

The SACGM Compendium of guidance

Part 3: Containment and control of activities involving
genetically modified microorganisms

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3.1 Containment and control of activities involving genetically modified microorganisms

Overview

1. The Genetically Modified Organisms (Contained Use) Regulations require that all activities involving genetically modified microorganisms (GMMs) in containment must use physical, chemical or biological barriers or any combination of these to limit contact with people and the environment. They also demand that certain standards of occupational and environmental safety be met. The following sections of guidance set out in broad terms the requirements of the Contained Use Regulations for the handling of GMMs in different situations. Plant growth facilities are not covered in this section and users are directed to Part 4 for guidance relevant to activities with GMMs associated with plants.
2. The guidance is divided into several sections and users will have to determine which guidance is relevant to the activity being undertaken and conform to the regulatory requirements covered by that guidance:
 - ***Containment and control measures for laboratory activities involving GMMs*** is intended to give guidance to help users determine the appropriate standards of containment and control that should be applied to work involving GMMs in *laboratories*, or *small-scale* activities.
 - ***Containment and control measures for large-scale activities involving GMMs*** is intended to give guidance to help users determine the appropriate standards of containment and control that should be applied to work involving GMMs at a *large-scale*, for example a biomanufacturing plant.
 - ***Containment and control measures for activities with GMMs that involves handling animals*** is intended to give guidance to help users determine the appropriate standards of containment and control that should be applied to animal studies involving GMMs. Users are directed to Part 5 for considerations relating to the risk assessment, containment and control of work involving GM or *transgenic* animals.
3. There is also more specific guidance relating to:
 - Inactivation.
 - Fumigation.
 - Microbiological safety cabinets.
 - Handling of oncogenic sequences.

Using the guidance

4. Any reference to regulations in the following guidance refers to the Contained Use Regulations.
5. The level of containment and control is required to be commensurate with, and determined by, the risk assessment. Regulation 18 requires that users compare the measures warranted by the risk assessment with the appropriate table of containment measures set out in Schedule 8. This determines the GM activity class, which in turn determines the minimum containment level needed for that activity and notification requirements.
6. The relevant Schedule 8 table is reproduced in each section. Control measures at every containment level has appropriate guidance associated with it. This guidance is intended to offer advice with respect to how to achieve the appropriate standards. Some of the listed control measures will not be required at the lower containment levels and, in some cases, the guidance will simply reflect this. The guidance may, however, also highlight other measures that are considered good practice or approaches that can be implemented to achieve the standards required by the legislation. These approaches are illustrative and users may adopt other methods so long as the standards set by the legislation are met.
7. The Schedule 8 tables describe containment for the use of GMMs in laboratories, animal houses and 'other' settings (which is interpreted as large-scale industrial research and production facilities). There are some settings for which these tables are not particularly appropriate, for example, a clinic. Users in such settings must execute containment and control, although it is acknowledged that application and interpretation of the Schedule 8 tables may be difficult and users in these situations should contact HSE for further guidance. Specific guidance for the use of GM organisms in a clinical setting can be found in Part 6.
8. This mechanism for determining the containment measures is underpinned by the requirements of regulation 17. This requires that exposure of humans and the environment, and the level of harm to humans as a result of activities involving genetically modified organisms, be reduced to the lowest level that is reasonably practicable. In addition to the containment measures set out in Schedule 8, the principles of *good microbiological practice* and *good occupational safety and hygiene* must be applied, to the extent to which they are appropriate. These principles are detailed in Schedule 7 of the regulations and are reproduced below. Additional guidance on each of these measures can be found in the HSE publication *A guide to the Genetically Modified Organisms*

- Keeping workplace and environmental exposure to any genetically modified micro-organism to the lowest reasonably practicable level.
 - Exercising engineering control measures at source and supplementing these with appropriate personal protective clothing and equipment where necessary.
 - Testing adequately and maintaining control measures and equipment.
 - Testing, where necessary, for the presence of viable process organisms outside the primary physical containment.
 - Providing appropriate training of personnel.
 - Formulating and implementing local codes of practice for the safety of personnel, as required.
 - Displaying biohazard signs where appropriate.
 - Providing washing and decontamination facilities for personnel.
 - Keeping adequate records.
 - Prohibiting in the work area eating, drinking, smoking, applying cosmetics or the storing of food for human consumption.
 - Prohibiting mouth pipetting.
 - Providing written standard operating procedures where appropriate to ensure safety.
 - Having effective disinfectants and specified disinfection procedures available in case of spillage of genetically modified microorganisms.
 - Providing safe storage for contaminated laboratory equipment and materials where appropriate.
9. The general requirements of regulation 17 and the application of the general principles of *good microbiological practice* (GMP) and of *good occupational safety and hygiene* (GOSH) from Schedule 7 are required for *all activities in any setting*. For example, activities involving self-cloned organisms are exempt from all the Contained Use Regulations, except for regulation 17.
10. It is important that users consider measures other than those set out in the tables, or those listed above. Some control measures deemed necessary by the risk assessment may not be specifically mentioned. For example, the use of sealed centrifuge rotor buckets or respiratory protective equipment might be required in certain circumstances to protect the user against aerosols. Furthermore, the measures required at each containment level may not provide appropriate protection for the environment. The risk assessment should be used to identify particular elements of containment that are lacking and the measures implemented accordingly to afford maximum protection for human health and the environment.

All measures shown to be necessary by the risk assessment must be implemented, but only those in the table will affect the GM activity classification and notification requirements.

Biosecurity

11. Where materials derived from certain hazardous pathogens are used and stored, it is important to consider any risks to national security. GM work with material derived from pathogens that appear on the Schedule 5 list from the Anti Terrorism, Crime and Security Act 2001 (ATCSA) may require a specific notification to the Home Office. Security assessments should be made in addition to risk assessments for the protection of human health and the environment, but many of the restricted access and safe storage measures required for human health and environmental protection will also be appropriate for biosecurity purposes. In some cases, laboratories will be using attenuated GMMs to study microorganisms that they are not equipped or permitted to handle. However the material could theoretically be used to reconstitute a hazardous pathogen and therefore precautions should be taken. In general, materials should be handled in an area with restricted access and stored securely in a section where admittance is controlled.

Organisational control

12. Another requirement of the Contained Use Regulations is that local rules are drawn up for the safety of personnel, making clear organisational responsibilities and duties. Items to be covered will depend on local circumstances and activities. In order to maintain standards within laboratories, standard operating procedures (SOPs) should be written with respect to the safe management of the work. New recruits and staff who have not previously been trained to handle GMMs should receive full instruction in order to prevent accidental misuse and records of their training should be made and retained. Compliance with SOPs and local rules should be rigorously enforced at the local level.

3.2 Containment and control measures for laboratory activities involving genetically modified microorganisms

Overview

1. The Genetically Modified Organisms (Contained Use) Regulations require that all activities involving genetically modified microorganisms (GMMs) in containment employ a combination of physical, chemical or biological barriers to limit contact with people and the environment. This general requirement is underpinned by specific regulations 17 and 18 and their associated schedules, which prescribe the minimum containment for particular activities.
2. Regulation 17 sets out the principles of occupational and environmental safety which aims to ensure that *the exposure of humans and the environment to genetically modified microorganisms is reduced to the lowest level that is reasonably practicable*. These principles (as outlined in the Section 3.1 and Schedule 7 of the regulations), are described as the general principles of *good microbiological practice* and of *good occupational safety and hygiene*, and apply to any activity involving the use of GMMs.
3. Regulation 18 requires that a person who undertakes an activity involving genetic modification of microorganisms shall apply the containment measures set out in the applicable Table in Schedule 8, where and to the extent required in the column of the appropriate containment level. The following guidance regards the minimum containment and control measures required by law for laboratory-scale activities with GMMs as well as methods that can be applied to aid the fulfilment of legal obligations. The Contained Use Regulations are not the only legislation that may impact upon the containment measures needed, and users must take this into account (further guidance can be found in Part 1 of the Compendium).

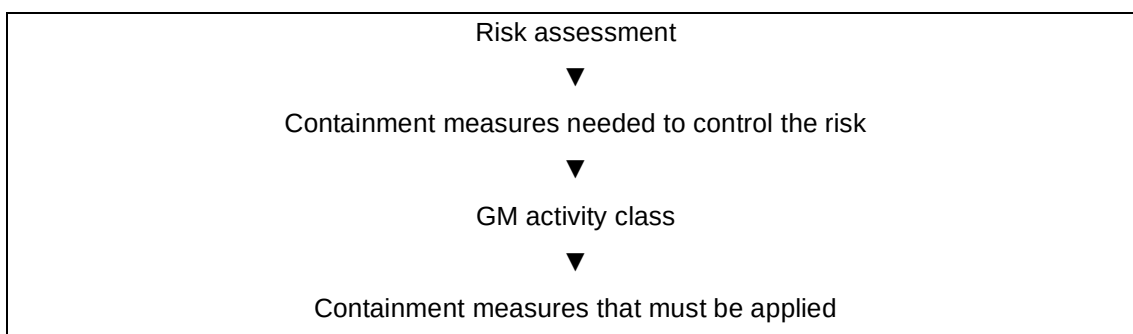
Scope

4. This section is intended to give guidance to help users determine the appropriate standards of containment and control that should be applied to work involving GMMs in *laboratory-scale* activities. Users will have to determine whether the activity being undertaken is laboratory-scale or whether guidance appropriate to large-scale activities is more appropriate. There is no absolute volume threshold at which a laboratory-scale activity will become a large-scale one but the primary factor will be the nature of the containment measures appropriate to the activity. It will normally be obvious from these what guidance users should follow. For instance, activities involving petri dishes, flasks

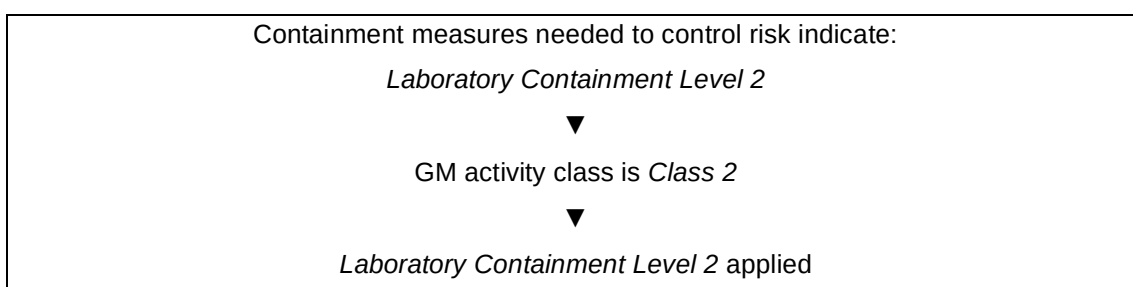
and culture bottles will normally indicate laboratory-scale activities, as would the use of small bench-top chemostats and fermenters. Once the scale has been decided, users should follow the appropriate guidance, although it may be necessary to consider multiple sets of guidance for operations that are not clearly covered by any one part. Users might find it helpful to refer to the ACDP publication *The management, design and operation of microbiological containment laboratories* Guidance HSE Books 2001 ISBN 978 0 7176 2034 0.

Selecting the minimum containment level

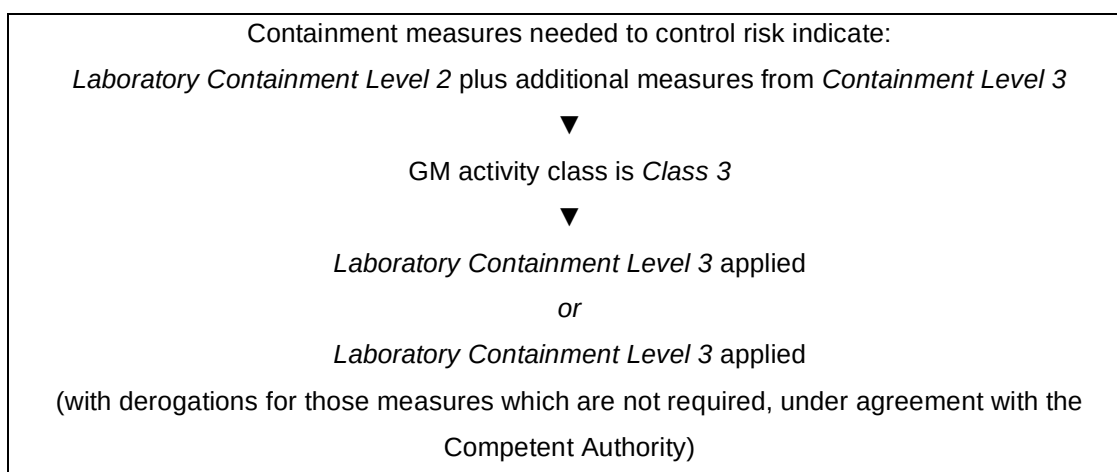
5. The risk assessment must be used to determine the appropriate control measures that are needed to afford maximum protection to both human health and the environment. Users should compare the measures warranted by the risk assessment with the table of containment measures (reproduced from Schedule 8) to determine the GM activity class. The GM activity class, in turn, determines the minimum containment level required for the activity.
6. Some control measures deemed necessary by the risk assessment may not be listed in the Schedule 8 containment level tables. The GM activity class is determined solely by those measures actually listed. The risk assessment must always take precedence and ALL measures identified as necessary must be applied.



7. In the majority of cases, the containment measures necessary to control the risk, the GM activity class and the minimum containment level to be applied will be the same.



8. The containment measures indicated by the risk assessment may consist of measures from two different levels. In these cases, the higher level of containment will determine the GM activity class and must be applied. A request can be made to the Competent Authority at the time of notification for permission to use the mixture of two levels identified. However, unless or until you have the agreement of the Competent Authority, you may not use a level of containment lower than that corresponding to the GM activity class.



Definitions

9. Legal requirements will be stated clearly as such, with the use of the words 'regulatory requirement', 'required' or 'must'. Otherwise in cases where the word 'should' is used the guidance highlights approaches that can be used to achieve the appropriate standards. These approaches are only illustrative, and users may adopt other approaches so long as the standards set by the Regulations are met.
10. The following terms do not have legal definitions. The explanations given below are intended to aid understanding:
- **Laboratory:** A room in which the organisms are handled.
 - **Laboratory suite:** One or more laboratories, not necessarily of the same discipline or containment level, and ancillary rooms within a section or department with shared use of equipment and facilities such as media preparation, autoclaves and centrifuges.
 - **Laboratory unit:** A separate building or self-contained suite within a building containing one or more laboratories and with ancillary rooms such as airlocks, changing rooms, showers or autoclave rooms.
 - **Required where and to the extent the risk assessment shows it is required:** This indicates that the need for a particular measure is determined by the risk assessment.

If the risk assessment specifies that a measure is needed for human health or environmental protection, its use is mandatory.

- **Competent authority:** The body with enforcing jurisdiction over the Contained Use Regulations. In England and Wales the competent authority includes the Health and Safety Executive (HSE), the Secretary of State and the Department for Environment, Food and Rural Affairs (Defra). In Scotland the competent authority includes HSE, the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the Scottish Ministers. Northern Ireland has its own separate competent authority. When seeking approval from the competent authority for particular actions, users should contact HSE in the first instance.

Containment measures for laboratory activities involving GMMs (Schedule 8 Table 1a)

CONTAINMENT MEASURES		CONTAINMENT LEVEL			
		1	2	3	4
M1	Laboratory suite: isolation	not required	not required	required	required
M2	Laboratory: sealable for fumigation	not required	not required	required	required
EQUIPMENT					
M3	Surfaces impervious to water and resistant to acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	required for bench	required for bench	required for bench and floor	required for bench, floor, ceiling and walls
M4	Entry to lab via airlock	not required	not required	required where and to extent the risk assessment shows it is required	required
M5	Negative pressure relative to the pressure of the immediate surroundings	not required	required where and to extent the risk assessment shows it is required	required	required
M6	Extract and input air from the laboratory should be HEPA filtered	not required	not required	HEPA filters required for extract air	HEPA filters required for input and extract air
M7	Microbiological safety cabinet/enclosure	not required	required where and to extent the risk assessment shows it is required	required, and all procedures with infective materials required to be contained within a cabinet /enclosure	Class III cabinet required
M8	Autoclave	required on site	required in the building	required in the laboratory suite	double ended autoclave required in laboratory
SYSTEM OF WORK					
M9	Access restricted to authorised personnel only	not required	required	required	required via air lock key procedure
M10	Specific measures to control aerosol dissemination	not required	required so as to minimize	required so as to prevent	required so as to prevent
M11	Shower	not required	not required	required where and to extent the risk assessment shows it is required	required
M12	Protective clothing	suitable protective clothing required	suitable protective clothing required	suitable protective clothing required; footwear required where and to extent the risk assessment shows it is required	complete change of clothing and footwear required before entry and exit
M13	Gloves	not required	required where and to extent the risk assessment shows it is required	required	required
M14	Efficient control of disease vectors (eg for rodents and insects) which could disseminate the GMM	required where and to extent the risk assessment shows it is required	required	required	required

CONTAINMENT MEASURES		CONTAINMENT LEVEL			
		1	2	3	4
M15	Specified disinfection procedures in place	required where and to extent the risk assessment shows it is required	required	required	required
WASTE					
M16	Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents	not required	not required	required where and to extent the risk assessment shows it is required	required
M17	Inactivation of GMMs in contaminated material	required by validated means	required by validated means	required by validated means, with waste inactivated in the laboratory suite	required by validated means, with waste inactivated within the laboratory
OTHER MEASURES					
M18	Laboratory to contain its own equipment	not required	not required	required, so far as is reasonably practicable	required
M19	An observation window or alternative is to be present so that occupants can be seen	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required	required
M20	Safe storage of GMMs	required where and to extent the risk assessment shows it is required	required	required	secure storage required
M21	Written records of staff training	not required	required where and to extent the risk assessment shows it is required	required	required

Laboratory Containment Level 1

11. Laboratory Containment Level 1 must be applied for Class 1 activities involving GMMs. All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.

M1. There is no regulatory requirement for a Containment Level 1 laboratory to be physically separated from other areas of the building. There should be adequate space provided and the laboratory should be a safe, comfortable working environment that takes full account of work practices and equipment present.

M2. Containment Level 1 laboratories do not need to be sealable for the purposes of fumigation. However, there may be a requirement for specific disinfection procedures to be in place (see M15).

Equipment

M3. There is a regulatory requirement for the laboratory bench surfaces to be easily cleaned, be impervious to water and resistant to acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. Hand washing facilities and a supply of soap should be provided.

M4. Not required.

M5. Not required.

M6. Not required.

M7. There is no regulatory requirement for the use of a microbiological safety cabinet or other similar equipment for worker protection and all work can take place on the open bench. However, procedures should be in place to limit the production and dissemination of aerosols. It is acknowledged that containment equipment such as a microbiological safety cabinet might be in use to prevent contamination of the GM work or products being handled.

M8. An autoclave is required to be available on the site, but not necessarily in the same building. At this level of containment, disinfection will usually be a satisfactory means of waste treatment and if no autoclave is available, derogation may be sought provided that a validated means of GMM inactivation is in place. Alternative methods, including waste

removal by contractors, may also be acceptable provided that the disposal method (eg incineration) is validated and that waste is stored and transported in a way that does not increase risk. *The site of disposal must also comply with the Contained Use Regulations for the handling of GMMs that have not been inactivated. Agreement for the disposal of waste by a third party must be obtained from the competent authority.*

System of work

M9. There is no requirement for access to Containment Level 1 laboratories to be restricted. However, those permitted to work in the laboratory should be competent, trained and properly informed (see M21).

M10. Procedures should be carried out in such a way as to keep aerosol production to a minimum but there is no requirement for specific measures to control aerosol dissemination.

M11. There is no requirement for a showering facility to be present and workers are not required to shower when entering or leaving the facility. However, hands should be washed immediately if contamination with a GMM is suspected, after handling viable GMMs or before leaving the laboratory.

M12. There is a regulatory requirement for suitable protective clothing to be worn. This would normally comprise laboratory coats or gowns that are worn when in the laboratory and removed upon exit. Eye protection might also be required if aerosols or airborne contamination is possible.

M13. There is no regulatory requirement for protective gloves to be worn. It is acknowledged that gloves might be used to prevent contamination of the GM work or to protect workers against other chemical or biological contaminants.

M14. Where the risk assessment shows that it is required, an efficient system for the control of disease vectors (eg for rodents and arthropods) that could disseminate the GMM is required. The precise control method will depend upon the nature of the vector.

M15. It is a regulatory requirement for specified disinfection procedures to be in place, where the risk assessment shows that they are required. Effective disinfectants should always be available for immediate use in the event of spillage (see Section 3.5). Bench tops and laboratory equipment should be cleaned or disinfected immediately after use, or on a regular basis and should be thoroughly decontaminated before removal, repair or servicing.

Waste

M16. Not required.

M17. All waste material containing viable GMMs, including spent culture fluid and other media, are required to be inactivated by validated means prior to disposal. Contaminated laboratory glassware and other materials awaiting disinfection should be stored in a safe manner and pipettes should be totally immersed in disinfectant, where necessary. Materials that require inactivation and disposal at a secondary facility (either within the building or off-site) should be transported in leak-proof containers to prevent spillage. Inactivation methods should be monitored to ensure that they are working correctly - the effectiveness of disinfection can be impaired by the presence of large amounts of organic matter or the use of buffered culture media (see Section 3.5).

Other measures

M18. There is no requirement to have equipment solely dedicated for use in Containment Level 1 laboratories. However, equipment should be cleaned and thoroughly decontaminated before removing the equipment from the laboratory for repair or servicing.

M19. An observation window or alternative method of visualizing the laboratory occupants might be required where the risk assessment indicates that it is necessary. It is unlikely that such a system will be required for safety reasons at Containment Level 1, although it may offer additional protection for lone workers.

M20. There may be a regulatory requirement to have a system for the safe storage for GMMs if the risk assessment indicates that it is needed. Cultures should be stored in appropriate vessels, be clearly labelled and be, so far as is reasonably practicable, stored within the laboratory or nearby.

M21. There is no formal requirement for written records of staff training, although the relevant personnel should receive information, instruction and training in the procedures conducted in the laboratory. All accidents and incidents should be recorded.

Laboratory Containment Level 2

12. Laboratory Containment Level 2 must be applied for Class 2 activities involving GMMs.

All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.

M1. There is no regulatory requirement for Containment Level 2 laboratories to be physically separated from other areas of the building. There should be adequate space provided and the laboratory should be a safe, comfortable working environment that takes full account of work practices and equipment present.

M2. Containment Level 2 laboratories do not need to be sealable for the purposes of fumigation. There may be a requirement for specific disinfection procedures to be in place (see M15).

Equipment

M3. There is a regulatory requirement for the laboratory bench surfaces to be easily cleaned, be impervious to water and resistant to acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. Hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap should be provided.

M4. Not required.

M5. It is a regulatory requirement to maintain the laboratory at a negative pressure with respect to the surrounding areas if the risk assessment shows that is necessary. This may be the case if the GMM can be transmitted via the air. This can be achieved by ensuring that the supply air is nominally 90% of the extract. In some cases, the laboratory could be maintained at positive air pressure (eg for good manufacturing practice purposes), provided the GMM is handled in a validated microbiological safety cabinet (see M6, M7; Section 3.7). In these circumstances, discussion with HSE is advised.

M6. There is no regulatory requirement for the laboratory input or extract air to be HEPA filtered at Containment Level 2. It is accepted that air extracted through a microbiological safety cabinet will be, but this in itself will not affect activity classification. When work is undertaken in facilities where air is extracted from the laboratory into the general ventilation system without filtration, then consideration should be given to the possibility of

airborne transmission of GMMs. Such a ventilation system may not be acceptable for some class 2 activities.

M7. It is a regulatory requirement to use a microbiological safety cabinet (or similar containment equipment) where the risk assessment shows that it is required. For example, if the GMM can be disseminated by aerosol then procedures that might generate aerosols (eg pipetting, vigorous shaking or sonication) should take place within a microbiological safety cabinet or similar containment equipment. Class I or II cabinets may be used as appropriate. Air passing through a microbiological safety cabinet will always be HEPA filtered. Procedures should be in place to limit the production and dissemination of aerosols and such equipment can be employed for this purpose (see M10).

M8. An autoclave is required to be available within the same building as the laboratory. At this level of containment, disinfection will often be a satisfactory means of waste treatment and, if no autoclave is available, derogation may be sought provided that a validated means of GMM inactivation is in place (see Section 3.5). Alternative methods, including waste removal by contractors, may also be acceptable provided that the disposal method (eg incineration) is validated and that waste is stored and transported in a way that does not increase risk.

System of work

M9. There is a regulatory requirement that access to a Containment Level 2 laboratory be restricted to authorised personnel only (ie laboratory personnel and other specified persons). This can often be achieved by the use of a suitable sign. The laboratory door should remain closed when work is in progress.

M10. Procedures to minimise aerosol dissemination are required. Some work at level 2 may be conducted on the open bench but procedures that may result in aerosol production (eg shaking, sonication or centrifugation) should be contained within suitable equipment. This may be a microbiological safety cabinet or, for centrifugation, sealed centrifuge buckets. Sealed buckets should be tested in accordance with BS EN 61010-2-20:1995.

M11. There is no requirement for a showering facility to be present and workers are not required to shower when entering or leaving the facility. Hands should be washed immediately if contamination with a GMM is suspected, after handling viable GMMs or before leaving the laboratory.

M12. There is a regulatory requirement for suitable protective clothing to be worn. This would normally comprise laboratory coats or gowns that are worn when in the laboratory

and removed prior to hand washing upon exit. Eye protection might also be required if aerosols or airborne contamination is possible.

M13. There is a regulatory requirement for protective gloves to be worn, where the risk assessment indicates that they are required.

M14. An efficient system for the control of disease vectors (eg for rodents and arthropods) that could disseminate the GMM is required. This will be especially important when working with GMMs derived from arthropod-borne pathogens.

M15. It is a regulatory requirement for specified disinfection procedures to be in place and effective disinfectants should be available for immediate use in the event of spillage (see Section 3.5). Bench tops and laboratory equipment should be cleaned or disinfected immediately after use or on a regular basis and should be thoroughly decontaminated before removal, repair or servicing.

Waste

M16. Not required.

M17. All waste material containing viable GMMs, including spent culture fluid and other media, are required to be inactivated by validated means prior to disposal. Contaminated laboratory glassware and other materials awaiting disinfection should be stored in a safe manner and pipettes should be totally immersed in disinfectant, where necessary. Materials that require inactivation and disposal at a secondary facility (either within the building or off-site) should be transported in leak-proof containers to prevent spillage (Carriage of Dangerous Goods Regulations will apply to viable GMMs transported off-site). *The site of disposal must comply with the Contained Use Regulations for the handling of GMMs that have not been inactivated and agreement for the disposal of untreated waste by a third party must be obtained from the competent authority.* Inactivation methods should be monitored to ensure that they are working correctly - the effectiveness of disinfection can be impaired by the presence of large amounts of organic matter or the use of buffered culture media (see Section 3.5).

Other measures

M18. There is no requirement to have equipment solely dedicated for use Containment Level 2 laboratories. However, equipment should be cleaned and thoroughly decontaminated before removal from the laboratory for repair or servicing.

M19. A window or alternative method of observing the laboratory occupants might be required where the risk assessment indicates that it is necessary. It is unlikely that this will be required in Containment Level 2 laboratories for the purposes of working with the GMM itself, but it might have other safety and security applications as well as reassuring staff that they can be seen.

M20. There is a regulatory requirement to have a system for the safe storage for GMMs at Containment Level 2. Cultures should be stored in appropriate vessels, be clearly labelled and be, so far as is reasonably practical, stored within the laboratory or nearby.

M21. Formal written records of staff training are required if the risk assessment indicates that it is necessary. Laboratory personnel should receive information, instruction and training in handling of GMMs. This will also satisfy the requirement for training and standards under COSHH and the Management Regulations (See Part 1 of the Compendium). All accidents and incidents should be recorded and immediately reported internally. If human health or the environment could have been harmed then this must be reported to HSE, in addition to any reports required under RIDDOR. Further guidance on this can be found in the publication *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* L29 (Third edition) HSE Books 2000 ISBN 978 0 7176 1758 6 and in Part 1 of the Compendium.

Laboratory Containment Level 3

13. Laboratory Containment Level 3 must be applied for Class 3 activities involving GMMs.

All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.

M1. There is a regulatory requirement for Containment Level 3 laboratories to be isolated from other activities within the same building. This means separation of the laboratory from other areas in the same building, or being in a separate building. Laboratory areas should be separated from offices or communal areas and sections frequented by non-technical staff. There should be adequate space provided and organised to take full account of work practices and equipment present.

M2. The laboratory is required to be sealable to permit fumigation. Validated fumigation procedures must be in place and should be used in the event of a major spillage. It may be necessary to fumigate prior to maintenance. The need for this can be determined by the risk assessments relating to particular activities. Laboratory ventilation and externally ducted safety cabinets should be controllable from outside the laboratory, so that fumigant can be vented safely without the need to re-enter the room. This is less applicable to hydrogen peroxide systems than it is to formaldehyde as the former degrades to harmless by-products (see Section 3.6). Further guidance can be found in the HSE publication *Sealability of Microbiological Containment Level 3 and 4 Facilities* available at: www.hse.gov.uk/biosafety/gmo/guidance/sealability.pdf.

Equipment

M3. There is a regulatory requirement for the laboratory benches and floor surfaces to be easily cleaned, be impervious to water and resistant to acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. Hand washing facilities should be provided, with taps that can be operated without being touched by hand and a supply of disinfectant soap.

M4. Where the risk assessment shows that it is required, entry to the laboratory must be via an airlock or lobby, ie a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably by interlocking doors (see M11). In practice, a lobby area rather than a mechanically ventilated airlock is sufficient at Containment Level 3 and it is unusual to have a dedicated showering facility.

M5. It is a regulatory requirement to maintain the laboratory at a negative pressure with respect to the immediate surroundings. In terms of regulatory requirements 'immediate surroundings' can be interpreted as being outside the building or in adjacent parts of the laboratory suite or building. Typically, the laboratory should be maintained at -30 to -50 Pa via a continuous inflow of air while work is in progress. If the airlock is also maintained at negative pressure, this should be an intermediate value (eg -15 Pa) to maintain the laboratory pressure differential with the surroundings. Negative pressure is often achieved via the implementation of a controlled supply and extract of air by mechanical ventilation systems. The supply and extract airflow should be interlocked to prevent positive pressurisation of the room in the event of failure of the extract fan. Negative pressure can also be provided by the operation of the safety cabinets or extract air alone, although in such systems the pressure differential is often compromised when the laboratory door is opened or the primary extract fan fails (see M6; M7). Further guidance can be found in the ACDP publication *The management, design and operation of microbiological containment laboratories* Guidance HSE Books 2001 ISBN 978 0 7176 2034 0.

M6. HEPA filtration of extracted air is required for Containment Level 3 laboratories. This is applicable to air extracted via mechanical room ventilation or via externally ducted microbiological safety cabinets or similar containment equipment (see M7).

M7. All work with infectious material must take place in a microbiological safety cabinet, isolator or similar containment equipment. Normally a Class I or Class II microbiological safety cabinet will be used, but a Class III cabinet (see Section 3.7) or an isolator might be required for work with organisms with an airborne route of transmission, or where a high degree of uncertainty exists (for example, with viruses with altered tissue tropism). Where re-circulating Class II cabinets are used, exhaust air should be passed through two HEPA filters in series and consideration given to heat and humidity build up and fumigation procedures.

M8. An autoclave is required to be available within the laboratory suite. Where the autoclave is outside the laboratory but within the laboratory suite, validated procedures for the safe transfer of material into that autoclave are required that provide a level of protection equivalent to having an autoclave in the laboratory. Although it is permissible for waste materials to be inactivated by chemical means prior to disposal, it is considered good practice to autoclave waste at Containment Level 3. Where chemical disinfection is used, the disinfection procedures must be validated under working conditions. Alternative methods, including waste removal by contractors, may be acceptable in certain circumstances provided that the disposal method (eg incineration) is validated and that waste is stored and transported in a way that does not increase risk. *The site of disposal must also comply with the Contained Use Regulations for the handling of GMMs that have*

not been inactivated and agreement for the disposal of waste by a third party must be obtained from the competent authority.

System of work

M9. There is a regulatory requirement that access to a Containment Level 3 laboratory be restricted to authorised personnel only (ie trained workers). This is often aided by the use of key-coded or card-swipe entry systems. The laboratory door should remain closed when work is in progress and be locked when the room is unoccupied or not in use.

M10. Measures to prevent aerosol dissemination are required. All work at Containment Level 3 involving infectious material must take place within a microbiological safety cabinet, isolator or similar (see Section 3.7). Centrifugation should take place within sealed containers that have been tested in accordance with BS EN 61010-2-20:1995.

M11. Where the risk assessment indicates that it is required, personnel must shower before leaving the laboratory. Hands should be washed immediately if contamination with a GMM is suspected, after handling viable GMMs or before leaving the laboratory.

M12. There is a regulatory requirement for suitable protective clothing to be worn. This would normally comprise laboratory coats or gowns (side- or back-fastening) that are worn when in the laboratory and removed prior to hand washing upon exit. Laboratory gowns should be changed on a regular basis and immediately if they become contaminated. Gowns must be autoclaved before laundering. Where the risk assessment shows it to be required, protective footwear (eg disposable overshoes) must also be worn. Eye protection might also be required if aerosols or airborne contamination is possible.

M13. There is a regulatory requirement for disposable protective gloves to be worn for all work involving infectious material. Routine wearing of double-gloves is suggested, so that contaminated outer gloves can be removed without loss of protection. Contaminated gloves should be removed for handling items that will be touched by others who may not be wearing gloves (eg telephone handsets, door-handles).

M14. An efficient system for the control of disease vectors (eg for rodents and insects) that could disseminate the GMM is required. This will be especially important when working with GMMs derived from arthropod-borne pathogens.

M15. It is a regulatory requirement for specified disinfection procedures to be in place and effective disinfectants should be available for immediate use in the event of a spillage (see Section 3.5). Bench tops and laboratory equipment should be cleaned or disinfected

immediately after use or on a regular basis and should be thoroughly decontaminated before removal, repair or servicing.

Waste

M16. Inactivation of GMMs in effluents from hand washing sinks and showers will be required prior to release if shown to be necessary by the risk assessment. Generally, this will not be required, as gloves should be worn. Contaminated gloves should be disposed of and inactivated using an autoclave or incinerator. Effluent should only need to be collected if a gross contamination of hands is suspected, although it is accepted that some facilities handling animal pathogens may be required to do so under license conditions.

M17. All waste material containing viable GMMs, including spent culture fluid and other media, will require inactivation by validated means within the laboratory suite prior to disposal. Contaminated laboratory glassware and other materials awaiting disinfection should be stored in a safe manner and pipettes should be totally immersed in disinfectant. Inactivation methods should be monitored to ensure that they are working correctly. The effectiveness of disinfection can be impaired by the presence of large amounts of organic matter or the use of buffered culture media (see Section 3.5), and reliance on such methods may not be appropriate for some Containment Level 3 work. Physical rather than chemical methods of inactivation (such as autoclaving) should therefore be used in preference. Chemically inactivated material should preferably be autoclaved prior to discharge, unless it can be demonstrated that complete inactivation has been achieved (note that chlorine-based disinfectants have the potential to cause damage to the autoclave).

Other measures

M18. There is a regulatory requirement for the laboratory to contain its own dedicated equipment, so far as is reasonably practicable. Where this is not reasonably practicable, material should be transported and stored without spillage in properly labelled robust containers that are opened only in the laboratory. Equipment that is to be removed from the laboratory for any reason (eg for repair or servicing) should be cleaned and thoroughly decontaminated.

M19. A window or alternative method of observing the laboratory occupants is required. A glass panel in the laboratory door will usually be adequate.

M20. There is a regulatory requirement to have a system for the safe and secure storage for GMMs at Containment Level 3. Cultures should be stored in appropriate vessels, be

clearly labelled and be, so far as is reasonably practical, stored within the laboratory or laboratory suite. Ideally, viable materials requiring level 3 containment should only be stored and handled within the Containment Level 3 laboratory itself. Fridges and freezers used for storage outside of the laboratory should be kept locked. There may be a need for safe storage for biosecurity purposes (see Anti Terrorism, Crime and Security Act 2001).

M21. Formal written records of staff training are required. Laboratory personnel should receive information, instruction and training in handling of GMMs. This will also satisfy the requirement for training and standards under COSHH and the Management Regulations (See Part 1 of the Compendium). All accidents and incidents should be recorded and immediately reported internally. If human health or the environment could have been harmed then this must be reported to HSE, in addition to any reports required under RIDDOR. Further guidance on this can be found in the publication *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* L29 (Third edition) HSE Books 2000 ISBN 978 0 7176 1758 6 and in Part 1 of the Compendium.

Laboratory Containment Level 4

14. Laboratory Containment Level 4 must be applied for Class 4 activities involving GMMs.

All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority. There are very few Containment Level 4 facilities in the UK. If the building of a new facility is proposed, it is advised that the details of containment requirements, management control and design of the facility is discussed in advance with HSE. Users should consult the ACDP/Defra/DH guidance on *The principles, design and operation of Containment Level 4 facilities* (see: www.hse.gov.uk/pubns/web09.pdf).

15. The requirements set down by Defra for the handling of Defra Group 4 pathogens (eg Foot and Mouth Disease Virus; Rinderpest Virus) will differ from those set out in this section as work with certain animal or plant pathogens will require less operator protection, but a high degree of environmental protection. Facilities designed to accommodate work with Defra Group 4 pathogens that represent little or no risk to human health will still require Containment Level 4 for the purposes of GM work, even though they may be subject to derogation (with agreement from the competent authority) from some of the measures set-out below. The Contained Use Regulations cover both human health and environmental safety and therefore the Defra level 4 classification leads to an automatic assignment to class 4. Furthermore, the prescribed conditions outlined in any relevant SAPO license must be adhered to.

M1. There is a regulatory requirement for Containment Level 4 laboratories to be isolated from other activities. Situating the laboratory suite in a separate building or a secluded part of a building would normally achieve this.

M2. The laboratory is required to be sealable to permit fumigation. Validated fumigation procedures must be in place and used in the event of a major spillage or prior to maintenance. Laboratory ventilation and externally ducted secondary containment devices should be controllable from outside the laboratory to permit the safe venting of fumigant without the need to re-enter the room (see Section 3.6). Further guidance can be found in the HSE publication *Sealability of Microbiological Containment Level 3 and 4 Facilities* available at: www.hse.gov.uk/biosafety/gmo/guidance/sealability.pdf.

Equipment

M3. Laboratory benches and floor surfaces, walls and ceilings must be easy to clean, be impervious to water and resistant to acids, alkalis, solvents, disinfectants and other

decontamination agents that may be in use. A high standard hygiene and cleanliness should be maintained.

M4. Entry to the laboratory must be via an airlock, which is a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably have interlocking doors (see M11).

M5. It is a regulatory requirement to maintain the laboratory at a negative pressure with respect to the immediate surroundings. Typically, the laboratory should be maintained at -30 to -50 Pa via a continuous inflow of air while work is in progress. The airlock should be at an intermediate value (eg -15 Pa) to maintain the laboratory pressure differential with the surroundings. Airflow should be achieved using a mechanical supply and extract ventilation system. The supply and extract airflow should be interlocked (to prevent positive pressurisation of the room in the event of failure of the extract fan) and the extract fan should be connected to an emergency power supply. An alarm system is to be present that is visible/audible from both inside and outside the laboratory and the ventilation system should also incorporate a mechanism that will prevent reverse airflow. There should be a telephone or intercom inside the laboratory suite, preferably with 'hands-off' controls. A fax or computer link can be useful for safe transfer of data.

M6. Both the supply and extract air must be HEPA filtered. A single HEPA filter should be present at the inlet and be protected by panel and bag filters. Extract air must be passed through two HEPA filters in series before discharge (a COSHH requirement for work with ACDP hazard-group 4 organisms).

M7. It is a regulatory requirement that a Class III microbiological safety cabinet, isolator or equivalent be present and that all procedures with infectious material must take place within such containment equipment (see Section 3.7). Exhaust air should be passed through two HEPA filters in series before it is vented to outside air or recirculated. An open-fronted (Class I or Class II) cabinet may be used with agreement of the competent authority if the hazards are entirely environmental and it is justified by the risk assessment.

M8. A double-ended autoclave is required to be installed within the laboratory. The equipment should have interlocking doors, and be loaded within the laboratory with inactivated waste removal in a clean area.

System of work

M9. There is a regulatory requirement that access to a Containment Level 4 laboratory be restricted to authorised personnel only and that a key procedure is established in order to

maintain this control at all times. It is good practice for there to be a second competent person present in the laboratory suite when it is occupied to assist in case of an emergency. Procedures should be drawn up for emergency evacuation (eg in case of a fire alarm or for the safe removal of an incapacitated worker).

M10. Measures to prevent aerosol dissemination are required and all work at Containment Level 4 involving infectious material must take place within a Class III microbiological safety cabinet, isolator or similar (see M7).

M11. It is required that a shower be installed and that all personnel must shower (including hair) before leaving the laboratory. A complete change of clothing is required (see M12).

M12. Suitable protective clothing and footwear must be worn. It is required that there should be a complete change of clothing and footwear. After work, clothing is to be removed on the dirty side of the airlock and placed in a container for autoclaving. High performance respiratory protective equipment (two or more units) needs to be available in the clean side of the laboratory suite for use in an emergency. Staff using the facility must have had the appropriate training in the use of respiratory protective equipment.

M13. There is a requirement for gloves to be worn for all work within the laboratory.

M14. An efficient system for the control of disease vectors (eg for rodents and insects) that could disseminate the GMM is required. This will be especially important when working with GMMs derived from arthropod-borne pathogens.

M15. It is a regulatory requirement for specified disinfection procedures to be in place and effective disinfectants should be available for immediate use in the event of spillage (see Section 3.5).

Waste

M16. Inactivation of GMMs in effluents from hand washing sinks and showers will be required prior to discharge.

M17. All material and waste must be inactivated and made safe by validated means within the laboratory suite prior to removal. This includes samples. Materials that cannot be autoclaved must be decontaminated by disinfection before removal. A double-ended dunk-tank with an effective disinfectant or an equivalent system may be required for this purpose. Disinfection tanks should be sealable if the disinfectant is incompatible with the fumigant used. Chemical inactivation should not be used as the sole inactivation method,

but may be used in conjunction with autoclaving (note that chlorine-based disinfectants have the potential to cause damage to the autoclave). Inactivation methods should be monitored to ensure that they are working correctly. The performance of the autoclave should be regularly tested using an independent thermocouple placed at the centre of the load and waste should only be discharged if the cycle was completed successfully. Waste should only be removed or handled by responsible or competent persons in accordance with defined local codes of practice.

Other measures

M18. There is a regulatory requirement for the laboratory to contain its own dedicated equipment. No equipment may be removed from the laboratory without disinfection by validated means. A secondary ventilated airlock that can be fumigated may be required to pass equipment into, and out of, the laboratory unit. Equipment should only be removed from the secondary airlock by responsible or competent persons and in accordance with defined local codes of practice.

M19. A window or alternative method of observing the laboratory occupants is required. A second competent person should be present in the laboratory suite when it is occupied (see M9).

M20. There is a regulatory requirement to have a system for the safe and secure storage for GMMs at Containment Level 4. All viable material must only be stored and handled within the Containment Level 4 suite. Fridges, freezers and storage containers should be kept locked when not in use. There may be a need for safe storage for biosecurity purposes (see Anti Terrorism, Crime and Security Act 2001).

M21. Formal written records of staff training are required. Laboratory personnel should receive information, instruction and training in handling of GMMs and specific rules and procedures should be drawn up for work in the laboratory. A high standard of supervision should be maintained. This will also satisfy the requirement for training and standards under COSHH and the Management Regulations (See Part 1 of the Compendium). All accidents and incidents should be recorded and immediately reported in accordance with locally specified procedures. All accidents at Containment Level 4 must be reported to HSE, in addition to any reports required under RIDDOR. Further guidance on this can be found in the publication *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* L29 (Third edition) HSE Books 2000 ISBN 978 0 7176 1758 6 and in Part 1 of the Compendium.

3.3 Containment and control measures for large-scale activities involving genetically modified microorganisms

Overview

1. The majority of large-scale manufacturing facilities in the UK operate at Containment Level 1 with few at Containment Level 2, which are principally for virus-based vaccine manufacturing purposes. If planning to build or adapt a large-scale facility to operate at Containment Level 3 or 4, advice should be sought from HSE.
2. The Genetically Modified Organisms (Contained Use) Regulations require that all activities involving genetically modified microorganisms (GMMs) in contained use employ a combination of physical, chemical or biological barriers to limit contact with people and the environment. This general requirement is underpinned by specific regulations, regulations 17 and 18 and their associated schedules, which prescribe the minimum containment measures for particular activities.
3. Regulation 17 sets out the 'principles of occupational and environmental safety' which aim to ensure that 'the exposure of humans and the environment to genetically modified micro-organisms is reduced to the lowest level that is reasonably practicable'. These principles (as outlined in the Section 3.1 and Schedule 7 of the regulations), are described as the 'general principles of good microbiological practice and of good occupational safety and hygiene', and apply to any activity involving the use of GMMs.
4. Regulation 18 requires that a person who undertakes an activity involving genetic modification of microorganisms shall apply the containment measures set out in the applicable Table in Schedule 8, where and to the extent required in the column of the appropriate containment level. The following guidance regards the minimum containment and control measures required by law for large-scale activities with GMMs as well as methods that can be applied to aid the fulfilment of legal obligations. The Contained Use Regulations are not the only legislation that may impact upon the containment measures needed and users must take this into account (further guidance can be found in Part 1 of the Compendium).
5. Many large-scale facilities will be required to operate in accordance with good manufacturing practice. The control measures required in order for the facility to comply with these standards are put in place to ensure the purity of the materials and prevent contamination, rather than to offer protection to the operator or environment. In some cases, there may be a conflict between the measures designed to offer protection to the operator/environment and those designed to protect the product. In most cases, however, it will be possible to assign equivalent protective controls that permit the maintenance of

good manufacturing practice (GMP – not to be confused with good *microbiological* practice). Furthermore, many of the measures required for manufacturing purposes will also offer operator/environmental protection and, in general, those measures are more stringent than those required by the Contained Use Regulations. GM activity classification and notification will only be affected by those measures required by the Contained Use Regulations.

Scope

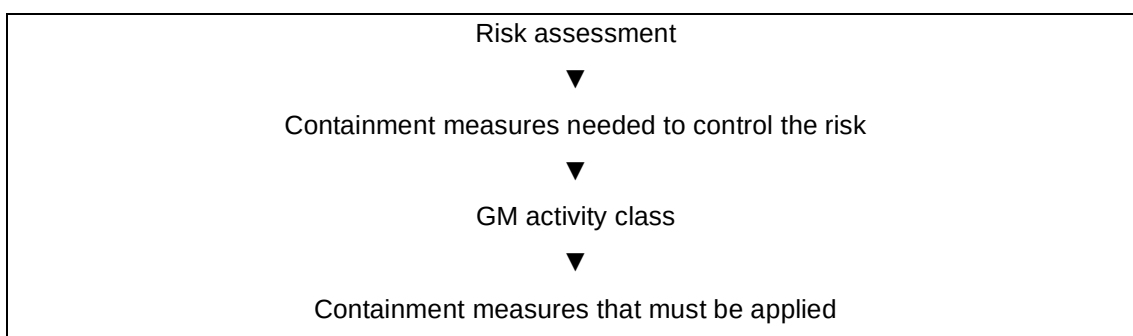
6. This section is intended to give guidance to help users determine the appropriate standards of containment and control that should be applied to work involving GMMs in *large-scale* activities. Users will have to determine whether the activity being undertaken is small or large scale. There is no absolute volume cut off to distinguish between small and large scale. The prime consideration has to be the nature of the containment and control measures appropriate to the activity. It will normally be obvious which guidance (both text and tables) users should follow for their particular activity. Highly engineered, closed systems involving the use of seals, pipework, measures to control exhaust gases and *in situ* probes are all factors found more normally in large-scale activities. The use of small bench-top chemostats and fermenters (typically up to around 50 litre capacity) are likely to constitute laboratory scale activities (see Section 3.2). Pilot plant facilities may also be more suited to large-scale control measures. Once the scale has been decided users should follow the appropriate guidance, although it may be necessary to consider both sets of guidance for operations of an intermediate nature.
7. Users might also find it helpful to refer to the ACDP publications *The management, design and operation of microbiological containment laboratories* Guidance HSE Books 2001 ISBN 978 0 7176 2034 0 and *The large-scale contained use of biological agents* Guidance HSE Books 1998 ISBN 978 0 7176 1544 5. The latter guidance is centred upon work with hazard group 2 biological agents in Level 2 containment. Guidance relating to the large-scale manufacture of influenza vaccine using embryonated eggs can also be found in the World Health Organisation Document *Production of pilot lots of inactivated influenza vaccines from reassortants derived from avian influenza viruses - Interim biosafety risk assessment* (see: www.who.int/csr/resources/publications/influenza/en/influenzaRMD2003_5.pdf).

Selecting the minimum containment level

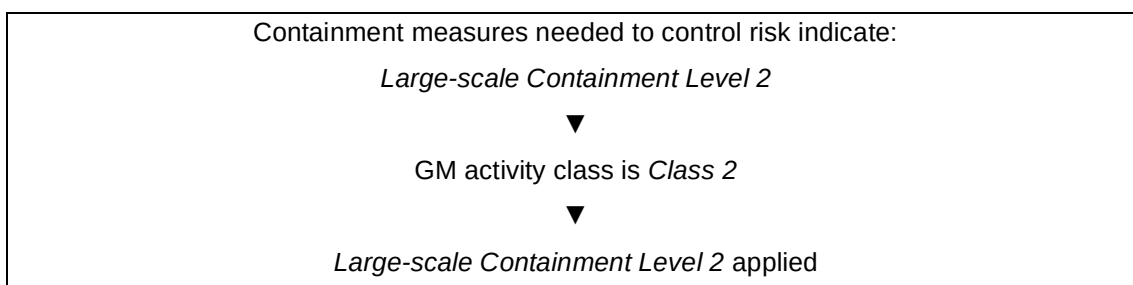
8. The risk assessment must be used to determine the appropriate control measures that are needed to afford maximum protection to both human health and the environment.

Users should compare the measures warranted by the risk assessment with the table of containment measures (which is reproduced below) to determine the GM activity class. The GM activity class, in turn, determines the minimum containment level required for the activity.

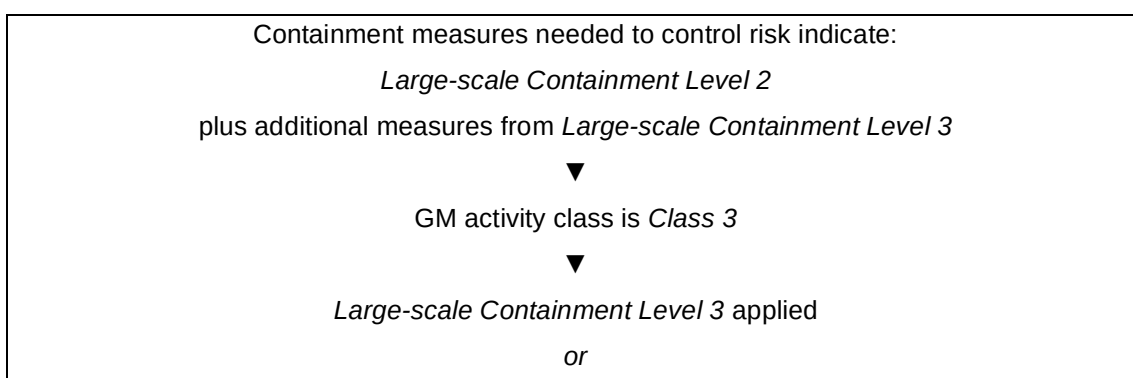
9. Some control measures deemed necessary by the risk assessment may not be listed in the Schedule 8 containment level tables. The GM activity class is determined solely by those measures actually listed. The risk assessment must always take precedence and ALL measures identified as necessary must be applied.



10. In the majority of cases, the containment measures necessary to control the risk, the GM activity class and the minimum containment level to be applied will be the same:

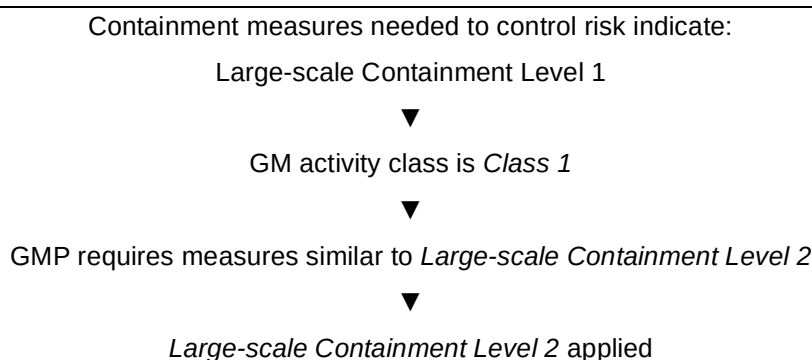


11. However, the containment measures indicated by the risk assessment may consist of a mixture of measures from two different levels. In these cases, the higher level of containment will determine the GM activity class and must be applied:



Large scale Containment Level 3 applied
(with derogations for those measures which are not required, under agreement with the
Competent Authority).

12. A request can be made to the Competent Authority at the time of notification for permission to use the mixture of two levels identified. However, unless and until you have the agreement of the Competent Authority, you may not use a level of containment lower than that corresponding to the GM activity class.
13. It is recognised that for lower risk GMMs the containment for process requirements (or good manufacturing practice (GMP)) are often more stringent than those needed for protection of human health and the environment. It is therefore possible for a Class 1 activities requiring Containment Level 1 to be undertaken at a level of containment similar to level 2, or even level 3, in order to protect the product:



14. It is only the containment requirements related to risk to humans and the environment from the GMM that are relevant to the activity classification (and hence notification requirements). Process requirements that deal with protection of the product should be excluded from these considerations.

Definitions

15. Legal requirements will be stated clearly as such, with the use of the words 'regulatory requirement', 'regulatory requirement', 'required' or 'must'. Otherwise, in cases where the word 'should' is used, the guidance highlights approaches that can be used to achieve the appropriate standards. These approaches are only illustrative and users may adopt other approaches so long as the standards set by the Regulations are met.
16. The following terms do not have legal definitions and the following explanations are intended to aid understanding:

- **Workplace:** Facilities where activities involving GMMs are undertaken (eg laboratories, factory premises, fermentation plants etc).
- **Controlled area:** Specific areas where activities involving GMMs are undertaken and where physical or chemical measures are, or can be, implemented to limit contact with people or minimise releases into the environment. Controlled areas should, in general, be separated from offices or communal areas and sections frequented by non-technical staff.
- **Limit contact:** Contact of people and the environment with the GMM must be reduced to the lowest level that is reasonably practicable. The control measures needed to achieve this will be determined by the risk assessment.
- **Minimise release:** It is acknowledged that the intention is to keep the GMMs under control, but that a number of GMMs may enter the work place and/or the wider environment. The risk assessment must be used to determine the number of GMMs released that is acceptable (this may be dependent upon the nature and properties of the GMM) and the containment and control measures that are needed to minimise the risk of harm to humans or the environment.
- **Prevent release:** It will be essential to prevent release in cases where the GMM is highly hazardous to humans and/or the environment. A range of methods may be required and the risk assessment should clearly demonstrate the efficacy of the intended control measures.
- **Closed system:** A system that physically separates the process/GMMs from the environment. A closed system might involve the use of a fermenter vessel that prevents any significant contact of the GMMs with the open air, workplace or wider environment. A closed system may consist of several stages and different pieces of equipment that are linked by a closed transfer system (for instance, a seed vessel leading to a fermentation vessel and, in turn, a downstream processing stage). Equipment that is 'open' but contained within a safety enclosure may also represent a closed system. The degree to which the system is 'closed' should be determined by the risk and, for lower levels of risk, the system may not need to be completely sealed. The integrity of a closed system should be routinely tested.
- **Required where and to extent the risk assessment shows it is required:** This indicates that the need for a particular measure is determined by the risk assessment. If the risk assessment specifies that a measure is needed for human health or environmental protection, its use is mandatory.
- **Competent authority:** The body with enforcing jurisdiction over the Contained Use Regulations. In England and Wales the competent authority includes the Health and Safety Executive (HSE), the Secretary of State and the Department for Environment, Food and Rural Affairs (Defra). In Scotland the competent authority includes HSE, the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the

Scottish Ministers. Northern Ireland has its own separate competent authority. When seeking approval from the competent authority for particular actions, users should contact HSE in the first instance.

Containment measures for large-scale activities involving GMMs

CONTAINMENT MEASURES		CONTAINMENT LEVELS			
		1	2	3	4
GENERAL					
M1	Viable micro-organisms should be contained in a system which separates the process from the workplace and wider environment (closed system)	required where and to extent the risk assessment shows it is required	required	required	required
M2	Closed systems located within a controlled area	not required	required where and to extent the risk assessment shows it is required	required	required and required to be purpose built
M3	Control of exhaust gases from the closed system	not required	required so as to minimise release	required so as to prevent release	required so as to prevent release
M4	Control of aerosols during sample collection, addition of material to a closed system or transfer of material to another closed system	required where and to extent the risk assessment shows it is required	required so as to minimise release	required so as to prevent release	required so as to prevent release
M5	Inactivation of bulk culture fluids before removal from the closed system	required where and to extent the risk assessment shows it is required	required by validated means	required by validated means	required by validated means
M6	Seals should be designed so as to minimise or prevent release	not required	required so as to minimise release	required so as to prevent release	required so as to prevent release
M7	The controlled area designed to contain spillage of the entire contents of the closed system	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required	required
M8	The controlled area sealable to permit fumigation	not required	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required
M9	Biohazard signs posted	required where and to extent the risk assessment shows it is required	required	required	required
EQUIPMENT					
M10	Entry via airlock	not required	not required	required where and to extent the risk assessment shows it is required	required
M11	Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	required for any bench	required for any bench	required for floor and any bench	required for bench, floor, ceiling and walls
M12	Specific measures to adequately ventilate the controlled areas in order to minimise air contamination	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required
M13	The controlled area maintained at an air pressure negative to the immediate surroundings	not required	not required	required where and to extent the risk assessment shows it is required	required
M14	Extract and input air from the	not required	not required	required for	required for input

CONTAINMENT MEASURES		CONTAINMENT LEVELS			
		1	2	3	4
	controlled area should be HEPA filtered			extract air, optional for input air	and extract air
SYSTEM OF WORK					
M15	Access restricted to nominated personnel only	not required	required	required	required
M16	Decontamination and washing facilities provided for personnel	required	required	required	required
M17	Personnel should shower before leaving the controlled area	not required	not required	required where and to extent the risk assessment shows it is required	required
M18	Personnel should wear protective clothing	work clothing required	work clothing required	required	complete change required before exit and entry
M19	Written procedures and records of staff training	not required	not required	required	required
WASTE					
M20	Inactivation of GMMs in effluent from handwashing sinks and showers or similar effluents	not required	not required	required where and to extent the risk assessment shows it is required	required
M21	Inactivation of GMMs in contaminated material and waste including those in process effluent before final discharge	required by validated means	required by validated means	required by validated means	required by validated means

Large-scale Containment Level 1

17. Large-scale Containment Level 1 must be applied for class 1 activities involving GMMs.

All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.

General

M1. Where the risk assessment shows that it is required, it is a regulatory requirement for viable GMMs to be contained in a closed system. The need for a closed system will depend upon the risk assessment, but for most activities at Containment Level 1 such a system will not be needed and fermentation processes may use GMMs in open vessels. The building itself will likely provide suitable limitation of contact with people and the wider environment and normal equipment used in the industry will probably be suitable (eg ventilated flasks, open top fermenters, open mixing vessels, baking tins and trays, moulds etc).

M2. There is no requirement for activities to be undertaken within controlled areas. However, it is sensible for production areas to be separated from offices, laboratories and other facilities.

M3. Not required.

M4. Where the risk assessment shows that it is necessary, measures to control aerosols during addition of material to a closed system, transfer of material to another closed system or sample collection will be required. The release of GMMs into the work place and wider environment should be minimised in any case. The stringency of these measures should be determined by the risk assessment. Where there is little risk of harm, elaborate methods for controlling escape will probably not be necessary.

M5. Bulk culture fluids that are removed from a closed system (eg for a further processing step) may require inactivation where the risk assessment shows that it will be necessary. Inactivation will not always be required but the requirement to inactivate all waste before final disposal remains (see M21).

M6. There is no requirement for seals (where they are present) to be specifically designed to minimise release of the GMMs. However, it is considered good practice to do so in order to limit the possibility of contamination.

M7. Where the risk assessment indicates that there is risk of harm arising from a catastrophic loss of primary containment, the controlled area must be designed to contain a spillage of the entire system. Some possible approaches include bunding at the periphery or the use of enlarged drainage channels. The containment method employed should allow for inactivation of the spillage and this could be achieved by direct drainage into an effluent tank.

M8. Not required.

M9. A biohazard sign must be displayed at the entrance to the controlled area if the risk assessment shows that it is necessary.

Equipment

M10. Not required.

M11. A good standard of hygiene should be maintained at all times and the Regulations require that bench surfaces in areas where GMMs are handled are easy to clean and resistant to water, acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use.

M12. Where the risk assessment shows it to be necessary, there is a requirement for adequate ventilation of the controlled area in order to minimise air contamination. Mechanical ventilation is not normally needed for activities requiring large-scale Containment Level 1, although the work place should be a safe and comfortable environment and good ventilation may be needed for the removal of heat from process operations and worker comfort.

M13. There is no requirement to maintain negative air pressure to contain airborne GMMs at large-scale Containment Level 1. Some processes might necessitate positive air pressure to maintain product integrity. This is acceptable provided that it does not conflict with the need to contain airborne GMMs in the event of an accident. The use of containment equipment such as a microbiological safety cabinet, or a bespoke isolator, could be considered to provide both product and operator protection (see Section 3.7).

M14. Not required. However, there is likely to be a need for input air to be HEPA filtered for GMP purposes.

System of work

M15. There is no requirement for access to controlled areas to be restricted at Large-scale Containment Level 1. However, those permitted to work in these areas should be competent, trained and properly informed (see M19).

M16. The provision of washing and decontamination facilities is required for all activities involving GMMs. For areas operating at large-scale Containment Level 1, this will probably consist of hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap. Hands should be washed immediately if contamination with a GMM is suspected, after handling viable GMMs or before leaving controlled areas.

M17. Not required.

M18. There is a regulatory requirement for suitable protective clothing to be worn. This will normally comprise disposable coveralls or gowns that are worn when in controlled areas and removed prior to hand washing upon exit. The wearing of dedicated work wear will often be enforced to protect the purity of the product in Large-scale containment facilities, and this will often obviate the need for specific protective clothing.

M19. Formal written records of staff training are not required. However, personnel should receive information, instruction and training in handling of GMMs and emergency procedures. Written standard operating procedures that include emergency spillage plans are recommended and all accidents and incidents should be recorded and immediately reported to a competent person.

M20. Not required.

M21. There is a regulatory requirement that contaminated material and waste (including culture fluids and other media) is inactivated before final discharge or disposal. Heat inactivation is generally considered more appropriate for large large-scale discharge of waste as chemical disinfectants may be environmentally damaging (waste discharge must comply with relevant environmental legislation – see Part 1 of the Compendium). If chemical inactivation is to be used, sufficient contact time should be given to afford adequate kill to minimise release of viable GMMs. The inactivation method chosen must be appropriate to the risk and its efficacy must be validated against the organism being used. Inactivation methods should be monitored to ensure that they are working correctly (see Section 3.5).

Large-scale Containment Level 2

18. Large-scale Containment Level 2 must be applied for class 2 activities involving GMMs. All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.

General

M1. It is a regulatory requirement for viable GMMs to be contained in a closed system and be physically separated from workers and the environment. The nature of the closed system will depend upon the hazards and be determined by the risk assessment, but for most activities a stainless-steel fermenter or chemostat with welded pipework will be suitable.

M2. Where indicated as necessary by the risk assessment, the Regulations require that activities be undertaken within controlled areas. These areas should be separated from offices, laboratories and other facilities and be away from access routes.

M3. Exhaust gases must be treated to minimise release of GMMs. Off-gases can be controlled using a variety of methods, either individually or in combination. This includes filtration, impingement filtration, cyclone separation, spraying gasses with hypochlorite in spray-towers or off-gassing through chemical disinfectants. This list of examples is not exhaustive and other techniques may be used, provided they are sufficient to control the risks. It is important to keep filters dry in order to maintain their efficiency and they should be easy to remove safely for maintenance purposes.

M4. Measures to control aerosols during addition of material to a closed system, transfer of material to another closed system or sample collection are required. Inoculation of seed vessels can be by direct injection using a sterile needle/septum technique or by using a transfer vessel that is part of the closed system. Where needles are used, procedures should be applied that minimise the likelihood of a needle stick injury. All potentially contaminated liquids should be transferred in closed piping and all pipework and valves should be leak-tight (see M6) and easily cleaned. Samples should be taken using aseptic techniques into a vessel designed to minimise aerosol generation and release. Running a mid-stream sample directly to drain is not acceptable - all material collected must be inactivated and the sampling valve/connection may need to be sterilised (see M5; M21).

M5. The Regulations require that viable GMMs in bulk culture fluids be inactivated by validated means prior to removal from the closed system. Chemical or physical methods

are acceptable, but must be appropriate to the organism and level of risk. All waste must be inactivated by validated means prior to final disposal (see M21).

M6. There is a requirement that all seals be designed to minimise release of GMMs. This includes static seals on equipment. Examples of typical types of seals appropriate for most level 2 work include single 'O' ring seals, flat gaskets, sealed couplings, single or double faced mechanical seals and sanitary couplings with gaskets. Where necessary, seals can be enclosed in HEPA (or equivalent) filtered housings (these examples are not exhaustive and alternatives may be used provided they are appropriate to control the risks). Where the equipment is connected to utility services, backflow should be prevented using a pressure differential, steam locks or 'double block and bleed' systems (non-return valves may be unreliable from a microbiological point of view and their use should be considered very carefully). It will be necessary to assess the operating pressure and the risk of the GMM being released through seal failure. Pressure-relief systems may be used, but the design needs to be considered carefully to prevent GMM release during venting and the requirements of Pressure Vessels Regulations met. Fixed or retractable sensors can be used.

M7. Where the risk assessment indicates that there is risk of harm arising from a catastrophic loss of primary containment, the controlled area must be designed to contain a spillage of the entire closed system. Some possible approaches include bunding at the periphery or the use of enlarged drainage channels. The containment method employed should allow for inactivation of the spillage and this could be achieved by direct drainage into an effluent tank.

M8. Controlled areas must be sealable for the purposes of fumigation where the risk assessment shows that it is required. Where necessary, validated fumigation procedures must be used in the event of a major spillage or prior to maintenance. Air handling and externally ducted safety cabinets should be controllable from outside the laboratory so that fumigant can be vented safely without the need to re-enter the room, although this is not applicable to hydrogen peroxide systems (see Section 3.6).

M9. A biohazard sign must be displayed at the entrance to the controlled area.

Equipment

M10. Not required.

M11. A good standard of hygiene should be maintained at all times and the regulations require that bench surfaces in areas where GMMs are handled are easy to clean and

resistant to water, acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use.

M12. Where the risk assessment shows it to be necessary, there is a requirement for adequate ventilation of the controlled area in order to minimise air contamination.

Mechanical ventilation is not normally needed for activities requiring large-scale Containment Level 2, although the work place should be a safe and comfortable environment and good ventilation may be needed for the removal of heat from process operations and worker comfort.

M13. There is no requirement to maintain negative air pressure to contain airborne GMMs at large-scale Containment Level 2. However, it is considered good practice if airborne dissemination of the GMM is a possibility and mechanical ventilation systems permit it (-15 Pa would be acceptable). Some processes might necessitate positive air pressure to maintain product integrity. This is acceptable provided that it does not conflict with the need to contain airborne GMMs in the event of an accident. The use of containment equipment such as a microbiological safety cabinet could be considered to provide both product and operator protection.

M14. Not required.

System of work

M15. Access to controlled areas at large-scale Containment Level 2 must be restricted to authorised personnel only. Swipe-card, key-coded or similar entry systems can aid the enforcement of this measure.

M16. The provision of washing and decontamination facilities is required for all activities involving GMMs. For areas operating at large-scale Containment Level 2, this will probably consist of hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap. Hands should be washed immediately if contamination with a GMM is suspected, after handling viable GMMs or before leaving controlled areas.

M17. Not required.

M18. There is a regulatory requirement for suitable protective clothing to be worn. This will normally comprise laboratory coats or gowns that are worn when in controlled areas and removed prior to hand washing upon exit. Clothing should ideally be kept in a dedicated locker and decontaminated prior to laundering where the risk assessment shows it to be necessary. The wearing of dedicated work wear will often be enforced to protect the purity

of the product in large-scale containment facilities, and this will often obviate the need for specific protective clothing.

M19. Formal written records of staff training are not required. However, personnel should receive information, instruction and training in handling of GMMs and emergency procedures. This will also satisfy the requirement for training and standards under COSHH and the Management Regulations (See Part 1 of the Compendium). All accidents and incidents should be recorded and immediately reported internally. If human health or the environment could have been harmed then this must be reported to HSE, in addition to any reports required under RIDDOR. Further guidance on this can be found in the publication *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* L29 (Third edition) HSE Books 2000 ISBN 978 0 7176 1758 6 and in Part 1 of the Compendium.

M20. Not required

M21. There is a regulatory requirement that contaminated material and waste (including culture fluids and other media) is inactivated before final discharge or disposal. Heat inactivation is generally considered more appropriate for large large-scale discharge of waste as chemical disinfectants may be environmentally damaging (waste discharge must comply with relevant environmental legislation – see Part 1 of the Compendium). If there is a risk to the environment from either the GMM or the disinfectant then the use of a validated autoclave will be preferable. If chemical inactivation is to be used, sufficient contact time should be given to afford adequate kill to minimise release of viable GMMs. However, consideration should be given to the use of chlorine-based disinfectants as they may damage stainless-steel process equipment. The inactivation method chosen must be appropriate to the risk and its efficacy must be validated against the organism being used. Inactivation methods should be monitored to ensure that they are working correctly (see Section 3.5).

Large-scale Containment Level 3

19. Large-scale Containment Level 3 must be applied for class 3 activities involving GMMs. All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.

General

M1. It is a regulatory requirement for viable GMMs to be contained in a closed system and be physically separated from workers and the environment. The nature of the closed system will depend upon the hazards and be determined by the risk assessment, but the GMMs must be physically contained such that release is *prevented*.

M2. The Regulations require that activities be undertaken within controlled areas. These areas should be separated from offices, laboratories and other facilities and be away from access routes.

M3. Exhaust gases must be treated to prevent release of GMMs. Off-gases can usually be controlled using filtration through 0.2µm cartridge HEPA filters (preferably two filters in series). It is important to keep filters dry to maintain their efficiency and prefilters may be necessary to prevent clogging and wetting. Filters should be easy to remove safely for maintenance purposes. Impingement filtration, cyclone separation, spraying gasses with hypochlorite in spray-towers or off-gassing through chemical disinfectants is not recommended at large-scale Containment Level 3.

M4. Measures to prevent release via aerosols during addition of material to a closed system, transfer of material to another closed system or sample collection are required. Inoculation of seed vessels should be done using a transfer vessel that is part of the closed system although direct injection using a sterile needle/septum technique may be adequate if well controlled. Where needles are used, procedures should be applied that minimise the likelihood of needle stick injury. All potentially contaminated liquids should be transferred in closed piping which is leak-tight (see M6). Sampling should take place using a closed aseptic technique. All material collected must be inactivated and the sampling valve/connection may need to be sterilised (see M5; M21).

M5. The Regulations require that viable GMMs in bulk culture fluids be inactivated by validated means prior to removal from the closed system. Chemical or physical methods are acceptable, but must be appropriate to the organism and level of risk. All waste must be inactivated by validated means prior to final disposal (see M21).

M6. There is a requirement that all seals are designed to prevent release of GMMs. Pipework should be welded wherever possible or practical and mechanical seals should be double faced with condensate fed to the interspaces (ideally with the condensate temperature being monitored and alarmed). If necessary, seals can be enclosed in HEPA (or equivalent) filtered housings. Where the equipment is connected to utility services, backflow should be prevented using a pressure differential, steam locks or 'double block and bleed' systems. Retractable sensors should not be used since duplicate sensors are safer for high-risk activities.

M7. The controlled area must be designed to contain a spillage of the entire closed system and the method employed should allow for inactivation of the spillage. This could be achieved by direct drainage into an effluent tank. Use of open drainage channels is not recommended at large-scale Containment Level 3.

M8. Controlled areas must be sealable for the purposes of fumigation where the risk assessment shows that it is required. Where necessary, validated fumigation procedures must be used in the event of a major spillage or prior to maintenance. Air handling and externally ducted safety cabinets should be controllable from outside the laboratory so that fumigant can be vented safely without the need to re-enter the room, although this is not applicable to hydrogen peroxide systems (see Section 3.6). Further guidance can be found in the HSE publication *Sealability of Microbiological Containment Level 3 and 4 Facilities* available at: www.hse.gov.uk/biosafety/gmo/guidance/sealability.pdf.

M9. A biohazard sign must be displayed at the entrance to the controlled area.

Equipment

M10. Where the risk assessment shows that it is required, access to controlled areas operating at large-scale Containment Level 3 must be via an airlock or a separate chamber.

M11. Good hygiene should be maintained at all times and the regulations require that bench and floor surfaces in areas where GMMs are handled are easy to clean and resistant to water, acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. Floors can be sprayed with epoxy resin or constructed of non-porous concrete to facilitate cleanability. Industrial wall cladding is not recommended for facilities operating at this level of containment, but walls can be sprayed with epoxy resin or covered with vinyl, resin-bonded fibre or gloss reinforced plastic (GRP). Vinyl coverings may not be appropriate if the controlled area is to be maintained at negative pressure as joints can open and the covering can detach from walls under these conditions. All penetration points (eg drains and cable entry points) must be sealed and tested.

M12. Where the risk assessment shows it to be necessary, there is a requirement for adequate ventilation of the controlled area in order to minimise air contamination. Whether or not this is shown to be necessary, the work place should be a safe and comfortable environment and good ventilation may be needed for the removal of heat from process operations and worker comfort.

M13. Where the risk assessment indicates that it is necessary, there is a requirement to maintain controlled areas at a negative pressure with respect to the immediate surroundings. In terms of regulatory requirements 'immediate surroundings' can be interpreted as being outside the building or in adjacent parts of the building. Typically, the controlled area should be maintained at -30 to -50 Pa via a continuous inflow of air while work is in progress. If an airlock is used, this should be maintained at negative pressure, this should be an intermediate value (eg -15 to -25 Pa) to maintain the laboratory pressure differential with the surroundings (see M10). Negative pressure is often achieved via the implementation of a controlled supply and extract of air by mechanical ventilation systems. The supply and extract airflow should be interlocked to prevent positive pressurisation of the room in the event of failure of the extract fan. Some processes might necessitate positive air pressure to maintain product integrity. This is acceptable provided that it does not conflict with the need to contain airborne GMMs in the event of an accident. The use of containment equipment such as a microbiological safety cabinet could be considered to provide both product and operator protection provided it is validated for use *in situ*.

M14. Where the activity requires that the control area be ventilated, extract air must be filtered through a HEPA filter. Input air can be optionally HEPA filtered, although it is not a requirement. Care should be taken, however, to ensure that contaminated air cannot be drawn into the ventilation inlets of another installation (recirculation of air is not recommended), or that contaminated air cannot be inadvertently expelled through input vents during a transient positive-pressurisation event. Easy and safe removal of HEPA filters should be possible for standard replacement and maintenance purposes.

System of work

M15. Access to controlled areas at large-scale Containment Level 3 must be restricted to authorised personnel only. Access to controlled areas can be via change facilities or airlocks. Card-swipe, key coded or similar entry systems can aid security.

M16. The provision of washing and decontamination facilities is required for all activities involving GMMs. For areas operating at large-scale Containment Level 3, this will probably consist of hand washing facilities with taps that can be operated without being

touched by hand and a supply of disinfectant soap. Hands should be washed immediately if contamination with a GMM is suspected, after handling viable GMMs or before leaving controlled areas (see M17).

M17. A showering facility may be required and workers will be required to shower when entering or leaving controlled areas where the risk assessment indicates that this is a necessary measure.

M18. Suitable protective clothing must be worn. This will normally comprise gowns and gloves that are worn when in controlled areas and removed prior to exit. Clothing should ideally be kept in a dedicated locker and decontaminated prior to laundering. Clothing that is being transported to an autoclave for decontamination should be suitably contained during transfer. The wearing of dedicated work wear will often be enforced to protect the purity of the product in large-scale containment facilities, and this will often obviate the need for specific protective clothing.

M19. Personnel should receive full instruction in handling of GMMs and emergency procedures and formal written records of staff training are required. This will also satisfy the requirement for training and standards under COSHH and the Management Regulations (See Part 1 of the Compendium). Written standard operating procedures that include emergency spillage plans are also required. All accidents and incidents should be recorded and immediately reported internally. If human health or the environment could have been harmed then this must be reported to HSE, in addition to any reports required under RIDDOR. Further guidance on this can be found in the publication *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* L29 (Third edition) HSE Books 2000 ISBN 978 0 7176 1758 6 and in Part 1 of the Compendium.

M20. If the risk assessment shows that it is required, all effluents from sinks and showers must be collected and inactivated prior to discharge (see M21).

M21. There is a regulatory requirement that contaminated material and waste (including culture fluids and other media) is inactivated before final discharge or disposal. Heat inactivation is generally considered more appropriate for large large-scale discharge of waste as chemical disinfectants may be environmentally damaging (waste discharge must comply with relevant environmental legislation – see Part 1 of the Compendium). If there is a risk to the environment from either the GMM or the disinfectant then the use of a validated autoclave will be preferable. If chemical inactivation is to be used, sufficient contact time should be given to afford adequate kill so as to prevent any release of viable GMMs. Consideration should be given to the use of chlorine-based disinfectants as they may damage stainless-steel process equipment. The inactivation method chosen must be

appropriate to the risk and its efficacy must be validated against the organism being used. Inactivation methods should be monitored to ensure that they are working correctly (see Section 3.5).

Large-scale Containment Level 4

20. Large-scale Containment Level 4 must be applied for class 4 activities involving GMMs. All the required measures, in addition to the principles of *good microbiological practice* and GOSH (see Section 3.1), must be applied unless agreement has been obtained from the competent authority.
21. Large-scale Containment Level 4 facilities are rare and extremely specialised. The guidance here is intended to be an outline of the regulatory requirements and users are advised to seek advice from HSE at an early stage should they wish to construct such a facility.

General

M1. It is a requirement for viable GMMs to be contained in a fully closed system that physically separates the GMMs from workers and the environment. The system should be designed to *prevent* release.

M2. The Regulations require that activities be undertaken within controlled areas that are separated from offices, laboratories and other facilities and are away from frequented access routes. Situating the controlled area in a separate building or a secluded part of a building will normally achieve this.

M3. Exhaust gases must be treated to prevent release of GMMs. Off-gases must be controlled using filtration through 0.2µm cartridge HEPA filters (preferably two filters in series). It is important to keep filters dry to maintain their efficiency and prefilters may be necessary to prevent clogging and wetting. Filters should be easy to remove safely for maintenance purposes. Impingement filtration, cyclone separation, spraying gasses with hypochlorite in spray-towers or off-gassing through chemical disinfectants are strongly discouraged at large-scale Containment Level 4.

M4. Measures to prevent release via aerosols during addition of material to a closed system, transfer of material to another closed system or sample collection are required. Inoculation of seed vessels should be done using a transfer vessel that is part of the closed system. Direct injection using a sterile needle/septum technique will not be acceptable at Level 4. All potentially contaminated liquids should be transferred in closed piping which is leak-tight (see M6). Sampling should only take place using a closed aseptic technique. All material collected must be inactivated and the sampling valve/connection may need to be sterilised (see M5; M21).

M5. The Regulations require that viable GMMs in bulk culture fluids be inactivated by validated means prior to removal from the closed system. Physical methods (such as heat treatment or autoclaving) are recommended as chemical methods vary in their efficiency and are less easy to validate. All waste must be inactivated by validated means prior to final disposal (see M21).

M6. There is a requirement that all seals are designed to prevent release of GMMs. Pipework should be welded and seals should be enclosed in HEPA (or equivalent) filtered housings wherever possible. The equipment should not be connected to utility services and retractable sensors should not be used since duplicate sensors are safer for high-risk activities.

M7. The controlled area must be designed to contain a spillage of the entire contents of the closed system and allow for inactivation of the spillage. This should be achieved by direct drainage into an effluent tank as the use of open drainage channels would not be acceptable at large-scale Containment Level 4.

M8. The controlled areas must be sealable for the purposes of fumigation. Validated fumigation procedures must be in place and used in the event of a major spillage or prior to maintenance. Laboratory ventilation and externally ducted secondary containment devices should be controllable from outside the laboratory to permit the safe venting of fumigant without the need to re-enter the room (see Section 3.6). Further guidance can be found in the HSE publication *Sealability of Microbiological Containment Level 3 and 4 Facilities* available at: www.hse.gov.uk/biosafety/gmo/guidance/sealability.pdf.

M9. A biohazard sign must be displayed at the entrance to the controlled area.

Equipment

M10. Access to controlled areas operating at large-scale Containment Level 4 must be via an airlock or a separate chamber. This must be maintained at a pressure negative to the surroundings (for example -15 to -25 Pa would be acceptable) but positive with respect to the controlled area itself.

M11. Scrupulous hygiene should be maintained at all times and the Regulations require that bench, floor, ceiling and wall surfaces in controlled areas are easy to clean and resistant to water, acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. Floors can be sprayed with epoxy resin or constructed of non-porous concrete to facilitate cleanability. Walls and ceilings can also be sprayed with epoxy resin or covered with resin-bonded fibre or glass reinforced plastic (GRP). Vinyl covering may not be appropriate as joints can open in negative air pressure conditions and the covering

can detach from walls under negative pressure conditions. All penetration points (eg drains and cable entry points) must be sealed and tested.

M12. Adequate ventilation of the controlled area to minimise air contamination is required at Large-scale Containment Level 4 and the area should be maintained at negative pressure relative to the immediate surroundings (see M13). The work place should be a safe and comfortable environment and good ventilation may be needed for the removal of heat from process operations and worker comfort.

M13. The controlled area must be maintained at negative pressure with respect to the immediate surroundings. In terms of regulatory requirements 'immediate surroundings' can be interpreted as being outside the building or in adjacent parts of the building. Typically, the controlled area should be maintained at -30 to -50 Pa via a continuous inflow of air while work is in progress. The airlock should also be at negative pressure but this should be an intermediate value to maintain the laboratory pressure differential with the surroundings (see M10). Negative pressure is often achieved via the implementation of a controlled supply and extract of air by mechanical ventilation systems. The supply and extract airflow should be interlocked to prevent positive pressurisation of the room in the event of failure of the extract fan. The ventilation system should be alarmed, connected to an emergency power supply and incorporate a system to prevent reverse air flux. Containment equipment such as a microbiological safety cabinet could be considered to afford product or operator protection, provided it is validated for use *in situ*.

M14. Input and extract air are required to be HEPA filtered. A single filter should be used on input air to prevent adventitious contamination if there is failure of ventilation. Extract air should be filtered through two HEPA filters mounted in series. Easy and safe removal of HEPA filters should be possible for standard replacement and maintenance purposes.

System of work

M15. Access to controlled areas at Large-scale Containment Level 4 must be strictly restricted to authorised personnel only. Access to controlled areas can be via change facilities or airlocks and card-swipe, key coded or similar entry systems can aid security.

M16. The provision of washing and decontamination facilities is required for all activities at large-scale Containment Level 4. This should include hand washing facilities with taps that can be operated without being touched by hand, a shower (see M17) and a supply of disinfectant soap.

M17. A showering facility is required and workers should shower (including hair) when leaving controlled areas.

M18. Suitable protective clothing must be worn. A complete change of clothing is required before entering and exit from controlled areas. This will normally comprise overalls and gloves that should be stored in the airlock or change facility in dedicated lockers. Protective workwear should be decontaminated by autoclaving prior to laundering. Contaminated clothing that is being transported to an autoclave for decontamination should be safely enclosed in secondary containment during transfer.

M19. Personnel should receive full instruction in handling of GMMs and emergency procedures and formal written records of staff training are required. Written standard operating procedures that include emergency spillage plans are also required. A high standard of supervision should be maintained. This will also satisfy the requirement for training and standards under COSHH and the Management Regulations (See Part 1 of the Compendium). All accidents and incidents should be recorded and immediately reported in accordance with locally specified procedures. All accidents at Containment Level 4 must be reported to HSE, in addition to any reports required under RIDDOR. Further guidance on this can be found in the publication *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* L29 (Third edition) HSE Books 2000 ISBN 978 0 7176 1758 6 and in Part 1 of the Compendium.

M20. All effluents from sinks and showers must be collected and inactivated prior to discharge (see M21).

M21. There is a regulatory requirement that contaminated material and waste (including effluents, culture fluids and other media) are inactivated before final discharge or disposal. Physical methods such as heat inactivation are considered more appropriate for large large-scale discharge of waste as chemical disinfectants vary in efficiency and may be environmentally damaging (waste discharge must comply with relevant environmental legislation – see Part 1 of the Compendium). The use of a validated autoclave will be preferable and is recommended. Furthermore, COSHH requires that physical methods be used for all ACDP hazard group 4 agents. If chemical inactivation is used for any procedure, then sufficient contact time should be given to afford adequate kill and waste should then be preferably autoclaved. Consideration should be given to the use of chlorine-based disinfectants as they may damage stainless-steel equipment. The inactivation method chosen must be validated against the organism being used and monitored to ensure that they are working correctly.

3.4 Containment and control measures for activities with GMMs that involves handling animals

Overview

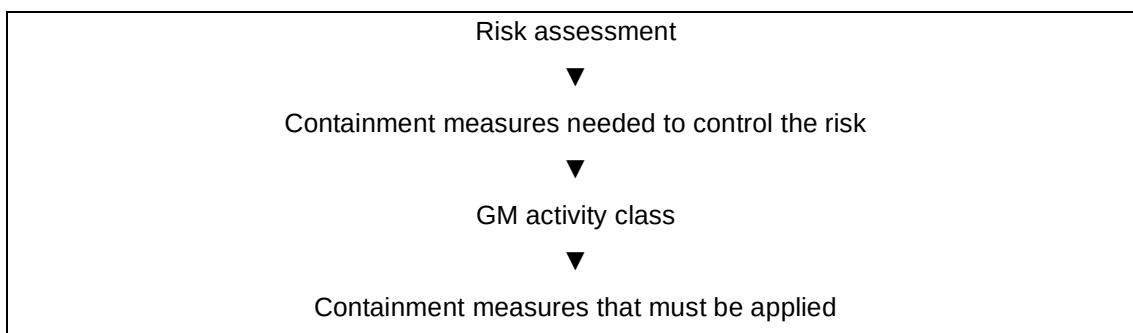
1. The Genetically Modified Organisms (Contained Use) Regulations require that all activities involving genetically modified microorganisms (GMMs) in containment employ a combination of physical, chemical or biological barriers to limit contact with people and the environment. This general requirement is underpinned by specific regulations 17 and 18 and their associated schedules, which prescribe the minimum containment for particular activities.
2. Regulation 17 sets out the principles of occupational and environmental safety which aims to ensure that *the exposure of humans and the environment to genetically modified micro-organisms is reduced to the lowest level that is reasonably practicable*. These principles (as outlined in the Section 3.1 and Schedule 7 of the regulations), are described as the general principles of *good microbiological practice* and of *good occupational safety and hygiene*, and apply to any activity involving the use of GMMs.
3. Regulation 18 requires that a person who undertakes an activity involving genetic modification of microorganisms shall apply the containment measures set out in the applicable Table in Schedule 8, where and to the extent required in the column of the appropriate containment level. The following guidance regards the minimum containment and control measures required by law for laboratory scale activities with GMMs as well as methods that can be applied to aid the fulfilment of legal obligations. The Contained Use Regulations are not the only legislation that may impact upon the containment measures needed, and users must take this into account (further guidance can be found in Part 1 of the Compendium).

Scope

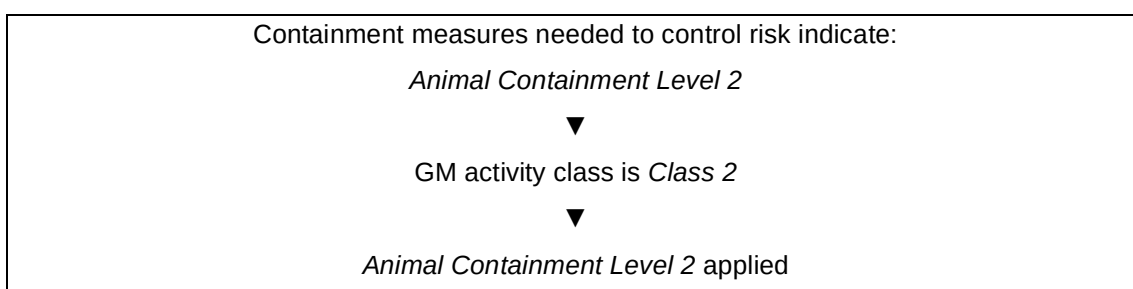
4. The section is intended to give guidance to help users determine the appropriate standards of containment and control that should be applied to work involving GMMs in conjunction with animals, for example within an animal facility. For activities with animals that involve handling GMMs, the relevant containment measures for *laboratory activities* must be applied and therefore users should read *Containment And Control Measures For Laboratory Activities Involving Genetically Modified Microorganisms* (Section 3.2) in conjunction with this guidance. Where GMMs and GM animals are both being handled,

users should also read Part 5, which covers activities involving the genetic modification of animals.

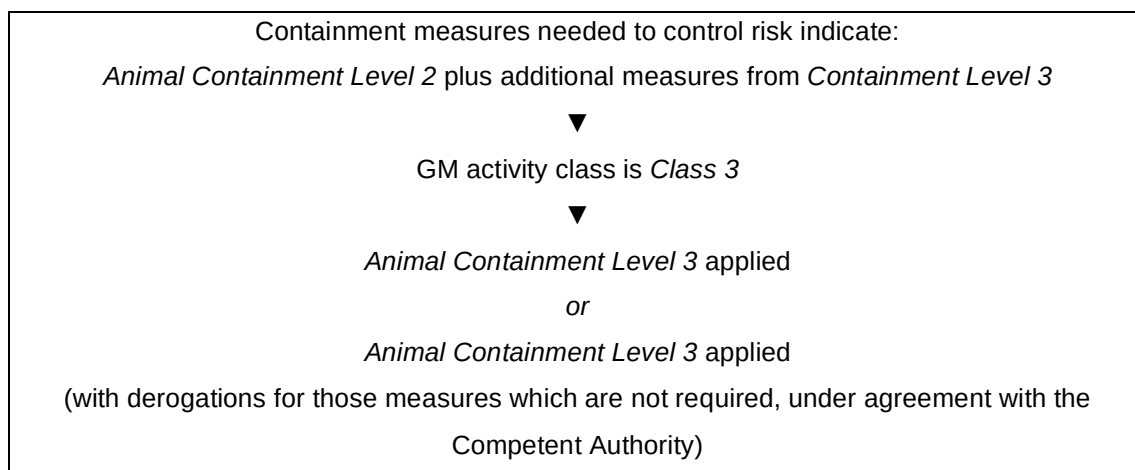
5. The Regulations set out certain specific requirements for activities being undertaken in Animal Units. In some cases the specifications *modify* those for laboratory containment, in others they impose *additional* requirements. The guidance in this section outlines the laboratory containment measures and the additional animal house requirements. Some control measures deemed necessary by the risk assessment may not be listed in the Schedule 8 containment level tables. The GM activity class is determined solely by those measures actually listed. The risk assessment must always take precedence and ALL measures identified as necessary must be applied (there is a general requirement for the exposure of humans and the environment to GMMs to be as low as reasonably practicable and the principles of good microbiological practice and of good occupational safety and hygiene must also be applied).



6. In the majority of cases, the containment measures necessary to control the risk, the GM activity class and the minimum containment level to be applied will be the same:



7. The containment measures indicated by the risk assessment may consist of a mixture of measures from two different levels. In these cases, the higher level of containment will determine the GM activity class and must be applied. A request can be made to the Competent Authority at the time of notification for permission to use the mixture of two levels identified, but unless and until you have the agreement of the Competent Authority, you may not use a level of containment lower than that corresponding to the GM activity class.



Definitions

8. The following terms do not have legal definitions and the explanations below are intended to aid understanding:

- **Animal unit:** A building, or separate area within a building, that contains an animal facility and includes other areas such as changing rooms, showers, autoclaves and food storage.
- **Animal facility:** A facility that is normally used to house stock, breeding or experimental animals or one that is used for the performance of minor surgical procedures on animals.
- **Isolator:** A transparent box used to contain small animals either within or outside a cage or isolated rooms for larger animals.
- **Required where and to extent the risk assessment shows it is required:** This indicates that the need for a particular measure is determined by the risk assessment. If the risk assessment specifies that a measure is needed for human health or environmental protection, its use is mandatory.
- **Competent authority:** The body with enforcing jurisdiction over the Contained Use Regulations. In England and Wales the competent authority includes the Health and Safety Executive (HSE), the Secretary of State and the Department for Environment, Food and Rural Affairs (Defra). In Scotland the competent authority includes HSE, the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the Scottish Ministers. Northern Ireland has its own separate competent authority. When seeking approval from the competent authority for particular actions, users should contact HSE in the first instance.

Animal welfare legislation

9. The Animals (Scientific Procedures) Act 1986 regulates any experimental or other scientific procedure applied to a 'protected animal' that may have the effect of causing that animal pain, suffering, distress or lasting harm. Codes of Practice relating to care and housing of laboratory animals, including breeding and humane killing are available from the Home Office (<http://scienceandresearch.homeoffice.gov.uk/animal-research/>).
10. The Act defines a 'protected animal' as any living vertebrate (other than man) and the invertebrate species *Octopus vulgaris*. Section 2(3) of the Act is of prime importance. This section states that *anything done for the purpose of, or liable to result in, the birth or hatching of a protected animal that may as a result of the procedure experience pain, suffering, distress or lasting harm is... a regulated procedure*.
11. **Home Office licensing.** A project licence that specifies the programme of work must be obtained before any procedures can be carried out. Applications for project licences are considered on a case-by-case basis and will only be authorised if there are no suitable alternatives that *replace* animal use, *reduce* the number of animals needed or *refine* the procedures used to cause less suffering. In addition, the likely benefits (to humans, other animals or the environment) must be weighed against the likely welfare costs to the animals involved.
12. Each person who undertakes the work must hold a *personal licence*. This lists the techniques and species they might use and the establishments where they work. The places where scientific procedures are carried out must be licensed under a *Certificate of Designation* unless the nature of the work requires that it is carried out in other places, such as farms or fields. Certain types of animal will need to be obtained from designated breeding or supplying establishments.
13. Guidance and application forms for obtaining licences and certificates can be obtained from the Home Office (<http://scienceandresearch.homeoffice.gov.uk/animal-research/>).
14. **Other legislation.** The welfare of domestic species is subject to the *Protection of Animals Act*, which makes it an offence to cause unnecessary suffering to any animals. Furthermore, the *Agriculture (Miscellaneous Provisions) Act* makes it an offence to cause unnecessary pain or distress to livestock on agricultural land. Detailed advice on the application of the relevant Code in individual circumstances is available through the official advisory services. The *Welfare of Animals (Transport) Order* is concerned with the welfare of all vertebrate animals in transit. Codes of Practice for the welfare of most species of farm livestock (cattle, sheep, pigs, poultry, etc) are published by Defra. Further information can be obtained from the Defra animal health and welfare web pages (www.defra.gov.uk/animalh/animindx.htm).

Containment measures for activities with animals involving GMMs

15. Note: This table contains requirements for containment measures for activities in animal facilities involving GMMs. The measures represent additions and modifications to the requirements for laboratory containment and must be read alongside the measures in the Laboratory activities containment table earlier in this document.

CONTAINMENT MEASURES		CONTAINMENT LEVELS				Additional/ Modification
		1	2	3	4	
M1	Isolation of animal unit	required where and to extent the risk assessment shows it is required	required	required	required	modification
M2	Animal facilities separated by lockable doors	required where and to extent the risk assessment shows it is required	required	required	required	additional
M3	Animal facilities (cages, etc.) designed to facilitate decontamination (waterproof and easily washable material)	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required	required	additional
M4	Floors and/or walls and ceiling easily washable	required where and to extent the risk assessment shows it is required	required for floor	required for floor and walls	required for floor, walls and ceiling	modification
M5	Appropriate filters on isolators or isolated rooms	not required	required where and to extent the risk assessment shows it is required	required	required	additional
M6	Incinerator for the disposal of animal carcasses	required to be accessible	required to be accessible	required to be accessible	required to be on site	additional
M7	Appropriate barriers at the room exit, and at drains or ventilation duct work	required	required	required	required	additional
M8	Animals kept in appropriate containment facilities, such as cages, pens or tanks but not isolators	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	additional
M9	Animals kept in isolators	required where and to the extent the risk assessment shows it is required	required where and to the extent the risk assessment shows it is required	required	required	additional
Other measures (From laboratory activities containment table)		other measures required at Containment Level 1	other measures required at Containment Level 2	other measures required at Containment Level 3	other measures required at Containment Level 4	

Containment Level 1 for GMMs in an animal facility

16. Containment Level 1 must be applied for class 1 activities with animals that involve GMMs. Required measures for Containment Level 1 laboratory activities must be applied, in addition to the measures set out for animal activities with GMMs and the application of the principles of good microbiological practice and GOSH (see Section 3.1), unless agreement has been obtained from the competent authority.

M1. The Regulations require that the animal unit be isolated from areas used for other activities, if the risk assessment shows that it is required. This means separation of the animal unit from other areas in the same building (or situated in a separate building) and away from offices, communal areas and sections frequented by non-technical staff.

M2. Animal facilities are required to be separated from other areas by lockable doors, where the risk assessment shows that this is required. Although not a regulatory requirement at this level, it is good practice to limit access to authorised persons only.

M3. Animal cages and other enclosure facilities must be designed to facilitate decontamination where the risk assessment shows this to be necessary. Good hygiene should be maintained at all times and the use of waterproof and easily washable materials will aid this.

M4. There is a regulatory requirement for the bench surfaces to be easy to clean and to be impervious to water and resistant to those acids, alkalis, solvents, disinfectants and other decontamination agents that may be expected in normal use (see Laboratory Containment Level 1 M3). Furthermore, the floors, walls and ceilings of animal containment facilities must be easily washable where the risk assessment shows that it is required. Hand-washing facilities and a supply of disinfectant soap should be provided.

M5. Not required.

M6. All waste material containing viable GMMs including animal waste and carcasses must be inactivated by validated means before final disposal (except effluent from hand wash basins). An incinerator must be accessible for the disposal of animal carcasses and an autoclave is required to be available on the site, but not necessarily in the same building. Waste removal by contractors may also be acceptable, provided that the final disposal method (eg incineration) is validated and that waste is stored and transported in a way that does not increase risk. The site of disposal must also comply with the Contained Use Regulations for the handling of GMMs that have not been inactivated. Agreement for the disposal of waste by a third party must be obtained from the competent authority (see Laboratory Containment Level 1 M8, M16, M17).

M7. There is a regulatory requirement for appropriate barriers to be placed at the exits and major penetration points (ventilation ducts and drains) within the facility. This may consist of a raised 'step' to be placed at the exit to the facility to prevent small mammals that are at large within the unit from escaping. In order to contain larger animals, it may be necessary to have a two-door entry system, separated by a small chamber or change area. Mesh barriers should be placed on ventilation ducts and drains and be sufficient to contain those species that are being handled within the facility.

M8. Where the risk assessment shows that it is required, animals must be kept in appropriate confinement equipment, such as cages, pens or tanks.

M9. The possibility of using an isolator (transparent boxes for the containment of small animals either in or out of a cage) must be actively considered. Where the risk assessment shows that it is required, animals must be kept in isolators.

Other measures (see *Laboratory Containment Level 1*)

17. There is a regulatory requirement for suitable protective clothing to be worn. The use of appropriate footwear should also be considered and it is good practice to cleanse or remove clothing and footwear when leaving the animal containment facility (Laboratory Containment Level 1 M12).
18. Specified disinfection procedures must be in place and effective disinfectants should be available for immediate use (see Laboratory Containment Level 1 M15).
19. Where the risk assessment shows that it is required, an observation window (or alternative) is required to be present to allow occupants to be seen. Such systems are particularly useful for the protection of lone workers (see Laboratory Containment Level 1 M19).
20. Where the risk assessment shows that it is required, there is a regulatory requirement for safe and secure storage for GMMs (see section Laboratory Containment Level 1 M20).
21. Where the risk assessment shows that it is required, controls must be put into place for disease vectors that could disseminate the GMMs (see Laboratory Containment Level 1 M14).
22. Laboratory personnel should receive information, instruction and training in handling of microorganisms and animals, and an appropriate standard of supervision of the work should be maintained.

23. All accidents and incidents should be recorded and immediately reported internally.

Containment Level 2 for GMMs in an animal facility

24. Containment Level 2 must be applied for class 2 activities with animals that involve GMMs. Required measures for Containment Level 2 laboratory activities must be applied, in addition to the measures set out for animal activities with GMMs and the application of the principles of good microbiological practice and GOSH (see Section 3.1), unless agreement has been obtained from the competent authority.

M1. The Regulations require that the animal unit be isolated from areas used for other activities. This means separation of the animal unit from other areas in the same building (or situated in a separate building) and away from offices, communal areas and sections frequented by non-technical staff.

M2. Animal facilities are required to be separated from other areas by lockable doors and access must be limited to authorised persons only (see Laboratory Containment Level 2 M9).

M3. Animal cages and other enclosure facilities must be designed to facilitate decontamination, where the risk assessment shows this to be necessary. Good hygiene should be maintained at all times and the use of waterproof and easily washable materials will aid this.

M4. There is a regulatory requirement for the bench surfaces to be easy to clean and to be impervious to water and resistant to those acids, alkalis, solvents, disinfectants and other decontamination agents that may be expected in normal use. The floors of animal containment facilities must be easily washable and, where the risk assessment indicates that it is necessary, so must the walls and ceilings. Hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap should be provided.

M5. Where the room itself is acting as an isolator, ventilated air must be HEPA filtered, if the risk assessment shows it is necessary. An isolator/cabinet may be required to prepare the GMM for inoculation into the animal, although this is often done in a separate laboratory. There is also a regulatory requirement for specific measures to minimise aerosol dissemination, which might necessitate the use of microbiological safety cabinets or isolators for procedures that generate aerosols (see Laboratory Containment Level 2 M6, M7 and M10).

M6. All waste material containing viable GMMs including animal waste and carcasses must be inactivated by validated means before final disposal (except effluent from hand wash basins). The legislation requires that an incinerator is accessible for the disposal of

animal carcasses but this can be off-site (see below). An autoclave is required to be available within the building. Other methods for the inactivation of contaminated carcasses may be suitable, for example Alkaline Hydrolysis (see Section 3.5), although if used in place of incineration, advice should be sought from the competent authority. Waste removal by contractors may also be acceptable, provided that the final disposal method (eg incineration) is validated and that waste is stored and transported in a way that does not increase risk. The site of disposal must also comply with the Contained Use Regulations for the handling of GMMs that have not been inactivated. Agreement for the disposal of waste by a third party must be obtained from the competent authority (see Laboratory Containment Level 2 M8, M16, M17).

M7. There is a regulatory requirement for appropriate barriers to be placed at the exits and major penetration points (ventilation ducts and drains) within the facility. This may consist of a raised 'step' to be placed at the exit to the facility to prevent small mammals from escaping. In order to contain larger animals, it may be necessary to have a two-door entry system, separated by a small chamber or change area. Mesh barriers should be placed on ventilation ducts and drains and be sufficient to contain those species that are being handled within the facility. Although not a regulatory requirement at this level, it is good practice to limit access to authorised persons only.

M8. Where the risk assessment shows that it is required, animals must be kept in appropriate confinement equipment, such as cages, pens or tanks.

M9. Where the risk assessment shows that it is required, animals must be kept in isolators. Isolators might be needed for work with GMMs derived from pathogens that could pose a hazard to humans, other animals in the facility or the wider environment. Guidance on the use, testing and maintenance of laboratory and animal flexible film isolators is available from HSE at: www.hse.gov.uk/biosafety/gmo/hseandgmos.htm.

Other measures (see *Laboratory Containment Level 2*)

25. There is a regulatory requirement for suitable protective clothing to be worn. The use of appropriate footwear should also be considered and it is good practice to cleanse or remove clothing and footwear when leaving the animal containment facility. Where the risk assessment shows that they are required, protective gloves must be worn (see Laboratory Containment Level 2 M12, M13).

26. Specified disinfection procedures must be in place and effective disinfectants should be available for immediate use (see Laboratory Containment Level 2 M15).

27. Where the risk assessment shows that it is required, an observation window (or alternative) is required to be present to allow occupants to be seen. Such systems are particularly useful for the protection of lone workers (see Laboratory Containment Level 2 M19).
28. There is a regulatory requirement for safe and secure storage for GMMs (see Laboratory Containment Level 2 M20).
29. The Regulations require that controls be put into place for disease vectors that could disseminate the GMMs (see Laboratory Containment Level 2 M14).
30. Laboratory personnel should receive information, instruction and training in handling of microorganisms and animals, and an appropriate standard of supervision of the work should be maintained. Where the risk assessment indicates that it is necessary, written records of training must be kept (see Laboratory Containment Level 2 M21).
31. All accidents and incidents should be recorded and immediately reported internally. The Regulations require that accidents whereby human health or the environment could have been harmed be reported to HSE.

Containment Level 3 for GMMs in an animal facility

32. Containment Level 3 must be applied for class 3 activities with animals that involve GMMs. Required measures for Containment Level 3 laboratory activities must be applied, in addition to the measures set out for animal activities with GMMs and the application of the principles of good microbiological practice and GOSH (see Section 3.1), unless agreement has been obtained from the competent authority.

M1. The Regulations require that the animal unit be isolated from areas used for other activities. This means separation of the animal unit from other areas in the same building (or situated in a separate building) and away from offices, communal areas and sections frequented by non-technical staff.

M2. Animal facilities are required to be separated from other areas by lockable doors. Access to the animal room itself should be via an airlock or separate chamber where the risk assessment shows this is necessary (see Laboratory Containment Level 3 M4). Access must be limited to authorised persons only and the room must be sealable for fumigation (see Laboratory Containment Level 3 M9 and M2).

M3. Good hygiene should be maintained at all times and animal cages and other enclosure facilities must be designed to facilitate decontamination, necessitating the use of waterproof and easily washable material.

M4. There is a regulatory requirement for bench and floor surfaces to be easy to clean and to be impervious to water and resistant to those acids, alkalis, solvents, disinfectants and other decontamination agents that may be expected in normal use (see Laboratory Containment Level 3 M3). The walls of animal containment facilities must also be easily washable and, where the risk assessment indicates that it is necessary, so must the ceilings. Hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap should be provided.

M5. A microbiological safety cabinet (or other enclosure/isolator) must be used for all activities with infective material. Air extracted from the animal room itself must be HEPA filtered in addition to the air from the enclosure/isolator. There is also a regulatory requirement for specific measures to prevent aerosol dissemination and the animal room must be maintained at a pressure negative to the immediate surroundings (see Laboratory Containment Level 3 M5, M6, M7 and M10).

M6. All waste material containing viable GMMs including animal waste and carcasses must be inactivated by validated means before final disposal (including effluent from hand wash basins and showers where the risk assessment shows this is necessary). The

legislation requires that an incinerator is accessible for the disposal of animal carcasses but this can be off-site (see below). An autoclave is required to be available within the building. Although it is permissible for waste materials to be inactivated by chemical means prior to disposal, it is normally more appropriate to autoclave waste at this level of containment. Where chemical disinfection is used, the disinfection procedures must be validated under working conditions. Other methods for the inactivation of contaminated carcasses may be suitable, for example Alkaline Hydrolysis (see Section 3.5). Waste removal by contractors may also be acceptable, provided that the final disposal method (eg incineration) is validated and that waste is stored and transported in a way that does not increase risk. The site of disposal must also comply with the Contained Use Regulations for the handling of GMMs that have not been inactivated. Agreement for the disposal of waste by a third party must be obtained from the competent authority (see Laboratory Containment Level 3 M8, M16, M17).

M7. There is a regulatory requirement for appropriate barriers to be placed at the exits and major penetration points (ventilation ducts and drains) within the facility. This may consist of a raised 'step' to be placed at the exit to the facility to prevent small mammals that are at large within the unit from escaping. It may be necessary to have two doors separated by a small chamber to contain larger animals. Mesh barriers should be placed on ventilation ducts and drains and be sufficient to contain those species that are being handled within the facility.

M8. Where the risk assessment shows that it is required, animals must be kept in appropriate confinement equipment, such as cages, pens or tanks.

M9. Animals must be kept in isolators. However, it is not necessary to use isolators for all class 3 activities. For example, using air-fed suits in conjunction with cages, pens and tanks for working with animals too large for isolators may be appropriate, although a derogation should be sought, providing full justification and an explanation as to why isolators are not practicable. Guidance on the use, testing and maintenance of laboratory and animal flexible film isolators is available from HSE at: www.hse.gov.uk/biosafety/gmo/hseandgmos.htm.

Other measures (see Laboratory Containment Level 3)

33. There is a regulatory requirement for suitable protective clothing to be worn and protective gloves must be worn for all work with infective materials. The use of appropriate footwear will be also be required if the risk assessment shows it to be necessary. Protective clothing and footwear should be removed when leaving the animal unit (see Laboratory Containment Level 3 M12, M13).

34. Where it is shown to be a requirement by the risk assessment, personnel are required to shower before leaving the animal facility (see Laboratory Containment Level 3 M11).
35. Specified disinfection procedures must be in place and effective disinfectants should be available for immediate use (see Laboratory Containment Level 3 M15).
36. There is a regulatory requirement for the facility to contain its own equipment, so far as is reasonably practicable (see Laboratory Containment Level 3 M18)
37. An observation window (or alternative) is required to be present to allow occupants to be seen (see Laboratory Containment Level 3 M19).
38. There is a regulatory requirement for safe and secure storage for GMMs (see Laboratory Containment Level 3 M20).
39. The Regulations require that controls be put into place for disease vectors that could disseminate the GMMs (see Laboratory Containment Level 3 M14).
40. Laboratory personnel should receive information, instruction and training in handling of microorganisms and animals, and an appropriate standard of supervision of the work should be maintained. Written records of staff training must be kept (see Laboratory Containment Level 3 M21).
41. All accidents and incidents should be recorded and immediately reported internally. The Regulations require that accidents whereby human health or the environment could have been harmed be reported to HSE.

Containment Level 4 for GMMs in an animal facility

42. Containment Level 4 must be applied for class 4 activities with animals that involve GMMs. Required measures for Containment Level 4 laboratory activities must be applied, in addition to the measures set out for animal activities with GMMs and the application of the principles of good microbiological practice and GOSH (see Section 3.1), unless agreement has been obtained from the competent authority.
43. Containment Level 4 animal facilities are rare and extremely specialised and there are very few in the UK. If the building of a new facility is proposed, it is advised that the details of containment requirements, management control, and design of the facility is discussed in advance with HSE. Users may wish to consult the ACDP/Defra/DH guidance on *The principles, design and operation of Containment Level 4 facilities* (see: www.hse.gov.uk/pubns/web09.pdf).
44. The requirements set down by Defra for the handling of Defra Group 4 pathogens (eg Foot and Mouth Disease Virus; Rinderpest Virus) will differ from those set out in this section as work with certain animal or plant pathogens will require less operator protection, but a high degree of environmental protection. Facilities designed to accommodate work with Defra Group 4 pathogens that represent little or no risk to human health will still require Containment Level 4 for the purposes of GM work, even though they may be subject to derogation (with agreement from the competent authority) from some of the measures set out below. The Contained Use Regulations cover both human health and environmental safety and therefore the Defra level 4 classification leads to an automatic assignment to class 4. Furthermore, the prescribed conditions outlined in any relevant SAPO license must be adhered to.

M1. The Regulations require that the animal unit be isolated. This is normally fulfilled by the animal facility being in a separate building or forming an isolated part of a building (see Laboratory Containment Level 4 M1).

M2. Animal facilities are required to be separated from other areas by lockable doors. Access to the animal room itself should be via an airlock or separate chamber and be limited to authorised persons only. This control should be maintained using a key procedure (see Laboratory Containment Level 4 M9). A second competent person should be available during work to assist in case of an emergency. The clean side of the airlock should be separated from the restricted side by changing and showering facilities with, preferably, interlocking doors (see Laboratory Containment Level 4 M4). The room must also be sealable for fumigation (see Laboratory Containment Level 4 M2).

M3. A scrupulously high standard of hygiene should be maintained at all times and animal cages and other enclosure facilities must be designed to facilitate decontamination, necessitating the use of waterproof and easily washable materials.

M4. There is a regulatory requirement for bench, floor, ceiling and wall surfaces to be easy to clean and to be impervious to water and resistant to those acids, alkalis, solvents, disinfectants and other decontamination agents that may be expected in normal use (see section Laboratory Containment Level 4 M3). The floor, walls and ceiling are also required to be easily washable.

M5. A class III microbiological safety cabinet (or enclosure/isolator of similar standard) must be used for all activities with infectious material. Air from the enclosure/isolator must be HEPA filtered. There is also a regulatory requirement for specific measures to prevent aerosol dissemination and the animal room must be maintained at a pressure negative to the immediate surroundings. Input and extracted air must be HEPA filtered (see Laboratory Containment Level 4 M5, M6, M7 and M10).

M6. All waste material containing viable GMMs including animal waste and carcasses must be inactivated by validated means before final disposal, including effluent from hand washbasins and showers. An incinerator must be accessible for the disposal of animal carcasses and a double-ended autoclave is required to be available within the animal facility. The autoclave should have interlocking doors, with entry in the restricted part of the animal facility and exit in a clean area. All material should be rendered non-infectious using a validated procedure before being removed from the restricted part of the animal facility (see Laboratory Containment Level 4 M8, M16, M17).

M7. There is a regulatory requirement for appropriate barriers to be placed at the exits and major penetration points (ventilation ducts) within the facility. Mesh barriers should be placed on ventilation ducts and drains and be sufficient to contain those species that are being handled within the facility. Note that drains are not appropriate at level 4.

M8. Where the risk assessment shows that it is required, animals must be kept in appropriate confinement equipment, such as cages, pens or tanks.

M9. Animals must be kept in isolators. It is likely that specialised air-fed isolators will be required, but it may not be necessary to use isolators for all class 4 activities. Using air-fed suits in conjunction with cages, pens and tanks for working with animals too large for isolators may be appropriate, although a full justification including an explanation as to why isolators are not practicable would be required. Guidance on the use, testing and maintenance of laboratory and animal flexible film isolators is available from HSE

(www.hse.gov.uk/biosafety/gmo/hseandgmos.htm).

Other measures (see *Laboratory Containment Level 4*)

45. There is a regulatory requirement for suitable protective clothing to be worn, including gloves. A complete change of clothing is required upon entry and exit from the containment area (see M2 and Laboratory Containment Level 4 M12, M13).
46. Personnel are required to shower before leaving the animal facility (see M2 and Laboratory Containment Level 4 M11). Hand washing facilities with taps that can be operated without being touched by hand and a supply of disinfectant soap should also be provided.
47. Specified disinfection procedures must be in place and effective disinfectants should be available for immediate use (see Laboratory Containment Level 4 M15).
48. There is a regulatory requirement for the facility to contain its own equipment (see Laboratory Containment Level 4 M18).
49. An observation window (or alternative) is required to be present to allow occupants to be seen (see Laboratory Containment Level 4 M19).
50. There is a regulatory requirement for safe and secure storage for GMMs. All viable GMMs requiring Level 4 containment must be stored in a level 4 facility and nowhere else (see Laboratory Containment Level 4 M20).
51. The Regulations require that controls be put into place for disease vectors that could disseminate the GMMs (see Laboratory Containment Level 4 M14).
52. The Regulations require that there be written procedures for staff training and that records are kept of all training undertaken. Specific rules should be drawn up for the work in the animal facility. Personnel should have specific training in the working of the animal house, use of safety equipment and information on the handling of the microorganisms and animals concerned (see Laboratory Containment Level 4 M21).
53. All accidents and incidents should be recorded and immediately reported internally. All accidents at Containment Level 4 must be reported to HSE, in addition to any reports required under RIDDOR.

3.5 Inactivation

Overview

1. The Contained Use Regulations require that waste contaminated with GMMs be inactivated prior to discharge or disposal. Inactivation is defined (as applied to treatment of waste prior to disposal) in the Regulations as *'the complete or partial destruction of GMMs so as to ensure that any contact between the GMMs and humans or the environment is limited to an extent commensurate with the risks identified in the risk assessment and to provide a high level of protection for humans and the environment'*. This means that the degree of 'kill' achieved by the inactivation must be related to the risk posed by the GMM. For the more hazardous GMMs, such as those assigned to activity classes 3 or 4, sterilisation will be required. For instance, physical methods and alkaline hydrolysis can be validated to effectively achieve a complete kill. For less hazardous GMMs, such as those assigned to GM activity class 1 or 2, a lower standard might be acceptable, provided that the risks are sufficiently reduced to a 'safe' level. For instance, chemical disinfection typically gives a 5-log reduction in viability of GMMs.
2. For some low risk activities, inactivation may not need to be a separate, discrete step in the procedure. Inactivation may be a consequence of other processing steps or experimental methods. For example, extraction methods frequently involve disruption or lysis steps that will achieve sufficient inactivation of the GMM. Care should be taken, however, to ensure that such methods are consistent and provide a level of inactivation that is adequate from a risk management perspective.
3. This guidance covers methods for the inactivation of waste. The majority of the guidance concerns chemical inactivation, as there are many different methods that vary in their effectiveness. Users should also refer to the British Standard (BS EN 12740: 1999 *Biotechnology. Laboratories for research, development and analysis. Guidance for handling, inactivating and testing of waste*) that gives guidance on the handling, inactivation and testing of waste, and details good practice in waste management.

Definitions

- **Disinfection:** This generally refers to the use of chemical agents to reduce the potential infectivity of a material, but does not imply the elimination of all viable microorganisms. The effectiveness of disinfection will be dependent upon the activity of the disinfectant used.

- **Sterilisation:** A process that renders an object effectively free of viable microorganisms, including those that may survive disinfection treatments (eg bacterial and fungal spores; Prions). A validated autoclave cycle represents the most effective way to achieve sterilisation of waste or equipment and must reduce the number of viable organisms by six logs.
- **Activity:** The effectiveness of a particular disinfectant, which varies with the target microorganism.
- **Concentration:** The 'working-concentration' or 'working dilution' refers to the correct dilution for effective disinfection in any given circumstance. This may be dependent on the intended use, for example, the dilution factor needed for tackling spillages, eg spillages may be different than for those required in discard jars. The effective concentration may also depend upon the age of the solution as some diluted disinfectants lose effectiveness over time.
- **Contact:** A sufficient period of time where the GMM is exposed to the disinfectant will be necessary to effect the required level of killing. This contact must also be effectively maintained between the disinfectants and the contaminated article, for example air-bubbles should be removed from submerged articles.
- **Validation:** Establishing documented evidence that a disinfection process will consistently inactivate target organisms under defined conditions of use.

Physical inactivation methods

4. Physical methods of inactivation are arguably the most reliable way to achieve a high kill rate. For large-scale operations, inactivation will usually involve heat inactivation of cultures. For other operations, the use of an incinerator (for example to dispose of infected animal carcasses) or rendering procedure might be appropriate or sufficient. However, the use of a validated autoclave cycle remains the most effective means of inactivating GMMs.
5. A range of autoclave cycle parameters are suitable for inactivating microorganisms and a typical cycle would be 121 °C, maintained for 15 minutes. Holding-time may need to be increased for work with particular organisms (for example spore-forming bacteria), large amounts of contaminated material or where steam penetration is inefficient. Users should consult the technical specifications for their particular autoclaves. A higher heat setting is recommended for work with TSEs (134-138 °C). Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection*.
6. The Regulations require that an autoclave be available for all laboratory-scale activities with GMMs and those that involve animals. The use of an autoclave should always be an

option for these activities, therefore, and it is recommended that one be used for waste inactivation, either by itself or in combination with chemical methods. Care should be taken, however, to ensure that chemical disinfectants do not damage the autoclave. The autoclave should be serviced regularly and its performance tested frequently in accordance with the manufacturer's recommendations and national standards (BS 2646-3: 1993).

7. Any inactivation method used should be validated or verified under working conditions.

Chemical inactivation methods

8. Chemical inactivation methods are commonly used in laboratory-scale operations to avoid the need to autoclave bulk waste (eg spent media and liquid cultures). Chemical disinfection is an inherently less reliable method of inactivation as there are many factors that can come to bear on the effectiveness of the chosen disinfectant. For example, the presence of organic matter can impede the performance of certain disinfectants. Similarly, the disinfection regime may be compromised if cultures are buffered or proteinaceous. Furthermore, the requirement to validate or verify the effectiveness of the procedure stands, and it can be technically difficult or impractical to adequately remove the disinfectant and screen for viable GMMs. For these reasons it is recommended that, for high-risk activities, chemical methods are not relied upon as a sole means of inactivation but are used in combination with physical methods. However, the sole use of chemicals does represent an acceptable means of inactivation for lower-risk activities.
9. When selecting a disinfectant, its toxicity to humans and the environment should be considered. Appropriate safety precautions should be adopted. Different disinfectants must not be mixed together or used in combination unless the possibility of hazardous reactions or the formation of toxic products has been properly assessed. Appropriate procedures should be used to ensure suitable disinfectants at the correct dilution are available at the point of need. Personnel should be trained in the correct use of disinfectants and in the emergency spillage protocols associated with them.
10. There are advantages in limiting the number of different disinfectants available in the workplace to the minimum necessary, in order to avoid confusion and to reduce costs. Once a disinfectant has been selected, in-use tests should be carried out to monitor both the performance of an individual chemical but also the way in which it is used (for example tests to detect incorrect dilution, old solutions and mixtures of incompatible reagents).

11. Disinfectants and chemical inactivation methods in common use are discussed below, including considerations relating to the characteristics, advantages and disadvantages of each.
12. **Hypochlorites.** Hypochlorites (eg bleach) have a wide spectrum of antimicrobial activity and are rapid in action. However, hypochlorites are corrosive and may damage equipment and stainless-steel surfaces. Furthermore, they are inactivated by organic matter and decompose in solution. Sodium hypochlorite can be used to inactivate Prions (20,000 ppm for 1 hour). Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection*.
13. **Chlorine releasing agents.** Chlorine releasing agents (eg sodium dichloroisocyanurate - NaDCC) are broad-spectrum and rapid acting biocides. Dry forms of NaDCC are stable but can be dissolved in water to generate chlorine, a process that is accelerated in an acidic environment. Chlorine releasing agents are corrosive and easily inactivated by organic matter but are widely recommended in conditions where corrosion or bleaching are not an issue.
14. **Phenolics.** Phenolics are non-corrosive and are not readily affected by organic matter. They have a wide range of antimicrobial activity (although they may be ineffective against non-enveloped viruses) but this may be impaired if diluted in hard water. Phenolics should not be stored diluted.
15. **Alcohols.** Alcohols (eg 70% ethanol; 60% isopropanol; Industrial methylated spirits) rapidly kill eukaryotic pathogens, bacteria and some viruses due to their dehydrating action. They are particularly useful for low-level contaminations and surface sterilisation. Alcohols are relatively volatile, however, and do not provide a sustained antimicrobial action. Furthermore, they are flammable, requiring appropriate precautions in storage and use.
16. **Aldehydes.** Formaldehyde vapour or aqueous solution (formalin) is highly toxic and unsuitable for general purposes. It is used for fumigating microbiological safety cabinets and sealed rooms (eg high-containment laboratories). During fumigation, containers of other disinfectants should be sealed if the disinfectant is incompatible with the fumigant. Glutaraldehyde solutions are also toxic and have a wide range of antimicrobial activity, including against bacterial spores. They are non-corrosive but are unstable once activated and do not readily penetrate organic matter (see Section 3.6). Furthermore, they are potent sensitisers and should not be used for routine laboratory work.

17. **Surface-active agents.** Quaternary ammonium compounds (QACs) are often sold in combination with other disinfectants and form the basis of the majority of cationic detergents. Only cationic and amphoteric detergents have any antifungal or antibacterial activity, and are regarded as being more bacteriostatic than they are bactericidal. They are less active against Gram-negative bacteria and vary in their antiviral performance. Furthermore, they appear to be inactive against mycobacteria and spore-forming bacteria. Soaps, anionic detergents and organic matter inactivate them, although they are relatively non-toxic and non-irritant.
18. **Iodophors.** Iodophors provide a sustained-release of antimicrobially active iodine from an inactive reservoir. They essentially consist of iodine in combination with a solubilising agent or carrier. They are bactericidal, fungicidal and virucidal, but show limited activity against spores. Iodophors are inactivated by organic matter (dependent upon preparation and concentration) and may corrode metals.
19. **Peroxygen compounds.** The antimicrobial properties of Peroxygen compounds are due to their oxidising activity, which damages cellular proteins, lipids and nucleic acids functions. They are effective against a wide range of microorganisms although this is impaired in the presence of organic matter. They can be used for routine laboratory disinfection but, before using on equipment, approval should be obtained from the manufacturer since corrosion of some metals and plastics may be a problem.
20. **Hydrogen peroxide.** Hydrogen peroxide owes its antimicrobial properties to its potent oxidising activity, which damages cellular proteins, lipids and nucleic acids. It is unstable when diluted and in the presence of microorganisms, this gives rise to highly oxidising hydroxyl radicals (such as those used by macrophages to destroy infectious organisms). Vapour-phase hydrogen peroxide has been developed as a fumigant (see Section 3.6) and diluted forms have also been used as a general disinfectant. The by-products of hydrogen peroxide decomposition are harmless oxygen and water, although concentrated solutions are irritating to the skin, eyes and mucous membranes and therefore care must be taken.
21. **Sodium hydroxide solutions.** Sodium hydroxide solutions (1%) may be used under certain circumstances as a laboratory disinfectant. Care must be taken with their use, due to the caustic nature of the chemical. Prions can be inactivated using 1M NaOH, which is less corrosive than the concentrated sodium hypochlorite alternative. This is particularly pertinent when steel or other metal surfaces require disinfection. Further information on the handling of TSEs can be found in ACDP/SEAC guidance document *Transmissible spongiform encephalopathy agents: Safe working and the prevention of infection*.

22. **Alkaline hydrolysis.** Alkaline hydrolysis uses high pH (pH14, usually from concentrated sodium or potassium hydroxide) in combination with high pressure and heat to reduce organic matter to liquid waste. The liquid waste, while still very alkaline (pH10.5), contains nothing more hazardous than short peptides, amino acids, sugars, salts and fatty acid residues. Due to the need for pressure and heat, alkaline hydrolysis requires proprietary equipment, and these systems have been developed for healthcare and tissue digestion applications and vary in scale from small laboratory units to those used for the disposal of large animal carcasses. The process can be validated and is effective against all infectious agents, including prions. The use of this system is particularly attractive for animal facilities as carcasses are reduced to harmless 'bone-shadow' powder and liquid waste. However, there are significant hazards associated with handling and storing large amounts of concentrated alkali.

3.6 Fumigation

Overview

1. On occasions it will be necessary to decontaminate laboratories, animal containment facilities and safety cabinets using a fumigation technique. This might be a routine procedure or performed if maintenance work is to be carried out. Alternatively, fumigation might be an emergency procedure if, for example, there has been a spillage of infectious material or an escape of an airborne pathogen. The following guidance offers advice for the safe and effective decontamination of microbiological safety cabinets (MSCs) and rooms using fumigation techniques. Information relating to commonly used fumigant chemicals and technologies is also included.
2. Fumigation should always be a planned exercise with suitable controls in place and with appropriate information and warnings provided for those who need to know. Only designated personnel, who are fully trained, should carry out fumigation operations. A standard procedure for routine decontamination and emergency fumigation plans should be agreed. Only a fumigant and method known to be effective in the circumstances of use should be used.

Fumigation of rooms

3. Where a room in a laboratory or animal containment unit is to be fumigated, the area should be checked to ensure that it is securely sealed so as not to allow the escape of fumigant to other parts of the building. The legal requirement that the room be sealable for fumigation can be interpreted as meaning that only the door should need sealing. It should not be necessary to enter the room in order to seal up gaps in the integrity of the laboratory prior to fumigation. Suspended ceilings can present a special difficulty as there may be a void above connecting with other rooms nearby. Careful thought also needs to be given to fumigation of any ductwork. Users should consult the HSE guidance *Sealability of Microbiological Containment Level 3 and 4 Facilities* (available at: www.hse.gov.uk/biosafety/gmo/guidance/sealability.pdf) for further information.
4. Fumigant concentrations can become stratified and may not penetrate completely to all parts of the room. It may be necessary to stir the air in the room to ensure even distribution of the fumigant. This can be achieved using desktop fans or, for newly-built facilities, integral fans can be fitted. The normal air-handling or air-conditioning systems should not be used as these could positively-pressurise the room and breach the door/vent seals.

5. Exposure to the residual effect of the fumigant after generation should take place for a time period appropriate to the chemical or system being used. Fumigant may be extracted from the area by the air handling system but only when that is a total loss system with no possibility of fumigant being conducted to other areas. More commonly, use is made of a microbiological safety cabinet or a fume cupboard as a means of extraction if one is situated within the area under treatment and if it exhausts to atmosphere. In all cases, an external switch should operate the ventilation system or equipment extracting the air so as to avoid entering the room.
6. Where possible, there should be a thorough check of the level of residual fumigant before anyone enters following extraction. This may be done most conveniently by, for example, sampling the air through a small port fitted in the door for this purpose. For example, meters and other assay devices are available to indicate the concentration of formaldehyde vapour remaining in the air.

Emergency room fumigation

7. Fumigation is an important component of the procedures used to treat spillages or escapes of GMMs in Containment Level 3 or 4 facilities. In the event of a major spillage, the safest and most logical procedure would be to:
 1. Evacuate the laboratory.
 2. Fumigate the laboratory.
 3. Disinfect the bulk spillage and remove any debris.
 4. Fumigate again, if deemed to be necessary.
8. Personnel should not enter an area when a major spillage of microorganisms has taken place, as they may remain suspended in the air for some time and there may be a great risk of exposure. The laboratory air handling system should be allowed to clear airborne particles before re-entering the laboratory. Users may wish to consult the ACDP publication *The management, design and operation of microbiological containment laboratories* Guidance HSE Books 2001 ISBN 978 0 7176 2034 0 for further information on how to calculate the appropriate time interval. Upon re-entry, the use of respiratory protective equipment may be necessary for the process of disinfecting the bulk spillage.
9. Emergency fumigation should be viewed as a way of inactivating small deposits of an infectious agent that may have been generated as a consequence of the splashes and aerosols that occur during a spillage. Fumigant will not penetrate throughout the entire volume of a large spillage and fumigation should not be seen as a way of inactivating the

bulk of the main spill. Thus, the main spill should be treated with an appropriate disinfectant and it may be necessary to fumigate again if the disinfection could have generated further infectious aerosols.

10. Personnel should not enter an area after the fumigant has been generated unless suitable breathing apparatus is worn. Only those trained in the use of breathing apparatus should use it. Respirators are not appropriate for use in the concentrations of formaldehyde vapour achieved when carrying out these procedures. Full breathing apparatus with an independent air source must be worn.

Fumigation of microbiological safety cabinets

11. Microbiological safety cabinets should be fumigated:

- Following a small spillage of infectious material within the cabinet.
- Before filters are changed.
- Before any maintenance work that requires access to the interior of the cabinet.

12. In the event of a large spillage, however, consideration should be given to the fumigation of the whole room (see above).

13. Fumigant should only be generated within a MSC with the sash/night door securely sealed and the non-return valve closed. Passive migration of the fumigant through the filter can occur but an alternative is to leave the valve open and the fan running for 10 to 15 seconds to ensure penetration of the filter medium. The valve should then be closed and the fan switched off while the remainder of the fumigant is left to disperse within the cabinet. After a time period appropriate for the chemical or system in use to be effective, the fumigant should be exhausted to atmosphere by switching on the fan. Air from the room must be allowed to enter the cabinet (for example through a large bunghole in the night door).

14. Before venting a hazardous fumigant to atmosphere (for example formaldehyde), it is essential to ensure that no personnel (for example engineers or contractors) are in the vicinity of the exhaust outlet and that the exhaust air does not enter nearby windows or ventilation air intakes.

15. If filters are to be changed after fumigation, the discarded filter unit should be bagged and disposed of. There are special difficulties if the cabinet is used with the agents causing transmissible spongiform encephalopathies, as they are resistant to fumigants.

Validation of fumigation

16. A test of the effectiveness of fumigation may be carried out by placing spore strips/discs carrying *Bacillus subtilis* var. *globigii* (filter paper inoculated with a suspension of the organism) at various points in the room to test penetration of the fumigant. Similarly, a standardised spore suspension may be painted onto small marked areas on surfaces that are later swabbed to recover any surviving organisms. As fumigant concentrations can often become stratified or not penetrate completely to all parts of the room, the positioning of spore strips/discs should be carefully considered and placed at a point where fumigant concentration is likely to be lowest or where contact time will be the least (for example, the floor, or beneath a piece of equipment).

Fumigants and fumigation methods

17. There are several different fumigant chemicals and fumigation technologies available which vary in their effectiveness and relative safety from a human health or environmental perspective. These are listed below:

- Formaldehyde.
- Hydrogen peroxide.
- Ethylene oxide.
- Propylene oxide.
- Peracetic acid.
- Chlorine dioxide.
- Ozone (O₃).

18. To date, the most commonly used fumigant in UK laboratories has been formaldehyde vapour. While it is a highly effective biocidal agent and is relatively easy to generate, it is also a Schedule 1 chemical under the COSHH Regulations and is both a sensitiser and a carcinogen. The harmful effects of formaldehyde have provided strong motivation for the development of alternative, less toxic gaseous fumigants. The requirement by COSHH to prevent or control exposure to hazardous chemicals as far as reasonable practicable confers a responsibility to replace formaldehyde with a less toxic alternative. Most technologies have struggled to match formaldehyde vapour for efficacy, economy and ease of use, although hydrogen peroxide now represents a realistic alternative and should be considered. The properties and limitations of formaldehyde vapour and hydrogen peroxide are discussed below.

19. **Formaldehyde.** Formaldehyde (CH_2O) acts as an alkylating agent, reacting with proteins and nucleic acids to inactivate a broad spectrum of microorganisms, including viruses and bacterial spores. There is more than one way of generating formaldehyde vapour, however, effective fumigation is typically achieved by heat-initiated vaporisation of a 40% solution of formaldehyde vapour in water (Formalin), usually performed at 70-80°C (note that formaldehyde vapour is also explosive at 7.75% in dry air with an ignition point of 430 °C). Formaldehyde vapour penetrates slowly into air spaces and porous surfaces. Therefore, a prolonged contact time of at least 12 hours is required.
20. A number of factors affect the efficiency of fumigation:
- The ratio of formalin to water used (and thereby the relative humidity created).
 - The volume of the space to be fumigated (Typically, 100ml of formalin diluted in 900ml of water is vaporised for every 28.3m³ of space).
 - The surface area exposed in that space and the presence of absorbent materials such as cardboard boxes.
 - Temperature - below 18 °C formaldehyde fumigation is less effective and below 9 °C formaldehyde sublimates and is less easy to vaporise.
21. Pre-cleaning will increase efficiency (provided it can be done without jeopardising safety) and it must be able to dissolve at adequate concentrations in the film of moisture in the immediate vicinity of the organisms to be inactivated. Water vapour generated in the process of dispersing formaldehyde provides is essential to achieve this (ie relative humidity between 35% and 80% is optimum). Too much formaldehyde will result in sticky deposits of paraformaldehyde.
22. It should be noted that any hydrochloric acid and chlorinated disinfectants should be removed from the room before fumigating with formaldehyde. This is to prevent the possibility of forming potentially carcinogenic bis (chlormethyl) ether. In high containment facilities, care must be taken where double-ended dunk tanks are present.
23. Formaldehyde fumigant is potentially harmful to health and will require strict controls to ensure the workplace exposure limit is not exceeded (see Table 3.6.1). The fumigant must therefore be fully evacuated from the room using the ventilation system before anyone re-enters the room unless suitable breathing apparatus or respiratory protective equipment (RPE) is worn.
24. Independent formaldehyde removal units are available and could be used to remove fumigant in large volume rooms or from a microbiological safety cabinet. Typically, such

equipment draws contaminated air over reusable carbon filter where chemical residues become bound are therefore immobilised for safe removal.

25. Formaldehyde vapour can be neutralised following the fumigation with either liquid ammonia in an open vessel or with carefully controlled ammonia vapour generation. The use of liquid ammonia is likely to be most appropriate for low-volume cabinet fumigation, where 300ml of ammonia is used in an open vessel for every 28.3m³ of treated space. Ammonia vapour, however, can be applicable to both whole room and cabinet fumigation. Chemical neutralisation procedures such as this might be particularly applicable to the fumigation of recirculating class II MSCs that cannot be vented externally to the atmosphere. This means that the exhaust of a fumigated cabinet can be recirculated back into the room. This will usually require charcoal filtration of the exhaust air to scrub any excess fumigant or neutraliser. Complete fumigation/neutralisation systems are available commercially.
26. **Hydrogen peroxide.** The broad antimicrobial activity of hydrogen peroxide is attributable to its powerful oxidising ability resulting in damage to proteins, lipids and nucleic acids. Several sterilisation systems that employ Vapour-phase hydrogen peroxide vapour (VPHP) and hydrogen peroxide plasma (HPP) technology is now commercially available and can be applied to whole room or cabinet sterilisation applications.
27. The use of VHP and HPP technology offers several advantages over traditional formaldehyde fumigation approaches:
- Hydrogen peroxide vapour is non-carcinogenic and environmentally friendly.
 - A much lower contact time is required (around 1-3 hours).
 - It is less sensitive to conditions (vapour phase temperature range is 4-80 °C).
 - Various hydrogen peroxide concentrations can be effective (0.3-12 mg/l).
28. Hydrogen peroxide decomposes when diluted into harmless by-products, oxygen and water. In the presence of metal transition ions (for example iron, copper, chromium, cobalt or manganese salts), highly toxic hydroxyl radical is generated, which is believed to be the strongest known oxidising agent. The microorganisms targeted for destruction provide these ions and therefore hydrogen peroxide vapour is only toxic at the specific point of action. HPP systems use various methods to generate plasma (for example direct current, radio- and microwaves) from hydrogen peroxide gas, this is thought to result in the generation of biocidal hydroxyl radicals and UV light without the need of transition ions.
29. Since hydrogen peroxide decomposes into harmless by-products, these systems should be considered to be a much safer alternative to the use of formaldehyde vapour.

However, like formaldehyde, hydrogen peroxide itself is also a hazardous chemical (see Table 3.6.1). Exposure to the vapour must be controlled and the laboratory must be sealed during fumigation.

30. Concentrated solutions of hydrogen peroxide are irritating to the skin, eyes and mucous membranes. Furthermore, VPHP can cause inflammation of the respiratory tract. Exposure to these substances is usually minimised through the use of cassette-loading systems when using commercially available equipment. However, gloves must be worn and rooms/cabinets will still require sealing during the application.

31. Currently, effective hydrogen peroxide fumigation systems are only available commercially. However, given the advantages over formaldehyde, their use may represent a safer option.

	WORKPLACE EXPOSURE LIMIT	
	Long-term exposure limit (8-hour reference period)	Short-term exposure limit (15-minute reference period)
FORMALDEHYDE VAPOUR	2 ppm 2.5 mg.m ⁻³	2 ppm 2.5 mg.m ⁻³
HYDROGEN PEROXIDE VAPOUR	1 ppm 1.4 mg.m ⁻³	2 ppm 2.8 mg.m ⁻³

Table 3.6.1: Workplace exposure limits for formaldehyde and hydrogen peroxide vapour.

Long-term exposure is an 8 hour time-weighted average exposure limit.

3.7 Microbiological safety cabinets

Overview

1. The following guidance provides a brief summary of the essentials of the design and function of Microbiological Safety Cabinets (MSCs) and offers practical recommendations for their safe use and operation. For full descriptions of the different types of MSC, reference should be made to British Standards BS EN 12469: 2000 and BS 5726: 2005. These define specifications for design, construction and minimum performance criteria. They also specify the test procedures that are required with respect to protecting the worker and cover aspects of installation and environmental factors that affect performance. For older equipment, reference should be made to BS 5726: 1992.
2. An MSC is a ventilated enclosure intended to offer protection to the user and the environment from infected and hazardous biological materials. They use a combination of airflow and filtration to contain airborne droplets and particles generated during handling, thus preventing their escape and exposure of workers and the local environment. All exhaust air discharged to atmosphere from an MSC is HEPA filtered. The use of an MSC is a regulatory requirement for laboratory activities involving genetically modified microorganisms (GMMs) at Containment Levels 3 and 4 and they are often required at lower containment levels, if not to protect the worker and environment, then to maintain sterility of the materials themselves. It should be remembered, however, that MSCs can only supplement containment afforded by the room/building and procedures in place that minimise exposure. It is not only vital that MSCs are correctly installed, but that attention is paid to the arrangement of the room and facilities present to ensure that all aspects of the MSC operation and maintenance are accounted for. Furthermore an MSC's performance can be affected by incorrect operation and users should be appropriately trained.

Choosing the correct equipment

3. MSCs vary in design, but they fall into three different classifications, each offering different levels of protection to workers and materials.
4. **Class I** cabinets have an aperture at the front through which the operator can carry out manipulations on potentially hazardous materials. They provide operator protection by maintaining an inward flow of air past the operator and over the work surface within. Exhaust air is HEPA filtered, although incoming air is unfiltered and, therefore, this type of cabinet is not designed to offer protection to material being handled (see Figure 3.7.1).

Hence, there is a risk that airborne organisms in the working environment will contaminate the work. Class I cabinets are appropriate for use with GMMs in activity classes 1 to 3.

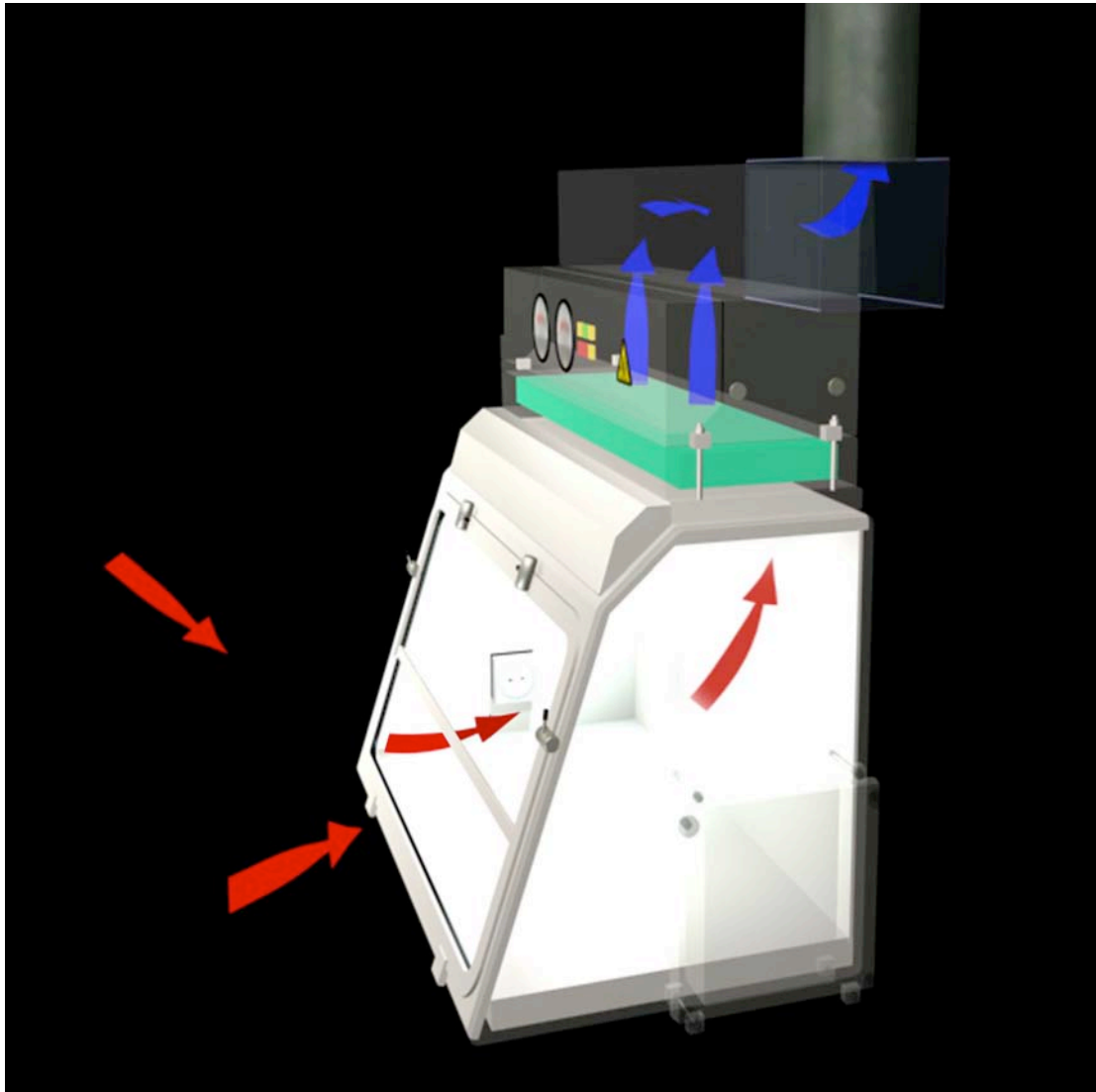


Figure 3.7.1 A class I microbiological safety cabinet. Coloured arrows indicate airflow – red arrows denote 'dirty', unfiltered air; blue arrows denote 'clean', filtered air.

5. **Class II** cabinets have an aperture at the front through which the operator can carry out manipulations on potentially hazardous materials. They provide protection to both the operator and the materials being handled as the inward airflow is diverted beneath the work surface and is HEPA filtered prior to circulation within the work area (see Figure 3.7.2). The downward airflow onto the work surface also minimises the possibility of cross-contamination within the cabinet. Exhaust air is HEPA filtered and this sort of cabinet is suitable for work with all GMMs, except those that are activity class 4 (unless derogation is successfully obtained from the competent authority).

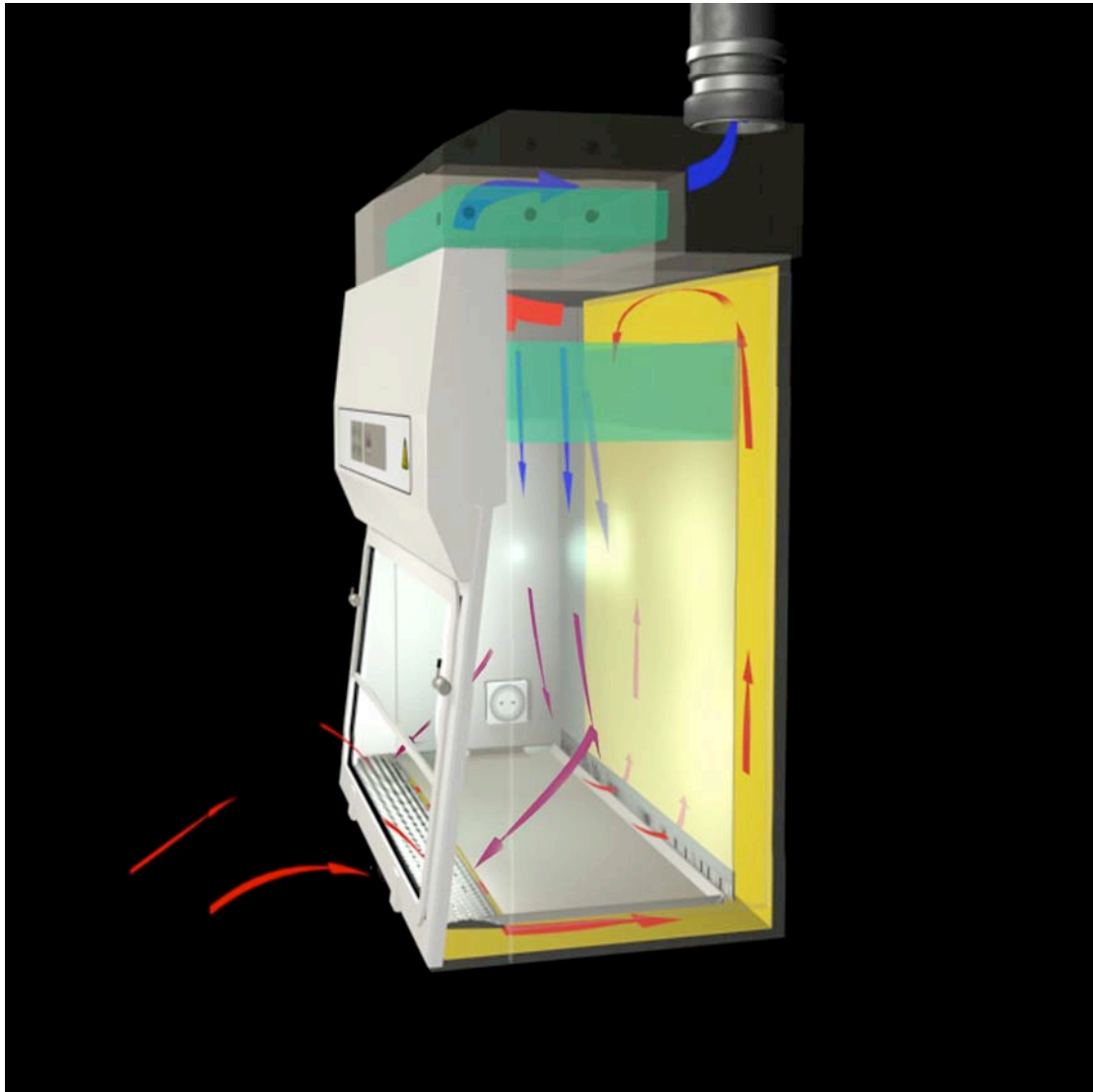


Figure 3.7.2 A class II microbiological safety cabinet. Coloured arrows indicate airflow – red arrows denote 'dirty', unfiltered air; blue arrows denote 'clean', filtered air.

Class I or Class II? Modern Class II MSCs will offer similar operator protection to a Class I MSC. However, Class I MSCs are less affected by external factors and internal flow rates than Class II. Users should consider the needs of the work and be aware of the limitations of the equipment before selection.

6. ***Class III*** cabinets are totally enclosed, providing maximum protection for the operator, the work and the environment. All inward and exhaust air is HEPA filtered and access to the work area is provided via the use of full arm-length gloves that are sealed to ports in the front of the cabinet (see Figure 3.7.3). Use of Class III cabinets is usually restricted to work with hazard group 4 biological agents or GM activity class 4 GMMS, but they may also be appropriate for work where there is a risk of generating large aerosols or where there is uncertainty as to the route of transmission of a highly hazardous virus, for example, following modification of viral tropism.

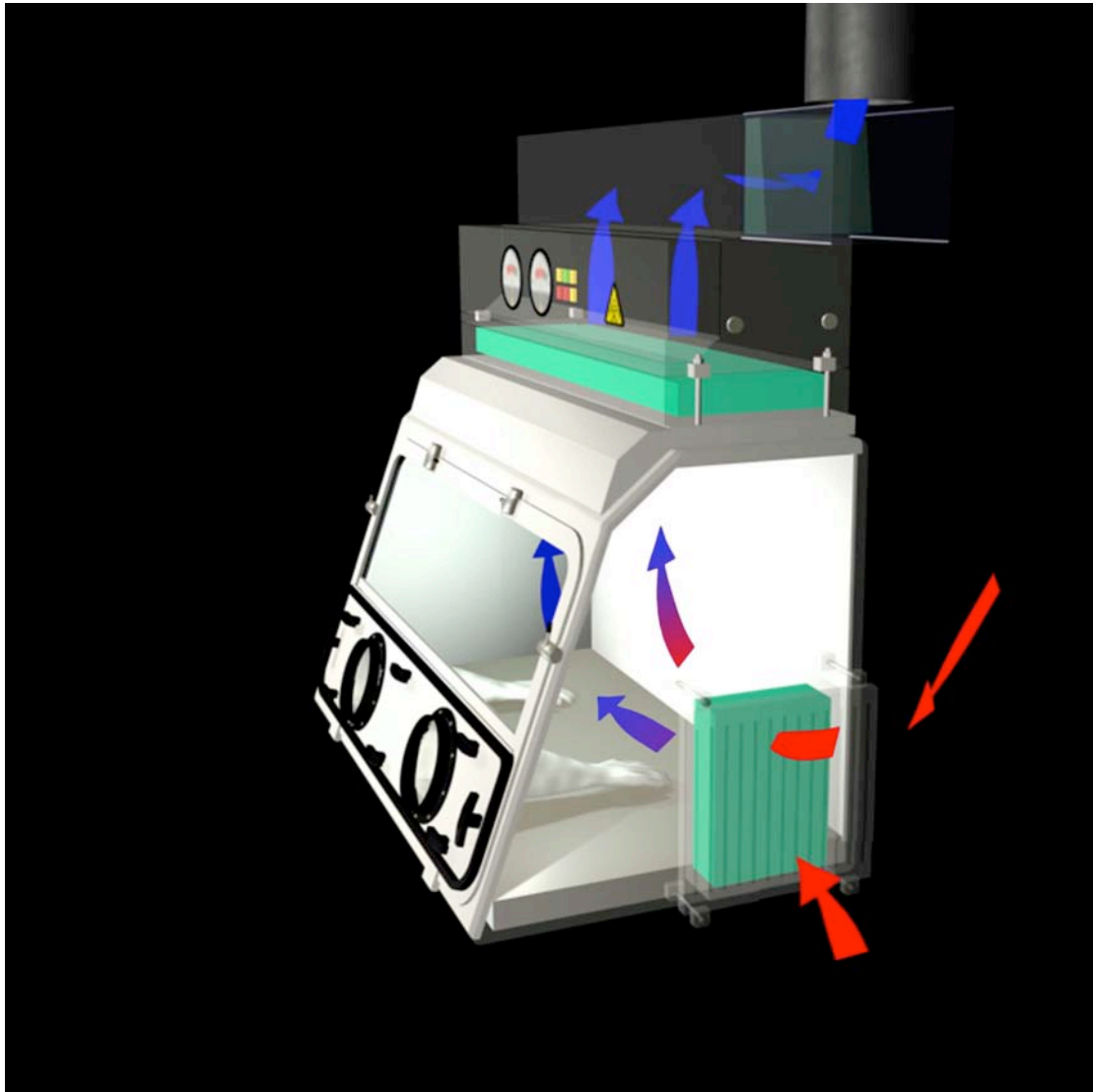


Figure 3.7.3 A class III microbiological safety cabinet. Coloured arrows indicate airflow – red arrows denote 'dirty', unfiltered air; blue arrows denote 'clean', filtered air.

Laminar flow cabinets or 'clean air' systems are NOT MSCs and should never be used when handling potentially infectious materials. These units are designed to deliver a stream of HEPA filtered 'clean' air over a working surface so as to prevent sterile materials such as culture media or drug preparations becoming contaminated. Any airborne droplets are actively directed at the operator and therefore the use of laminar flow cabinets with any material that is potentially infectious for humans is hazardous.

Installation

7. The effectiveness of an MSC will depend upon (1) its design; (2) correct use/maintenance and (3) suitable installation. All newly purchased and supplied cabinets should be compliant with the standards set by BS EN 12469: 2000 and BS 5726: 2005 for design,

construction and installation. Older cabinets should comply with the standards set by BS 5726: 1992. It is important to choose a cabinet that is appropriate for the work. Class I and II cabinets should not be used for class 4 GMMs unless the competent authority agrees derogation.

8. Under normal operational circumstances, it is considered good practice for filtered exhaust air to be discharged to the atmosphere via a dedicated extraction system. This is not always reasonably practicable or feasible and the recirculation of discharge air back into the laboratory can be considered. If being used in recirculation mode at Containment Level 3, a double HEPA filter should be used. It is also important in these cases to consider factors such as cabinet fumigation and how the fumigant is to be safely extracted from the room. For instance, temporary ducting could be used to extract fumigant to an air outlet or vent. Alternatively, the fumigant could be neutralised inside the sealed cabinet. For example, formaldehyde fumigant can be neutralised using ammonia vapour prior to recirculating the contents of the cabinet back into the room. This will usually require charcoal filtration of the exhaust air to scrub any excess neutraliser. Complete fumigation/neutralisation systems are available commercially (see Section 3.6).
9. It is important to consider the optimum position of the cabinet within the room, taking into account any factors that may affect performance (the installer of a newly purchased MSC should discuss these issues with the customer). Factors to be considered are the proximity of the MSC to doors, windows ventilation ducts and personnel movement routes. Standards relating to installation and positioning of an MSC are set out in BS 5726: 2005 and BS 5726: 1992.
10. Once installed, commissioning tests should be conducted to verify the performance of the cabinet *in situ*. This includes verification of the operator protection factor (OPF - this is referred to in the standard as the aperture protection factor). which is related to the inward airflow through the front aperture of a Class I or Class II cabinet and is defined in BS EN 12469: 2000. The importance of these tests cannot be over-emphasised as they demonstrate the cabinet's performance and the level of protection in practice rather than on paper. Commissioning tests should also be performed if the MSC is moved within the room, moved to a new location or where changes have been made to the laboratory that may impact upon factors that affect the cabinet's performance.

Factors affecting MSC performance

11. The OPF should be verified at installation and thereafter on a regular basis (see Table 3.7.1). The inward airflow, and therefore the level of protection afforded by a Class I or Class II MSC, can be disturbed by a number of factors. These include:

- Sudden movement at the aperture, eg sudden movements of operator's arms.
 - Turbulence generated by air movement around items on the work-surface.
 - Movement of people in the vicinity of the cabinet.
 - Changes in pressure or air movements in the room, eg opening doors or switching on another cabinet.
12. Modern Class II MSCs built to the correct standards will offer a similar level of operator protection as a Class I MSC. Tests have shown, however, that Class I MSCs are less affected by external factors and internal flow rates than Class II. Centrifuges should not be placed inside the cabinet, unless 'in use' tests show that the overall protection factor is unaffected. Similarly, the use of bunsen burners is not recommended as localised heat sources may affect airflow patterns and should only be used following verification from an 'in use test'. If used, they should be placed toward the back of the work surface.

'In use' operator protection factor testing

13. To test containment afforded by a Class I or Class II MSC *in situ*, it may be necessary to carry out 'in use' operator protection factor tests. This may be particularly appropriate when working with GM activity class 3 GMMs, particularly if there are local factors that may affect the cabinet's performance. The tests should be performed under conditions that are as representative as possible to the working conditions expected.
14. The ventilation system, laboratory equipment and working practices should be normal. The laboratory should not be modified for the tests. It may be that two or more scenarios should be tested where use of the room will vary.

Maintenance

15. Fumigation and decontamination procedures (see Section 3.6; Section 3.5) should be in place. Where the unit has been used to handle an organism that may be hazardous to human health, full decontamination should take place before engineers carry out maintenance. Recommendations as to the type and frequency of the tests can be found in Table 3.7.1. It should be noted that MSCs are considered to be Local Exhaust Ventilation (LEV) systems under the COSHH Regulations and are subject to a statutory requirement for maintenance and testing annually.

Training

16. All users of MSCs should be fully instructed on the correct and safe use of an MSC and how to decontaminate them after use. It is also important that users understand the principles of airflow and how the cabinet affords them and their work protection in order that they can operate safely within and around them (see Figure 3.7.1, Figure 3.7.2 and Figure 3.7.3).

TEST	TYPE OF TEST	CLASS I	CLASS II	CLASS III
Installation testing	Manufacturers specification check; volumetric airflow rate; airflow patterns; OPF test (optional); 'In use' OPF test (optional)			
Routine maintenance	OPF test*	Annually		NA
	'In use' OPF test*	As required		NA
	Alarms and indicators check	Every use		
	Face velocity/inflow	Monthly		
	Inflow/downflow	NA	CL2: annually; CL3: every six months	CL3&4 every six months

Table 3.7.1 Type and frequency of tests for MSCs. OPF – Operator Protection Factor (equivalent to aperture protection factor); CL – Containment Level; NA – not applicable *OPF test is normally Potassium iodide (KI) discus or microbiological test

3.8 Containment and control measures for work with DNA encoding oncogenic sequences

Overview

1. DNA sequences are regarded as oncogenic if they are able to make cells tumorigenic. Potentially oncogenic sequences, particularly where they are handled as preparations of naked DNA or in viral vectors with a human host range, may be carcinogens as defined under the COSHH Regulations. Further guidance relating to oncogenic sequences vectored by GMMs can be found in Part 2, Sections 2.2 and 2.3. The guidance given here should not preclude assignment of a particular experiment to a higher standard of containment where that is appropriate.
2. It is prudent to adopt a precautionary approach to work with potentially oncogenic sequences, irrespective of whether they are COSHH carcinogens or not. It is arguably a substance hazardous to health and the general provisions of COSHH will therefore apply. This approach is recommended in the COSHH General Approved Codes of Practice (ACOP), and is endorsed by SACGM.

Naked DNA: possible hazards and routes of transmission

3. Handling naked oncogenic DNA may involve a potential risk to the laboratory worker. Although there is no direct evidence as yet that contact with DNA can lead to tumours in humans, this possibility cannot be discounted, as evidence does exist for animals. Possible routes of transmission of naked DNA sequences to laboratory workers will primarily be inoculation or entry through broken skin. Other possible routes of transmission such as inhalation, ingestion and eye splashes may be less likely to lead to tumourigenesis.
4. Workers with unprotected skin lesions on the hands or forearms should have their suitability for work reviewed. Where a worker has active eczema, chapping or sepsis, they should consult a competent person (eg a medical professional or occupational health provider) before embarking upon, or continuing activities. The use of suitable personal protective clothing such as gloves and laboratory clothing may be sufficient to prevent exposure.
5. GMSCs should consider any risk to workers in handling oncogenes and potentially oncogenic sequences as naked DNA, particularly if linked to strong promoters or enhancer sequences that function in mammalian cells. The GMSC should be satisfied that

local rules and standard procedures give effective guidance on laboratory discipline and on avoiding accidental inoculation of workers.

Control measures

6. COSHH requires that exposure to any substance hazardous to health is prevented, or where this is not reasonably practicable, adequately controlled. In cases where the oncogenic DNA is clearly a COSHH carcinogen and it is not reasonably practicable to prevent exposure (eg by substitution with a less hazardous substance), COSHH sets out a series of control measures that must be applied.
7. Given the uncertainty about the hazards of most potentially oncogenic sequences and the small quantities used, prevention of exposure or total enclosure will rarely be 'reasonably practicable' because the costs will outweigh any benefits such as a reduction in exposure. The minimum requirements for handling such sequences should therefore include:
 - ***The wearing of protective gloves.*** Gloves should be worn for all work with naked oncogenic DNA sequences. The use of gloves should not preclude the covering of cuts by suitable dressings.
 - ***Avoiding the use of sharps.*** Sharps should not be used for work with naked oncogenic DNA, unless it is essential (eg for animal inoculation). Glassware should not be used where plastic alternatives exist.
8. All experimental procedures involving naked oncogenic DNA should be performed so as to minimise aerosol production. Procedures likely to generate aerosols such as the use of blenders, sonicators, vigorous shaking and mixing etc, must be conducted under effective containment. This might include the containment of the equipment in a sealed box or the use of a microbiological safety cabinet. The suitability of such systems should be decided after a risk assessment as required under the COSHH Regulations, although the control measures implemented must not increase risk in other workplaces or the environment.

Further information

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This document contains notes on good practice which are not compulsory but which you may find helpful in considering what you need to do.

This document is available web only at: www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp

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