GENETICALLY MODIFIED MICRO-ORGANISM (GMMs)

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Scope and definitions

The work carried out in biological risk environments may involve the contained use of genetically modified micro-organisms (GMMs). This type of work is regulated by Legislative Decree 206/2001, pursuant to Legislative Decree 81/08, repealing Legislative Decree no. 91 dated 03 March 1993.
The law defines a genetically modified micro-organism (GMM) as a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Within the terms of this definition:

1) Genetic modification occurs at least through the use of the techniques listed in Annex I, Part A, i.e.

- Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.
- Techniques involving the direct introduction into a micro-organism of heritable material prepared outside the micro-organism, including micro-injection, macro-injection and micro-encapsulation.
- Cell fusion or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

2) The techniques listed in Annex I, Part B, i.e.

- in vitro fertilisation
- natural processes such as: conjugation, transduction, transformation
- polyploidy induction

are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified micro-organisms.

The law also defines the installation owner as the employer, within the meaning of Legislative Decree 81/08, as amended, and the user as the scientific supervisor and manager of the contained use of GMMs. Within the deadlines provided, the user or installation owner must submit a notification to the Ministry of Health, together with the documentation containing the information required pursuant to Legislative Decree 206/01.

Exclusions from the scope of Legislative Decree 206/01

The following are excluded from the scope of Legislative Decree 206/01:

1) Genetic modification resulting from any of the following techniques:

- Mutagenesis
- Cell fusion (including protoplast fusion) of prokaryotic species that exchange genetic material through known physiological processes
- Cell fusion (including protoplast fusion) of eukaryotic species, including the production of hybrids and the fusion of plant cells
- Self-cloning consisting in the removal of nucleic acid sequences from a cell of an organism which may or may not be followed by reinsertion of all or part of that nucleic acid (or a synthetic equivalent) with or without prior enzymic or mechanical steps, into cells of the same species or into cells of phylogenetically closely related species which can exchange genetic material by natural physiological processes where the resulting micro-organism is unlikely to cause disease to humans, animals or plants. Self-cloning may include the use of recombinant vectors with an extended history of safe use in the particular micro-organisms

on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified micro-organisms.

2) Contained GMM uses that meet the following requirements, i.e. can be considered safe:

GENERAL CRITERIA

Strain verification/authentication

- Identity of the strain must be precisely established
- Modification must be known and verified

Documented and established evidence of safety

- Documented evidence of the safety of the organism must be provided

Genetic stability

- Where any instability could adversely affect safety, evidence of stability is required

SPECIFIC CRITERIA

Non-pathogenic

- The GMM should not be capable of causing disease or harm to a healthy human, plant or animal. Since pathogenicity includes both toxigenicity and allergenicity, the GMM should therefore be:

- Non-toxigenic: The GMM should not produce toxigenicity as a result of the genetic modification nor be noted for its toxigenic properties
- Non-allergenic: The GMM should not produce allergenicity as a result of the genetic modification nor be a noted allergen, having, for example, allergenicity comparable in particular with that of the micro-organisms identified in Legislative Decree 81/08, as amended

No harmful adventitious agents

- The GMM should not harbour known harmful adventitious agents such as other micro-organisms, active or latent, existing in the culture medium or inside the GMM, that could cause harm to human health and the environment

Transfer of genetic material
- The modified genetic material must not give rise to harm if transferred; nor should it be self-transmissible or transferable at a frequency greater than other genes of the recipient or parental micro-organism

Safety for the environment in the event of a significant and unintended release
- GMMs must not produce adverse effects on the environment, immediate or delayed.

The aim of these requirements is to establish the safety of GMMs for human health and the environment. The GMMs that meet all of these criteria will be added on a case-by-case basis to a list excluded from the scope of Legislative Decree 206/01.

3) The transport of GMMs by road, rail, inland waterway, sea or air

4) The storage, culture, transport, destruction, disposal or use of GMMs which have been placed on the market in accordance with Legislative Decree 92/93 (Implementing Directive 90/220/EEC on the deliberate release into the environment of genetically modified organisms) and Regulation (EEC) No 2309/93 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Agency for the Evaluation of Medicinal Products.

Risk assessment

The risk assessment requires consideration of potentially harmful effects to human health and the environment, taking into account both the nature and scale of operations, to determine the final containment facilities required. 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 degree of risk arising from contained uses with a genetically modified micro-organism, 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 full risk assessment process consists of two procedures outlined below (1 and 2), to be carried out one after another.

Procedure 1

Identify potential harmful properties (hazard) of the GMM and allocate the GMM to an initial class (1 to 4) taking into account the potential harmful effects. This allows for the assessment of the 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.
Identification of harmful properties (hazard) of the GMM

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.
Consideration will be given to:

- The recipient organism
- The donor organism
- The characteristics and location of inserted genetic material and any vectors:

The recipient organism

- Nature of pathogenicity and virulence, infectivity, allergenicity, toxicity and vectors of disease transmission
- Nature of indigenous vectors and adventitious agents, where they could mobilise the inserted genetic material, and the frequency of mobilisation
- Nature and stability of disabling mutations, if any
- Host range (if relevant)
- Any significant physiological traits which may be altered in the final GMM and if relevant their stability; natural habitat and geographic distribution
- Significant involvement in environmental processes (such as nitrogen fixation or pH regulation)
- Interaction with, and effects on, other organisms in the environment (including likely competitive, pathogenic or symbiotic properties)
- Ability to form survival structures (such as spores or sclerotia).

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

- Nature of pathogenicity and virulence, infectivity, toxicity and vectors of disease transmission
- Nature of indigenous vectors
- Sequence
- Frequency of mobilisation and specificity
- Presence of genes which confer resistance to anti-microbials including antibiotics
- Host range
- Other relevant physiological traits

The insert

- Specific identity and function of the insert (genes)
- Level of expression of inserted genetic material
- Source of the genetic material, identity of the donor organism(s) and characteristics where appropriate
- History of prior genetic modifications if appropriate
- Location of inserted genetic material (possibility of insertional activation/deactivation of host genes).

The vector

- Nature and source of the vector
- Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified micro-organism

- (If present in the final GMM) frequency of mobilisation of inserted vector and/or capability for transfer of genetic material

The resulting GMM:

1) Human health considerations

- Expected toxic or allergenic effects of the GMM and/or its metabolic products
- Comparison of the modified micro-organism to the recipient or (where appropriate) parental organism regarding pathogenicity
- Expected capacity for colonisation
- If the micro-organism is pathogenic to humans who are immunocompetent
- Diseases caused and mechanism of transmission
- Invasiveness and virulence
- Infective dose
- Possible alteration of route of infection or tissue specificity
- Possibility of survival outside of human host
- Biological stability
- Antibiotic-resistance patterns
- Allergenicity
- Toxigenicity
- Availability of appropriate therapies and prophylactic measures

2) Environmental considerations

- Ecosystems to which the micro-organism could be unintentionally released from the contained use
- Expected survivability, multiplication and extent of dissemination of the modified micro-organism in the identified ecosystems
- 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
- 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
- Known or predicted involvement in biogeochemical processes.

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.

Initial classification of the GMM

Following the assessment of risks associated with the recipient organism, donor organism, vector, etc., considering the estimated harmful effects independently of the possibility of the harmful effect occurring, the GMM can be allocated to an initial class (1 to 4). At this stage, 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. Then, the possibility of harmful effects occurring will be assessed.

Assessment of possibility of harmful effects occurring

The key factor to be considered in assessing the possibility of a harmful event occurring is the level and nature of exposure of humans or the environment to a particular GMM.
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. 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.

Nature of activities to be undertaken
For laboratory scale work where the effect of standard laboratory procedures on exposure is 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.

Concentration and scale
The density of a culture can lead to a risk of exposure to high concentrations of the GMM, so the effect of concentration on the possibility of a harmful event occurring must be considered. Another important factor is scale, which 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.

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. In addition to using the most suitable work equipment, consideration must be given to reliability and possible failure rates for such equipment, 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 (SOPs) 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.
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. Other aspects that should be considered, as appropriate, when assessing how potentially harmful effects may impact the environment, and therefore the level of risk and the choice of containment measures, are listed below.

Environment likely to be exposed
In some cases, a wider environment than the workplace and the immediately surrounding area may need to be considered as likely to be exposed. The estimate of the exposure may be

influenced by the nature and scale of the activity, as well as by 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).

Presence of susceptible species
The possibility of harm occurring can also depend on the presence of susceptible species (including humans, animals or plants) in the environment that is likely to be exposed.

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

Effects on the physical environment
Both direct and indirect harmful effects of a GMM from significantly altering the physico-chemical properties and/or ecological balance of the soil or water components of the environment must be considered.

Procedure 2
When all effects 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. A comparison of the initial classification and associated containment measures with the final classification and containment can give rise to three results:

- 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. The application of additional containment measures and possibly revision of the classification of the activity are required
- The initial classification was correct and the attendant containment measures adequately prevent or minimise harm to human health and the environment
- The initial classification is higher than the activity warrants and accordingly a lower classification with its attendant containment conditions would be appropriate.

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, is acceptably low. When this has been done the risk assessment process has been completed.

Classification

After completing both risk assessment procedures, the GMM in question will be allocated to a certain risk class and the corresponding containment measures will apply. Pursuant to the law, classification is as follows:
Class 1: Contained uses of no or negligible risk, that is to say activities for which level 1 containment is appropriate to protect human health and the environment;

Class 2: Contained uses of low risk, that is to say activities for which level 2 containment is appropriate to protect human health and the environment;
Class 3: Contained uses of moderate risk, that is to say activities for which level 3 containment is appropriate to protect human health and the environment;
Class 4: Contained uses of high risk, that is to say activities for which level 4 containment is appropriate to protect human health and the environment.

Where there is doubt as to which class is appropriate, the higher class will be applied.

Installation notification and contained use notification

An installation owner who intends to carry out activities using GMMs for the first time must, before commencing such use, submit a notification to the Ministry of Health (and, for information, to the concerned Region or Autonomous Province) containing at least the following information:

- Name and qualifications of installation owner
- Name of Prevention and Protection Service Manager, information on their training and qualifications
- Details of the institution and, within the Prevention and Protection Service, of any biological committees or subcommittees or of the person responsible for biological safety
- Address and description of the installation, with an indication of the number or rooms and the corresponding ID numbers, as well as, for uses in classes 2, 3 and 4, a map of the premises
- Class(es) of contained uses that can be carried out at the installation, with an indication of the containment level of each room
- List and technical description of the equipment
- Only for class 1 contained uses, a summary of the risk assessment and information on waste management

In the absence of any indication to the contrary from the Ministry of Health, the approval for a class 1 GMM installation will be deemed as granted 45 days after notification, while class 2, 3 and 4 installations will require prior and express consent from the Ministry.

Contained use notifications vary depending on the GMM class to be used at the installation. Contained use notifications must be submitted by the user to the Ministry of Health and, for information, to the installation owner.

Information required for the contained use notification of class 2 GMMs

- Date of submission and approval of the installation notification
- Name and curriculum vitae of user
- Recipient and donor micro-organism(s) and, where applicable, host-vector system(s)
- Source(s) and the intended function(s) of the genetic material(s) involved in the modification(s)
- Identity and characteristics of the GMM
- Approximate culture volumes to be used
- Description of the containment and other protective measures to be applied, including information about waste management (treatment, final form and destination)

- Purpose of the contained use, including the expected results
- Expected duration of the contained use
- Summary of risk assessment
- Information necessary for preparing emergency response plans
- Proof of disclosure by the user of the contained use notification to the installation owner, unless they are the same person

Information required for the contained use notification of class 3 and 4 GMMs
In addition to the above information:

- Description of the parts of the installation to be used for contained uses
- Information about fire prevention and emergency response plans, if any (any specific hazards arising from the location of the installation, the preventive measures applied, such as safety equipment, alarm systems, containment methods, etc., the procedures and plans for verifying the continuing effectiveness of the containment measures, a description of information provided to workers, the information necessary for preparing emergency response plans)
- Copy of risk assessment

While in the absence of any indication to the contrary the approval for class 2 contained uses will be deemed as granted 60 days after notification, class 3 and 4 contained uses will require prior and express consent from the Ministry of Health, which will also forward such approval to the installation owner as well as to the concerned Region or Autonomous Province.

Obligations and formalities

All the assessments carried out must be gathered in a risk assessment document prepared by the user (head of research) and handed out to the installation owner (head of structure), who must keep it on the premises.

Furthermore, the user must:

- Make sure that the provided containment measures are applied
- Keep the record of the operations carried out
- Review the containment and other protective measures applied periodically – i.e. every three years for class 1 and 2 contained uses and every year for class 3 and 4 contained uses –, and forthwith if there is reason to suspect that the containment measures applied are no longer appropriate judged in the light of new knowledge, or in the event of an accident, or on the reasoned request of the Ministry of Health. For class 3 and 4 contained uses, the Ministry of Health must be notified of the review. Following the review, the user must prepare a document and hand it out to the installation owner.

The installation owner must:

- Retain the review document referred to above
- Make the document available on request to the Ministry of Health, Ministry of the Environment, Ministry of Labour and Social Security, and supervisory bodies.