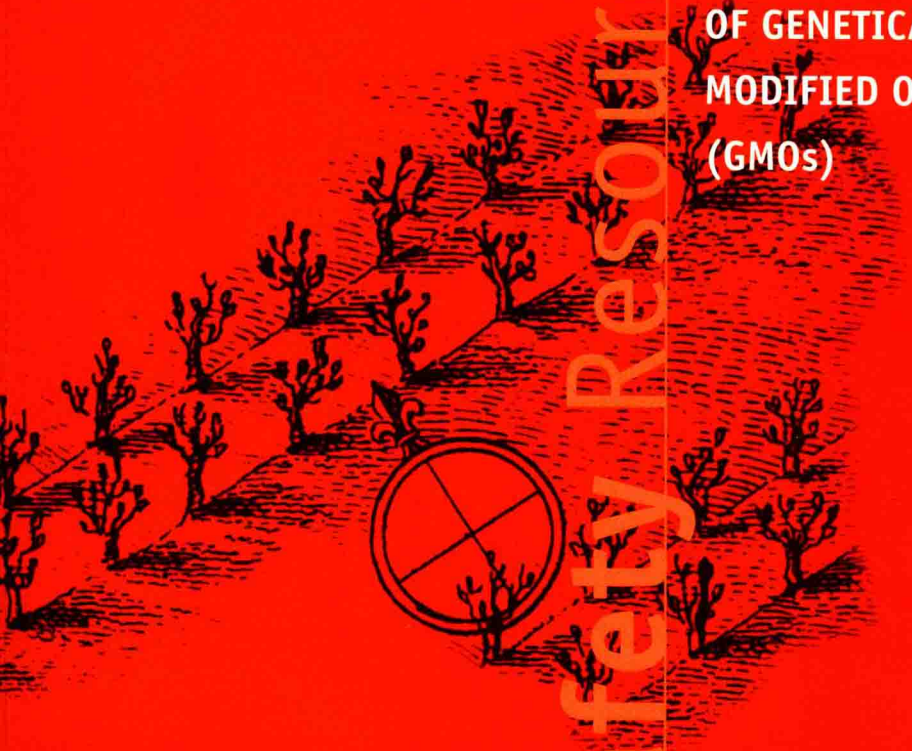
MODULE

d

Biosafety Resource Book

TEST AND POST-RELEASE MONITORING OF GENETICALLY MODIFIED ORGANISMS (GMOs)



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LIST OF ABBREVIATIONS

BLP	biosafety level for plants
Bt	*Bacillus thuringiensis*
CBD	Convention on Biological Diversity
CPB	Cartagena Protocol on Biosafety
DNA	deoxyribonucleic acid
EU	European Union
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
ERA	environmental risk assessment
GMHP	genetically modified higher plant
GMM	genetically modified micro-organism
GMO	genetically modified organism
GPS	global positioning system
GURT	genetic use restriction technology
HEPA	high efficiency particulate air
mRNA	messenger RNA
NIH	National Institutes of Health
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
pH	logarithmic measure of acidity/alkalinity of a solution
RNA	ribonucleic acid
SOP	standard operating procedures
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

From the initial research and development of a genetically modified organism (GMO) to its commercial release and placing on the market three different stages, each with specific **biosafety requirements**, can be defined and need to be passed. Namely, these include use of the GMO under containment, confined and limited field trials, and post-release monitoring of the GMO. The specific objectives, procedures and requirements of each of these three areas will be described in detail in this module.

BIOSAFETY REQUIREMENTS
Specific biosafety requirements exist for each stage of a GMO operation; biosafety can be defined as "the avoidance of risk to human health and safety, and the conservation of the environment, as a result of the use for research and commerce of infectious or genetically modified organisms." (FAO, 2001).

GMOs are not static entities, but are living organisms and as such show all attributes of life: they interact with their environment in a variety of ways, they might show unanticipated effects, they are subject to evolutionary processes, and they follow ecological and biological rules in the same way as every other living organism. The behaviour and attributes of a GMO as well as its interaction with the environment must therefore be considered as dynamic and subject to change over time. This requires careful assessment and evaluation of the potential risks posed by the release of a GMO.

Spanning the entire process from the initial research and development of a GMO to its commercial release and placing on the market, a huge amount of information on the GMO needs to be gathered and evaluated. Detailed information is required in order to assess and predict the (agricultural) performance and benefits of the GMO and, most importantly, the risks it poses to human health and environment. A list of recommendations concerning information that should be collected prior to the commercial release of a GMO is provided in Annex 11.

This extensive evaluation and assessment procedure is a bottom-up, iterative process:

» At early research and development stages, no evidence regarding the behaviour and performance of the engineered GMO is available. However, it might be possible to predict to a certain extent such information, including on potential risks, based on the characteristics of the non-modified, recipient organism and the traits encoded by the inserted transgene(s). Once the GMO has been obtained, it can be subjected to laboratory tests to gain information on its characteristics and behaviour under controlled conditions. All research, development and laboratory or greenhouse testing procedures are performed under *Containment*. Containment means that all contact of genetically modified material or organisms with the external environment is prevented, to the extent required by the risks posed by that material or organism. This is usually achieved by a combination of physical and biological barriers.

» If the performance of the GMO under containment is promising and the potential risks it poses are found to be manageable, the testing can proceed to *confined field trials*. Here, the GMO is tested in the open environment, preferably under conditions that resemble its future area of use. However, stringent measures are put in place to confine the release, i.e. to prevent any escape of the GMO or the transgene into the environment and to prevent genetically modified (GM) material from entering human or animal food supplies. Confined field trials are repeated at different scales until all the needed information is acquired.

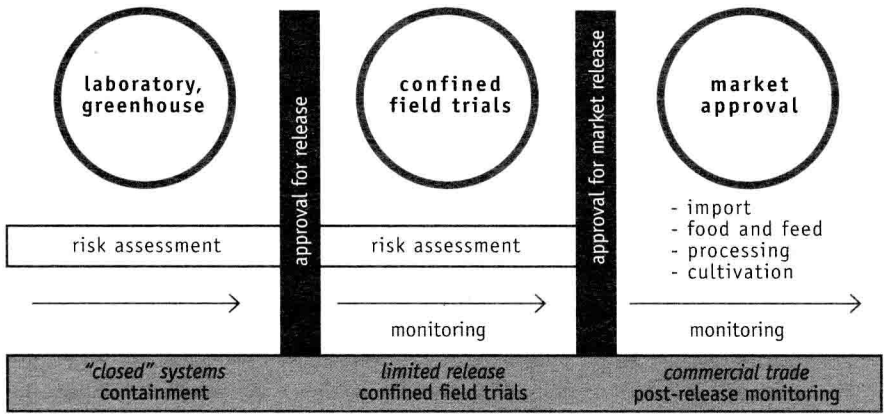
» Once a GMO has passed all testing stages, the risk analysis has been performed with a positive outcome and the approval from the responsible national or international authority has been granted, it may be placed upon the market and released into the environment. From this point on, no measures are put in place that limit the contact between the GMO and the receiving environment, even if specific risk management measures can be requested by the national biosafety authorities. However, it is important to implement *post-release monitoring* procedures to monitor the risks identified in the risk assessment of the GMO, recognize possible new, unanticipated risks and adverse effects, and to quantify the performance and benefits of the GMO. The overall goal of a monitoring programme should be the protection of the productivity and ecological integrity of farming systems, the general environment and human and animal health.

It should be noted that the objectives and procedures as well as the requirements (in terms of financial and organizational inputs, human capacity, infrastructure and equipment) of the three stages can be very different. As mentioned above, the evaluation of a GMO is a **bottom-up, iterative process**: each stage builds upon the information obtained in the previous stages, and possibly provides information that feeds back into these previous stages (Figure 1.1). The ultimate goals of the entire process are to reduce potential risks and prevent potential adverse effects of a GMO on human health and the environment to the maximum extent possible while the risks are not fully understood, to assess and evaluate the risks once they have been identified, and to monitor the manifestation of those risks and potential adverse effects as well as the occurrence of novel, previously unidentified risks once the GMO is released. The objectives, procedures and requirements of each stage are presented in detail in the following chapters. In addition, two small chapters introduce concepts and procedures for GMO traceability, labelling, import and transboundary movements. Thus, all major aspects of GMO deployment, from research and development to market release and international trade, are covered and introduced within this module.

BOTTOM-UP, ITERATIVE PROCESS
The evaluation of a GMO can be described as a bottom-up, iterative process: each evaluation stage during the development, testing and commercial release of a GMO builds upon information obtained during the previous stages, and generates information that feeds back into these previous stages.

Figure 1.1 | **The relation between containment, confined field trials and post-release monitoring of GMOs**

This module will focus on the technical aspects of these processes; for a detailed introduction to the legal background and extensive international frameworks that regulate these processes please refer to Module E: Legal Aspects.



Adapted from: Züghart *et al.*, 2008.

CHAPTER 2

TESTING OF GMOs UNDER CONTAINMENT

Containment, or **contained use**, refers to measures and protocols applied to reduce contact of GMOs or pathogens with the external environment in order to limit their possible negative consequences on human health and the environment (FAO, 2001). Containment measures have to be adjusted to the highest level of risk associated with the experiment, especially when the risk category of the material being worked with is not certain. The risk associated with each GMO should be assessed on a case-by-case basis; accordingly, GMOs are classified into four different risk groups in relation to the risks they pose (see below).

CONTAINED USE
Contained use means any activity in which organisms are genetically modified or in which such GMOs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment (EU, 1998).

Containment can be achieved by a combination of physical containment structures and safe work procedures (also referred to as good laboratory practices). As an additional feature, biological containment can be included, i.e. "built-in" features of the organism being worked with that prevent its spread, survival or reproduction in the external environment (see Box 2.2). Appropriate containment measures should be applied at each stage of an experiment involving GMOs to avoid release into the external environment and prevent harmful events. This overall objective of a containment system is always the same, however the actual measures that are required can differ, depending on the organisms being worked with (micro-organisms, plants, animals), the scale of the application (large-scale versus small-scale), the research setting (laboratory, greenhouse) and of course the risk classification of the GMOs.

CONTAINMENT FACILITY
The containment facility is the primary structure that ensures containment, by providing physical barriers that limit dissemination of GMO material into the environment into the extent required by the risk posed by the material.

The basic structure of a **containment facility** must meet minimum standards appropriate for the category of risk of the work being conducted. Establishment of the basic minimum structure, adherence to general safety requirements and adoption of good laboratory practices specified for a certain risk group enable any work identified as part of that risk group to be performed within that facility. Therefore, the first step in any operation dealing with GMOs is to classify the GMO and the associated work procedures into one of the four risk groups. Subsequently, one can easily identify the required minimum facility features and good laboratory practices associated with that risk group, and check if the facility that is designated to be used and the standard operating procedures (SOP) for the personnel that are in place comply with these requirements.

2.1 RISK CLASSIFICATION

RISK CLASSIFICATION
A risk classification is the first step that should be performed prior to any GMO operation under containment: The GMO should be classified into one of four risk classes, which dictate the required containment level.

The most common risk classification system is based on four different risk groups, associated with four different biosafety levels (WHO, 2004; NIH, 2009; please refer to Module C: Risk Analysis for a detailed introduction to the topic). Risk groups 1 to 4 represent increasing risk to human health and the environment, similarly biosafety levels 1 to 4 represent increasing strength in the containment measures required to prevent dissemination and spread of the organisms being worked with.

To establish the classification of a GMO, a comprehensive risk assessment should be performed on a case-by-case basis. An initial assessment can be made by classifying an organism according to the following criteria (NIH, 2009):

» Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans.
» Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.
» Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

» Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Subsequently, a comprehensive **risk assessment** should take a detailed look at the organism and the type of genetic manipulation that it is subjected to; factors to be taken into consideration include virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, laboratory operations, quantity being worked with, availability of vaccine or treatment and gene product effects such as toxicity, physiological activity, and allergenicity (NIH, 2009). Such considerations should result in a classification of the organism/project into one of the four risk groups, which also defines the containment level that applies (usually the containment level is the same as the risk group). It should be noted that, to a certain extent, this is a subjective process dependent on the individual researcher/biosafety manager performing the classification.

RISK ASSESSMENT
In order to establish the GMO risk classification a risk assessment needs to be performed, taking into account all relevant characteristics of the organism being worked with and the intended genetic modification(s).

Furthermore, the above-listed criteria are only of limited value when GMOs with a proposed use in agriculture need to be evaluated, because in those cases the potential adverse effects on the environment need to be taken into consideration, in addition to the effects on human health. Detailed lists of factors that need to be evaluated for each organism group (micro-organisms, plants and animals) in order to establish a risk group classification and also define appropriate containment levels can be found in the sections on each organism group below.

2.2 ALTERNATIVE RISK CLASSIFICATION SCHEMES

ALTERNATIVE RISK CLASSIFICATION SCHEMES
Several alternative GMO risk classification schemes exist; however, the four-risk-class system is nowadays widely recognized for classifying GMO operations under containment.

An alternative GMO classification scheme, which is often found in older legislative documents (e.g. EU, 1990) is based on the classification of GMO operations as either type A or type B. Type A is defined as small-scale operations (generally less than 10 litre culture volume) of a non-commercial, non-industrial type, although they can include research and development processes necessary for

BOX 2.1

GENETIC MODIFICATION TECHNIQUES THAT REQUIRE CONTAINMENT

In general, all work that involves recombinant DNA molecules should be performed under containment. For example, the scope of the NIH guidelines is defined as "to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules."

In this sense, recombinant DNA molecules are defined as "(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above." (NIH, 2009).

Similarly, Council Directive 2001/18/EC (EU, 2001) defines genetic modification, and thus the need for containment measures, as a result of the following techniques:

"(1) 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;

(2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;

(3) cell fusion (including protoplast 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."

subsequent industrial exploitation. All activities that are not considered to be of type A are automatically classified as type B. This generally implies that the activities take place on an industrial scale and involve production processes and large volumes of material.

In addition to the classification of operations into types A and B, GMOs can be classified into Groups I and II. Group I GMOs are those that meet the following criteria:

» the donor organisms from which the gene or genes derive (parent) do not cause diseases in humans, animals or plants;
» the nature of the vector used in the transformation process is such that it is unlikely to acquire the capacity to produce disease;
» it is unlikely that the resulting GMO can cause disease or adverse effects on the environment.

All GMOs that do not fall into Group I are automatically included in Group II. Such organisms are intrinsic pathogens or have been modified so that they are potential pathogens of humans, animals or plants. However, it is recommended that the risk classification scheme based on the four risk groups described above, together with the four resulting biosafety levels, should be applied. This system is the internationally recognized and accepted system to classify the risks and containment measures for any operation involving recombinant DNA molecules and GMOs.

2.3 NOTIFICATIONS, RECORDS AND EMERGENCIES

2.3.1 Notifications and records

NOTIFICATIONS AND RECORDS
Any GMO operation under containment should be notified to the relevant national competent authority; detailed records of such operations should be prepared and kept.

Any operation that falls under the categories specified in Box 2.1 should be notified to the competent national authority, if such an authority exists. It is recommended that the person wishing to perform operations involving GMOs under containment submits a notification to the competent authority before undertaking such an operation for the first time. This should allow the competent authority to verify that the proposed facility to carry out the operation is appropriate, i.e. that the relevant containment measures are met. The competent authority should confirm that the containment measures and SOPs proposed for the operation limit the hazard to human health and the environment to the required extent.

Any GMO operation should be well documented and the records need to be kept and made available to the competent authority on request. A time span of ten years of record-keeping after the operation has finished is suggested.

2.3.2 Accidents and emergencies

ACCIDENT
An unintentional release of GMOs which presents an immediate or delayed hazard to human health and the environment.

In the event of an **accident**, defined as an unintentional release of GMOs which presents an immediate or delayed hazard to human health or the environment, during the course of the operation, the responsible person should immediately notify the competent authority and provide information that is required to evaluate the impact of the accident and to adopt appropriate counteractions. The information that should be provided includes (EU, 1990):

- the circumstances of the accident;
- the identity and quantities of the released GMO(s);
- any information required to evaluate the effects of the accident on human health and the environment;
- the emergency measures taken.