Example of a Risk Assessment for Class 2 GMMs

The following constitutes an example of a Risk Assessment for a GMM falling within Class 2.

It is designed as a guide to how a Risk Assessment should be conducted, the elements which should be considered during a Risk Assessment (the recipient organism, the insert, the vector, the host, the final GMM, human health and environmental considerations, containment measures) identification of possible harmful effects and the possibility of those effects occurring. The Risk Assessment process culminates in the determination of the final classification of the GMM and the appropriateness of the corresponding containment measures.

Description of each GMO

Recombinant Adenovirus viral vectors containing a gene encoding a therapeutic protein VEGF- which promotes the growth of vascular endothelial tissue.

Purpose of the contained use

The aim is to produce live intact Adenovirus viral vectors. The Adenovirus vector contains a gene which codes for human vascular endothelial growth factor. This protein has the potential to be effective in the treatment of coronary artery disease and peripheral vascular disease.

Procedure 1

1. Identification of harmful properties (hazard) of the GMM

1.1. The recipient organism

The recipient organism is human Adenovirus type 5. It is ubiquitous, causes only a mild respiratory disease in humans which is self-limiting and does not require any specific treatment. Similarly, there is no association with allergic or toxic effects. The complete E1 region and the majority of the E3 region of the genome have been removed thereby rendering the viral vector replication deficient.

1.2. The insert

The genetic insert (VEGF) is human in origin and poses no risk to animal health or the environment.

1.3. The vector

Construction of recombinant Adenovirus is a two-step process in which the desired expression cassette is first assembled into a pUC vector and subsequently transferred into the Adenoviral genome by homologous recombination. pUC vectors have a history of safe use. The Adenovirus vectors are replication defective by virtue of deletion of the E1 and E3 regions.

1.4. The host

The host cell is PER.C6 cell line which is derived from human embryonic Retin oblasts transformed with the E1 region of Adenovirus 5. Since the Adenoviral vector is replication deficient recombinant Adenovirus can only grow in complementing cells such as PER.C6 which contain the appropriate E1 sequences.

PER.C6 cells die rapidly outside the artificial environment created within the laboratory. There is little likelihood of the recombinant PER.C6 cells proliferating or surviving in the environment and therefore poses little risk to animal/plant health or the environment

1.5. The resulting GMM

Recombinant Adenovirus vector contains the gene for human vascular endothelial growth factor. The recombinant Adenovirus is replication deficient and therefore can only replicate in cells which carry complementing regions of the E1 genes. It will not replicate in other in vivo or in vitro cells. Furthermore, since the modified virus is replication deficient, it is less pathogenic than the wild type and there is minimal capacity for colonisation. If it is exposed to the environment, it is unlikely to survive for extended periods.

A replication competent Adenovirus has the potential to be produced; however, this is unlikely given that the E1 sequences in the PER.C6 cells do not overlap with the deleted E1 region. Therefore, in order to generate a replication competent Adenovirus, two non-homologous recombination events would have to occur. A revertant regaining the E1 gene would still be devoid of the E3 gene since PER.C6 does not contain the E3 gene. The absence of the E3 gene would reduce the fitness of the virus as an infective agent

1.5.1. Human Health Considerations

Wild type human Adenovirus type 5 is ubiquitous and causes self-limiting infections of the upper respiratory tract and the common cold. There may be a possibility that the modified Adenovirus may mimic some of the characteristics of the wild type, however, recombinant Adenovirus can only replicate in complementing cells such as PER.C6. Even if replication competent Adenovirus type 5 were generated, the risk associated is low since human adenoviral infection is very common and the majority of adults have already been infected.

1.5.2. Environmental Considerations

There may be a possibility that the modified Adenovirus may mimic some of the characteristics of the wild type. However, recombinant Adenovirus can only replicate in complementing cells such as PER.C6 and consequently poses a low risk to animal/plant health and the environment.

Level 2 containment measures will be in operation, and the principles of Good Microbiological Practice will be applied. In conjunction with this access to the laboratory will be restricted when work with infectious agents is in progress. Persons at increased risk of acquiring infection or for whom infection may have serious consequences will not be allowed to enter the laboratory. A biohazard sign will be posted at the laboratory entrance bearing appropriate information including the agent(s) in use, containment level, the investigator's name and telephone number, personal protective equipment requirements and exiting procedures if any. Biosafety procedures will be incorporated into Standard Operating Procedures (SOPs) or the biosafety manual and personnel will be advised of special hazards. All work will be done with the approval of the safety sub-committee.

1.6. Initial classification of the GMM

Human Adenovirus type 5 is ubiquitous, causes only a mild respiratory disease in humans which is self-limiting and does not require any specific treatment. The Adenovirus viral vectors are replication defective owing to the removal of the E1 and the E3 regions. The genetic insert (VEGF) is human in origin and poses no risk to animal/plant health or the environment. The pUC vectors have a history of safe use. Modified Adenovirus may mimic some of the characteristics of the wild-type or may pose a risk to immune- compromised individuals. It is therefore allocated to Class 2, GMM activities of low risk for which level 2 containment is appropriate to protect human health as well as the environment.

1.7. Assessment of possibility of harmful effects occurring

1.7.1. Nature of activities to be undertaken

The experiments entail standard molecular biology techniques which carry no special risk, and all are conducted in accordance with safety SOPs held in house.

1.7.2. Concentration and scale

A typical viral preparation involves propagation in 30 flasks each containing 30ml of culture medium The cell pellet is harvested from these flasks, and the virus is released by repeated freeze thawing. The virus is isolated, and a typical yield is 1 x 10^{10} pfu/ml.

1.7.3. Culture conditions

Adenoviral vectors will be propagated in PER.C6 cells. Culture conditions are as per 1.7.2 above. The GMM is incubated at 32°C for 36 – 48 hours.

1.7.3.1. Environment likely to be exposed

Only the immediate laboratory environment is likely to be exposed and as outlined in section 1.5.2 considerable care is taken to ensure that the GMM is contained and that areas/personnel beyond the laboratory are not exposed to the GMM. Furthermore, it is expected that the GMM will not survive for significant periods in the environment.

1.7.3.2. Presence of susceptible species

Neither animals nor plants are susceptible to human Adenovirus type 5. Laboratory staff and/or immune-compromised persons constitute those most at risk and as outlined in section 1.5.2 considerable care is taken to ensure that the GMM is contained.

1.7.3.3. Whether the environment can support the survival of the GMM

Recombinant Adenovirus is replication incompetent by virtue of the fact that the E1 and the E3 gene sequences have been removed. It is therefore only capable of replication in complementing cells such as PER.C6.

1.7.3.4. Effects on the physical environment

Since the GMM is considered incapable of survival in the environment no effects on the physical environment are expected.

1.7.4. Waste treatment provisions

Waste from GMM work is segregated into liquid and solid waste. Liquid waste is treated primarily with 1%W/V Virkon solution and then with Presept chlorine tablets. Inactivated liquid waste is disposed of down the sink with multiple volumes of water. It is expected that 5L of liquid waste will be produced per month.

Contaminated, solid, GMM waste such as plastic disposables, tissue paper, culture flasks, etc. is double bagged and autoclaved in-house. Waste autoclave runs are carried out in accordance with in-house Standard Operating Procedures (SOPs). Class 2 GMM waste is autoclaved at 123℃ for 30 minutes, 2 bar pressure, prior to disposal to landfill by a registered waste contractor (provide name of waste contractor). Spore strips are included in each Class 2 GMM waste load for purposes of validation of destruction. The autoclaved waste is then stored in a sealed 240L wheelie bin with biohazard symbols displayed, for 48 hours, while the spore strips are incubated to validate destruction. After 48 hours and a positive destruction result with the spore strips, the inactivated waste is removed by a registered waste contractor to landfill. The autoclave is validated annually.

Class 2 sharps containers are autoclaved in accordance with an SOP for Class 2 GMM waste inactivation before being removed by a registered waste contractor (name of waste contractor) for disposal by (how the waste is treated and disposed of by the waste contractor).

PROCEDURE 2

2.1 Determination of final classification and containment measures

Wild type human Adenovirus type 5 is classified as Class 2. It is ubiquitous, causes only a mild respiratory disease in humans which is self-limiting and does not require any specific treatment. There is no association with allergic/toxic effects. The genetic insert (VEGF) is human in origin and poses no risk to animal/plant health or the environment. pUC vectors have a history of safe use and Adenoviral vectors are replication defective by virtue of deletion of the E1 region. Modified Adenovirus may mimic some of the characteristics of the wild-type virus or may pose a risk to immune-compromised persons. It is therefore allocated to Class 2, GMM activities of low risk for which level 2 containment is appropriate to protect human health as well as the environment

The GMM activity is therefore classified as Class 2.

2.2 Confirmation of adequacy of final containment measures

Principles of Good Microbiological Practice and Good Occupational Safety and Hygiene in accordance with Part A of the Fourth Schedule of the GMO (Contained Use) Regulations, 2001 to 2010.

The requirements of Containment Level 1 as given in table 1A- 'containment measures for contained use of GMOs in a laboratory'- of the GMO (Contained Use) Regulations, 2001 to 2010.