Annex 3 of Government Decree No. 132/2004. (IV. 29.)

Principles to be followed for the risk assessment on the contained use

This Annex describes in general terms the elements to be considered and the procedure to be followed to perform the assessment.

A) Elements of assessment

1. The following should be considered as potentially harmful effects:

a) disease to humans, including allergenic or toxic effects,

b) disease to animals or plants,

c) deleterious effects due to the impossibility of treating a disease or providing an effective prophylaxis,

d) deleterious effects due to establishment or dissemination in the environment,

e) deleterious effects due to the natural transfer of inserted genetic material to other organisms.

2. The assessment should be based on the following:

a) the identification of any potentially harmful effects, in particular those associated with:

aa) the recipient micro-organism,

ab) the genetic material inserted (originating from the donor organism),

ac) the vector,

ad) the donor micro-organism (as long as the donor micro-organism is used during the operation),

ae) the resulting GMM,

b) the characteristics of the activity,

c) the severity of the potentially harmful effects,

d) the likelihood of the potentially harmful effects being realised.

B) Procedure

1. The first stage in the assessment process should be to identify the harmful properties of the recipient and, where appropriate, the donor micro-organism, and any harmful properties associated with the vector or inserted material, including any alteration in the recipient's existing properties.

2. In general, only GMMs which show the following characteristics would be considered appropriate for inclusion in class 1 as defined in paragraph 3 of Article 4:

a) the recipient or parental micro-organism is unlikely to cause disease to humans, animals or plants,

b) the nature of the vector and the insert is such that they do not endow the GMM with a phenotype likely to cause disease to humans, animals or plants, or likely to have deleterious effects on the environment,

c) the GMM is unlikely to cause disease to humans, animals or plants and is unlikely to have deleterious effects on the environment.

3. In order to obtain the necessary information to implement this process the user may firstly take into account relevant Community legislation. International or national classification schemes (e.g. World Health Organisation, National Institutes of Health) and their revisions due to new scientific knowledge and technical progress may also be considered.

These schemes concern natural micro-organisms and as such are usually based on the ability of micro-organisms to cause disease to humans, animals or plants and on the severity and transmissibility of the disease likely to be caused. Furthermore the user may take into consideration micro-organisms, as biological agents, classified into four classes of risk on the basis of potential effects on a healthy human adult. These classes of risk can be used as

guidance for the purposes of categorisation of the contained use activities in the four classes of risk referred to in Article 4(3). The user may also take into consideration classification schemes referring to plant and animal pathogens (which are usually established on a national basis). The abovementioned classification schemes give only a provisional indication of the risk class of the activity and the corresponding set of containment and control measures.

4. The hazard identification process carried out in accordance with points 1 to 3 should lead to the identification of the level of risk associated with the GMM.

5. Selection of the containment and other protective measures should then be made on the basis of the level of risk associated with the GMMs together with consideration of:

a) the characteristics of the environment likely to be exposed (e.g. whether in the environment likely to be exposed to the GMMs there are known biota which can be adversely affected by the micro-organisms used in the contained use activity),

b) the characteristics of the activity (e.g. its scale and/or nature),

c) any non-standard operations (e.g. the inoculation of animals with GMMs; use of equipment likely to generate aerosols).

Consideration of points *a)* to *c)* for the particular activity may increase, reduce or leave unaltered the level of risk associated with the GMM as identified under point 4.

6. The analysis carried out as described above will finally lead to the assignment of the activity to one of the classes described in paragraph 4 of Article 3.

7. The final classification of the contained use should be confirmed by reviewing the completed assessment referred to in paragraph 3 of Article 3.

C) Guidance notes for risk assessment

1. Introduction

The elements of the risk assessment outlined in sections A and B requires consideration of potentially harmful effects to human health and the environment. Potentially harmful effects are defined as those effects which may give rise to disease, render prophylaxis or treatment ineffective, promote establishment and/or dissemination in the environment which gives rise to harmful effects on organisms or natural populations present or harmful effects arising from gene transfer to other organisms. The assessment requires that the risk of these potentially harmful effects are considered for each activity and allocated to a class as defined in this regulation, taking into account both the nature and scale of operations, to determine the final containment facilities required. The degree of risk arising from contained uses with a genetically modified micro-organism (GMM), and their construction, is determined by consideration of the severity of the potential harmful effects, to human health or the environment, with the possibility of those effects occurring. The risk assessment considers the exposure of humans or the environment to GMMs during the operation of, or possible unintended release from, a contained use facility. The classification level determined by the risk assessment defines the containment requirements for the activities involving GMMs in accordance with Annex 2.

2. Risk assessment

The full risk assessment process consists of two procedures outlined below:

2.1. Procedure 1

Identify potential harmful properties (hazard) of the GMM and allocate the GMM to an initial class (class 1 to class 4) taking into account the severity of the potential harmful effects.

and

Assessment of possibility of harmful effects occurring by consideration of exposure (both human and environmental) taking into account the nature and scale of the work, with containment measures appropriate to the initial class allocated.

2.2. Procedure 2

Determination of final classification and containment measures required for the activity. Confirm final classification and containment measures are adequate by revisiting Procedure 1.

3. Procedure 1

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

The risk assessment process requires the identification of any potentially harmful properties of the GMM as a result of the genetic modification or any alteration of the recipient organisms' existing properties. Potentially harmful properties associated with the GMM must be determined. This should be done by consideration of the recipient organism, the donor organism, the characteristics and location of the inserted genetic material and any vector. It is important to appreciate that the genetic modification of a micro-organism can affect its ability to cause harm to human health and the environment. Genetic modifications can result in a decreased, unchanged or increased ability to cause harm.

3.2. Aspects that should be considered where relevant are:

3.2.1. The recipient organism

a) nature of pathogenicity and virulence, infectivity, allergenicity, toxicity and vectors of disease transmission,

b) nature of indigenous vectors and adventitious agents, where they could mobilise the inserted genetic material, and the frequency of mobilisation,

c) nature and stability of disabling mutations, if any,

d) any prior genetic modifications,

e) host range (if relevant),

f) any significant physiological traits which may be altered in the final GMM and if relevant their stability,

g) natural habitat and geographic distribution,

h) significant involvement in environmental processes (such as nitrogen fixation or pH regulation),

i) interaction with, and effects on, other organisms in the environment (including likely competitive, pathogenic or symbiotic properties),

j) ability to form survival structures (such as spores or sclerotia).

3.2.2. The donor organism (for fusion experiments or "shotgun" experiments where the insert is not well characterised)

a) nature of pathogenicity and virulence, infectivity, toxicity and vectors of disease transmission,

b) nature of indigenous vectors,

c) sequence,

d) frequency of mobilisation and specificity,

e) presence of genes which confer resistance to anti-microbials including antibiotics,

f) host range,

g) other relevant physiological traits.

3.2.3. The insert

a) specific identity and function of the insert (genes),

b) level of expression of inserted genetic material,

c) source of the genetic material, identity of the donor organism(s) and characteristics where appropriate,

d) history of prior genetic modifications if appropriate,

e) location of inserted genetic material (possibility of insertional activation/deactivation of host genes).

3.2.4. The vector

a) nature and source of the vector,

b) structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified micro-organism,

c) if present in the final GMM frequency of mobilisation of inserted vector and/or capability for transfer of genetic material.

3.2.5. The resulting GMM

3.2.5.1. Human health considerations

a) expected toxic or allergenic effects of the GMM and/or its metabolic products,

b) comparison of the modified micro-organism to the recipient or (where appropriate) parental organism regarding pathogenicity,

c) expected capacity for colonisation,

d) if the micro-organism is pathogenic to humans who are immunocompetent,

e) diseases caused and mechanism of transmission including invasiveness and virulence,

f) infective dose,

g) possible alteration of route of infection or tissue specificity,

h) possibility of survival outside of human host,

i) biological stability,

j) antibiotic-resistance patterns,

k) allergenicity,

l) toxigenicity,

m) availability of appropriate therapies and prophylactic measures.

3.2.5.2. Environmental considerations

a) ecosystems to which the micro-organism could be unintentionally released from the contained use,

*b) e*xpected survivability, multiplication and extent of dissemination of the modified micro-organism in the identified ecosystems,

c) anticipated result of interaction between the modified micro-organism and the organisms or micro-organisms which might be exposed in case of unintentional release into the environment,

d) known or predicted effects on plants and animals such as pathogenicity, toxicity, allergenicity, vector for a pathogen, altered antibiotic-resistance patterns, altered tropism or host specificity, colonisation,

e) known or predicted involvement in biogeochemical processes.

3.3. Initial classification of the GMM

Points 3 to 5 of Section B indicate that the first stage of the risk assessment process for a GMM is to identify the potential harmful properties of the GMM, to determine an initial classification for the GMM. This is achieved by the identification of hazards associated with the recipient, donor organism, vector and insert where appropriate. The corresponding set of containment and other protection measures indicated in Annex 2 are used as a reference set of measures to determine whether more stringent containment and control measures are required to control identified harmful effects.

The risk of harm arising from any harmful property of the GMM is obtained by the consideration of the severity of the harm and any biological properties (e.g. disabling mutations) which limit the possibility of harm occurring. The estimation of the severity of the harmful effects is performed independently of the possibility of the harmful effect occurring. The severity of any possible harm is determined by considering what the result could be, not

whether it is likely to occur in the particular case. For instance, for a pathogen you would estimate how serious the disease would be assuming that the susceptible species was infected. The allocation of the GMM to an initial class includes consideration of severity. Classification schemes take severity into account. However many schemes are based only on either human health or environmental considerations. Care must be taken to ensure that the severity of harmful effects on human health and the environment from the GMM have been fully considered.

3.4. Assessment of possibility of harmful effects occurring

The key factor that affects the possibility of a harmful event occurring is the level and nature of exposure of humans or the environment to a particular GMM. Exposure is, in most cases, of primary importance to risk assessment as it will often determine whether a harmful effect could occur. The possibility of humans or the environment being exposed to a GMM depends upon what operations are being carried out (for example the scale of the operations) and the containment measures appropriate to the initial classification applied to the work.

The characteristics of the operation be taken into account when making the final classification and selection of control measures. The nature and scale of the activity need to be considered in order to estimate the possibility of exposure of humans and the environment and will also affect the choice of appropriate risk management procedures.

The characteristics of the operation that could affect the risk assessment and so should be taken into account as appropriate include the actual activities to be undertaken, work practices, scale and containment measures applied.

The assessment should especially take into account the question of disposal of waste and effluents. Where appropriate, the necessary safety measures should be implemented in order to protect human health and the environment.

3.4.1. Nature of activities to be undertaken

The degree of risk and application of control measures to reduce that risk from the GMM to an appropriate level will be influenced by the nature of the activities to be undertaken, since these will affect human and environmental exposure and hence possibility of harm occurring.

The nature of the activities will also determine which table in Annex 2 has the most appropriate containment and control measures to be considered.

In practice, for laboratory scale work where the effect of standard laboratory procedures on exposure are well known, detailed risk assessment of each individual procedure would be unlikely to be required unless a highly hazardous organism was being used. More detailed consideration however may be necessary for non-routine procedures or procedures which might have a significant effect on the degree of risk, for example, procedures which generate aerosols.

3.4.2. Concentration and scale

The density of a culture can lead to a risk of exposure to high concentrations of the GMM, particularly in downstream processing operations. The effect of concentration on the possibility of a harmful event occurring must be considered.

Scale is also a factor that must be taken into account in the risk assessment. Scale may be in terms of the absolute volume of a single operation or the frequent repetition of a process, because both could give rise to an increased possibility of exposure if the containment and control measures failed and thus affect the possibility of a harmful event occurring.

While large scale does not necessarily mean high risk, increased scale may lead to an increased possibility of exposure both in terms of the number of humans and the amount of environmental exposure that might occur in the event of containment failure.

Scale will also influence which table in Annex 2 has the most appropriate containment and control measures to be considered.

3.4.3. Culture conditions

In many contained use activities, the culture conditions are rigorously contained to protect the work, however, the nature and design of the growth vessels or other culture equipment will also influence the degree of risk to human health and the environment. Highly engineered and sealed fermentation vessels can significantly reduce exposure and hence risk from a GMM. Consideration of reliability and possible failure rates for such equipment is important where failure could lead to high levels of exposure to harmful GMMs. Where such loss is reasonably foreseeable, additional containment measures may be required. The standard operating procedures of individuals undertaking work with cultured GMMs such as centrifugation or sonication will have a significant impact on the effectiveness of any containment measures employed.

In combination with physical culture conditions that act as containment measures, both biological and chemical measures that are employed to protect the work can also contribute significantly to the containment measures that may be required. Examples of biological containment could well be auxotrophic mutants that require specific growth factors to be supplied to grow. Examples of chemical containment measures could be disinfectant solutions maintained in drainage systems.

The characteristics of the environment likely to be exposed and the severity of the effect be taken into account when assessing the possibility of harmful effects occurring and their severity.

There are a number of aspects to this consideration of the environment that are important, such as the extent and nature of environmental exposure and whether there are biota which can be adversely affected by the particular GMM in the area exposed.

The following factors should be considered, as appropriate, when assessing how the characteristics of the receiving environment will affect the possibility that the potentially harmful effect will be realised and hence the level of risk and selection of control measures.

3.4.3.1. Environment likely to be exposed

The environment likely to be exposed will in most cases probably be limited to the workplace environment and the area immediately surrounding the facility, but depending on the specific characteristics of the contained use and the facility, a wider environment may need to be considered. The extent of the environmental exposure may be influenced by the nature and scale of the activity, but consideration should also be given to all possible modes of transmission into the wider environment. These can include physical modes (such as local drains, watercourses, waste disposal, air movement) and biological vectors (such as movement of infected animals and insects).

3.4.3.2. Presence of susceptible species

The possibility of harm actually occurring will depend on whether there are susceptible species, including humans, animals or plants, in the environment that is likely to be exposed.

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

The extent to which the GMM can survive and persist in the environment is a strong consideration in the risk assessment. The possibility of harm occurring will be significantly reduced if a GMM cannot survive in the environment to which it might gain access.

3.4.3.4. Effects on the physical environment

In addition to direct harmful effects of a GMM, indirect harmful effects from significantly altering the physico-chemical properties and/or ecological balance of the soil or water components of the environment must be considered.

4. Procedure 2

4.1. Determination of final classification and containment measures

When all potentially harmful characteristics have been reviewed for their severity and possibility of occurrence, with the effect of the containment and control measures indicated by the initial classification of the recipient considered, the final classification and containment

measures for the GMM can be determined. In considering the final classification and containment measures, the initial classification should be revisited to determine if it was correct bearing in mind the activities and characteristics of the operations proposed. A comparison of the initial classification and associated containment measures with the final class and containment requirements can give rise to three results:

a) there are harmful effects which are not adequately taken into account in the initial classification, these would not be adequately contained by the provisional containment considered under Procedure 1. This would require the application of additional containment measures and possibly revision of the classification of the activity,

b) the initial classification was correct and the attendant containment measures adequately prevent or minimise harm to human health and the environment,

c) the initial classification is higher than the activity warrants and accordingly a lower classification with its attendant containment conditions would be appropriate.

4.2. Confirmation of adequacy of final containment measures

Once the proposed final classification and containment conditions have been determined, the level of human and environmental exposure should be reassessed (Procedure 1). This should confirm that the possibility of any harmful effects occurring, taking into account the nature and scale of the work and the proposed containment conditions are acceptably low. When this has been done the risk assessment process has been completed.

If the nature or scale of the work changes significantly or new scientific or technical knowledge becomes available, such that the risk assessment is no longer adequate, the risk assessment must be reviewed in the light of the changes. Any alteration in containment conditions indicated as a result of the review of the risk assessment must be applied forthwith to maintain adequate protection for human health and the environment.

The classification and the containment and control measures identified in the risk assessment as required to adequately contain the GMM during the proposed operations, leads to the classification of the contained use activities into classes 1 to 4. The containment and control measures for each class of contained use are detailed in Annex 2.

The classification of the contained use activities for the GMM defines the administration requirements.

If there are any uncertainties in the final classification and containment conditions, it is advisable to contact the competent authority.