UNIVERSITY OF SUSSEX

SPG-29-09

GENETIC MODIFICATION

Safety procedures and guidance for the implementation of

Aknowledgment:-Drafted by Dr. Stephen Pearce 0
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Aknowledgment:-

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 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**

Aknowledgment:-

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. Pal CO

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 -
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 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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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).

- 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.

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

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(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 infringemen3.75 745.66-39(i)5(r

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