Guidelines for the large-scale use of genetically manipulated organisms / Advisory Committee on Genetic Manipulation.

ACGM/HSE/NOTE 6

ADVISORY COMMITTEE ON GENETIC MANIPULATION

GUIDELINES FOR THE LARGE-SCALE USE OF GENETICALLY MANIPULATED ORGANISMS

INTRODUCTION

1. In 1979 the Genetic Manipulation Advisory Group (GMAG) issued guidance on the large-scale use of genetically manipulated organisms and this was revised in February 1982. The guidance set out factors to be considered in risk assessment and laid down a procedure for the notification to HSE and GMAG of large-scale work.

2. GMAG Note 12 has now been reviewed by ACGM as part of its programme of work and in response to:

2.1 increasing experience of large-scale fermentation of genetically manipulated organisms;

2.2 the need to provide more detailed guidance on risk assessment and physical containment;

2.3 the recommendations and conclusions of a major international study on rDNA safety considerations set up by the OECD (Reference).

This guidance supersedes and replaces GMAG Note 12.

3. This guidance should be read in the context of:-

3.1 The legal responsibility of employers under Section 2(1) of the Health and Safety at Work etc Act 1974 to ensure, so far as is reasonably practicable the health, safety and welfare at work of their employees;

3.2 The Health and Safety Commission's booklet on safety representatives and safety committees, 1977; and

3.3 GMAG Note 1, which sets out the constitution and functions of local genetic manipulation safety committees.

DEFINITION

4. The definition of large-scale widely accepted in the UK and elsewhere is fermentation in volumes of 10 litres or more. Although this is a useful and well understood definition it involves only the fermentation volume and makes no reference to the number of recombinant organisms present and GMAG tried to move away from this concept in its revised Note 12 (1982). ACGM believes that a better definition for the purposes of the guidelines would be the use of a cell or organism constructed by genetic manipulation for example, in a laboratory scale reaction vessel, for pilot plant work or commercial manufacture.

NOTIFICATION

5. The existing notification arrangements should continue. Information on the detail required is given in paras 21-24. Notification of individual projects is required to enable HSE and ACGM to monitor developments and hence to update guidance to ensure that this important and relatively new application of genetic manipulation continues to develop with public confidence.

6. These notification arrangements are not at present mandatory. However the Health and Safety (Genetic Manipulation) Regulations 1978 are under review and proposals for revision will be issued for public consultation in due course.

HAZARDS

7. When genetic manipulation techniques were first introduced there was concern that they could give rise to potential hazards. After more than a decade of experience these hazards have remained conjectural and not based on incident. There are no known health hazards specific to genetic manipulation although hazards other than those associated with non-manipulated micro-organisms may be envisaged.

8. The hazards posed by large-scale fermentation of genetically manipulated organisms are expected to be of the same nature as for other biological agents, namely:

8.1 infection hazard - the potential for disease following exposure to the organism;

8.2 toxic, allergenic or other biological effects of the non-viable organism or cell, its components or its naturally occurring metabolic products;

8.3 toxic, allergenic or other biological effect of the product expressed by the organism.

However, it can be postulated that genetic manipulation may give rise to complications of these hazards eg enhanced immune response to human proteins expressed as fusions with bacterial proteins.

9. Relative to the construction of genetically manipulated organisms, there is nothing intrinsically more hazardous about their large-scale use; it is the scale of operation and hence the potential for a greater degree of exposure to an organism and its biologically active products that is increased.

PROCESS RISKS

10. There are discrete stages (unit operations) such as the steps involved in downstream processing each of which require individual assessment (see para 19). For example, any consideration of risk must take account of factors such as whether the organism is killed in the fermenter before downstream processing. Methods of processing micro-organisms on a large-scale such as cell separation by centrifuging or otherwise concentrating and washing cells, in breaking them and in extracting the required products, have the potential for widespread contamination and aerosol generation unless appropriate precautions are taken.

APPROACH TO RISK ASSESSMENT AND CONTAINMENT

11. All work involving the construction and large-scale use of genetically manipulated organisms should continue to be considered by the local genetic manipulation safety committee (see GMAG Note 1). Each proposal should be treated on a case by case basis using the recommendations below.

12. The majority of organisms used in traditional manufacturing industry have rarely given rise to safety problems. Modified organisms constructed by inserting segments of DNA that are well characterised and free from known harmful sequences to improve their performance are unlikely to pose significant risk. Where traditional micro-organisms are modified by inserting segments of DNA to facilitate the manufacture of new products there should not be any safety considerations beyond those that might be posed by the product itself.

13. ACGM recognises that the vast majority of large-scale applications of genetic manipulation will use organisms of intrinsically low risk which warrant only minimal containment. It is proposed that in accord with the OECD report this level of minimal containment be known as Good Large Scale Practice (GLSP). GLSP will involve no containment measures beyond those required for process needs. For GLSP as well as all levels of containment, the following fundamental principles of occupational safety and hygiene should be applied:

(i) to keep workplace and environment exposure to any physical, chemical or biological agent to the lowest practicable level;

(ii) to exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary;

(iii) to test adequately and maintain control measures and equipment;

(iv) to test when necessary for the presence of viable process organisms outside the primary physical containment;

(v) to provide training of personnel;

(vi) to formulate and implement local codes of practice for the safety of personnel.

14. GLSP can be considered as analogous to the concept of "minimal risk" used for toxic substances. In that context minimal risk is defined as the "level [of exposure] above which adverse effects may start to become apparent. Exposure below this level is not without risk, but the risks appear to merge with, and are comparable to, the general risks to which all workers are routinely exposed".

Criteria for GLSP

15. The local genetic manipulation safety committee should use the following criteria to allow the designation of GLSP:-

15.1 The host organism should not be a pathogen, should not contain known adventitious agents, and should have an extended history of safe use, or have built-in environmental limitations that permit optimum growth in the bioreactor but limited survival without adverse consequences in the environment.

15.2 The vector/insert should be well-characterized and free from known harmful sequences; should be limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the recombinant in the environment unless that is a requirement of the intended function; should be poorly-mobilisable; and should not transfer any resistance markers to micro-organisms not known to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

15.3 the genetically manipulated organism should not be a pathogen; and should be assessed as being as safe in the bioreactor as the host organism, and without adverse consequences in the environment.

16. There are two clear examples of other classes of organisms that warrant the GLSP designation unless they are pathogenic:

16.1 those constructed entirely from a single prokaryotic host (including its indigenous plasmids and viruses) or from a single eukaryotic host (including its chloroplasts, mitochondria or plasmids - but excluding viruses); and

16.2 those consisting entirely of DNA segments from different species that exchange DNA by known physiological processes.

These criteria are summarised in Appendix I.

Assessment and Physical Containment of non-GLSP organisms

17. It must be recognised that it may in some cases be necessary to use some physical containment to match the assessed risk. Evaluation of potential risk should include consideration of the organism's biological containment and potential adverse effects. Points to consider in evaluating such organisms and the effect of the introduced DNA are outlined in Appendix II. It is anticipated that much of the necessary information will have been generated during the organism's construction and characterisation. The points to consider are not expected to be applicable in all cases. The level of containment used in the construction of the organism (See GMAG Note 14) is one factor to be taken into account when assigning the appropriate level of containment for large-scale work.

PHYSICAL CONTAINMENT

18. The primary objective in selecting containment is to match an appropriate level of physical measures and associated safety procedures to the conclusions of the risk assessment. In essence the considerations that should influence selection of containment are:

(i) the nature of the modified organism;

(ii) the nature of the process; and

(iii) the nature of the product.

In some cases, an evaluation of the modified organism may indicate that the containment level appropriate for the construction of the organism is not appropriate to the large-scale process. For example, the laboratory level of physical containment may be high if the donor organism is a pathogen; however, the resulting modified rDNA-containing organism may be a non-pathogen which contains donor DNA sequences not associated with the pathogenic phenotype (eg, E.coli host-vector systems expressing hepatitis B surface antigen). Lower containment levels might then be appropriate for any studies subsequent to construction of this organism. Application of a lower physical containment level to the large-scale use of this modified organism might also be appropriate. Aspects of large-scale work differ from laboratory research. The modified organism should be re-evaluated and appropriate containment selected at the time of transfer to large-scale processes.

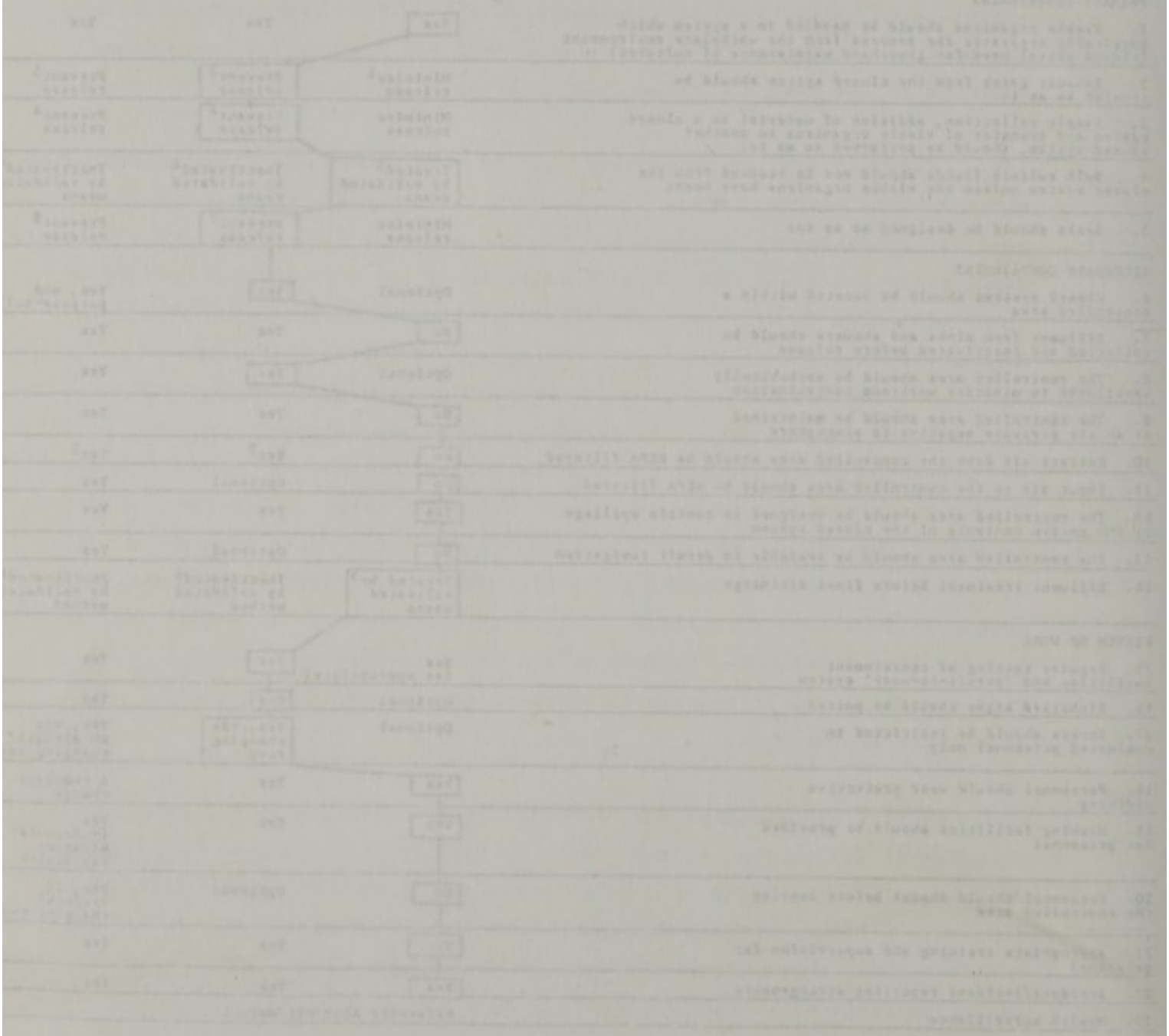
19. It should also be recognized that in some cases, the risks presented by other aspects of the process and by the product may dictate the level of physical containment. Large-scale process plants and equipment are diverse in application and scale and the methods selected for the physical control of risks will also be diverse. In addition, large-scale processes should be considered in terms of their unit operations (see para 10). The characteristics of each operation will dictate the physical containment to be used in that stage. This will allow selection and design of process, plant and operating procedures best fitted to assure adequate and safe containment. Two important factors to be considered when selecting the equipment needed to implement the containment are the risk of, and the effects consequent on, equipment failure. Engineering practice may require increasingly stringent standards to reduce the risk of failure as the consequence of that failure becomes less tolerable. Because of the rapid advance of knowledge, precise risk assessment as it relates to physical containment may be revised as experience accumulates.

20. As part of this approach, flexibility in selection of containment measures is desirable. It may, thus, be appropriate to select and combine containment on the basis of a unit operations assessment rather than to implement a fixed category of containment for a whole process. Examples of possible containment categories are given in Appendix III. However it is emphasised that this approach does not call for strict adherence to all the requirements of any particular category. For example, the local genetic manipulation safety committee, having carried out a full risk assessment, may

advise that a particular fermentation be carried out basically under conditions of Large Scale Containment Category 1. However, if the organism concerned has a known potential to cause allergenic effects following airborne exposure, then selection of Category 2 precautions for the enclosure of sample ports, seals etc. may be appropriate. This example is expressed in the Table below. In other words it is recommended that these categories should be flexibly applied. HSE's specialist microbiology inspectors are available for advice on risk assessment and physical containment.

Specimen example of Process Containment (see Appendix III

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:	Minimise[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:	Minimise 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[6] by validated means	Inactivated[6] by validated means
5. Seals should be designed so as to:	Minimise release	Prevent[7] release	Prevent[8] release
SECONDARY CONTAINMENT			
6. Closed systems should be located within a controlled area	Optional	Yes	Yes, 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 minimise 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 (+ 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/HSE/Note 4		



NOTIFICATION PROCEDURES

21. Although similar to the arrangements set up by GMAG, it should be noted that the procedures below are related to the large-scale risk assessment rather than the laboratory categorisation.

Procedure A

The following types of work should be notified to HSE with sufficient information for HSE and ACGM to review the local risk assessment.

(i) procedures that involve the use of recombinants made by self-cloning in systems exempt from notification when conducted on the laboratory scale (at present including E. coli, Saccharomyces, B. subtilis), and other systems listed in GMAG Note 8.

(ii) procedures that utilise recombinants which can be designated as GLSP with due consideration to the factors outlined above.

For work complying with (i) and (ii) above, large-scale work can proceed after notification alone.

Procedure B

The proposer should notify HSE and await comments before commencing work which involves the use of:

(i) recombinants assessed as outside GLSP designation

(ii) non-disabled host-vector systems

(iii) genetic factors determining pathogenicity

(iv) recombinant organisms coding for or producing a substance with a pharmacological effect, as discussed under 'Damage' on page 4 of GMAG Note 14.

22. Each notification to HSE under either procedure should include the following information:

(a) the nature of the product

(b) the host-vector system to be used

(c) the risk assessment and comments of the local genetic manipulation safety committee

(d) the scale of operation proposed

(e) the safety precautions proposed - for non GLSP organisms the proposed process containment with reference to Appendix III.

(g) the name of the BSO and the composition of the local genetic manipulation safety committee (and who its members represent).

23. It is anticipated that a response will be given within one month of receipt. ACGM and its Secretariat in HSE are available for advice on risk assessment and notification.

Commercial Confidentiality

24. There are arrangements for handling commercial-in-confidence information established by the Health and Safety Commission for advisory committees such as ACGM. Where a notification is to include such information initial contact should be made with the ACGM Secretariat for full details.

APPENDIX I

CRITERIA FOR rDNA GLSP MICRO-ORGANISMS

Host Organism	rDNA Organism	Vector/Insert
Non-pathogenic	Non-Pathogenic	Well characterised 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 not transfer any resistance markers
Built-in environment limitations permiting 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 acquistion could compromise use of drug to control disease agents)

APPENDIX II

GENERAL SCIENTIFIC CONSIDERATIONS

This Appendix attempt to set out basic scientific considerations that may be relevant in assessing the possible risks associated with the use of rDNA organisms. Although the list attempts to be comprehensive as far as present knowledge allows, not all the points included will apply to every case. It is to be expected therefore that individual proposals will address only those issues that are relevant to the proposed work. The level of detail required is also likely to vary according to the nature of the proposal.

A. Characteristics of Donor and Recipient Organisms

1. Taxonomy, identification, source, culture

(a) Names and designations.

(b) The degree of relatedness between the donor and recipient organisms and evidence indicating exchange of genetic material by natural means.

(c) Characteristics of the organism which permit identification and the methods used to identify the organisms.

(d) Techniques employed in the laboratory and/or environment for detecting the presence of, and for monitoring, numbers of the organism.

(e) The sources of the organisms.

(f) Information on the recipient organism's reproductive cycle (sexual/asexual).

(g) Factors which might limit the reproduction, growth and survival of the recipient organism.

2. Genetic characteristics of donor and recipient organisms

(a) History of prior genetic manipulation.

(b) Characteristation of the recipient and donor genomes.

(c) Stability of recipient organism in terms of relevant genetic traits.

3. Pathogenic and physiological traits of donor and recipient organisms

(a) Nature of pathogenicity and virulence, infectivity, or toxicity.

(b) Host range.

(c) Other potentially significant physiological traits.

(d) Stability of these traits.

B. Character of the Modified Organism

(a) Description of the modification.

(b) The nature, function and source of the inserted donor nucleic acid, including regulatory or other elements affecting the function of the DNA and of the vector.

(c) The method(s) by which the vector with insert(s) has been constructed.

(d) Methods for introducing the vector-insert into the recipient organism and the procedure for selection of the modified organism.

(e) The structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism.

(f) Characterization of the site of modification of the recipient genome. Stability of the inserted DNA.

(g) Frequency of mobilisation of inserted vector and/or genetic transfer capability.

C. Expression and properties of the gene product

(a) Rate and level of expression of the introduced genetic material. Method and sensitivity of method.

(b) Activity of the expressed protein.

(c) Allergenic hazard of the product.

(d) Toxic hazard of the product.

APPENDIX III

EXAMPLES OF CONTAINMENT APPROACHES FOR LARGE SCALE APPLICATIONS OTHER THAN GLSP

For explanation of the application of these containment categories see para 20

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 growthand maintenance of cultures)	Yes	Yes	Yes
2. Exhaust gases from the closed system should be treated so as to:	Minimise[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:	Minimise 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[6] by validated means	Inactivated[6] by validated means
5. Seals should be designed so as to:	Minimise release	Prevent[7] release	Prevent[8] release
SECONDARY CONTAINMENT			
6. Closed systems should be located within a controlled area	Optional	Yes	Yes, 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 minimise 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 (+ 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/HSE/Note 4		

KEY

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 sterilisable 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 housing that are exhausted by a HEPA filter.

ADVISORY COMMITTEE ON GENETIC MODIFICATION: APPENDIX IV OF ACGM/HSE/NOTE 6 - LARGE SCALE USE OF GENETICALLY MANIPULATED ORGANISMS

Environment aspects to risk assessment

1 In arriving at a Good Large Scale Practice (GLSP), designation for work involving Genetically Manipulated Organisms (GMOs) it is essential that environmental considerations are taken into full account.

2 It is an inherent feature of large scale work, especially at GLSP, that microorganisms will be released incidentally at various stages of the fermentation process and at early stages of down stream processing. It is therefore important that in any large scale use of genetically manipulated organisms, an environmental assessment be carried out before work commences. Such assessment should take into account the following factors, where these are relevant or appropriate and where appropriate information is known or can be obtained;

i the volume/biomass of organisms likely to be released;

ii known or predicted behaviour of the organism, including factors affecting the survival, multiplication and dissemination of the organism;

iii description of the ecosystems to which organisms could be disseminated and known or predicted impact in such ecosystems including effects on plants, animals and micro-organisms eg pathogenicity, toxicity, virulence, allergenicity, colonisation;

iv the availability of techniques for the detection, identification and monitoring of the organism and for detecting transfer of new genetic material to other organisms;

3 The continued use by industry of microorganisms that meet the GLSP criteria will help to ensure that the environment is protected from potential harm that might otherwise be caused by the discharge of large numbers of viable organisms.

4 Whenever genetically manipulated organisms are being disposed of after use, fundamental principles of good occupational safety and hygiene should be applied based on the outcome of risk assessment. Methods should always be employed that avoid harm to people and the environment.

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