

RISK ASSESSMENT of WORK with GENETICALLY MODIFIED ORGANISMS

The requirements of Genetically Modified Organisms (Contained Use) Regulations 2000 are reflected in the University Health and Safety Policy which requires that risk assessment of all work with Genetically Modified Organisms **must** be carried out in advance of work commencing and, in addition, **must be scrutinised and approved** by the University's relevant Safety personnel. The tables at the end of this document are drawn from the current legislation and the appropriate table **must** be completed as part of the assessment. Finally, **WORK MUST NOT BEGIN** until the proposal has been **approved** and clearance has been given via Health and Safety.

Date submitted		Date approved	
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Please provide the following general information:

School/Department	AACME
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Principal investigator	<u>Karen Coopman</u>	Position	Lecturer
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Please give a brief and descriptive title for this risk assessment

Title	Creation of bio-artificial kidney with renal cells (immortalised) as a model of renal transport
Please provide a brief description of the nature of the work, identifying any GMMs produced (e.g. virus vector with insert), and their use to transform cells. Please identify the components of the project for which this risk assessment is carried out.	
A bioreactor system utilising polysulfone hollow fibres has previously been developed at Loughborough University. The hollow fibres will be made using wet spinning and their porosity and properties such diameter and thickness will be measured by SEM.	
Transfected HEK and MDCK cells will be used to determine the optimum conditions in terms of cell culture medium composition, cell density, flow rate, cell culture surface on the polysulfone fibres. The expression and function of renal transporters (uptake and efflux of substrates), as well as, the formation of tight cellular monolayer will be tested. In addition, both apical and basolateral compartments of the cellular monolayer should be available and sampled separately.	

Donor	
Name of gene/nucleic acid sequences	Over-expression of MDR1a transporter
Vector	Received from AstraZeneca. Details not provided
Host	MDCK MDR1a clone 5K11
ACDP category* of host (where appropriate)	Category 1

*The ACDP categorisation of biological agents can be found in the *Approved List of Biological Agents* published by the Health and Safety Executive.

Note: The questions in this proforma are designed to ensure that all the relevant issues have been addressed for the majority of Risk Assessments for work involving Genetic Modification at the University of Loughborough. However in the interests of streamlining the majority of applications, and because not all possible applications of genetic modification may have been anticipated, there may be instances in which answer of these questions alone may not be sufficient for a full risk assessment. The Genetic Modification Safety Committees reserve the right to request additional information. For a more complete description of the requirements of a Risk Assessment, refer to ACGM notes and newsletters, and the Guidelines to the 2000 Regulations. Less detail will be required for commonly used and familiar host/vector systems than for those less widely known or characterised. References may be helpful in some instances.

It may be appropriate to write the assessment to cover a range of closely related GMOs, e.g. a defined family of genes, a range of vectors with similar properties, complete and partial sequences, with and without insertion; however the assessment and containment conditions proposed must reflect the greatest potential hazard of any of the range of GMMs covered by the assessment.

Do not feel constrained by the box sizes, in some cases considerably greater amounts of information may be required. The box sizes should expand to accommodate your text. To add further rows to a table, use tab key when cursor is in the last box.

Any potentially confidential information should be highlighted, e.g. by use of **red text**. This will include all personal information, and possibly e.g. commercially sensitive information, which the applicant wishes **NOT TO APPEAR ON THE PUBLIC REGISTER**. NB There are tight restrictions on what will be accepted as confidential. The remainder of the risk assessment must be understandable without the confidential information.

It may be possible for outside bodies to access information in this form under the Freedom of Information Act, unless it can be categorised as an exemption. Furthermore, work with organisms listed in Schedule 5 of the Anti-terrorism, Crime and Security Act 2001, or genetic material from those organisms, may be notifiable to the Home Office.

Characteristics of the Donor, Insert, Vector and Host

Name (species/strain if appropriate) and characteristics of the source of the nucleic acid sequences ("the donor")

Dog

Note: Species from which the nucleic acid sequences were obtained, whether a pest or pathogen, tissue (normal, tumour, healthy or diseased), health status of the donor, etc.

Name, description and function of the gene/nucleic acid sequences involved ("the insert")

MDR1 (multidrug resistance gene): A transporter protein expressed at the apical side of renal cells. They are responsible for the active uptake or efflux of substrates.

Note: Biological function of the intact, natural gene; whether protein-coding sequence complete, partial, unknown, or known to be absent in construct; whether or not interrupted by introns etc; whether wild type or mutant; known, suspected or intended function of mutants; any other biological activities e.g. antisense, ribozyme, replication origin, mobilisation functions, etc. Genomic or cDNA library (consider the properties of the library as a whole; separate assessment is required for the specific clones you intend to isolate from the library).

Name and characteristics of the "vector"

CMV promoter controlled expression of MDR1 gene.

Note: Name of parental plasmid, bacteriophage, etc; characteristics, i.e. mobilisable, mobilisation defective, non-mobilisable; host range; presence of drug resistance markers or other sequences of potential clinical or environmental significance. Whether constructs transferred into host cells e.g. as non-mobilisable DNA; presence of replication origins, conditional (e.g. SV40, EBV) or otherwise. Involvement of viral vectors (e.g. retrovirus, baculovirus); name, characteristics, whether replication defective and the basis of this (e.g. deletion); host range; pathogenicity; potential for complementation by products expressed in the host, or by superinfection, etc.

Name and characteristics of the "host"

MDCK: Canine kidney epithelial cells. The sample was confirmed to be of canine origin and no mammalian interspecies contamination was detected. A genetic profile was generated for the sample by using a panel of STR markers for genotyping.

Note: Species/strain etc, whether disabled/ highly disabled; presence of other agents which may e.g. assist transmission; or affect pathogenicity; any history of safe use; whether an intact multicellular organism is produced at any stage (e.g. transgenic animals, plants); if host is (a) cell line(s) derived from multicellular organisms, the species, any potential for harm to humans or the environment; presence of other agents which are themselves transmissible or may assist the mobilisation of the transferred sequences e.g. as a result of recombination.

Characteristics of the Genetically Modified (Micro)Organism

Will there be expression of the protein (or other functional product) encoded by the insert, in the genetically modified organism?

Constitutive MDR1a over-expression under the action of the CMV promoter

Note: Provide details, e.g. of the promoter, level of expression, secretion, presence of introns within the coding region which might preclude expression of a functional product in *E. coli*, or other specific hosts, etc.

Specify any known or expected characteristics of the GMO which pose a risk to human health and safety and assess the severity and likelihood of such effects

Effects on human health (include colonisation, infection, allergy, toxin-mediated disease)

NONE

Humans at increased risk of the above effects (e.g. immunocompromised, pregnant or breastfeeding women)

NONE

Note: Characteristics which might increase the pathogenicity of the GMO relative to the unmodified host, or decrease susceptibility to control measures, e.g. alteration in susceptibility to clinically relevant drugs or to immunological or other natural defences; any other potentially significant biological activities of encoded products, e.g. potential toxicity, allergenicity, growth promotion/inhibition, oncogenicity, other pharmacological activity, etc.

Does this project involve work with animals? Provide details

NO

Either use of transgenic animals or work with GMMs in animal models

Quantity of organisms to be used

400 000 cells per well (24 well plates). 1 ml of media

Specify volumes and concentrations/culture density

Interim Assignment of Containment Conditions to Protect Human Health

Using the appropriate table(s) in Annex 1 of this form please select your control measures (you may place a **X** alongside each appropriate control measure to indicate that you have considered each one) and assign an interim level of containment for the work, i.e. ACGM containment level, (taking into account the hazard grouping of any biological agent). Please justify your decision to use this level of containment.

NB CLASSIFICATION OF THE PROJECT IS DEPENDENT ON ONLY THOSE CONTROL MEASURES THAT ARE SHOWN BY THE RISK ASSESSMENT TO BE NECESSARY TO PROTECT HUMAN HEALTH OR THE ENVIRONMENT. MEASURES THAT RESULT FROM CONVENTION, CONVENIENCE OR ARE REQUIRED FOR PRODUCT PROTECTION ARE NOT RELEVANT TO THE CLASSIFICATION See ACGM Newsletter 27/ACGM Compendium of guidance for further information

Interim containment level and corresponding Class (classes) of GMO(s) involved in the work (& explanation)

For operational purposes, all procedures will be carried out under Containment Level 2 within the CBE laboratory.

Note: You will need to consider the containment level necessary to control the risk of the host and then make a judgement as to whether the modification will result in a GMO more hazardous/less hazardous/about the same

Please provide the following information for the Committee:

Are any of the work procedures likely to generate aerosols? If so, is the work to be undertaken in a safety cabinet?

Aerosols may be generated when pipetting or manipulating solutions and as such, manipulations will always be conducted in Biological Safety Cabinets as detailed in the attached biological risk assessment.

Identify any use of sharps in the work; justify their use and specify control measures

No

Protective equipment and clothing to be used

Lab coat and nitrile gloves

Transport and storage arrangements

Refer to section C1.2.3, C1.2.4 and C1.2.5 on the associated biological risk assessment CBE/BRA/142 for transport and safe storage procedures

Specify arrangements for safe storage; whether, and if so how, materials are likely to be transported between buildings, on public roads, or posted)

Disinfection

Small spills on surfaces will be decontaminated immediately using 1% Virkon according to local procedures. Large accidental spills will be sprinkled with powdered Virkon before cleaning according to local procedures. General disinfection on a daily basis will be with IMS and 1:20 chemgene with weekly deep clean using 1% Virkon in addition to the above.

Refer to section C.1.2.9 for further disinfection directions.

Specify disinfectant(s) to be used, and their dilution. Have these been validated for use with the relevant organism?

Inactivation of GMMs in waste, and subsequent disposal

All disposable culture and labware from the CBE laboratory will be autoclaved within the CBE unit and properly labelled before leaving the building for incineration.

The Contained Use Regulations 2000 require that GMMs in contaminated material and waste are inactivated by validated means. You must specify the METHOD of inactivation of the GMMs, the expected DEGREE OF KILL of the GMM achieved by that method, and the VALIDATION of that method.

Monitoring of Containment and Control Methods

Monitoring of containment at point of use

Not required as cells are unable to survive or propagate outside of laboratory culture. Engineering controls in the CBE (e.g. BSCs) are monitored according to SOPs outlined in the accompanying BRA

Monitoring of waste inactivation methods

Waste inactivation is monitored according to COPs for maintenance of autoclaves and disposal of biological waste outlined in the accompanying BRA.

Emergency procedures - Is an emergency plan required? Provide details (or attach)

An emergency plan is not required as small scale activities will not result in significant spills and consequent significant release of the GMO. The risk of any low level release to health and safety and/or the environment is considered effectively zero. However, emergency response procedures for dealing with biological spills and reporting incidents are in place as part of the CBE CL2 laboratory unit operational code of practice.

Note: In the event of a reasonably foreseeable accident where the health and safety of people outside the premises is liable to be seriously affected or where there is a serious risk of damage to the environment then an emergency plan is required. This plan may need to be communicated to the emergency services and other relevant bodies. In most cases this will only be required for Class 3 and 4 projects (See ACGM Newsletter 27/Compendium of Guidance for further information). However, details of accident/spillage procedures should be provided for all projects.

Occupational Health issues

Health forms are submitted and monitored by the occupational health office in the university as part of the authorisation process for entry into the CBE CL2 laboratory unit.

Specify any requirements for immunisation, chemoprophylaxis or health monitoring, and any special requirements for record keeping

Environmental Considerations

ANSWERS MUST BE JUSTIFIED IN SOME DETAIL, i.e.- IT IS NOT ACCEPTABLE TO SIMPLY STATE THAT THERE IS NO RISK TO THE ENVIRONMENT.

Risk to animals, fish, plants etc

If the recipient microorganism is controlled by DEFRA, do you have a DEFRA licence? (delete as appropriate)

N/R

Approval will not be granted until a copy of the DEFRA licence (if applicable) has been submitted to both the local GMSC and the Advisory Group for the Control of Biological Hazards

Identify any identifiable potential hazards to the environment, which might occur if the genetically modified organism were to be accidentally released. Classify the potential hazard as Severe, Medium, Low or Negligible.

Negligible

Cell lines are unable to propagate or survive outside of the laboratory environment. They are unlikely to cause disease in a healthy human following accidental needlestick injury. There is a significant risk to the original donor if cells were introduced to his system but there is no situation in which this would occur.

Note Potential hazards might be identified, and their severity assessed, dependent upon: the host species, the vector or the insert; or phenotypic changes caused by the genetic modification; the presence of host or susceptible species in the environment; the potential for survival, multiplication and dissemination in the environment; the stability of the GMO in the environment; the possibility of gene transfer to other species, etc. Refer to ACGM Compendium of guidance for further information

In view of the characteristics of the GMO, specify the likelihood of accidental release and occurrence of the above mentioned potential harmful effects, if the work were to be performed at the interim containment level specified above. Classify this as High, Medium, Low or Negligible.

Effectively zero

Note: This includes the wider as well as the local environment in which the activity is to be carried out. Consideration should be given to any potential exposure of the environment to the GMMs and the magnitude and duration of such exposure. Refer to ACGM guidance for further information

Grade the overall Risk to the environment (= Potential harm x Likelihood) as High, Medium, Low or Effectively Zero.

Effectively Zero

Additional Containment

If, in considering the potential for harm to the environment, you have concluded that the Risk to the environment is high or medium, then the containment conditions previously specified may need to be modified to reduce the risk to an acceptably low level. Use these considerations to revise your provisional containment level so that all risks are controlled to low or effectively zero.

Additional containment provisions for environmental protection

None

Assign your final containment level.

CL2

Are all hazards now controlled by this proposed level of containment?

Yes

Final classification of the activity, i.e. Class 1/2/3/4. Is the activity notifiable to HSE?

No notification required

Where the containment and control measures fall between two levels, e.g. where level 1 is appropriate with some control measures from level 2, the classification for the activity is equivalent to the HIGHER containment level. All Class 2,3 and 4 projects are notifiable to the Health and Safety Executive through the Health and Safety Unit

Do you intend to apply all control measures from your highest selected level of containment (See Annex 1)? If not, please justify the exclusion of any control measures not used.

Yes

Formal application to the Health and Safety Executive is required for derogation from the full containment level for all Class 2, 3 and 4 projects.

***EC Regulation requires notification of transboundary movements of Class 3 GMMs to the Biological Clearing House and European Commission (*transboundary movements are those entering or leaving the EC*). If your work involves Class 3 GMMs please indicate below whether they will be subject to transboundary movements.**

N/R

Workers Involved in the Project and Facilities Used for the Work

Please indicate the areas where work will be carried out (including Room No. and Designation):	
Room No. and designation	ACGM Categorisation
CBE Laboratory Suite	CL2

Workers initially involved in work:	Post/experience/training:
Alexandros Englezakis	Training recorder in training file
Training and assessment of competence for existing and future personnel <i>Specify arrangements for provision for existing and future personnel</i>	

Authorisation and Notification

The work proposed should be discussed with the Departmental Biological Safety Officer.

Signature of proposer Date

Please print name Alexandros Englezakis.....

Other Signature (s) *R. Temple* SSO & BSO Date *21/05/2019*

Please print name *R I Temple*

Signature of Biological Safety Officer or authorised Deputy *[Signature]* Date *9-9/19*

Please print name *JULIE TURNER*

NB The Approval of the University's relevant Safety Committee is required before work starts.

APPROVAL of the RELEVANT SAFETY COMMITTEE

On behalf of SC *[Signature]* Approval Date *9-9/19*

ANNEX 1

TABLES OF CONTROL MEASURES AND CONTAINMENT LEVELS

The basic principles of classification are that you:

1. Determine the containment and control measures required by the risk assessment to control the risk of the activity;
2. Where this corresponds to a single containment level this will read across directly to give you the activity class, i.e. level 1 = class 1, level 2 = class 2, etc;
3. Where the measures identified correspond to measures from two different levels of containment the class corresponds to the higher of the two levels.

Further information can be found in the guide to the Contained Use Regulations and in the ACGM Compendium of guidance

Please consider the table(s) overleaf. Select the appropriate table for the work you are involved in. In most cases this will be **Table 1A (Laboratory Activities)**. **Where your project involves the use of GMMs in plant growth facilities or animal facilities, you should consider Table 1B or 1C in conjunction with table 1A.** (In the final column of Tables 1B and 1C "additional" specifies use of that control measure in addition to the measures in Table 1A, while "modification" specifies that this measure shall be substituted for the relevant measure in Table 1A).

Large scale activities should be classified using **Table 2**.

Select your control measures. You should place a **X** in the appropriate box on each row to indicate whether that containment measure is required or not.

Determine the corresponding level of containment and hence the class of GMO. Where controls are selected from more than one containment level the Class corresponds to the higher of the containment levels.

FOR FURTHER INFORMATION PLEASE REFER TO ACGM NEWSLETTER 27 OR THE ACGM COMPENDIUM OF GUIDANCE

Please delete tables not relevant to your risk assessment. You may also delete this explanatory page from your final risk assessment

TABLES OF CONTAINMENT MEASURES

TABLE 1A: LABORATORY ACTIVITIES

TABLE 1B: PLANT GROWTH FACILITIES

TABLE 1C: ANIMAL FACILITIES

TABLE 2: OTHER ACTIVITIES (LARGE SCALE)