) and disposal (paras 10.9, 10.10 and 11.54) needs to be thoughtfully considered in assessing the hazards of the process as a whole. However, what they do not seem to have considered is the unavoidable discharge of organisms resulting from the normal operation of almost any process.

The inconsistency lies not in what the large-scale containment and the deliberate release are trying to achieve but in the different attitudes to their regulation. I believe that this reflects a fundamental misconception about the physical containment of micro-organisms. It is a rather obvious statement to make that the micro-organisms in a process are either contained within some physical boundaries or they are not. However, their absolute containment is very difficult, if not impossible, to achieve and anything short of it amounts to release.

The Asilomar Conference specifically recognized this, and only the most stringent of the containment measures which they described required the inactivation, as distinct from the disinfection, of waste (*Table 2*). Disinfection would merely kill the growing organism, allowing its regrowth from spores or from some latent state (Colwell *et al.*, 1985), while inactivation would ensure that this was impossible, and would completely prevent its release from the

process. Yet in the intervening decade these measures have become more generally codified into large-scale guidelines, which, like those suggested in the OECD Report, have two upper levels at which release of the organism is to be prevented (*Table 7*). How is that possible, if containment itself cannot be graded? There should be no difference between two categories both of which are designed to prevent release.

The realistic answer accepts that these categories do not amount to an increasingly stringent set of containment measures at all, but are operational guidelines which ensure ever smaller levels of release. Their nearest counterpart lies in the graded set of basic environmental standards for sterile manufacture (*Table 9*), particularly in the way those standards define the permitted concentration of particles and micro-organisms in the air. This approaches but does not fall to zero.

This is not a captious argument. It underlies a fundamental, if unconscious, difference in outlook between those of us who have been involved in the writing of guidelines for the large-scale use of recombinant organisms, and those others who are now considering their deliberate release. It may be uncomfortable, but we must accept that any use will allow release. We can only control the scale of the release, which, in truth, may be very small. It is why biological containment is so important. The difference between the recombinant organisms which are designed for large-scale manufacture and those which are designed for deliberate release to the environment is that their biological containment is different. Only the latter group must survive, albeit in a controlled and limited way, after they are released (Bishop *et al.*, 1988; Dwyer, Rojo and Timmis, 1988; Lindow and Panopoulos, 1988). This is why the regulations controlling the two groups should be different; not because one group can be physically contained while the other is not.

A change in emphasis, from a hierarchy of increasing containment to one of decreasing release is necessary and useful for the control of large-scale manufacture, even if it is socially uncomfortable. It can help us to write more effective sets of guidelines, and to analyse the differences between one set and another. In order to understand why this is true it is necessary to examine the nature of the large-scale processes to which the guidelines apply and to define some of the terms that are used to describe them. Otherwise phrases such as 'secondary containment' and 'minimize release' will carry meanings that are too vague to be of practical value in designing a process.

Definitions

OPEN SYSTEMS

Industrial processes, such as the recovery of penicillin G (*Figure 1*), are made up of a sequence of unit operations which transform the input materials into the recovered product. These input materials are either derived from earlier unit operations, for example the broth from a large-scale fermentation of *Penicillium chrysogenum*, or they will enter the sequence from the external

Table 9. Basic environmental standards for the manufacture of sterile products (from Sharp, 1983 Appendix 1, p. 88, courtesy of HMSO, London)

Grade	Final filter efficiency (as determined by BS 3928)*	Recommended minimum air changes per hour	Max. permitted number of particles per cubic metre equal to or above:†		Max. permitted no. of viable organisms per cubic metre†,‡	Nearest equivalent standard classification		
			0·5 micron	5 micron		BS 5295§	US Fed. Std. 209B‖	VDI 2083, P.1¶
1/A (unidirectional air flow work station)	99·997%	flow of 0·3 m s^{-1} (vertical) or 0·45 m s^{-1} (horizontal)	3000	0	less than 1	1	100	–
1/B	99·995%	20	3000	0	5	1	100	3
2	99·95%	20	300000	2000	100	2	10000	5
3	95·0%	20	3500000	20000	500	3	100000	6

Air pressure should always be highest in the area of greatest risk to product. The air pressure differentials between rooms of successively higher to lower risk should be at least 1·5 mm (0·06 inch) water gauge.

* BS 3928: Method for Sodium Flame Test for Air Filters, British Standards Institution, London, 1969.

† This condition should be achieved throughout the room when unmanned, and recovered within a short 'clean up' period after personnel have left. The condition should be maintained in the zone immediately surrounding the product whenever the product is exposed. (Note: It is accepted that it may not always be possible to demonstrate conformity with *particulate* standards at the point of fill, with filling in progress, due to generation of particles of droplets from the product itself.)

‡ Mean values obtained by air sampling methods.

§ BS 5295: Environmental Cleanliness in Enclosed Spaces, British Standards Institution, London, 1976.

‖ US Federal Standard 209B, 1973.

¶ Verein Deutscher Ingenieure 2083, P.1.

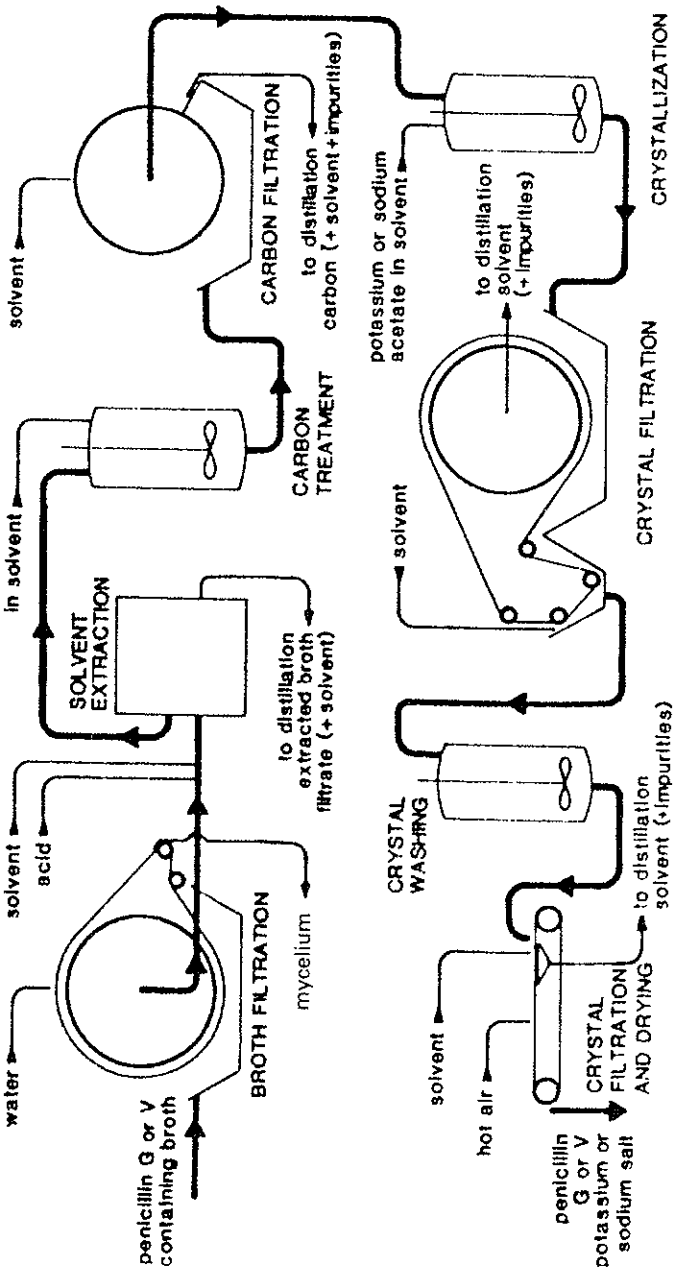


Figure 1. The penicillin purification process of Gist-Brocades (from Hersbach, van der Beek and van Dijck, 1984, courtesy of Marcel Dekker Inc., New York).

environment, for example the water used to wash the mycelial felt or the various other reagents used throughout the process (*Figure 1*). The output materials can either pass on to the next operation, be recycled to an earlier one, or leave the sequence altogether. In the latter case they return to the external environment.

The whole process can be represented in a simplified block diagram (*Figure 2*) with a number of unit operations (A–G) connected to the external environment through its inputs (*I*) and outputs (*O*). At some point the recovered product (*P*) will itself become an output from the process, either as a crude intermediate or as the finished material. The transfer of material through the process, from one unit operation to the next, may be a continuous flow, but it may also be held for batch processing. This does not affect the block diagram, except that the transfer of materials through the necessary holding tank becomes an extra unit operation in the process. Even the laboratory purification of a protein can be represented in this way.

Each of the unit operations has inlets (i), which receive the input materials, and outlets (o), which pass material to the next stage. Where these outlets discharge directly to the external environment the unit operation is *open*. These latter outlets (o) do not necessarily carry a main part of the process stream; solvent vapour emerging from the top of an open vessel, or an aerosol formed at a centrifuge, both of which could discharge to the external environment, would be enough to render the operation open. If the process stream that leaves a unit operation through one of the outlets (o) contains viable micro-organisms, they may be transferred to one of the inlets (i) of the next operation; alternatively they could be released to the external environment as outputs (*O*), either purposefully as waste or products, or incidentally as losses.

CLOSED SYSTEMS–PRIMARY CONTAINMENT

A process which is *closed* is built from unit operations in the same way as one which is open, except that it has no outlets to the external environment (*Figure*

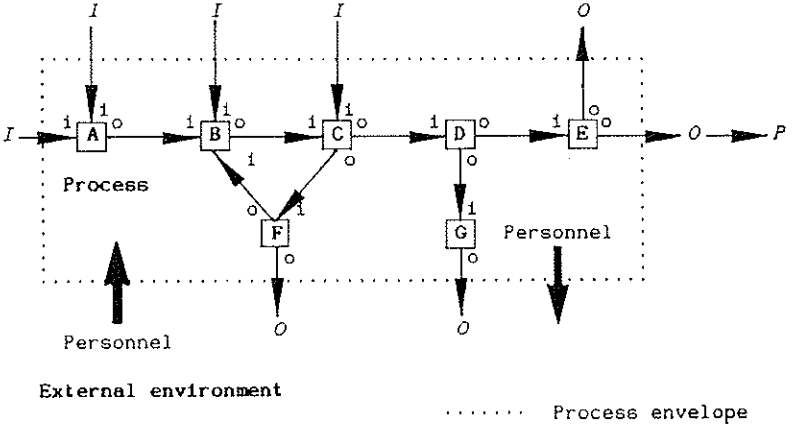


Figure 2. An open system of unit operations.

3). Every outlet (o) from each unit operation must flow to the inlet (i) of a subsequent one. Although there are inputs (*I*) from the external environment no outputs (*O*) return to it.

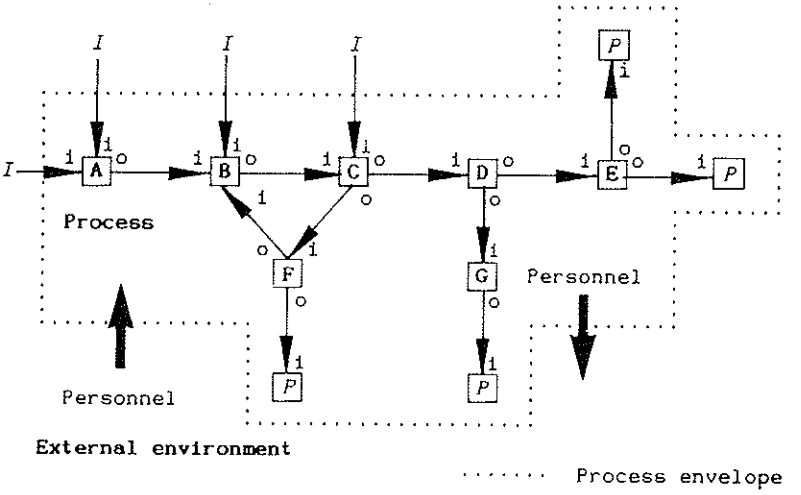


Figure 3. A closed system of unit operations—primary containment.

Clearly the closed system cannot be sustained indefinitely; there has to be an overall materials balance, and products of some sort (*P*) must emerge from the system. These products bear the same relationship to the system as do the materials held for batch processing at an intermediate stage. They can leave the immediate vicinity of the process but they must remain separate from the external environment. They can do this by entering a unit operation which has inlets (i) but no free outlets (o). In essence the system will remain closed if the products leave in suitable closed containers, such as a vaccine leaving a process in closed ampoules.

The closure of the process does not just extend to the main process streams. Nothing must be allowed to escape from the process if it is to remain closed; this includes the vapour, aerosol or dust which may emerge in small quantities from pump glands, centrifuges or powder filling stations. This is difficult, if not impossible, to achieve in practice, but, for the sake of this definition let us assume that it can be done. Under these circumstances it will be fully contained. Where the closure is achieved through the use of the appropriate equipment, and the operating procedures needed to ensure its safe operation, the method of closure is defined as *primary containment* (*Table 10*).

CLOSED SYSTEMS–SECONDARY CONTAINMENT

If an outlet from one unit operation cannot be directly connected to the inlet of another, then some output will be released into the environment in the vicinity of the operation. Under these circumstances it is inevitable that the unit operation will remain *open*. The only way to close the process as a whole is to

run the open operation within a *secondary containment* barrier (*Table 10*). This is a physical and operational barrier erected around the process to isolate it from the external environment (*Figure 4*).

The extent of this barrier will depend on the number of unit operations which remain open and on the nature of the output. The release of vapour from a number of pumps and valves could only be contained with a rather extensive barrier, but individual open operations within the process could be contained within more limited barriers (*Figure 5*). Inputs (*I*) can cross the containment barrier from the external environment to enter the process, and outputs (*O*) can emerge from the process inside the barrier. However, if the system is to remain closed any products (*P*) which are released must do so through a *closed* process. They must emerge through a unit operation which has inlets (i) but no free outlets (o).

Secondary containment barriers are even more difficult to seal than are those for primary containment. If the barrier encloses a limited volume, then only materials will need to cross it, but if the volume is large, personnel will also be

Table 10. Primary and secondary containment (after OECD Report, 1986)

Primary containment	The protection of personnel and the immediate vicinity of the process from exposure to process materials is provided by appropriate equipment and the use of safe operating procedures
Secondary containment	The protection of personnel and the environment external to the facility from exposure to process materials is provided by a combination of facility (building) design and operating practices

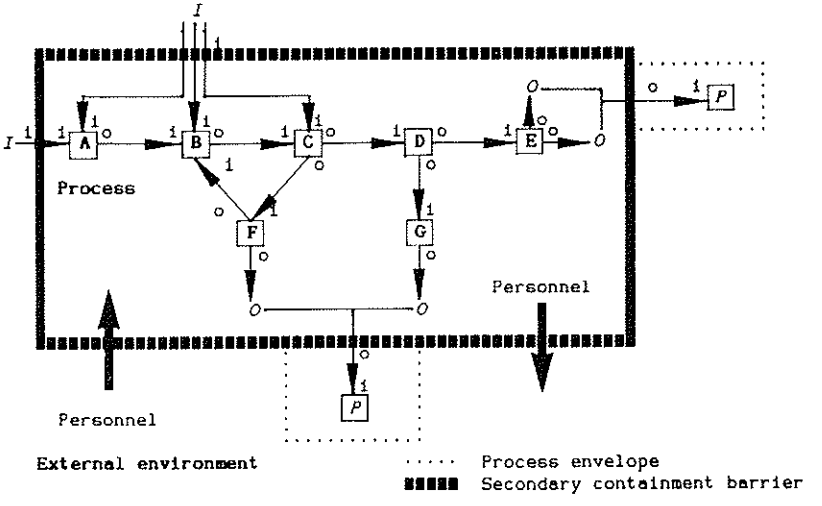


Figure 4. A closed system of unit operations—secondary containment.

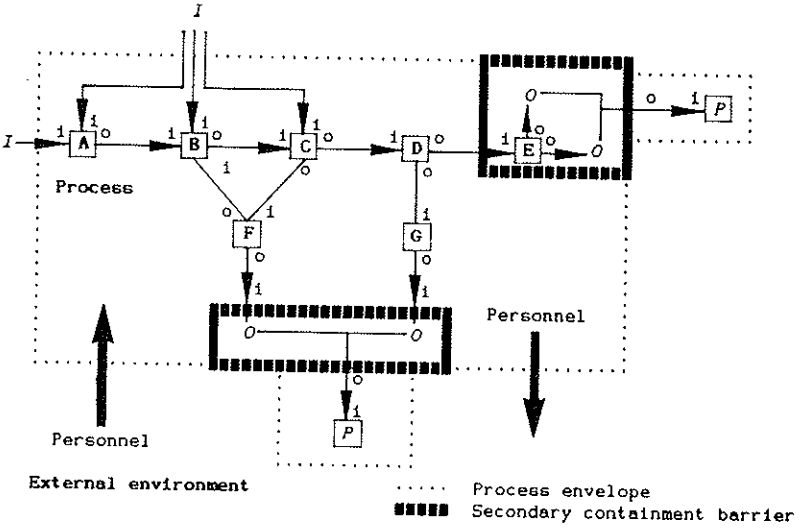


Figure 5. A closed system of unit operations—limited secondary containment.

involved (*Figure 4*). This traffic is almost certain to break the barrier to some degree, but, again for the purpose of these definitions, we can assume that the system will remain closed.

CONTAINMENT OF MICRO-ORGANISMS

So far these definitions have been applied to the process as a whole, and to all of the materials which flow through it. In practice, it can also apply to just one subset of those materials. The same principles will apply except that it is only necessary to trace one, or a few, components of the system, rather than considering its total mass balance. Tracing the viable micro-organisms in a system would be a good example, and the system could be *closed* to the micro-organisms while remaining *open* to the other materials in the process streams.

In this case the viable micro-organisms must be removed from the process streams before they enter the external environment. They can either be passed through a selective physical barrier, such as a filter, or they can be sterilized at high temperature or with a chemical (*Figure 6*). Either way the viable micro-organism will be removed, although in the former case it will remain within the closed system. This is true whether the containment is achieved at a primary or a secondary level.

These selective barriers effectively enhance the level of primary containment, and the secondary containment can be limited to those operations for which primary containment is impractical (*Figure 6*). How much more of the process envelope should then be enclosed is a matter of judgement rather than necessity.

It is important to remember that the aim of the containment in this sense is to

retain the viable micro-organism within the system. It is not necessary for the micro-organism that is being traced to be the only one that is present within the containment boundary. Although these other organisms might not easily leave the system independently of the viable organism they could freely enter it without compromising the containment. This may not be desirable, for reasons of Good Manufacturing Practice (GManP), but that is another issue.

This selective containment of viable organisms must not be confused with *biological containment.*

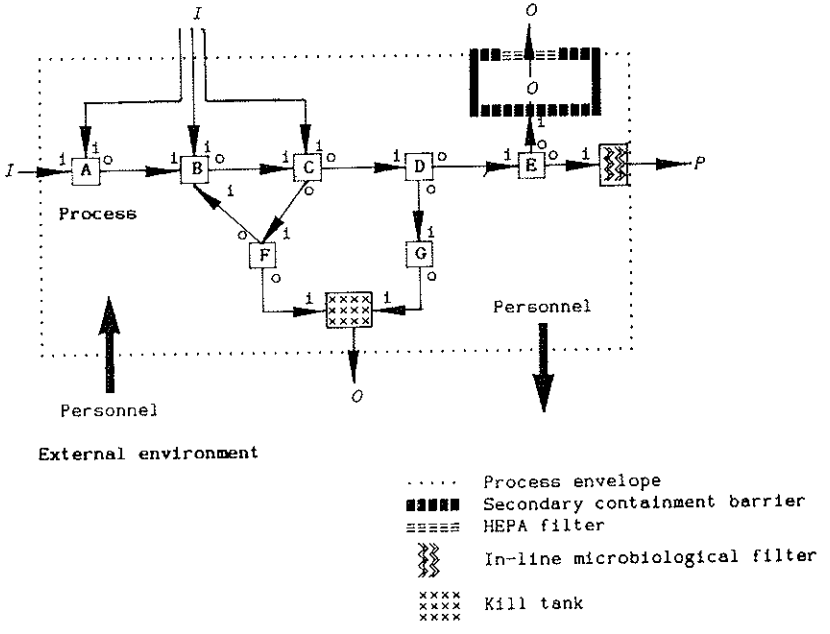


Figure 6. An open system of unit operations closed to viable micro-organisms—limited secondary containment.

TEMPORAL CONTAINMENT

The space that is contained, whether by primary or secondary containment, does not need to remain closed at all times. The containment is necessary because of some particular danger associated with the process. The danger may be a temporary one; a toxic chemical used in the process may have been eliminated or diluted to a safe concentration; a micro-organism may have been destroyed by sterilization or fumigation; or a radio-isotope may have decayed to a safe level. Once that danger is past, there is no reason to retain the containment barrier and it can be removed. There is then a *temporal* element to containment (cf. Sharp, 1983 para. 9.27). It is particularly important where the closed system relies heavily on secondary containment.

BIOLOGICAL CONTAINMENT

All of the foregoing definitions relate to the ability of the viable organisms to

leave the manufacturing process. Where the system is open it can do so, where it is closed it cannot. The aim of these containment techniques is to prevent the organism or the genetic elements it contains from reaching the external environment. Once there both may survive and could affect the environment to a greater or lesser extent. To prevent this, either or both can be constructed with an intrinsic *biological containment* which limits their survival outside of the laboratory or the manufacturing process (Curtis, 1988).

Closed systems in practice

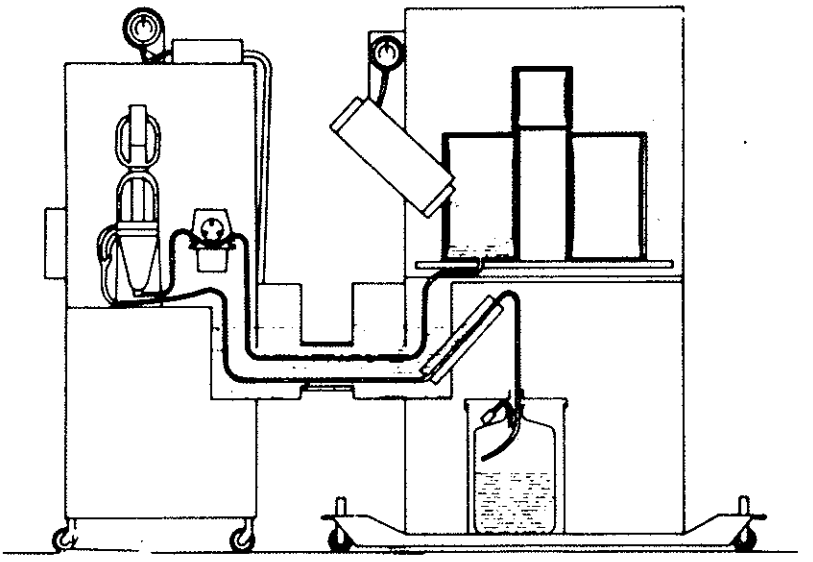
Complete closure is difficult if not impossible to achieve. In normal operation it is a limit which is more or less closely approached rather than actually reached. Good equipment and safe operating procedures are both needed to achieve a high standard of primary and secondary containment. Much of the equipment needed to achieve a closed system is mechanically complex, and will not provide the necessary containment unless it is kept in good repair and is operated correctly (van Hemert, 1982; Walker *et al.*, 1987).

A process that is engineered with primary containment throughout is more likely to approach the limit of closed system, than is one that relies on secondary containment. The enclosed space will be smaller, and it will be much easier to operate the inlets and outlets. However, there are many small-scale operations in microbiology that are open processes and which therefore must rely on secondary containment to give a closed system. They include some of the laboratory stages that precede a large-scale fermentation, and which are usually operated under GMicP. An example is the transfer of small quantities of spore suspensions. The problem can be reduced but not eliminated by good manipulative technique.

A useful booklet produced at the University of Utrecht (Winkler and Hoekstra, 1985) outlines these problems of laboratory microbiology. It makes a careful distinction between GMicP and Safe Microbiological Technique (SMicT). The former is designed to protect the microbiological integrity of the work itself, while the latter, which includes a considerable element of secondary containment, is designed to protect the microbiologist as well. For this reason the concept of GMicP is likely to fall in favour of descriptions of SMicT. The latter is very similar to ACDP (ACGM) Containment Level 1 (ACGM, 1988a, 1988b). This emerged as the lowest level of containment for laboratory work when the original GMAG categories were realigned with the ACDP categories, and GMAG 0 and GMicP were dropped (ACGM, 1988c).

Other operations that fall into this open category include a number of machines for breaking micro-organisms. Frequently the problem is not so much that the machine could not be engineered differently to ensure complete closure, but that the expense of doing so outweighs any practical advantage.

Some secondary containment is therefore inevitable, but there are real operational problems with this method of closing a system. Materials, and more importantly personnel, will need to cross the containment barrier. If the unit operation is of a small scale, the traffic of personnel can be avoided; for example open centrifuges can be installed in closed cabinets (*Figure 7*),



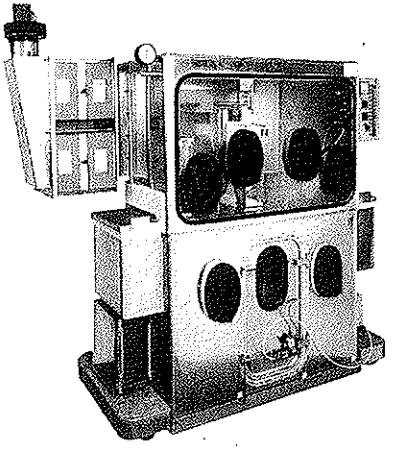


Figure 7. General arrangement of two contained cabinets housing a chemostat fermenter linked to a continuous centrifuge through a disinfectant liquid lock. The centrifuge cabinet itself, with a disinfectant lock to its left, is also shown. Items inside the cabinets were handled with gloves sealed into the ports in their front faces. (Reprinted from Evans *et al.*, 1974; photograph kindly provided by Dr P. Hambleton, Centre for Applied Microbiology and Research, Porton Down, Salisbury SP4 OJG, UK.)

ensuring an effective secondary containment (Evans *et al.*, 1974; Flickinger, 1979). However, for large-scale operations personnel will need to move across the secondary containment boundary, and it will not be easy to maintain a closed system (e.g. *see* Flickinger and Sansone, 1984).

One way of improving the secondary containment is to introduce some temporal containment as well, so that the operators do not need to enter when the space inside the barrier is in an unsafe condition. A unit operation can only discharge an aerosol of viable organisms or a toxic substance into its immediate environment while the process itself actually contains these materials (e.g. E in *Figure 6*). The operator can enter the contained area to set up the process equipment while it is empty and in a safe condition. He can then leave the area, sealing the secondary containment barrier as he goes. He can then start the process from a remote station outside the area.

At the end of the process the contaminated area will need to be made safe; if the danger arises from viable organisms, the equipment itself will need to be sterilized, and the contained area fumigated. Fortunately, the engineering principles underlying containment are similar to those which underlie sterilization procedures (Brooks and Russell, 1986). Once the area is sterile it should then be possible to break the containment barrier providing normal access to an area now rendered safe. This is a complex arrangement, and it implies the need for strict adherence to operating procedures. Any deviation from the procedure could lead to a loss of the containment; the procedures for handling a breakdown of the processing equipment while it contains viable micro-organisms also need to be thought through. On the other hand, it is still easier than trying to put personnel into the contained area as a routine while viable organisms are present, and then to bring them out from the area after they have been contaminated. If the area is to remain closed, that becomes a game played with airlocks and airsuits (Flickinger and Sansone, 1984).

Standards of primary containment

From these ideas it is possible to develop a consistent set of containment standards. However, this is only possible if it is accepted that the closed system is a figment of our imagination. It may be a useful figment, but that does not make it any more real. In practice all systems are more or less open and we can realistically grade their containment only if we grade the degree to which they are open.

The engineering standards implicit in the primary containment of the micro-organisms in a closed system (*Figure 3*) are best described by the leakage rate from each of the unit operations. This will depend on two factors, the first is the effectiveness of the seals, while the second is the risk of failure. These need to be treated separately.

EFFECTIVENESS IN NORMAL USE

A static seal, the turning of a valve on a pipline, the rotating shaft on a pump or the overall movement of materials through a unit operation are all likely to

allow the release of small quantities of micro-organisms (Lawrence and Barry, 1982; Cameron, Hambleton and Melling, 1987, *see Figure 8*; Houwink, 1988). The actual levels of release are difficult to estimate precisely; one calculation suggests that a 250 l fermenter with an exhaust filter which is 99.99% efficient will release 2–3 $\times$ 10^6 cells per day from a fermentation containing 10^9 cell ml^{-1}, but that this could rise to 3 $\times$ 10^9 cells for the harvesting of 10 l fermenters in an NIH category BL1 laboratory (Lincoln, Fisher and Lambert, 1985; Strauss, 1987). The same 250 l fermenter with no filtration on the exhaust gases could release as many as 3.5 $\times$ 10^{11} viable cells per day (Evans, Preece and Sargeant, 1981). This suggests a need for a 10^5 reduction by filtration rather than 10^4 to reach the lower level of 3 $\times$ 10^6 per day unless the method of release decreases the viability of the organisms (Evans, Preece and Sargeant, 1981).

The filters, seals and valves, and microbiological technique can all be improved to the point where this release becomes negligible, or at least undetectable (East, Stinnett and Thoma, 1984; Robertson and Frieben, 1984; Muijs, 1987; Walker *et al.*, 1987). The point is not that they then become closed systems while the others are open, but that these devices could be graded according to the quantities of viable organisms which they release in normal use. This would provide one criterion for their assignment to the various OECD categories of containment (OECD, 1986). Only those with a leakage rate which fell below a certain level in standard tests would qualify for use in Categories 2 and 3 (*Table 7*), which demand that the release should be prevented (Items 2 and 3).

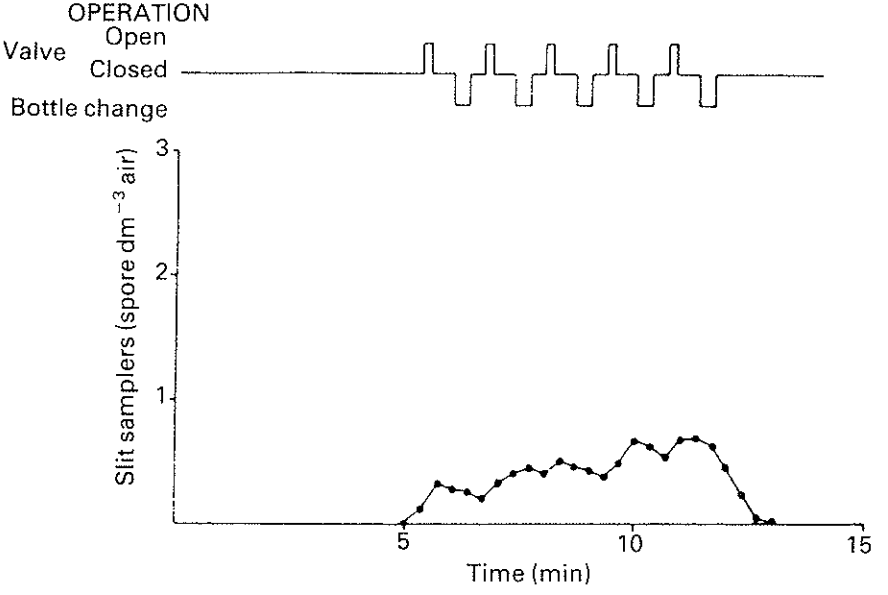


Figure 8. Release of *Bacillus subtilis* spores during the operation of a sampling device attached to a fermenter containing 2·3 $\times$ 10^9 spores ml^{-1}. The sample valve was opened to fill a sample bottle, which was then closed and the bottle changed. This operation was repeated several times. The spores released into a class III safety cabinet which surrounded the fermenter were detected with a slit sampler. (Reprinted from Cameron, Hambleton and Melling, 1987, courtesy of Ellis Horwood, Chichester.)

This is an obvious way in which to categorize the engineering equipment that makes up the unit operations, but the actual categories are likely to depend on the nature of the organism that is being contained. A virus particle, or microbial spores, are likely to require more stringent standards than yeasts, if only because of the different sizes and viabilities of the particles. In some respects the problem is similar to the specificiation of electrical equipment for work in flammable gases, for which the standards are more stringent where the atmosphere contains hydrogen or diethyl ether than they are where it contains ethanol or acetone, for example (BS 5345, 1976); or for GManP of pharmaceuticals, where sporogenic organisms should be processed in separate suites (Sharp, 1983 para. 9.27).

RISK OF FAILURE

One problem about a discussion of the risk of failure lies in our use of the word risk itself. To the general public the 'risk of failure' could encompass the environmental impact of the failure; to an insurer it could mean the insured hazard or the expected claims arising from the failure; to an operator of the plant it could mean the chance of injury, or more simply the frequency of failure. The resolution of this problem lies outside the scope of this article; useful discussions which each come to different conclusions can be found in two books on hazard control (Firenze, 1978; Marshall, 1987).

For the purposes of categorizing biological containment the most useful definition of *risk* is the restricted one of the likelihood or frequency of the failure. However, the nature of the failure itself is also important. Some failures have insignificant consequences, while the outcome of others is very serious. The reason is that the risk of failure, taken simply as the frequency of some event, lies between a hazard, taken as 'a physical situation with a potential for human injury, damage to property, damage to the environment or some combination of these' (Marshall, 1987), and the realization of that potential (*Figure 9*). The public perception of this sequence of hazard, risk and event is notoriously irrational (Morgan, 1989), but it lies at the heart of any debate about the safety of new technology. It makes a prospective analysis of an acceptable risk of failure very difficult, particularly when the risk can only approach zero as a lower limit.

Even to reduce the risk of failure to a very low level is not easy, nor, necessarily desirable. '. . . there are limits to technological management of risks external to technology. These limits include: *first* the potentiality of their effectiveness; *second* high and often unlimited costs; and *third* and most important, the amount of technical paternalism individuals and society are willing to accept as a reward for better and safer products' (Sass, 1987). The Advisory Committee on Genetic Manipulation (ACGM) Large-Scale Working Group endorsed a concept of minimal risk which is 'the point at which [the] risk . . . under consideration appears to merge with the general average risk to which all workers are inevitably subjected' (ACGM, 1987b para. 14). These quotations use the word 'risk' in a broad sense, but that does not affect their relevance. The particular risk that the report considered was that from a toxic

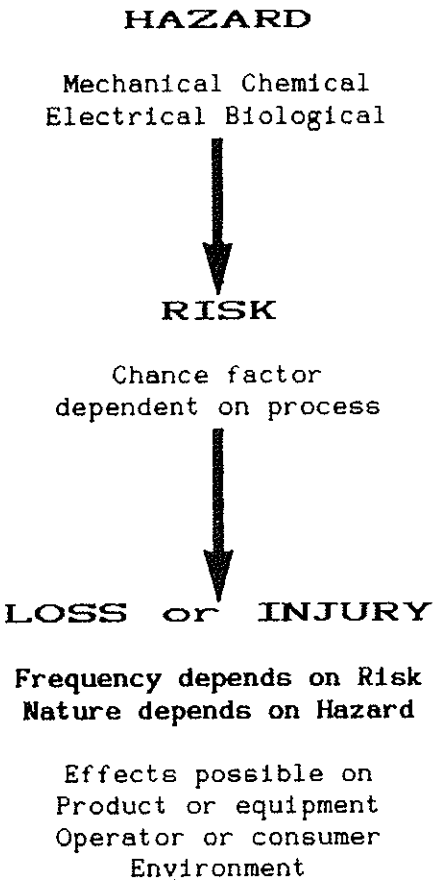


Figure 9. Relationship between hazard and risk, and their effects in causing loss or injury.

substance, but it is helpful as a starting point for any discussion of safety. It implicitly recognizes that safety is a comparative rather than an absolute concept and it avoids the need to define a safe substance or, indeed, a safe process.

The difficulty with a new technology, particularly one handling living organisms, is that the public perception of its hazards is very acute. This distorts the concept of 'minimal risk' and the point at which it 'appears to merge with the general average risk' is actually much lower than the point at which it does merge (Morgan, 1989). In short, the public demands a greater security from the dangers of the new technology than is really necessary on the basis of a risk assessment.

For this reason, if for no other, the equipment used for the highest category of containment ought to have the lowest practical risk of failure. It is difficult to identify precisely what that risk might be. The emphasis in the HASAW Act (1974) on what is reasonably practical ought to allow the development of sensible standards, but there can be no excuse, at the highest level of large-scale

Table 11. Suggested comparison of primary containment standards for unit operations to be used at OECD Categories 2 and 3

	Release of viable organisms per hour of normal operation	Risk of failure of unit operation per 10^6 hours of normal operation
Category 2	$100E$	$100R$
Category 3	E	R

containment (Category 3 in OECD, 1986), in failing to achieve this lowest practical risk. At the lower OECD Category 2 a less stringent target could apply (*Table 11*; cf. *Table 9*).

The risk of failure of each unit operation could be related to its incidental emission of organisms. Assuming that there is some steady incidental emission from a particular operation (E organisms per hour), it would be possible to set a risk of failure (R per 10^6 hours) such that the total number of organisms released through failure equals the total number lost to the external environment through the incidental emission between failures ($E \times 10^6$).

$$R = \frac{E \times 10^6}{\text{Vol. (m}^3\text{) lost in failure} \times \text{viable cells per m}^3}$$

If $R = 1$ (i.e. the complete loss of the containment of the particular unit operation every 114 years) then E would be equivalent to the loss of, for example, 1 ml culture per hour from a 1 m^3 fermentation process. If the fermenter contains 10^9 cells per ml this is at least an order of magnitude larger than the highest of the previously quoted figures for the steady emission of viable cells from a fermenter (Robertson and Frieben, 1984; Lincoln, Fisher and Lambert, 1985; Strauss, 1987). This suggests that no more than 10% of the viable cells should be allowed to escape to the environment following any catastrophic failure. If the figures for steady emission from some of the more highly contained processes quoted above are correct, then no more than 0·1–1% should be allowed to escape in the event of a major accident.

The emission of viable organisms equivalent to less than 1 µl from 1 m^3 process fluids per day, which the lowest of the figures for steady emission implies, is very small bearing in mind the transfer of fluids between the various unit operations that make up a manufacturing process. If it is realistic, then it is clearly more important to protect against a catastrophic failure than it is to worry about the low level of constant emission from the normal process. Either the risk of failure must be reduced to very low levels, which would seem impractical, or the failure must be contained. That will depend far more on effective secondary containment and on good working practices than it will on the good primary containment which normally has the highest priority in process design.

While there is logic in this concept that the losses of micro-organisms are inevitable, it ought not to be pursued to the point where either category allows the process to be engineered as if it were open. On the contrary, it is essential to presume that, in any category which presently demands that loss should be 'prevented', the process should be built as if all of the unit operations were closed. The release of viable organisms from such categories ought to be

treated either as accidental emissions, dependent on the failure of otherwise closed processing equipment, or as incidental emissions which are below the limits set for release in normal operation (*Table 11*).

Standards of secondary containment

The risk of a single catastrophic failure immediately raises the problem of the standard of secondary containment. At OECD Category 3 the system of primary containment must be operated in a controlled area (Item 6, *Table 7*), which is effectively a secondary containment boundary. If there were to be a spillage of the entire process fluids, this must remain within this boundary (Item 6j). This implies that the controlled area is a closed system, which would retain not only any major spillage but also any emission of viable organism from the process in its normal operation. Ignoring, for the moment, the impossibility of maintaining a totally closed system, the description of the primary and secondary containment for OECD Category 3 amounts to a closed system within a closed system. It is essentially a double barrier against release.

OECD Category 2 makes this closure of the secondary containment optional. The controlled area need not contain the spillage. Nor does the input and output air need to be filtered, allowing any aerosols released from the primary containment system free access to the external environment. Clearly this is an open system, and it is the essential difference between the two most stringent categories (*Table 12*).

It is fair to say that most of the differences in the secondary containment of these categories are optional. However, only Category 3 envisages entry to the controlled area through the airlock (Item 6b, *Table 7*) which would be essential for the area to be properly decontaminated after a spill without loss of the secondary containment barrier. It is only necessary to ask how the operators would leave the area after they had been contaminated to see the logic behind this airlock. Even then they would need to be fully contained within airsuits to allow for their complete decontamination.

The methods of work, and careful design of the building beyond any details laid down in the guidelines would also have a large effect on the risk and outcome of a failure of the primary containment even at OECD Category 3. What procedures will be laid down for maintaining the equipment? If, due to its

Table 12. Suggested comparison of containment standards to be used at OECD Categories 2 and 3

	Primary	Secondary
Category 2	Build unit operations as if CLOSED allowing some release (100x lowest practical limit)	OPEN controlled area
Category 3	Build unit operations as if CLOSED with lowest practical release	Build controlled area as if CLOSED with lowest practical release

breakdown, one of the operators were injured and contaminated, which should have priority, treatment of their injury or their decontamination? What design of the drainage system and the airlocks is best suited to the necessary decontamination of the area itself? Should there be floor drains that transfer the spillage direct to an inactivation tank (Flickinger and Sansone, 1984); or should the spill be directed to an open pit within the contained area, whence it can be pumped back into one of the process tanks? In the first instance the spillage can be flushed away with disinfectant, but the drains may be difficult to clean; in the second the actual removal of the spillage may be a little more difficult, but only the room will be contaminated.

These detailed questions of design and operation in relation to spills are important because the quantities likely to be released are large compared to the amount released through incidental emissions in normal operation. At OECD Category 3 these procedures and the design ought to be based on the assumption that the secondary containment barrier will remain intact. For a similar reason the idea that the controlled area at OECD Category 2 is open must not be used to argue either that there should be free access to it, or that there is no point in the minimum level of secondary containment.

The definition of open refers to the process and not to personnel. The fact that the process uses some complex equipment whose safety and closure depends on the use of trained personnel to operate it correctly and to keep it in good repair should be good enough reason to restrict access. As for the minimum level of containment, that depends on a hierarchy of release standards. I have already suggested such a hierarchy in the primary containment at OECD Categories 2 and 3. There seems no reason why such a hierarchy should not also exist for secondary containment. The point is that the secondary containment need not be built as if it were closed but rather to 'minimize release'.

This now raises a difficult question; what is meant by 'minimize release'? In logic it is no more than a good description of OECD Category 3, but that is obviously not what is implied.

Containment in open systems which 'minimize release'

OECD LARGE-SCALE CATEGORY 1

The description of OECD Category 1 is the least successful of the OECD categories (OECD, 1986). The notes clearly imply a closed system of primary containment (Item 1, *Table 7*), and the viable organisms in the bulk culture fluids should be inactivated before release. Yet the exhaust gases and sampling routine both anticipate some release beyond that encompassed by the accidental or incidental emissions which accompany OECD Categories 2 and 3. So does the body of the report itself (OECD, 1986, see final section of Chapter 4). The European Commission, in the preamble to their draft directive for controlling the use of recombinant organisms (CEC Draft Directive, 1988), also stated that:

> Genetically modified microorganisms can be released into the environment in the course of their contained use in two different ways:
> —routine release in normal operating conditions, e.g. as wastes or in airborne emissions;
> —accidental release in abnormal operating conditions, i.e. significant release to the environment following an event which causes the activity to get out of control.

It is clearly confusing to pretend that the containment at OECD Category 1 describes a closed system. Nor does it seem sensible at this stage to propose that the unit operations should be built as if they were closed, but with a less rigorous set of engineering standards allowing, say, 100 times the release allowed at OECD Category 2. However, there is no reason why the process overall could not be a mixture of unit operations, some of which are open and others closed, in their design at least (e.g. *Figure 2*). It would then be sensible to ensure that in this process the closed operations met the criteria for OECD Category 2 (*Table 11*) if only to provide some standardization of the equipment.

A similar approach could be used to define the secondary containment at OECD Category 2, where the external environment could be protected from some of the incidental or accidental emissions of viable organisms but not from others. However, in both cases, one further point would need to be established. It relates to the need expressed in the Robens' Report (1972) to assess the hazards in advance and to translate these into actions. There must be some logic behind the need to close some of the operations but not others. It certainly cannot be simply a matter of convenience.

The key to the division between the open and closed operations must surely depend on the size of the emission and where it is directed. The Royal Commission on Environmental Pollution has suggested that our 'ability to predict the outcome of a release is likely to be greater if the genetically engineered organism is a modified version common in the locality of the release' (Report, 1989 para. 5.14). We could reasonably predict a much safer outcome from discharging a disabled strain of *Escherichia coli* into a foul sewer compared to its discharge to surface water. Other strains of the same organism have been safely grown for about 30 years for the isolation of penicillin amidohydrolase (EC 3.5.1.11) (Savidge, 1984).

The discharge of recombinant organisms to the environment in this way is emotive. However, that is not the issue; it is enough to recognize that under OECD Category 1 discharges of this sort will take place almost as a matter of routine through their normal use. While there may be no intention under this OECD category to 'minimize release' in the strict sense, at least the scale and site should be controlled so as to allow the biological containment to operate. This is surely important; if we really believe in biological containment we ought to allow it to work for us. OECD Category 1 would allow the discharge of the organism from some open unit operations in a controlled manner such that the biological containment is effective (*Table 13*). The choice of which operations are open and which are closed will depend on the nature of the biological containment.

Table 13. Comparison of containment standards for recombinant organisms to be used for GILSP and OECD Category 1

	Primary containment	Biological containment
GILSP	Dependent on process and product constraints rather than on the recombinant organism	Reliable—no special precautions beyond those required for comparable non-recombinant organism
Category 1	Controlled release	Reliable if scale and site of emission are controlled

The problem with this approach is the suggestion in the OECD Report (1986) that OECD Category 1 is a closed system. It is almost as if, having raised the concept of biological containment, we are not prepared to believe in it as a practical method of control. In fact, it is the only sure method of control available, and the concept of a controlled release to a safe place where the biological containment can take effect could replace 'minimize release'.

GOOD INDUSTRIAL LARGE-SCALE PRACTICE (GILSP)

While the three containment categories could be uniformly applied over a variety of industries, GILSP is characteristic of each separate industry, or even of each separate process. Its description in a process which employs a micro-organism in a manufacturing role will certainly depend on the nature of that organism, but it will also depend on the product. Given the pharmacological activity of many of the products of genetic engineering they could well have a more important influence on the containment of their manufacturing processes than the organism responsible for their synthesis.

The GILSP of the pharmaceutical industry will draw heavily on its experience with GManP (Sharp, 1983), and it will be quite different from that of the water-treatment industry (Fannin *et al.*, 1976; Wheatley, 1984). The common feature of them all is that they are processes where any containment of a micro-organism is essential for the safe operation of the process and the quality control of the product rather than to prevent the release of the micro-organism as an end in itself.

The containment which exists at GILSP can arise for several reasons. A fermenter that is used to grow a micro-organism may need to run under sterile conditions which are best achieved in a process which, if not closed (OECD Category 2), will at least only release the organisms in a controlled manner (OECD Category 1). The harvesting of these micro-organisms may release a hazardous aerosol of microbial particles which must be contained within a closed secondary containment barrier (Dunnill, 1982) while allowing the cell paste to be handled in an open operation (OECD Category 1). The level of secondary containment of flammable solvents that is now required under the new COSHH Regulations (Health and Safety Commission, 1988) could well affect the solvent extraction of an antibiotic such as penicillin G from whole fermentation broth to such an extent that it becomes a closed operation both for the flammable solvent and for the producing organism (OECD Category 2), even though the fermentation may remain at the level of controlled release

Table 14. Examples of containment approaches for large-scale applications other than GLSP (from ACGM, 1987b, courtesy of the Health and Safety Executive, London)

Specifications	Containment categories		
	1	2	3
PRIMARY CONTAINMENT			
1. Viable organisms should be handled in a system which physically separates the process from the workplace environment (closed vessel used for growth and maintenance of cultures)	Yes	Yes	Yes
2. Exhaust gases from the closed system should be treated so as to:	Minimize[1] release	Prevent[2] release	Prevent[3] release
3. Sample collection, addition of material to a closed system and transfer of viable organisms to another closed system, should be performed so as to:	Minimize release	Prevent[4] release	Prevent[4] release
4. Bulk culture fluids should not be removed from the closed system unless the viable organisms have been:	Treated[5] by validated means	Inactivated by[6] validated means	Inactivated[6] by validated means
5. Seals should be designed so as to:	Minimize release	Prevent[7] release	Prevent[8] release
SECONDARY CONTAINMENT			
6. Closed systems should be located within a controlled area	Optional	Yes	Yes, and and purpose-built
7. Effluent from sinks and showers should be collected and inactivated before release	No	Yes	Yes
8. The controlled area should be mechanically ventilated to minimize workroom contamination	Optional	Yes	Yes
9. The controlled area should be maintained at an air pressure negative to atmosphere	No	Yes	Yes
10. Extract air from the controlled area should be HEPA filtered	No	Yes[2]	Yes[3]
11. Input air to the controlled area should be HEPA filtered	No	Optional	Yes

12. The controlled area should be designed to contain spillage of the entire contents of the closed system	Yes	Yes	Yes
13. The controlled area should be sealable to permit fumigation	No	Optional	Yes
14. Effluent treatment before final discharge	Treated by[5] validated means	Inactivated[6] by validated method	Inactivated[6] by validated method
SYSTEM OF WORK			
15. Regular testing of containment facilities and 'permit-to-work' system	Yes (as appropriate)	Yes	Yes
16. Biohazard signs should be posted	Optional	Yes	Yes
17. Access should be restricted to nominated personnel only	Optional	Yes, via changing room	Yes, via an airlock/ changing room
18. Personnel should wear protective clothing	Yes	Yes	A complete change
19. Washing facilities should be provided for personnel	Yes	Yes	Yes (and decontamination facilities)
20. Personnel should shower before leaving the controlled area	No	Optional	Yes, in airlock/ changing room
21. Appropriate training and supervision for personnel	Yes	Yes	Yes
22. Accident/incident reporting arrangements	Yes	Yes	Yes
23. Health surveillance	Reference ACGM.1987a		

Examples of containment:

1. Exhaust gases discharged to a safe place or treated by a microbiologically competent HEPA filter or other equivalent procedure.
2. Exhaust gases treated by a microbiologically competent HEPA filter or other equivalent procedure.
3. Exhaust gases treated by double microbiologically competent HEPA filters in series or other equivalent procedure.
4. Steam sterilizable sample ports.
5. Or discharge to a safe place (subject to any Local Authority or Regional Water Authority requirements—in Scotland, Regional and Island Councils for discharges to sewers and River Purification Authorities for streams and controlled waters).
6. A method which has been demonstrated to be effective against the organism in question.
7. Designed to prevent leakage *or* fully enclosed in ventilated housings that are exhausted by a HEPA filter.
8. Designed to prevent leakage *and* fully enclosed in ventilated housings that are exhausted by a HEPA filter.

(OECD Category 1). Finally, there are many examples of vaccine manufacture (e.g. Lawrence and Barry, 1982; Walker *et al.*, 1987) that are already operated under a combination of GManP and SmicT that requires a system of closed primary containment within closed secondary containment (OECD Category 3).

At the other extreme, GILSP may require the controlled release of the organisms into a fermented food or drink, or their dispersal in water treatment systems (Wheatley, 1984) or with agricultural crops (Beringer and Bale, 1988). This wide variety of industrial practice reflects the need to protect the environment and the product from the predictable effects of some microbial hazard, while allowing the process organism to fulfil its manufacturing role. It also acknowledges the wide variation in the level of the biological containment that manufacturing organisms possess.

In any of these processes GILSP defines the standards of working practice and containment that are essential to its proper and safe operation. Where the level of the biological containment for a recombinant organism is at least as great as for a comparable non-recombinant organism, GILSP will be enough to contain a manufacturing process based on either of them. Even the principles specifically set out for GILSP with recombinant organisms (*Table 6*) are little different from those that should apply to any microbiological process. Some of the testing may not be routine, but it would be useful if it were. GILSP should certainly not be seen as a fixed standard; it will change, it is to be hoped for the better, as research and the continuing analysis of accidents show the failings of current practice.

Taken in this sense the extra containment required for some recombinant organisms is simply a part of GILSP. It is Good Industrial Large-Scale Practice to provide the level of containment that a process requires. The guidelines for OECD Categories 1 to 3 simply show how that containment can be built up as it is required.

Comparison of OECD, US and UK guidelines

The sets of large-scale guidelines from the US National Institutes of Health (NIH, 1986) and the UK (ACGM, 1987b) are similar in outline to those from the OECD (OECD, 1986). All are structured into a set of three increasingly stringent levels of containnment (Categories 1, 2 and 3).

The Advisory Committee on Genetic Manipulation (ACGM) placed these large-scale categories (*Table 14*) above Good Large Scale Practice (GLSP); this is the equivalent of the OECD GILSP, the adjective 'Industrial' being dropped to emphasize the need for its application wherever large-scale processes are carried out. The ten-litre rule was also considered arbitrary and the guidelines apply to 'use . . . in a laboratory scale reaction vessel, for pilot plant work or commercial manufacture'.

In the US the situation is a little different. There is no specific designation of GLSP, although a subsequent modification of the guidelines (NIH, 1987) does allow a lower category for which 'the appropriate physical containment conditions need be no greater than those for the host organism unmodified by

recombinant DNA techniques . . .'. The three NIH containment categories themselves (BL1–LS, BL2–LS and BL3–LS in ascending stringency) are matched with the equivalent laboratory categories (BL1, BL2 and BL3). They are unfortunately not tabulated, but they are described in detail in Appendix K of the guidelines (NIH, 1986). Work can be transferred from the laboratory to a matching –LS category once a ten-litre scale is exceeded. Although the NIH laboratory categories rise to a most stringent level of BL4 'no provisions are made for large-scale research or production of viable organisms containing recombinant DNA molecules which require BL4 containment at the laboratory scale. If necessary these requirements will be established by NIH on an individual basis.' This implies the need for a category more stringent than BL3–LS.

Leaving that stringent, if undefined, category on one side for the moment, how do the others compare through the three sets of guidelines, and how does an analysis of open and closed systems help with this comparison?

COMPARISONS AT LARGE-SCALE CATEGORY 3

At Large-Scale Category 3 all three guidelines specify a closed system of primary containment for the process itself. A summary of Appendices K-IV-A, K-IV-D, K-IV-C, K-IV-B and K-IV-G in the NIH guidelines (NIH, 1986) would be little different from items 1–5, respectively, from the OECD (1986) and ACGM (1987b) guidelines (*Table 7* and *Table 14*). The one difference appears in the ACGM guidelines in the footnotes to items 2 and 5; the exhaust gases from the fermenter are to be passed through two layers of filtration to remove viable organisms (footnote 3 in *Table 14*), while rotating seals should be designed to prevent leakage and must be fully enclosed in a housing which is exhausted through a high efficiency particulate air (HEPA) filter (footnote 8 in *Table 14*). In each case these footnotes imply the need for a closed system (the inner filter or the rotating seal) operating within a closed system (the outer filter in each case). In both instances the NIH guidelines only specify a single layer of closure (Appendices K-IV-D and K-IV-G). This difference is not apparent in the OECD guidelines because they are not so specific in their description of how the release of the viable organisms is to be prevented.

The difference is similar in the descriptions of the secondary containment barrier. All three specify:

1. that the closed system of primary containment should be located within a controlled area (OECD and ACGM item 6, NIH Appendix K-IV-M) complete with airlock and changing room (OECD items 6b and 6c, ACGM item 17, NIH Appendix K-IV-M-1); and
2. that the controlled area must, in each case, be designed to contain an accidental spillage of the process fluids (OECD item 6j, ACGM item 12, NIH Appendix K-IV-M-7);

yet the air-handling plant to this controlled area is specified differently.

The OECD and ACGM both specify HEPA filtration on the input and extract air (OECD item 6i, ACGM items 10 and 11), but with two layers of

protection on the exhaust in the ACGM guidelines (footnote 3 in *Table 14*). They are closed systems (*Figure 10*). In sharp contrast, in the NIH guidelines both input and extract air are unfiltered (Appendix K-IV-M-8). The extract can 'be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes'; in short it is an open system because there is an unimpeded path to the external environment (*Figure 10*). Should there be an accidental spillage of the process fluids into the controlled area, the air movement would discharge a large volume of aerosol.

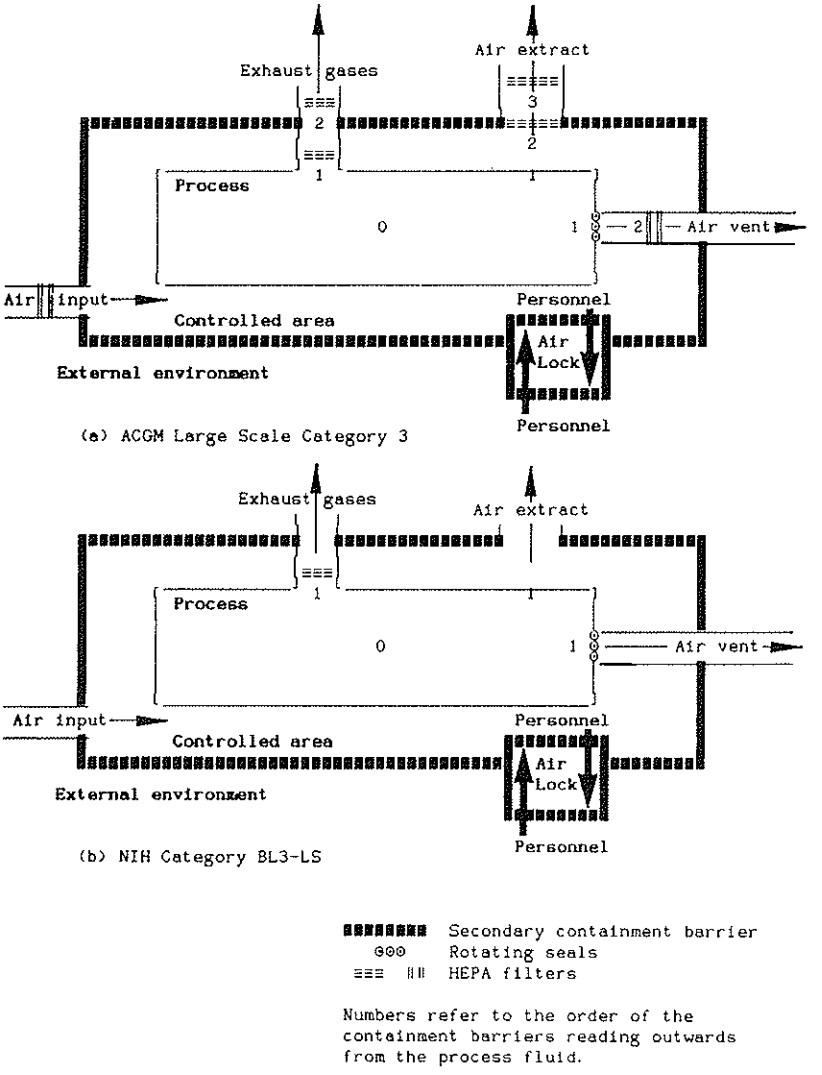


Figure 10. A comparison of the number of levels of containment at large-scale containment categories ACGM-3 and NIH-BL3-LS.

The essential difference between these large-scale categories in the ACGM and NIH guidelines is that the former describes the closed system within a closed system which was implied by the OECD (1986), while in the latter it is a closed system within an open one (cf. OECD Category 2; *Table 12*). The difference is further emphasized by the need for two layers of HEPA filtration on the exhaust air from the process itself which is specified by the ACGM. Since this exhaust will penetrate the secondary containment barrier, thus avoiding one of the levels of containment, the relevance of the second filter is clear. Any rupture of the inner containment will be held within the outer, either in the secondary containment of the controlled area or by the second HEPA filter. However, if the rotating seals which provide the first layer of containment are all within the controlled area which provides a second, does not the contained housing (footnote 8 in *Table 14*) simply replace one method of containment with another? If those housings were to exhaust directly into the controlled area they would provide a third layer of containment (*Figure 10*), which is what the double filtration on the air extracted from the secondary containment actually provides. On the other hand, if they are seen as a source of weakness in the primary containment it is sensible that their emissions should be trapped by a filter so as to prevent contamination of the working environment of the controlled area.

In fact it is probably unnecessary for the guidelines to provide this much detail unless they are going to fix some standard engineering practices. The comparison describes the NIH category of BL3-LS as being less stringent than the ACGM Large-Scale Category 3 but the NIH guidelines envisage, but do not describe, a more stringent category of BL4-LS. However, it does make sense to ask of each of these containment categories, how many layers of closed systems there are, like so many Russian dolls, one inside the other, and then to design the containment consistently on that basis. The ACGM Large-Scale Category 3 has two or three, the OECD two, while the NIH Category BL3-LS has one inside a second from which controlled release is allowed.

COMPARISONS AT LARGE-SCALE CATEGORY 2

The differences at large-scale category 2 are even more pronounced. All three guidelines define a closed system of primary containment for the process in much the same terms as for large-scale category 3. However, the secondary containment is absent from the NIH BL2-LS, is optional for the OECD Category 2 (Item 6 in *Table 7*), and required for ACGM Large-Scale Category 2 (Item 6 in *Table 14*).

The NIH guidelines do require emergency plans for handling a large loss of the culture fluids (Appendix K-III-K), but, without any secondary containment even to control emissions from the loss, the accident would entail a total loss of containment. In contrast, at ACGM Large-Scale Category 2 it would be almost as well contained as at Category 3; the differences in the secondary containment of the controlled area are the replacement of an airlock by a changing room (Item 17 in *Table 14*), and options on HEPA filtration of the input air (Item 11) and on fumigation (Item 13), which would have only a small

effect on the outcome of the incident. There is only a single layer of filtration on the exhaust air from the controlled area (Footnote 2 *Table 14*) and no added protection on the rotating seals, but these changes would not affect the integrity of the closed secondary containment. They only affect its *risk* of failure.

COMPARATIVE RANKINGS AT LARGE-SCALE CATEGORIES 2 AND 3

All of these systems of containment at categories 2 and 3 specify a closed system of containment for the unit operations that go to make up the process itself. There has been an implicit assumption that this would be provided as primary containment, but for some operations, particularly those whose scale is small, this may be impractical. Indeed, under NIH BL2-LS any part of the process that falls below a ten-litre scale can operate under circumstances which 'minimize release' (Appendices K-III-A, G-II-B-1-g and G-II-B-2-k). Nevertheless, every effort would surely be made to provide effective secondary containment for these unit operations. Assuming that the process itself is satisfactorily closed, it is possible to rank the various descriptions of categories 2 and 3 according to the stringency of the secondary containment of the controlled area (*Table 15*).

The differences between the ACGM, NIH and OECD guidelines do not really matter if we are to match the containment to the needs of the process. The operator ought to choose whatever level of containment is necessary. 'The nature of the problems must be methodically assessed and the assessments translated into practical objectives and courses of action'. The guidelines ought to be an aid, not a prescription. In any case, the quality of the training given to the operators and the details of good working practice and functional design will influence the outcome of the containment quite as much as the guidelines themselves. However, at a practical level there is a good reason for trying to define the objectives of the containment in terms of closed systems and controlled emission. It would be unfortunate if the guidelines, from whichever source, became a description of the methods by which the containment for a particular process should be achieved.

Such a view is almost set out in the ACGM guidelines, which do envisage that the conditions for one category of containment do not all need to be taken together. If, to improve the secondary containment at ACGM Large-Scale Category 2, some of the working practices at Category 3 need to be used, so be

Table 15. Comparison of containment levels amongst ACGM, NIH and OECD categories with closed primary containment

Primary containment	Increasing secondary containment			
	No secondary containment	Controlled emission from secondary containment	Closed secondary containment	
			One layer	Two layers
Closed	NIH-BL2-LS	NIH-BL3-LS OECD-2 ACGM-2	(NIH-BL4-LS?) OECD-3 ACGM-3	

it. That does not mean that all of the other features of Category 3 need to be taken as well. It may even be that it is the operators who need protection from an allergenic reaction to large doses of the recombinant organism, while the environment is easily protected by its biological containment. Under these circumstances the working practices of Category 3 might even be joined to some of the primary containment of Category 1. The particular containment objectives should drive the use of the guidelines, not vice versa.

COMPARISONS AT LARGE-SCALE CATEGORY 1

At category 1 all three sets of guidelines allow an open system for the process itself, but one which either 'minimizes release' or directs any effluents to a safe place. Only the ACGM guidelines specify 'safe place' (footnotes 1 and 5 *Table 14*) but I have already suggested why this is the only sensible interpretation of 'minimize release'.

The fact that these are all open categories makes any comparison between them very difficult; the stringency of the containment will depend almost entirely on the nature of the biological containment, which is a property of the organism and not of the engineering or methods of working that the guidelines can describe. In so far as the emissions are controlled, ACGM Large-Scale Category 1 (ACGM, 1987b) specifies that the controlled area should be designed to contain a spillage (item 12 in *Table 14*). This would seem to be covered by GILSP as much as by the principles underlying the containment of the recombinant organism, not least to ensure that the spill can be directed to the correct drain or kept out of the drains altogether.

What is more pertinent than the comparison is the recognition that the bulk of all manufacture with recombinant organisms will be done at or below this category of containment (ACGM, 1987b *see* para. 13). If the containment of these organisms is crucial, it is perhaps surprising that so much time and effort should have been spent on the definition of the containment categories, rather than on the nature of the biological containment. Where a higher category of containment is required it is at least as likely to be due to the nature of the product as it is to the organism itself. In a recombinant organism the two hazards are intimately linked, but it is worth reminding ourselves of those experiments which were to be deferred under the Asilomar classification (*Table 5*) if only to realize that this point is not a new one.

Validation of containment

One of the principles of GILSP tests for the presence of viable process organisms outside of the primary containment (*Table 6*). Where the process is closed this will have some value, and, taken at face value, it would provide a useful validation of the containment. It is quite likely that the process organisms would not be detected if the primary containment is closed, although the environment will not be sterile. However, if there is some low background level of release at OECD Category 2, for example, some upper limits could be placed on the numbers of organisms allowable in some standard test.

If accidental release is the most likely route for a breach of the containment, then this microbiological testing could well be less important than the strict adherence to validated methods of work. Ensuring that trained staff know how to operate the equipment, and that they have done so correctly, is, in the end, more important than a reliance on the microbiological tests that would demonstrate that an accidental release had taken place. The defined working practice will need to extend through to the final cleaning and sterilization, so that the plant is left in a safe condition at the end of the process.

Validation is an important feature of temporal containment. If, as seems likely, many of these plants run a number of processes, with the unit operations assembled in different sequences for each process (*see* Gailliot *et al.*, 1987), then it is also likely that the processes will operate at different containment categories. It would be possible to run the plant always at the highest likely level of containment, but this would be very restrictive. It is much more likely that the level of containment will be adjusted to suit the immediate process. However, this does mean that after a process at a high level of containment the plant will need to be downgraded to a lower level. This is only possible if the plant can be validated as safe after the highly contained process is complete.

The working practices need to be effective, well documented and kept under review (*see* Sharp, 1983). They need to ensure, not only that the organism remains within the containment boundaries, but that, at the end of the process, any left within the boundaries are killed (see appendices K-IV-E and K-IV-I, etc. in NIH, 1986). If the plant has been operated correctly, and the microbiological tests confirm the absence of the process organism, then it should be safe.

The working practices will need to be equally effective in the event of an accidental release or some other plant failure. There can be no doubt that it will be much more difficult to validate a plant as safe after an accident than following a normal process. This points to the need for equipment that is reliable above all else, even to the point of sacrificing some containment in normal operation.

Although the objectives of the working practices can be defined for an open system from which controlled release is allowed, it is not easy to set down the criteria which would ensure that they had been achieved. The validation of these lower categories of containment is important if only because of the expectation that they will cover the bulk of the manufacturing processes with recombinant organisms. Once the processes are established it would be possible to ensure that they were operated correctly. It would also be possible to ensure that the process organism had been removed from the products which leave the contained process, or at least from those that do have to be free of the organism. However, the objectives should ensure that the biological containment of the organism is effective, and that will be difficult to validate.

Fitting organisms and processes to categories

Reviewing the categories of containment is a straightforward exercise when

compared to the problem of assigning the particular category needed for an organism and the process which uses it. The hazards of the micro-organisms that are used in large-scale manufacture (Sargeant and Evans, 1979) are a good guide to the hazards to be expected from recombinant organisms. Many of the former are insignificant. The same will be true of the latter, but the organisms are novel and there is no long history of their use. The lack of any historical evidence makes it possible to argue convincingly that recombinant organisms will bring unpredictable effects, particularly in the environment (Sharples, 1987), or that they will not (Davis, 1987). This is one of the difficulties with which prospective safety legislation must come to terms.

Accidents are concrete events whose causes can be analysed to provide the basis for retrospective safety legislation. The prospective analysis of hazards and their potential effects contains a much greater element of theory, not to say hypothesis. It may be difficult even to identify the hazards that a new process contains, let alone calculate the risks of their manifestation in an accident. Furthermore, we do not actually want to put any hypothesis to an experimental test. It is therefore inevitable that our current attitude to legislation will cause us to err on the side of caution.

One attempt to predict the hazards of recombinant organisms assigns

Table 16. Factors for calculating the hazards associated with recombinant organisms (adapted from ACGM, 1988a)

Access—Probability that a manipulated organism can enter the human body and survive	
Certain (wild-type *Salmonella* or *E. coli*)	1
Little-known ability (*Streptomycetes* spp., *E. coli* K12)	10^{-3}
Disabled host/vector system	10^{-6}–10^{-9}
Expression—Measure of the anticipated or known level of expression of the inserted DNA	
Deliberate intention to maximize expression	1
Insertion without maximizing expression	10^{-3}
Insertion at site which does not facilitate expression	10^{-6}
Non-expressible DNA	10^{-9}
Damage—Probability of gene product resulting in ill-health of exposed worker	
Toxin or pathogenic determinant	1
Biologically active substance	10^{-3}
Biologically active substance below 10% of normal body burden	10^{-6}
Biological effect unlikely	10^{-9}
No biological effect (non-coding DNA)	10^{-12}

Table 17. Assignment of recombinant organisms to containment categories based on the scores calculated as the product of Access, Expression and Damage. The ACDP/ACGM categories have now superseded the old GMAG categories

Score	Containment category	
	ACDP/ACGM	GMAG
10^{-15} or lower	1	0 or GMicP
10^{-12} to 10^{-14}	2	1
10^{-9} to 10^{-11}	3	2
10^{-6} to 10^{-8}	Case-by-case to 3 or 4	3
10^{-5} or greater	4	4

numerical values to their properties under three headings, Access, Expression and Damage (ACGM, 1988a; *see Table 16*). These aim to calculate the likely hazard that the recombinant organism poses to a worker who is using it. The numerical product of the values assigned under each of the three headings is a score which dictates the ACGM Laboratory Containment Category (*Table 17*). These Laboratory Categories of 1 to 4 (ACGM, 1988b) do not necessarily fix the containment levels that would be used in large-scale manufacture. However, they provide a starting point for the necessary discussion that needs to be held on a case-by-case basis. It will take a more cautious account of the product because of the larger quantities involved. The level of biological containment will also be important.

There is no point here in opening up the debate on biological containment. Strains released by human activities do survive for extended periods (Evans, Preece and Sargeant, 1981; Levy, 1987; Beringer and Bale, 1988) and they can be difficult to remove (Manchee and Stewart, 1988) but biological containment can be engineered into organisms just as any other property (Molin *et al.*, 1987; Curtis, 1988), and it may be possible to test its effect in the laboratory (Skinner, 1988). Until there is more information we shall need a careful analysis of the likely hazards.

The Royal Commission (Report, 1989) have suggested that GENHAZ, which is an as yet unpublished version of the Hazard and Operability Study (HAZOP), might be useful. HAZOP itself is one of a number of methods that are routinely used in chemical engineering to identify likely causes of failure in new process plants (Firenze, 1978; Marshall, 1987). It will be very useful if the losses from the more stringent categories of containment are largely the result of accidental failure. Where, at lower categories of containment, the emissions from the primary or secondary containment need to be controlled, these same procedures will help to ensure that they remain so. However, hazard analysis relies on comparisons with available information; even for chemical plants it is of limited use in predicting failures that involve unforeseen chemistry (Marshall, 1987). The techniques will therefore be less useful in predicting the outcome of the failure if it occurs.

The prospective analysis of the hazards of recombinant organisms and of the loss or injury that they could cause is difficult. We ought not to pretend that because we develop systematic procedures for the analysis that it becomes less speculative. Nor ought we to presume that the actions we have taken have actually contained the hazards, and have, as a consequence, prevented accidents. There may have been no effects because the actions were not needed. The hazards themselves may have been imaginary. On the other hand, a public debate of the issues with the involvement of national and local safety committees does create an environment in which the moral risk (Sass, 1987) can be properly recognized.

In this debate there is a balance to be drawn between common sense and a healthy respect for the limits of our knowledge and technology. To hedge in the large-scale work with too many restrictions does not solve the problems, it simply refuses to address them. To provide too few could release a pathogen. It is a far more serious problem than H.G. Wells imagined (1894).

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