UNIVERSITY OF SUSSEX

SPG-29-09

GENETIC MODIFICATION

Safety procedures and guidance for the implementation of

Genetically Modified Organisms (Contained Use) Regulations 2000 (GMO(CU))
(Ctrl + click on title above to open hyperlink)
and the ammendments
Genetically Modified Organisms (Contained Use) (Amendment) Regulations 2002
(Ctrl + click on title above to open hyperlink)
Genetically Modified Organisms (Contained Use) (Amendment) Regulations 2005.
Guidance on the 2005 regulations
(Ctrl + click on title above to open hyperlink)

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Uploaded to the University Safety Office website June 2010

1. INTRODUCTION

This document provides guidance on the contained use of genetically modified organisms (GMOs) at the University of Sussex. For further advice contact the University Health and Safety office.

Abbreviations

ACGM	-	Advisory Committee on Genetic Modification
BIOL GMSC	-	Biological and Genetic Modification Sub-Committee
COSHH	-	Control of Substances Hazardous to Health Regulations
GM	-	Genetic Modification/Genetically Modified
GMM	-	Genetically Modified Micro-organism
GMO	-	Genetically Modified Organism
HSE	-	Health and Safety Executive
SBSO	-	School Biological Safety Officer
TSM	-	Technical Services Manager
UBSO	-	University of Sussex Biological Safety Officer

The Genetically Modified Organisms (Contained Use) Regulations 2000 replaced earlier legislation in this field, and were brought into force in the United Kingdom to enact the European Community Directive 98/81/EC of 26 October 1998 which amended Directive 90/219/EEC on the contained use of genetically modified organisms. These regulations place a number of statutory duties on employers in relation to both human health and environmental safety. The full details can be found in the regulations and guidance but the main duties can be summarised as follows:

- To undertake a risk assessment covering both human health and safety and environmental safety. Whoever undertakes a risk assessment shall appoint a genetic modification safety committee to advise them.
- To ensure that adequate containment facilities and procedures are in place to control any risks to workers and the environment.
- To maintain and test containment equipment at appropriate intervals and where necessary to monitor for the presence of viable process organisms outside of containment.
- To provide adequate training commensurate with the level of risk.
- To formulate and implement local rules.
- To formulate and implement emergency plans and procedures.

Other regulations, the **Genetically Modified Organisms (Deliberate Release) Regulations 1992** as amended by the **Genetically Modified Organisms (Deliberate Release) Regulations 1995** (made under the Environmental Protection Act 1990) covers the deliberate release of genetically modified organisms (GMOs) into the environment. These regulations require that anyone intending to release or market a GMO has first to obtain consent through the Department of the Environment, Transport and the Regions.

If you have any plans to deliberately release GMOs then contact Director of Health and Safety, Hastings Building (x8376) or SBSO at earliest opportunity.

Definitions

"genetic modification" in relation to an organism means the altering of the genetic material in that organism by a way that does not occur naturally by mating or natural recombination or both. Examples given in Appendix 1 illustrate techniques which are considered to result in genetic modification and those which are not.

"micro-organism" means a microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, and includes a virus, a viroid and an animal or plant cell in culture.

"organism" means a biological entity capable of replication or of transferring genetic material and includes a micro-organism, but does not include a human or a human embryo.

"contained use" means any activity in which organisms are genetically modified or in which GMOs are cultured, stored, used, transported, destroyed or disposed of and where barriers are used to limit contact of the GMOs with humans and the environment.

Advisory Committee on Genetic Modification (ACGM)
Set up by the Health and Safety Executive (HSE), the ACGM publishes advice and guidance on all aspects of genetic modification. The ACGM has a World Wide Web site at http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp

The ACGM Compendium of Guidance (Ref. 1) gives detailed information on risk assessment for genetic modification activities, and is a key document which must be referred to before filling in any GM risk assessment form. It is available from HSE Books or can be viewed on the World Wide Web at http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp.

HSE inspectors have powers under the Health & Safety at Work Act 1974 to inspect facilities, issue Statutory Notices or, in appropriate cases, bring prosecutions for breaches of the Genetic Modification regulations or the Health and Safety at Work Act.

Local Biological and Genetic Modification Sub-Committee (BIOL GMSC)
The Genetically Modified Organisms (Contained Use) Regulations 2000 place a statutory obligation on anyone carrying out an activity involving genetic modification to establish a genetic modification safety committee. Within the University, GM work is monitored by the BIOL GMSC, a local Sub-Committee of the University Safety Management Group which is chaired by the University Biological Safety Officer (UBSO). The Sub-Committee's primary role is to review all experimental proposals, and to advise on risk assessment. Secondary roles include the discussion of safety, training and laboratory discipline. The overall objective is to ensure that the University complies in all respects with national GM rules and good practice. **New projects can only commence after approval by the Sub-Committee.**

University Biological Safety Officers

The University must appoint a **University Biological Safety Officer (UBSO)** who will usually be a member of academic faculty or technical staff who have experience and competence in the area of biological safety. The UBSO's duties are to act as adviser to the University in all matters relating to the containment of genetically modified organisms and the safety of staff as described in guidance published from time to time by the Advisory Committee on Genetic Modification. In addition the UBSO will attend meetings of the University Safety Committee and will chair the Biological and Genetic Modification Sub-Committee. The UBSO keeps records of projects, provides information and, in general, acts as a focal point and co-ordinator for the administrative aspects of GM work at the University of Sussex and point of communication to Senior Management and external agencies. (**Duties are defined in Appendix 2A)**

School Biological Safety Officers (SBSOs)
Are appointed in each school where GM work is being undertaken. The SBSO will be a member of the BIOL GMSC **(Duties are defined in Appendix 2C).**

2. CATEGORISATION AND NOTIFICATION OF PROJECTS

- **Applications to carry out work involving GMOs** should be submitted for evaluation on the proposal/risk assessment form. Forms should be used which are most relevant to the study organism. http://www.sussex.ac.uk/hso/1-2-4-1.html. If the category is not clear then consult the BSO for advice.
- **NO GM WORK MAY START BEFORE APPROVAL IS RECEIVED**
- The proposer should consult the ACGM Compendium of Guidance to help with filling out the form http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/
- The proposer is responsible for the contents of the application form/ risk assessment (consult your SBSO for advice).
- Incomplete forms will be returned to the proposer for completion.
- The PI should keep a copy of the risk assessment and should review it as necessary. Regulations require that these are kept for at least 10 years after work on the project ceases.

Notification Requirements
Notification is required for work involving construction of genetically modified organisms, or storage, use, transportation, destruction or disposal of genetically modified organisms under conditions of containment. Depending on the nature of the project, notification may be:

Local: A completed University of Sussex GM project application/risk assessment form is submitted to the BIOL GMSC for approval. The project may commence when the proposer has been notifed of approval by the Sub-Committee. The UBSO submits an annual return of the total number of such projects to the HSE.

OR

To HSE: The individual notification is submitted in advance to the HSE.

In both cases the applications are first forwarded to the UBSO prior to consideration by the GMSC. The UBSO will be responsible for the forwarding of notifications to the HSE.

Risk assessment and Classification of Genetically Modified Organisms and Genetic Modificiation Activities
Classification of GMOs is based on risk assessment; this determines the containment measures required to control the identified risks. There are four tables of containment measures, for laboratories, plant growth facilities, animal units and other premises. These are clearly explained in the ACGM Compendium of Guidance. The containment measures required decide the classification of the activity and it is this classification which determines notification requirements. ***[The former classification of GMMs into Group I or II organisms used in Type A or B activities no longer applies].***

The classes of contained use activity are

Class 1. Activities of no or negligible risk, for which **containment level 1** is appropriate to protect human health and the environment.

Class 2. Activities of low risk, for which **containment level 2** is appropriate to protect human health and the environment.

Class 3. Activities of moderate risk, for which **containment level 3** is appropriate to protect human health and the environment.

Class 4. Activities of high risk, for which **containment level 4** is appropriate to protect human health and the environment.

Note that the final classification is of the *activity*, not the organism.

For activities involving GMOs that are not micro-organisms (ie animals and plants) there are no standard containment levels. Such activities are simply classified as 'notofiable' or non-notifiable ' according to the nature of the GMO itself.

Notifiable = Activity involving a GM organism which poses a *greater risk* to human health than its unmodified parental organism.

Non-notifiable = Activity involving a GM organism which poses *no greater risk* to human health than its unmodified parental organism.

Application and Approval Procedure
For all GM experiments, a written protocol in typescript, accompanied by risk assessments for human health and safety and environmental safety, must be submitted to the SBSO on the officially approved form, which is available from the following link http://www.sussex.ac.uk/hso/1-2-4-1.html. For Class 1 activities and those involving the use of GMOs as safe as their parents in containment, local approval by the BIOL GMSC will suffice. In all other cases, in addition to local approval it will be necessary to notify the HSE in advance, using the appropriate HSE form which can be obtained from the UBSO; a fee is payable to the HSE which will be charged to the PI. Notification to the HSE must be made through the UBSO and the BIOL GMSC.

Connected Programmes
A single proposal can be made for a connected programme of work covering more than one activity. Work covered by such a proposal must form a coherent and

integrated programme and must not be comprised of a collection of unrelated projects that happen to be carried out by a single department or research group. A connected programme notification has the advantage of minimising the number of notifications to be made thereby saving time and, in the case of notifications to HSE, money. Note that the same level of detail about the individual activities should be included as would be the case if they were being notified separately.

Requirement for risk assessment
The proposal form used at the University of Sussex, correctly completed, fulfils the requirements of all the relevant regulations.

Work with disabled *E. coli* and other attenuated hosts is generally assessed as low risk, provided that no hazardous inserts such as bacterial toxins are being cloned. In recognition of this, the University of Sussex risk assessment form allows the project proposer to assign work with non-hazardous hosts and inserts to Class 1 without in-depth assessment.

Transport of GMMs is subject to the requirements for risk assessment. This will need to take particular account of risks associated with transport itself. Key aspects are appropriate packaging and labelling, the supply of information where appropriate to the person transporting the micro-organisms, and consideration of action to be taken in the event of a release to the environment.

Other genetic modification activities
If any GM work is planned which falls outside the scope of this guidance, for example with GM plant pathogens or with animals infected with GMMs, consult the UBSO who will advise on risk assessment and notification requirements.

3. EMERGENCY PLANS

The Regulations state that: " Where the assessment made for the purposes of regulation 6(1) or 7(1) shows that as a result of any reasonably foreseeable accident –

(a) the health or safety of persons outside the premises in which an activity involving genetic modification is carried on is liable to be seriously affected; or
(b) there is a risk of serious damage to the environment,

the person undertaking that activity shall ensure that, before the activity to which the assessment relates begins, a suitable emergency plan is prepared with a view to securing the health and safety of those persons and the protection of the environment." If this applies to any work proposed, the proposer must append an emergency plan to the risk assessment when the project proposal is submitted.

4. WASTE DISPOSAL

The Contained Use Regulations require that waste containing GMMs must be inactivated **by validated means** before disposal. Under exceptional circumstances, the Competent Authority (HSE, the Secretary of State and the MAFF Minister) will agree to derogation from this requirement, but would require considerable supporting data to justify such a request. Inactivation is defined 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". The degree of inactivation required will therefore vary depending on the nature of the organisms being used.

Validation

"Validation" essentially means that users must confirm or corroborate that the chosen method will work under the conditions of use. Validation of a disinfection procedure requires knowledge of the concentration and contact time required, and confirmation that the chemical agent will act in a satisfactory manner in the exact conditions of use (e.g. in the presence of proteins.) It may be possible to rely on data provided by the manufacturer of the disinfectant, provided that the conditions under which the disinfection is used correspond to those applied during the manufacturer's validation. If in-house validation is needed, measurements should be undertaken to quantify the efficacy of the procedures being used.

Validation studies undertaken for inclusion with risk assessments should use the parental or host micro-organism; once the study is under way the procedure should be checked using the actual GMM. A short report of the validation study, with supporting operating and analytical records, will give documentary evidence that the inactivation procedure is effective and reproducible. The report should indicate the percentage kill or log reduction in viability. If viability is reduced to below detectable limits, the limit of detection should be stated.

When there is difficulty in validating chemical inactivation, consideration should be given to the use of autoclaving as an alternative. It is well established that a cycle of 121ºC for 15 minutes, with full steam penetration throughout the load, is sufficient to render most materials sterile. Validation of this method will rely on the annual testing of the autoclave to demonstrate, using independent thermocouples placed throughout a typical load, that the correct temperature and pressure have been reached for the required time. Verification that the correct conditions are reached during each run should be obtained by the use of, for example, chart recorders or appropriate indicators autoclaved with the load.

If liquid waste is autoclaved, it is advisable to disinfect bulk liquid that is awaiting autoclaving. Under these circumstances, the use of disinfectant is a precautionary measure rather than the primary means of waste treatment, and as such does not need to be validated. Care should be taken when autoclaving materials that have been treated with disinfectants, as the high temperature can cause release of toxic or harmful substances (e.g. chlorine from hypochlorite disinfectants.)

The efficacy of waste treatment procedures should be monitored.

Risk assessments must include the identification of appropriate containment and control measures; control measures must include in all cases include the use of validated inactivation methods for the treatment of waste. On the GM project proposal form, the method of inactivation and the degree of kill that will be achieved should be stated. If validated autoclaving and incineration are being used, it is reasonable to state in the risk assessment that 100% kill will be achieved. For chemical disinfectants, manufacturers' data can be given; where these are not applicable, results of a validation study should be supplied.

5. WORK WITH CELL LINES

http://www.hse.gov.uk/biosafety/biologagents.pdf

When planning genetic modification work involving cell lines, two risk assessments should be undertaken in parallel. The GM risk assessment should focus only on the hazards associated with the cells and their modifications, and the COSHH assessment should then take into account the possibility that adventitious agents might be present. The ACGM Compendium gives guidance on selection of containment level for cell culture work based on the GM risk assessment. For low-hazard cell culture work, Containment Level 1 is deemed to be acceptable. Such work can therefore be classified as Activity Class 1. However, cell culture work is routinely carried out at ACDP Containment Level 2. Most users always use a microbiological safety cabinet and restrict access to cell culture facilities to protect the cells from contamination. This is a separate issue from the containment required to protect human health and the environment from the risks associated with the GMM, which forms the basis of classification and notification requirements under the Contained Use Regulations and, therefore, it is not necessary to classify a project as Activity Class 2 on this basis. If a higher level of containment is being applied than the GM risk assessment indicates, then this should be explained in the project application.

6. WORK WITH ONCOGENIC NUCLEIC ACID SEQUENCES

There is no precise definition of an oncogene, but DNA sequences are regarded as oncogenic if they are able to make cells tumorigenic. Oncogenes can be identified by induction of a growth advantage in cultured calls, however such phenotypes are not always associated with tumorigenicity. Growth advantages include growth at confluence, focus formation, growth in low serum medium, growth in suspension and immortalisation.

Oncogenes and COSHH

Potentially oncogenic sequences may be carcinogens as defined under the COSHH Regulations and the requirements detailed in the Carcinogens ACoP must be met, e.g. prevention or control of exposure and staff training. The nature of the hazard depends on the gene, control sequences and how it is handled e.g. as naked DNA, in a bacterial host or in a eukaryotic virus. Compliance with the Contained Use Regulations and the Biological Agents provisions of COSHH will satisfy the carcinogens requirements under COSHH.

Health surveillance

If an oncogene is considered to be a carcinogen under COSHH, particularly if handled as naked DNA or in viral vectors with a human host range, special health surveillance may be required. The collection, maintenance and review of health records will always be required. The details to be kept in the health record are in the appendix to the General COSHH ACoP. The project application/risk assessment forms, provide a historical record of occupational exposure. It is therefore important that all new staff working on GM projects are added to the documentation and that copies of the amended forms are forwarded to the SBSO and the UBSO. It is also important that staff are removed when they have finished working on the project.

Guidance on using oncogenic sequences

Work on oncogenes requires special consideration. The introduction of one oncogene into a small number of cells is unlikely to cause cancer as this represents

only one of the steps involved in tumorigenesis; the risks to human health from cloned oncogenes cannot be generalised. Cloning of oncogenic sequences in prokaryotic or lower eukaryotic cells often results in a GMM that does not express a harmful product. Such a GMM may represent a low risk to human health and safety, however the possibility of the oncogene being transferred to other cells where it could have a harmful effect should be considered. Potentially harmful DNA sequences should whenever possible be cloned using especially disabled or disabled hosts and poorly mobilisable vectors. This also applies to cloning eukaryotic virus genomic DNA. Oncogenes that induce tumorigenicity only in immortalised cell lines, and those that give a growth advantage to cells without inducing tumorigenicity, are generally low risk. Oncogenes that immortalise primary cell lines are higher risk. Further guidance on oncogenes can be found in Part 2A, Annex III of the ACGM Compendium (ref.1.) Almost any gene encoding a protein involved in cell-to-cell or intracellular signalling, interaction with the environment, cell cycle control, differentiation or programmed cell death (apoptosis) could be regarded as potentially oncogenic in some circumstances (e.g. if expressed constitutively at high levels.) For example, expression of a growth factor gene can allow proliferation of cells which otherwise would not grow in culture. If there is any doubt as to whether a particular sequence could be oncogenic, the UBSO should be consulted and will obtain specialist advice from experts and the HSE. Combinations of oncogenes should be treated with particular caution. Sequences that inactivate tumour suppressor genes may also co-operate with oncogenes.

Naked oncogenic DNA

Oncogenic DNA sequences, whether or not they can be classified as carcinogens by definition, should be regarded as substances hazardous to health and are subject to the COSHH Regulations. The most likely route for transmission of naked DNA sequences to workers is injection or entry through broken skin. Results from experiments on DNA immunisation have demonstrated gene expression from injected naked DNA. Workers with unprotected skin lesions on the hands or forearms, such as active eczema, chapping or sepsis, should not work with oncogenic DNA. The risk from oncogenic sequences is higher if the sequence is linked to strong promoters or enhancer sequences that function in mammalian cells. The risk assessment should therefore take this into consideration.

The following control measures are recommended in the ACGM Compendium:

a) All designated workers should be trained in good laboratory techniques before starting work with oncogenic DNA. They must be made fully aware of the potential hazards.

b) Access to the laboratory where naked oncogenic DNA is handled should be restricted to authorised personnel.

c) Laboratory bench space should be designated for work with oncogenic DNA, and the local rules for such work must be followed in the designated area.

d) Gloves (chosen for resistance to any chemicals in use) should always be worn when handling oncogenic DNA, and should not then be used elsewhere. They should be examined for punctures and changed regularly and disposed of carefully.

e) Sharps must be avoided except where absolutely essential, for instance for animal inoculation. Plasticware should be used instead of glassware if possible.

f) Aerosol production should be minimised. Use of blenders, sonicators, vigorous shaking and mixing, and so on must be conducted using suitable local exhaust ventilation systems, or in equipment designed to contain the aerosol.

g) Where there may be an additional microbiological hazard, a microbiological safety cabinet should be used (see Appendix 4).

7. WORK WITH EUKARYOTIC VIRAL VECTORS

The risk assessment forms should be used for proposing work using viral vectors. The procedure is the same as that described in section 2. As with any other GM risk assessment, it is necessary to consider risks posed by the viral vector, the insert and the resultant GMM. Particular care must be given to the assessment of vectors with an actual or potential ability to infect humans or human cells. Whenever possible, use of a vector without a human host range should be considered. For genetic modification work using viruses with a human host range, there is a general requirement under COSHH to use disabled or attenuated viral vectors. When considering the level of hazard, the hazard group of the wild-type virus as defined by the Advisory Committee on Dangerous Pathogens (see ref. 8) should be taken as a starting point. The effects of disabling mutations and the properties of the insert should then be considered, as should the possibility of rare events such as recombination and reversion events leading to the production of replication competent viruses. Having considered theoretical scenarios, it will be necessary to evaluate the likelihood that the GMM virus could *actually* cause harm to human health; in some cases this may be judged to be extremely low. When in doubt the advice of the UBSO should be sought and, if necessary, they will ask advice from a specialist HSE inspector or other expert.

Viral vectors which are disabled or attenuated derivatives of human pathogens may be placed into a provisional class lower than the hazard group of the parental virus. For example, well-characterised replication defective vectors such as E1a-deleted adenovirus can be considered as “unlikely to cause disease” for the purpose of re-categorisation into a biological agent hazard group. Hence a provisional class of 1 would be appropriate even though wild-type adenovirus falls into Hazard Group 2. Similarly, replication-defective ecotropic non-primate retroviral vectors containing an insert unlikely to be harmful in the target species can be handled at ACGM level 1 containment. Experiments using viral vectors that do not normally infect human cells in culture and for which there is no evidence of human infection are considered to represent a minimal risk to the operator and can be conducted as Activity Class 1, unless a higher standard of containment is indicated as a result of a potential for harm to other species (this will be determined by the environmental risk assessment.) Experiments which involve DNA (or RNA) vectors derived from viruses and cells in culture as hosts (even if the cells contain viral sequences) and in which no infective virus is involved or can be produced, are considered to represent minimal hazard and can be carried out as Activity Class 1**.**

Particular attention should be paid to experimental procedures that might activate an endogenous or latent virus capable of acting as a helper. To minimise the risk of accidental colonisation with infected cell lines, users should not infect cultures of their own cells, or those of their immediate family or other

members of the laboratory. The final activity Class should be determined in the normal way, as described in section 3. Detailed technical guidance on risk assessment for work with specific viral vectors (such as adenoviruses, retroviruses and herpes simplex viruses) is contained in the ACGM Compendium of Guidance, Part 2B. Refer also to the section above on oncogenes (section 8.)

It should be noted that work with plant and animal pathogens cannot be classified as Class 1. These activities will therefore always need to be notified to HSE as Class 2 or above. In such cases it will be possible to ask for the competent authority's agreement not to apply some of the measures. Human pathogens are all in Hazard Groups 2-4 in any case, hence containment level 2 or above is assumed. In the case of viruses that can only infect man, the risks to the environment can be assumed to be negligible. In all other cases, hazards to the environment should be considered and the risks assessed.

Viral vectors and oncogenes / other harmful sequences
The general requirement under COSHH to use disabled or attenuated viral vectors is particularly relevant when oncogenic DNA is being inserted. The risk assessment should confirm that the virus is adequately disabled, and the possibility of reversion or complementation should be considered. It may be necessary to test for adventitious agents and recombination-competent virus. Proposers must be certain of the nature of the disabling mutation(s). The possibility of transfer of harmful sequences to related viruses should also be considered, for instance if a worker who is carrying an infection with the wild-type virus becomes exposed to the disabled virus carrying a harmful insert. The gene should be inserted into the site of a disabling mutation to reduce the likelihood that recombination events could result in the generation of replication competent virus expressing the gene. Where it is proposed to insert a potentially harmful gene into a site other than the site of a disabling mutation, full justification must be given in the risk assessment.

8. WORK WITH TRANSGENIC ANIMALS

A suitable & sufficient assessment of risks to human health & safety is required under the Contained Use Regulations (Schedule 4.) Assessment of risk to the environment is required under the Environmental Protection Act 1990 and associated regulations. The proposal form used at the University of Sussex, correctly completed, fulfils the requirements of the relevant regulations (forms GManimal and GMORAanimal.) For detailed guidance on risk assessment refer to Part 2E of the ACGM Compendium. If the GM animal is more likely to cause harm to humans than the non-modified parental organism, the activity has to be notified to HSE before commencing via the UBSO.

- The Contained Use regulations include both vertebrates and invertebrates.
- All proposed work with vertebrates and *Octopus vulgaris* requires application to the Home Office for a Project Licence in the normal way. With regard to the welfare of transgenic animals, they are not considered differently from any other laboratory or domestic species.
- These regulations do not cover deliberate releases, or work on transgenic animals that might be used for food.
- Not all cells of an animal need to be genetically modified for the work to fall within the scope of these regulations; mosaics, with only a proportion of the
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- cells carrying the modification, are considered to be GMOs. The modification does not have to involve modification of the germline.
- The breeding on of GM animals, and the crossing of a GM animal with a non-GM animal, falls within the scope of these regulations.
- Activities involving GM animals supplied by others, such as GM mice, falls within the scope of these regulations even if there is no intention to further genetically modify them.
- Some GM animals may be exempt from control under these regulations. This includes those produced by cell fusion between any eukaryotic species, as long no recombinant nucleic acid or other GMOs are involved, and "self-cloned" animals where the GMO is unlikely to cause harm to humans. The Guide to the Genetically Modified Organisms (Contained Use) Regulations 2000 (ref. 2) contains further information.
- Transport of GM animals is subject to the requirements for risk assessment.This will need to take particular account of risks associated with transport itself. Key aspects are appropriate packaging and labelling, the supply of information where appropriate to the person transporting the animals, and consideration of action to be taken in the event of a release to the environment.

Containment

Animal containment is divided into two categories, Containment A being the minimum level. Containment A can be used when the GM animals

- are incapable of surviving in the environment in the UK, **or**
- have limited ability to transfer genetic material to UK animal species, **or**
- are female farm animals which are easily recalled, e.g. transgenic sheep, **or**
- have a genetic modification that does not increase the level of risk to human health or the environment above that of the non-modified parental organism,
- **AND** have NOT been inoculated with GMMs or other pathogens.

Containment A is only suitable if the final risk assessment is "low" or "effectively zero", for example for "knockout" mice, tropical fish, or large mammals expressing pharmacologically active proteins in their milk. Containment B would be used when the GM animals

- could cause harm to humans or the environment if they escaped from the containment facility, **and** have the ability to transfer novel genetic material to UK animal species, **or**
- could establish outside of the containment facility, **or**
- have a genetic modification that increases the risk to human health or the environment above that of the non-modified parental organism, **or**
- **AND** have not been inoculated with GMMs or other pathogens.

Containment B must be used when Containment A is insufficient to reduce all risks to "low" or "effectively zero".

9. WORK WITH TRANSGENIC PLANTS

Assessment of risk to human health is required under the Contained Use Regulations 2000. Assessment of risk to the environment is required under the Environmental Protection Act 1990 and associated Regulations. Both requirements are covered by the University of Sussex GM risk assessment forms.

If the GM plant is more likely to cause harm to humans than the non-modified parental organism, the activity has to be notified to HSE before commencing (see UBSO).

- The term “plant” includes mosses and ferns as well as vascular flowering plants. It includes all vegetative and reproductive organs, including spores, seeds, pollen, bulbs, rhizomes, tubers etc., as well as undifferentiated plant tissue i.e. callus cultures.
- Plant tissue cultures are not included as they are classified as GMMs for the purposes of the Contained Use Regulations.
- Plant viruses and viral vectors are covered in part 2C of the ACGM Compendium. Proposers should consult the BSO before submitting proposals for work involving plant pathogens and viral vectors.
- Proposals for deliberate release of GM plants into the environment are outside the scope of this guidance. Consult the UBSO if any work is planned which might involve application for consent to deliberately release GM plants.
- Consumption of GM plant material is only permitted after evaluation for safety by the Advisory Committee on Novel Foods and Processes, and cleared by MAFF and Department of Health ministers.
- Work with vector systems derived from plant pests, and plant material that has been modified to contain genetic material derived from a plant pest or pathogen, are covered by the Plant Health (Great Britain) Order 1993. This work will require notification to MAFF and, if appropriate, authority from MAFF in the form of a licence.
- Plant pests that have been modified to eliminate all pathogenic sequences e.g. disarmed *Agrobacterium*, and the cauliflower mosaic *caulimovirus* 35S promoter, are exempt from the Plant Health Order.
- Transport of GM plants, e.g. from glasshouses off the main site to laboratories on the main site, is subject to the requirements for risk assessment. This will need to take particular account of risks associated with transport itself. Key aspects are appropriate packaging and labelling, the supply of information where appropriate to the person transporting the plants, and consideration of action to be taken in the event of a release to the environment.

Project proposals

When planning transgenic plant work, preparative cloning should be proposed as a GMM project and stages involving gene transfer to plants should be proposed as a GMO project. Hence both the GMM and GM plant forms will need to be completed. Where *Agrobacterium tumefaciens* is being used, the same form can be used for the *E. coli* work and the *Agrobacterium* work.

Risk assessment

Consult Part 2D of the ACGM Compendium for guidance. Possible harmful effects to consider include:

- hazards to humans or animals, such as increased allergenicity or toxicity (e.g. increase in biologically active compounds such as alkaloids);
- adverse effects on plants; ability to survive, establish or disseminate in the environment and cause adverse effects;
- adverse effects arising from natural transfer of genetic material to other organisms.

As for transgenic animal work, the risk assessment focuses on the possible risks to the environment, since transgenic plants are unlikely to pose a risk to human health

except in special circumstances (e.g. when a pharmacologically active compound is being expressed in plants.)

Environmental protection
The risk assessment form for GM plant work has an emphasis on the risk of environmental harm in the event of escape into the environment. Generally the containment will be required to prevent the dissemination of seed and pollen, and may combine physical methods and procedural controls. Survival is the key consideration. If the GM plant is not able to survive in the wider environment, it can normally be considered to be safe. Some GM plants will be capable of completing a full life cycle, in which case all of the other possible hazards listed on the risk assessment form should be considered. Some plants might have limited survivability, for example they might be able to take root and grow through the spring and summer, but may not flower, produce seed or survive the winter. Even where limited survivability is expected, the other hazards should be considered.

Containment
It is assumed that work will generally be undertaken in purpose-built plant growth facilities, i.e. greenhouses or plant growth rooms. Containment is divided into two categories, **Containment A** being the minimum level which can be used when the risk is negligible or low, **and** the GM plants or the control measures have one or more of the following features:

- the plants are incapable of surviving outdoors in the UK, **or**
- the plants have limited ability to transfer genetic material to UK plant species (this can be achieved artificially, e.g. by removal of flower buds or bagging of flowerheads to prevent pollen dispersal), **or**
- the plants were transformed using a disarmed plant pest e.g. *Agrobacterium* that was subsequently removed during the plant regeneration procedure (as long as a harmful phenotype has not been conferred on the plants.)

Containment B would be used for GM plants which:

- could transfer genetic material to UK plant species, **or**
- could establish outside the containment facility and cause harm to the environment, even if such harm is not a direct result of the genetic modification, **or**
- express plant pest-derived sequences, if the risk assessment indicates that harm could occur if there was an escape of viable material from the containment facility, **or**
- are modified to express hazardous substances (including toxic, allergenic or otherwise biologically active substances, which could cause harm to humans or the environment.)

Containment B **MUST** be used when containment A is insufficient to reduce all risks to “low” or “effectively zero”. Where a MAFF licence is required, containment measures must be agreed with MAFF. Part 3B of the ACGM Compendium sets out the A and B containment measures. Different containment levels (Plant Growth Facilities Levels 1-4) apply when plants are infected with GMMs contact UBSO for advice.

10. HEALTH SURVEILLANCE OF PERSONS INVOLVED IN GENETIC MODIFICATION

There is no specific requirement for health surveillance for GM work; the requirements are those of COSHH and the Management of Health & Safety at Work Regulations 1999 (ref. 6.) There is no longer a requirement to appoint a Supervisory Medical Officer (SMO) for the surveillance of the health of workers involved in genetic modification. Any need for health surveillance should therefore be determined on a case-by-case basis, by considering whether the genetic modification aspects of the work involve a significant risk to health and whether health surveillance is appropriate.
COSHH regulation 11 requires health surveillance where it is appropriate for the protection of the health of workers exposed to a substance hazardous to health (which includes biological agents and therefore some GMMs). Health surveillance is appropriate when:

- an identifiable health effect may be related to exposure; **and**
- there is a reasonable likelihood that the disease or effect may occur under the conditions of the work; **and**
- there are valid techniques for detecting indications of the disease or health effect.

Worker Records for every worker engaged in genetic modification are kept by the UBSO via the project risk assessment/proposal forms. Supervisors should ensure that all workers engaged in genetic modification are entered in the documentation and that amended copies are sent to the UBSO.
Evaluation of risks

- For low risk work with GMMs with no identifiable risk to human health (e.g. plant pathogens), there is unlikely to be a requirement for health surveillance. It may however be necessary to identify workers who may be at greater risk because of pre-existing illness or an underlying medical condition, such as impaired immunity.
- Where there is a risk of ill-health resulting from work exposure to a GMM and there are methods available to detect disease, some form of health surveillance may be required under COSHH or MHSWR. Local arrangements should include a health questionnaire and health record. Medical examinations, or medical contact cards are unlikely to be needed. Baseline serum samples are of little value.

Health surveillance requirements for work with pathogenic GMMs and/or oncogenic sequences will be determined by the BIOL GMSC at the notification stage.

11. REFERENCES AND FURTHER READING

1. ACGM Compendium of Guidance, Health & Safety Commission 2000. ISBN 0 7176 1763 7. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp
2. A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000, HSE 2000. ISBN 0 7176 1758 0.
3. Biotechnology: Health and safety in education. Education Service Advisory Committee, Health & Safety Commission, 1996. ISBN 0 7176 0724 0.
4. HSE Leaflet: Contained Use of Genetically Modified Organisms, IND(G)86(L). http://www.hse.gov.uk/pubns/indg86.htm
5. Control of Substances Hazardous to Health Approved Codes of Practice, Health & Safety Commission 1999. ISBN 0 7176 1670 3.
6. Management of Health & Safety at Work Regulations Approved Code of Practice & Guidance, Health & Safety Commission 2000. ISBN 0 7176 2488 9.
7. Collins, C.H. & D.A. Kennedy 1999. Laboratory-acquired infections: History, incidence, causes and preventions (4th edition.) Butterworth-Heinemann, ISBN 0 7506 4023 5
8. Categorisation of biological agents according to hazard and categories of containment, Advisory Committee on Dangerous Pathogens 1995 (4th edition.) ISBN 0 7176 1038 1.
9. Biological agents: Managing the risks in laboratories and healthcare premises http://www.hse.gov.uk/biosafety/biologagents.pdf

University of Sussex documents – available from the Safety Office Website

Biological Safety including GM Project risk assessment/proposal forms
http://www.sussex.ac.uk/hso/1-2-4-1.html

University of Sussex Safety Procedures and Guidance Documents
SPG-20-09 Safe Working with Microbiological Material
SPG-24-09 Control of Biological Agents (COSHH)
SPG-31-09 Disinfection
SPG-01-09 Local Exhaust Ventilation (LEV)
http://www.sussex.ac.uk/lifesci/1-4-1-2-1.html

Useful WWW sites

Advisory Committee on Genetic Modification
http://www.hse.gov.uk/aboutus/meetings/sacgmcu/
Advisory Committee on Releases to the Environment
http://www.detr.gov.uk/environment/acre/
Department of the Environment, Transport and the Regions
http://www.local.doe.gov.uk/struct/reorg.htm

APPENDIX 1: Definition of Genetic Modification

(Extracted from Schedule 2 of the Genetically Modified Organisms (Contained Use) Regulations 2000)

Part I - Examples of techniques constituting genetic modification Examples of the techniques which constitute genetic modification which are referred to in sub-paragraph (a) of the definition of genetic modification in regulation 2(1) are- (a) recombinant DNA techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules, produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not occur naturally but in which they are capable of continued propagation; (b) techniques involving the direct introduction into an organism of heritable genetic material prepared outside the organism, including micro-injection, macro-injection and micro-encapsulation; and (c) cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Part II - Techniques which are not considered to result in genetic modification The following techniques are not considered to result in genetic modification if they do not involve the use of genetically modified organisms made by techniques other than those listed in Part III or the use of recombinant nucleic acid molecules, namely - (a) in vitro fertilisation; (b) natural processes including conjugation, transduction or transformation; (c) polyploidy induction.

Part III - Techniques to which these Regulations do not apply These Regulations shall not apply to the following techniques of genetic modification, if they do not involve the use of recombinant-nucleic acid molecules or of genetically modified organisms other than those recombinant-nucleic acid molecules or genetically modified organisms produced by one or more of the following techniques of genetic modification - (a) mutagenesis; (b) cell fusion (including protoplast fusion) of prokaryotic species which can exchange genetic material through homologous recombination; (c) cell fusion (including protoplast fusion) of cells of any eukaryotic species, including production of hybridomas and plant cell fusions; (d) self-cloning, where the resulting organism is unlikely to cause disease to humans, animals or plants, and in this sub-paragraph, “self-cloning” means the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by re-insertion of all or part of that nucleic acid (or a synthetic equivalent), whether or not altered by enzymic or mechanical processes, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by homologous recombination, and self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular organism concerned.

APPENDIX 2: DUTIES OF PERSONELL REGARDING THE CONTROL OF GENETICALLY MODIFIED ORGANISMS

A Vice Chancellor

- Will appoint the University Biological Safety Officer (UBSO)

B UNIVERSITY BIOLOGICAL SAFETY OFFICER (UBSO)

(1) The UBSO acts as adviser to the Vice Chancellor and Heads of Schools in all matters relating to the containment of biological hazards.

(2) Has overall responsibility for ensuring that the University local rules and policy are in line with current policy and that all activity on site is compliant with current GMO(CU) regulations.

(3) Will ensure (in consultation with the Dean/Head of School or Director of research units) that a School Biological Safety Officer (SBSO) is appointed in each school or non-academic unit where work with genetically modified organisms is taking place or is planned to occur.

(4) Will chair the BIOL GMSC and appoint the membership which will include the USRPA, all appointed SBSOs and other invited experts.

(5) Is responsible for maintaining records of projects for 10 years after activity has ceased. Is responsible for submitting notifications to the HSE and Director of S of new projects and when particular GMO projects have ceased. Copies of the annual returns/reports of class I activities/projects must be sent to USRPA and the Secretary of the BIOL GMSC.

(6) Must notify the HSE, with copies to the USRPA and Dean/Head of School or Director of research unit of any accident*.

C HEAD OF SCHOOL /DIRECTOR OF A RESEARCH UNIT

(1) Has overall responsibility for all aspects of safety in the school/unit. The Head or Director has the authority to order (at any time) that any GMO project may not proceed. In taking the decision he/she would be expected to first consult with the UBSO and SBSO. In some circumstances consultaton with the HSE may be appropriate.

(2) Will appoint the School Biological Safety Officer (SBSO) in consultation with the UBSO.

D SCHOOL BIOLOGICAL SAFETY OFFICER (SBSO)

(1) Will be a member of the BIOL GMSC.

(2) Through providing relevant advice and monitoring activity will ensure that work in the school is compliant with the university policy and local rules regarding the control of genetically modified organisms.

(3) Will keep a copy of the Regulations and the ACGM Guidance Notes.

(4) Will receive and archive copies of proposals for GMO projects from the principal investigators and will evaluate the risk assessment for each project, inspect the facilities and present the proposal to the secretary of the BIOL GMSC for consideration and comment as they arise.

(5) The SBSO must keep records of all risk assessments for 10 years. Copies must be sent to the Head of School / Director of a non-University Research Unit, and to the UBSO.

(6) Will maintain records of approved and ongoing projects and will notify the UBSO and relevant Head of School/Director of any changes of activity, infringements of procedure or local rules or accidents* involving work with GMOs.

E PRINCIPAL INVESTIGATOR /SUPERVISOR/LAB HEAD

(1) Has responsibility for all aspects of laboratory safety and for ensuring that the University Local rules and policy regarding the use of GMOs is carried out.

(2) Is the responsible for the training and supervision of workers.

(3) Is responsible for the drafting and submission of project proposals/risk assessment documentation with advice from the SBSO.
(4) Is responsible for informing the SBSO of changes in workers or cessation of project.
(5) Will monitor that regular safety inspections and fumigations are carried out and that when exhaust protective cabinets and HEPA filters are part of the laboratory equipment that these are included in the programme. They should alert SBSO or TSM where there is a problem.
(6) Is responsible for the safe execution of the work in progress and ensuring the day-to-day cleanliness of the laboratory and reporting any accidents* or infringements of policy to the SBSO.

F WORKERS

(1) Have responsibility to the safety of themselves, others and the environment and must adhere to the programme of work as set out by their PI/Supervisor/Lab Head and University local rules.
(2) Will report any accidents* or infringements of local rules immediately.
(3) Will read and sign the project proposal/risk assessment form.

*****An **'accident**' is an incident involving a significant and unintended release of GMO which presents a hazard to either (or both) human health or the environment.

APPENDIX 3: Notification of Individual Genetic Modification

Activities to HSE

The University premises have been notified to HSE as a GM centre. Any queries regarding centre notification should be directed to the Safety Office. Notification of activities to the HSE must be made through the BSO and the GM Sub-Committee.

Activities involving GMMs

Class 1

- no notification to HSE or consent required – local Genetic Modification Sub-Committee approval will suffice.
- apply Level 1 containment

Class 2

- notify to HSE. Work can start on receipt of the acknowledgement, unless informed otherwise.
- apply level 2 containment

Class 3 & 4

- First use at the University of Sussex: notify to HSE. Work may not start until consent is received. This will be decided within 90 days.
- Not first use at the University of Sussex: notify to HSE. Work may not start until consent is received. This will be decided within 45 days.
- apply level 3 or 4 containment.

The notifier can seek permission from the competent authority to omit certain containment measures if this is justified by risk assessment. Application must be made at the time of notification.

Animals and plants

- If the GMO has no greater potential to cause harm to humans than the equivalent non-modified organism, no notification to HSE required.
- If the GMO does have greater potential to cause harm, notify to HSE. Work can start 45 days after notification is acknowledged, unless informed otherwise. Apply appropriate containment for human protection.

Guidance on notification, and the appropriate forms, will be provided by the Biological Safety Officer when a notifiable GM project is proposed. A fee is payable to HSE and must be sent with the notification.

APPENDIX 4: Use of class II microbiological safety cabinets (see SPG-1-09 Local Exhaust Ventilation)

A microbiological safety cabinet (MSC) is a device intended to offer protection to the user and the environment from airborne droplets or particles generated in handling infected and other hazardous biological material. The design, construction, installation, use, maintenance and testing of microbiological safety cabinets is the subject of British Standard 5726:1992.Air discharged from a MSC to the atmosphere is always filtered. A high level of filtration efficiency is achieved through use of HEPA filters (High Efficiency Particulate Absorption.) Two of the three types of cabinet specified by the BS also provide protection against contamination of the materials manipulated in them. For further information about MSCs see ref. 7.

Class I MSCs provide operator protection by maintaining an inward flow of air past the operator and over the work surface inside the cabinet. As the incoming air is unfiltered, this type of cabinet does not provide protection to the material being worked on in the cabinet.

Class II MSCs offer protection to both the operator and the material being worked on. The inflow of air at the front of the cabinet, which is filtered before circulation within it, discourages emission of airborne particles generated by the work while the downflow of filtered air over the working surface protects the work. However, Class II cabinets are susceptible to disruption of the airflow pattern which may compromise operator protection. This can be caused by movement of people in the vicinity of the cabinet, sudden movement of the arms of the operator, or changes in air pressure (e.g. when a door is opened.)

Class III cabinets are totally enclosed, manipulation being carried out via glove ports. These cabinets provide the maximum protection for the operator, the environment and the work. Both incoming and outgoing air is filtered.

Testing. It is essential that full commissioning tests are carried out by the supplier when a cabinet is installed or moved to a new location. The airflow of safety cabinets should be checked at regular intervals, particularly those of Class II cabinets. The minimum inward airflow through the front aperture of a Class I or Class II cabinet is defined in BS 5726. This is necessary to provide containment and is related to the “operator protection factor”, for which the minimum is $1.0 \times 10^{5.}$ This means that for every 100,000 particles used in a test as a challenge to the inward flow of air at the working aperture, not more than one should escape. Regulation 9 of COSHH requires a thorough examination and testing of MSCs at intervals not exceeding 14 months.
Maintenance and safety testing should be carried out by specialist contractors. Records of testing must be kept. Safety cabinets must be fumigated regularly and before repair or maintenance. Safety cabinets must be used in accordance with manufacturers' instructions. Staff must receive appropriate training before being allowed to use safety cabinets. In particular, staff using Class II cabinets must be made aware of the factors that may adversely effect operator protection, such as air movement at the aperture and obstruction of air intake slots.

NOTE:
Laminar flow cabinets are NOT safety cabinets although some superficially resemble Class II MSCs. These cabinets must never be used for handling infectious or potentially infectious materials.
Fume cupboards are not recommended for any type of work with hazardous biological agents, including genetically modified organisms.