

Example of a Risk Assessment for Class 1 GMMs

The following constitutes an example of a Risk Assessment for a GMM falling within Class 1.

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

Cloning of structural/non-structural gene sequences from *Pseudomonas aeruginosa* into *E.coli* XL-1-Blue using standard molecular biology techniques.

Purpose of the contained use

To investigate the opportunistic pathogenicity capability of *Pseudomonas* spp.

PROCEDURE 1

3.1 Identification of harmful properties (hazard) of the GMM

3.2.1 The recipient organism

The recipient organism is *E.coli* XL-1-Blue, which is a commercially available *E.coli* strain derived from *E.coli* K12 and used for routine and widespread molecular biology studies. Derivatives of K12 are avirulent and may be considered to be equivalent to Directive 90/679/EEC (and amendments) and ACDP biological agents hazard group 1 because they have an established record of safety in the laboratory with no adverse effects on human, animal or plant health or the environment. Furthermore *E.coli* XL-1-Blue is incapable of survival outside the laboratory.

3.2.2 The donor organism

The donor organism is *Pseudomonas aeruginosa*. It is categorised as Class 2 according to Directive 90/679/EEC and amendments. However *Ps. aeruginosa* is not considered a primary pathogen but rather an opportunistic agent involved in hospital acquired infection. It exists as a normal inhabitant of human intestinal flora and is associated with infection in the immunocompromised host. It is widely dispersed in the hospital environment, has no known involvement in environmental processes and is non-spore forming.

3.2.3 The insert

Inserts constitute structural and non-structural gene sequences encoding transmembrane receptor molecules, outer membrane proteins, binding proteins. These proteins will be expressed at high levels in *E.coli* in order to obtain proteins for functional analyses. None of these proteins are known to be involved in pathogenicity but all are anticipated to play physiological roles.

3.2.4 The vector

The vectors used for this project are commercially supplied, pUC derived (history of safe use), are unlikely to endow any pathogenic traits and are rendered non-mobilisable by virtue of the fact that they lack *bom*, *mob* and *tra* genes.

3.2.5 The resulting GMM

The host organism is classified as belonging to Class 1. The insert and the expressed gene product are not considered harmful or capable of altering the pathogenicity/ survivability /fitness of the recipient organism. On this basis the GMM is deemed incapable of causing disease in humans, animals or plants and does not cause adverse effects in the environment.

3.2.5.1 Human Health Considerations

E.coli XL-1-Blue (the recipient organism) is not known to cause disease in animals or plants or cause any adverse effects in the environment. The genetic modification described will not increase the ability of the GMM to cause disease. No enhanced pathogenicity or virulence is expected.

3.2.5.2 Environmental Considerations

The recombinant *E.coli* will be cultured at volumes not exceeding 50ml at a time. Precautions will be taken to avoid the generation of aerosols. Personnel are fully trained in aseptic techniques, are required to wear white coats while working in the laboratory and gloves while carrying out aseptic techniques.

The presence of the *Ps aeruginosa* characters described, in a laboratory strain of *E.coli* are not considered likely to enhance its survival in the wild.

3.3 Initial classification of the GMM

E.coli X-L1-Blue has an established record of safety in the laboratory and therefore is unlikely to cause disease to humans, animals or plants. Furthermore the genetic modification is not expected to alter this. The pUC derived vectors have a similar history of safe use and the inserts will not endow the recipient organism with a phenotype likely to cause disease or to have deleterious effects in the environment. Therefore the GMM is unlikely to cause disease or to have deleterious effects in the environment and is allocated to Class 1 GMM - activities of no or negligible risk for which level 1 containment is appropriate to protect human health as well as the environment

3.4 Assessment of the possibility of harmful effects occurring

3.4.1 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.

3.4.2 Concentration and scale

E.coli XL-1-Blue is cultured on LB solid media (approximately 10 petri dishes at a time) or in LB broth at typical volumes of 50ml or less.

3.4.3 Culture conditions

Culture conditions are as per 3.4.2 above. The GMM is incubated at 37°C for 24 hours.

3.4.3.1 Environment likely to be exposed

Only the immediate laboratory environment is likely to be exposed and as outlined in section 3.2.5.2 considerable care is taken to ensure that the GMM is contained and that areas 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.

3.4.3.2. Presence of susceptible species

Neither humans, animals nor plants are susceptible to *E.coli* XL-1-Blue and the insert and the expressed gene product are not considered harmful or capable of altering the pathogenicity/survivability/fitness of the recipient organism.

3.4.3.3 Whether the environment can support the survival of the GMM

E.coli XL-1-Blue is a disabled strain and it is expected that the organism would not survive in the environment.

3.4.3.4 Effects on the physical environment

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

3.4.4 Waste treatment provisions

Accidental spillages will be dealt with by swabbing with disinfectant 10% Ethanol which is bactericidal. Contaminated, solid, GMM waste such as plastic disposables, tissue paper, culture plates, etc., are double bagged and autoclaved at 121°C for 20 mins, 1 bar pressure. The autoclave is located in the building and is validated on an annual basis. The autoclaved waste is stored in a sealed 240L wheelie bin with biohazard symbols displayed until removal by a registered waste contractor (**name of waste contractor**) for disposal.

Approximately 2L of liquid culture medium is treated by adding to Virkon to achieve a final concentration of 10% and left to stand overnight before discharge.

Sharps material is disposed of in cin-bins. Cin-bins are removed by a registered waste contractor (**name of waste contractors**) for disposal by (**how the waste is treated and disposed of by the waste contractor**).

PROCEDURE 2

4.1 Determination of final classification and containment measures

E.coli XL-1-Blue is non-pathogenic, has a history of safe laboratory use and is classified as Class 1. The inserts, vectors and expressed gene product are not considered harmful or capable of altering the pathogenicity/survivability/fitness of the recipient/host organism. On this basis the GMM does not pose a risk to human/animal/plant health or the environment. Therefore the containment level required to control that risk is Level 1, the same as unmodified *E.coli* XL-1-Blue.

The GMM activity is therefore classified as Class 1.

4.2 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.