

## RISK ASSESSMENT EXAMPLE

### ***E. COLI* K-12 DERIVATIVE EXPRESSING HUMAN GROWTH HORMONE.**

#### **Overview**

The aim of the project is to clone and express the human growth hormone gene in an *E. coli* K-12 derivative, DH5 $\alpha$ . The insert will be carried on the vector pUC 18. The construct will be grown at a pilot plant scale of 200 litres

**(i) Consideration of the predicted properties of the GMM to determine if there are any potential mechanisms by which it could represent a hazard to human health.**

#### ***(a) Hazards associated with the recipient micro-organism***

*E. coli* K-12 derivatives such as, DH5 $\alpha$  are recognised as non-colonising and disabled, and may be considered to be equivalent ACDP hazard group 1. They are not considered pathogenic to humans or animals. They are expected to have limited survivability in the environment and often have auxotrophic requirements, which are unlikely to be satisfied outside of laboratory culture.

#### ***(b) Hazards arising directly from the inserted gene product (e.g. cloning of a toxin gene or oncogene)***

The human growth hormone is expressed as a fusion protein, which forms insoluble inclusion bodies within bacterial cells (reference or data required). The initially expressed product is thus biologically inactive and it requires treatment by several in vitro laboratory steps to produce active protein.

Therefore, while human growth hormone could exert harmful effects if delivered in a biologically active form, for the purposes of the risk assessment the expressed gene product can be considered non-harmful.

#### ***(c) Hazards arising from the alteration of existing pathogenic traits (e.g. alteration of host range or tissue tropism)***

The cloned protein is unlikely to alter the pathogenicity of the cloning host.

#### ***(d) The potential hazards of sequences within the GMM being transferred to related micro-organisms***

The vector pUC 18 is considered to be non-mobilisable. Gene transfer is thus a remote possibility. In any case there are no specific environmental concerns relating to the foreign gene as it will be continually being released into the environment as the result of natural decay processes.

**(ii) Consideration of the likelihood that, in the event of exposure, the GMM could actually cause harm to human health.**

No significant hazards have been identified above and so it is unnecessary to consider the predicted properties of the GMM any further in relation to human health issues.

#### **(iii) Assignment of a provisional containment level.**

*(This step will often involve considering the containment level necessary to control the risk of the recipient micro-organism and making a judgement about whether the modification will result in a GMM which is more hazardous, less hazardous or about the same. Sometimes it may help to compare the GMM with the relative hazard presented by other organisms.)*

No significant hazards have been identified above and so it is appropriate to assign a provisional containment level of 1.

**(iv) Consideration of the nature of the work to be undertaken and a detailed review of the control measures to safeguard human health.**

The genetically modified micro-organism (GMM) is being grown at large scale (200 litres), under mono-septic conditions. It is being grown in a closed stainless steel fermenter, and will be harvested by centrifugation. The paste will be passed through a cell disrupter, and the insoluble inclusion bodies harvested. The centrate, containing cell debris and a low titre of viable cells will be heat inactivated and discharged.

Although the fermenter will be completely contained, with appropriate seals being used and with off gases being filtered, these measures are primarily to prevent contamination, and are in excess of what would be required for the purposes of protection of human health or the environment.

In the event of spillage from the fermenter, the area can be effectively disinfected. The fermenter is housed within a process building, and the wider environment is unlikely to become contaminated.

For laboratory operations a standard containment level 1 facility, and the use of good microbiological practice will be sufficient to limit contact with humans and the environment. For the large scale operations, the process equipment used will be sufficient to limit contact. The organism is unlikely to cause harm to either workers or the environment, so filtration of the off-gasses, and the use of a closed system is not required as a safety measure. None of the measures in Containment level 2 of Table 2 (of Schedule 8) are required for safety reasons, even though they will be used for process reasons.

**(v) The identification of any hazards to the environment and the assignment of any additional control measures to protect the environment.**

The GMM would not itself survive and become established in the environment (reference required). In any case it would not, in itself, be hazardous to the environment. When considering the possibility of gene transfer giving rise to environmental hazards the same arguments apply as used in section (i)(b).

Since it would be predicted that there would be negligible consequences, even if containment were to be breached, the environmental risk can be judged as effectively zero.

**(vi) Assignment of the activity class (1, 2, 3 OR 4)**

*(This is done by comparing the containment and control measures identified as necessary to control the risk with the tables of containment in Schedule 8 of the new Regulations.)*

Based on the above the work can be assigned to Class 1.