MODULE d

Biosafety Resource Book

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

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Rome, 2011

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MODULE d

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

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.

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.

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).

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.

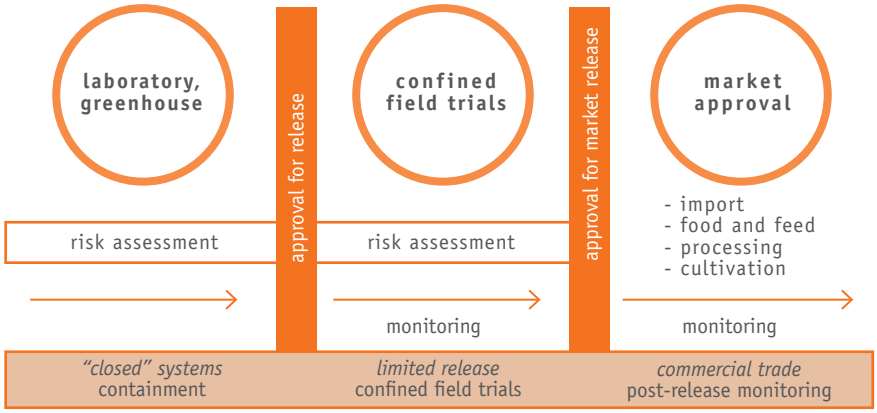
» Once a GMO has passed all testing stages, the risk analysis has been performed with a positive outcome and the approval from the responsible national or international authority has been granted, it may be placed upon the market and released into the environment. From this point on, no measures are 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).

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.

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 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.

Information on the occurrence of an accident and the required countermeasures should also be distributed to the general public. Subsequently, an analysis of the causes of the accident as well as of the effectiveness of countermeasures taken should be performed, in order to avoid similar accidents in the future and improve, if necessary, the available countermeasures.

Emergency plans should be developed prior to starting any operation in order to effectively deal with any possible accident and limit the hazard to human health and the environment to the maximum extent possible. The competent authority should ensure that such emergency plans are prepared prior to the operation, that information on safety measures in case of an accident are supplied to persons likely to be affected by the accident and that such information is publicly available (EU, 1990).

EMERGENCY PLANS
In order to react quickly and effectively in case an accident occurs, emergency plans should be developed prior to any GMO operation under containment.

Specifically, the plan should indicate:

- procedures to control the GMO in case of unexpected spread;
- methods to decontaminate or eliminate the effects of an accident;
- methods for disposal or sanitation of plants, animals, soils, etc. that were exposed during the accident or spread.

2.3.3 Other administrative tasks and procedures

In order to allow quick and reliable analysis of whether or not the required safety standards for the biological agent/GMO in question are being followed and met, a checklist should be developed that includes all necessary protocols, safety procedures and facility design parameters. This checklist, or questionnaire, should be prepared in relation to the prescribed biosafety level of the operation. Careful use of such a checklist by the operating personnel and entry of all relevant information should help to maintain the required containment level, avoid unsafe working procedures and identify safety gaps in the experimental design or the design of the facility.

All stipulated regulations, if they are followed properly, will result in meeting the required containment level. An assessment of the training of workers and managers of the containment facility should also be included.

Furthermore, it is recommended that the risk assessment of the GMO operation be revised and updated on a regular basis or when the initial risk assessment is no longer valid. Reasons for this could include changes in the operation (e.g. the scale, available containment measures, changes in work procedures) or the accumulation of new information concerning the organism being worked with that may have significant impact on the risk assessment. Records of the new risk assessment should be kept and the competent authority should be informed of any changes regarding the risk assessment and the applied containment measures.

TRAINING AND SUPERVISION
To ensure safety of personnel working in a containment facility and prevent accidents, regular training and detailed supervision of personnel should be provided.

Regular **training and supervision** should be provided to all personnel involved in the GMO operation. Personnel should be competent to safely perform all working procedures and special care should be taken to ensure that new personnel are made familiar with all working procedures and use of laboratory equipment prior to commencing any work. Training should specifically focus on areas of potential risk as identified in the risk assessment of the GMO being worked with. In addition, all personnel working within the containment facility should be provided with regular health checks.

2.4 CONTAINMENT OF GENETICALLY MODIFIED MICRO-ORGANISMS (GMMS)

GMMS
Specific requirements exist for the risk assessment and containment measures when work with GM micro-organisms is performed.

For the scope of this document, micro-organisms shall be defined as “any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture“ (EU, 1998). This definition, therefore, includes bacteria, fungi, protozoans, algae and viruses as well as eukaryotic cell cultures, amongst others.

The general containment strategies and procedures described above also refer to micro-organisms. The characteristics of each GMM operation should be evaluated and result in a risk classification, which then dictates the containment measures required to ensure the protection of human health and the environment. In cases of uncertainty regarding the risk classification of a GMM operation higher containment measures, corresponding to a higher risk classification, should be applied.

The procedure for the risk assessment of GMMs is described in detail in Annex 1. The ultimate result of such a classification is the assignment of the operation to one of the four risk groups described below:

Class 1: Activities of no or negligible risk, that is to say activities for which level 1 containment is appropriate to protect human health as well as the environment.

Class 2: Activities of low risk, that is to say activities for which level 2 containment is appropriate to protect human health as well as the environment.

Class 3: Activities of moderate risk, that is to say activities for which level 3 containment is appropriate to protect human health as well as the environment.

Class 4: Activities of high risk, that is to say activities for which level 4 containment is appropriate to protect human health as well as the environment.

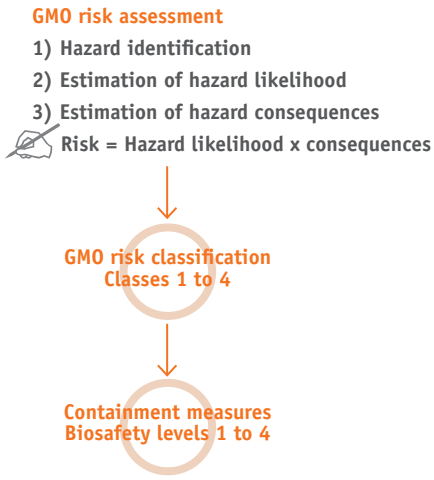
The assessment should also take into account the disposal of waste and effluents, and establish adequate safety measures to control these emissions. The containment levels and physical containment measures (often referred to as biosafety levels), which are appropriate for and correspond to each of the four risk classes described above, are described in detail in Annex 2. In addition to the physical containment measures, principles of good laboratory practice should be put in place and followed by all staff involved with the operation. Guidance for such principles is provided in Annex 3.

Furthermore, considerations concerning the characteristics of the likely receiving environment in case of an accident, the scale of the operation and employment of non-standard operations or equipment may alter the risk class of the operation and similarly affect the containment measures that need to be in place to control that risk level.

It is recommended that the GMM risk assessment and the applied containment level be reviewed on a periodic basis, especially if the containment measures employed are no longer suitable or the risk class of the operation has changed. This may also be the case when new scientific knowledge suggests that the initial risk assessment may be no longer correct.

Figure 2.1 | **The general workflow of the risk assessment, risk classification and adoption of the suitable containment level**

It should be noted that this scheme is not only valid for GMMs, but for every GMO operation that falls under containment requirements (i.e. including genetic modification of plants and animals).



2.5 CONTAINMENT OF GM PLANTS

In this document, plants shall be defined in a broad sense and include higher (vascular) plants, including their reproductive organs such as spores, pollen, seeds, tubers, bulbs, rhizomes, as well as mosses, ferns, algae and aquatic species. In general, the same principles for the risk assessment and containment classification that were laid out in the introduction and for GMMs are also valid for plants. However, the actual risks posed by GM plants and the required containment measures to control and limit these risks and potential hazards are, at least partially, different. The process of risk assessment and the implementation of appropriate containment measures for GM plants are described below.

GM PLANTS
In this document, plants shall be defined in a broad sense and include higher (vascular) plants, including their reproductive organs such as spores, pollen, seeds, tubers, bulbs, rhizomes, as well as mosses, ferns, algae and aquatic species.

2.5.1 Risk assessment for GM plants

In the case of GM plants, the risks posed to the environment are, in most cases, at least equally as important as the risks posed to human health. This is probably because most genetic modifications of plants, especially for envisaged use in agriculture, target growth, survival, herbicide tolerance or pest resistance characteristics, which usually have no implications for human health. Therefore, the risks posed to the environment if an escape of the GM plant were to occur need to be carefully assessed. However, if genetic modifications that target characteristics with possible implications on human health (toxic compounds, allergenic compounds, bioactive compounds in biopharming) are introduced, the risk assessment must pay due attention to these potential hazards.

GM PLANT RISK ASSESSMENT
The general principle for a GM plant risk assessment is identical to other GMO operations; however, for plants the potential adverse effects on the environment are in many cases the primary source of concern, which needs to be taken into account during the risk assessment.

The comprehensive **GM plant risk assessment** should consist of the following steps (Health and Safety Executive, 2007; see also Figure 2.1):

- identification of potential hazards and evaluation of the likelihood that these hazards are realized;
- evaluation of the consequences should these hazards be realized;

- assessment of the risk, i.e. the likelihood of hazard realization and estimated consequences;
- assignment of a risk group and assignment of containment measures appropriate for that risk group.

The detailed procedures and parameters to be taken into account when performing the risk assessment for GM plants are laid down in Annex 4. The ultimate objective of the risk assessment procedure is the assignment of the specific activity with a GM plant to one of four risk classes, and the concomitant definition of containment measures required to control and minimize the risks associated with that risk class.

BIOSAFETY LEVELS FOR PLANTS
Specific biosafety levels for plants, providing detailed information on required containment measures for GM plants, have been defined.

The four risk classes and associated containment measures, also known as **biosafety levels for plants** 1 to 4 (BL1-P to BL4-P) have been defined by NIH (NIH, 2009); brief descriptions of each level are provided below (adapted from Adair and Irwin, 2008). Biosafety levels constitute a combination of facility features and equipment, work practices and procedures, and administrative measures required to maintain a specified level of containment, with the aim of preventing contact between the material being worked with and the outside environment to the appropriate extent. A detailed table summarizing the exact containment measures associated with each biosafety level for plants is provided in Annex 5.

BL1-P: The lowest level of containment is recommended for GM plants for which evidence suggests that they are unable to survive and spread in the environment, and therefore do not pose an environmental risk.

BL2-P: Recommended for GM plants and associated organisms that could be viable in the receiving environment, but are assumed to have a negligible impact or could be easily managed; this includes GM plants with weedy characteristics or capable of interbreeding with related species in the environment.

BL3-P: Recommended for GM plants or associated organisms, including plant pathogens, that have a recognized potential for significant detrimental impact on the environment; this includes genes from exotic infectious agents, gene coding for vertebrate toxins, and plant-associated GM micro-organisms capable of causing environmental harm.

BL4-P: Recommended for readily transmissible exotic infectious agents, possibly in the presence of their arthropod vector, that are serious pathogens of major crops; also included are certain biopharming experiments in which bioactive compounds (e.g. vaccines) are produced in GM plants.

2.5.2 Containment measures for plant research facilities

Research on plants is regularly conducted in **greenhouses** – specialized structures with a transparent or translucent covering enabling the growth of plants inside a controlled environment. Such structures, and the concomitant work procedures, differ significantly from typical laboratory settings and require special considerations regarding containment.

GREENHOUSES
Research and testing of GM plants is regularly performed in greenhouses, specialized structures that allow plants to be grown inside and that require specific containment measures.

The primary objective of plant containment is environmental protection – at least when no risks to human health have been identified. In order to achieve this goal it is recommended to carefully consider all factors that might interfere with containment, including characteristics and behaviour of the organisms being worked with, organism interactions, conduct of experiments, facility (greenhouse) design and limitations, escape routes, and social (personnel-related) factors. A large variety of transport mechanisms for organisms – ranging from micro-organisms to plants – into and out of a containment facility exists, and likewise many opportunities for breaches of containment. These routes include air, water and soil, as well as via personnel (clothing, shoes, etc.), equipment, waste, or via small animal intruders.

Containment measures specifically for greenhouses directed against those factors are briefly described below, while the exact requirements for each plant biosafety level can be found in Annex 5.

- All personnel working in the facility should be familiar with the containment requirements and the work procedures to be followed; SOPs and a reference manual should be established and followed. Problems should be noted and investigated as soon as they become apparent. Routine access should be restricted.
- Care should be taken that dissemination of organisms through clothing, shoes etc. is prevented. Wearing laboratory coats and gloves is recommended even at lower biosafety levels where such measures are not compulsory.
- Physical containment is provided by the facility itself and by equipment employed within that facility; correct handling of the facility and the equipment is required to maintain containment.
- Signs advising of restricted experiments in progress, limited access, potential hazards and contact details of responsible persons should be in place.
- The capability of a greenhouse to isolate organisms from the surrounding environment, as well as to limit entrance of undesired organisms, is strongly affected by the type of glazing, sealing, screening, airflow system, air filtration and air pressure employed.
- Layering of containment measures, i.e. combining several physical measures or combining physical with biological containment measures, can significantly enhance containment (see Box 2.2).
- Special care should be taken when work involves plant-associated micro-organisms, whether or not they are genetically modified themselves. In such cases, the containment measures for micro-organisms should additionally be consulted.
- Storage of material (plant parts, cell culture, seeds) should preferably be performed in lockable repositories.

- Specific requirements exist for safe transfer of material into or out of the facility (use of closed containers, possibly in two layers).
- Prior to disposal, biological material (including soil) must be rendered inactive by validated means (autoclaving recommended).
- Periodic cleaning, as well as disinfection or decontamination of all surfaces or the entire facility should be performed, by means that are efficient for the target organism.
- A pest and undesired organism control programme should be in place; traps or bioindicators can be employed to monitor spread of pollen, insects or viruses etc.
- Alarm systems should be operational to indicate system failures due to technical, human or weather-caused errors and malfunctions.
- Records of experiments should be kept; greenhouses should be inspected periodically.
- Security measures to limit access of unauthorized persons should be in place (fencing, self-locking doors, sensors, security cameras, safety personnel, etc.).
- Researchers should be involved in the planning and design process of a greenhouse facility, since they have the most profound knowledge of the biological aspects of the work to be performed within that facility.
- The site of the facility should be chosen carefully, ideally in an environment that provides the lowest chance of survival and spread of escaped organisms.
- The most suitable greenhouse design offers good security, is long-lasting, easy to clean, withstands repeated disinfection and minimizes hiding places for pests and other organisms.

A detailed description of these points and further helpful information regarding design and maintenance of containment greenhouses are provided by Adair and Irwin, 2008.

BOX 2.2

BIOLOGICAL CONTAINMENT/CONFINEMENT STRATEGIES

BIOLOGICAL CONTAINMENT/ CONFINEMENT STRATEGIES Are highly useful for complementing physical containment measures and thus ensuring effective containment of GMOs.

As pointed out in the text, layering of physical and biological containment measures is considered a most efficient means of achieving containment. Biological containment refers to all measures that directly target the organism being worked on with the aim of preventing sexual or vegetative reproduction and reducing its capability of transgene spread and dissemination, instead of simply providing the physical barriers that contain it in a given area. This can include specific agricultural, horticultural or other work techniques as well as genetic manipulation of the organism to alter its dissemination abilities. These techniques are not only important for research under contained conditions, e.g. in laboratories and greenhouses, but also at later stages of GMO development and commercialization, such as confined field trials or even at the market release stage.

Some of the most common biological containment techniques are listed below.

Horticultural/agricultural management strategies:

- reproductive isolation by removal of flowers prior to anthesis (pollen shed);
- cover flower or seed heads (bagging) prior to pollen or seed release;
- ensure spatial isolation from sexually compatible relatives; specific isolation distances for each crop should be maintained (see Annex 8);
- ensure temporal isolation from sexually compatible relatives, i.e. grow experimental plants in such a way that flowering takes place at different times than that of sexually compatible relatives in the receiving environment;
- stop experiments and destroy plant material prior to flowering;

- if seeds are produced, stringent measures to collect seed, minimize seed dissemination and prevent seed germination in the receiving environment should be in place.

Genetic modification/breeding strategies:

- use male-sterile lines, or sterile triploid lines or interspecific hybrids;
- introduce the transgene into the chloroplast genome; chloroplasts are usually maternally inherited, i.e. no transgene spread via pollen takes place;
- employ cleistogamy, i.e. flowers that do not open, resulting in self-pollination;
- employ genetic use restriction technology (GURT) to yield plants with sterile seeds, or seeds where expression of the engineered trait is repressed (highly controversial due to the implications for farm-saved seeds).

For micro-organisms or insects:

- avoid creating aerosols when working with micro-organisms;
- genetically modify micro-organisms so that survival and replication outside of the experimental setting and/or pathogenicity are compromised;
- when challenging plants with pathogens: use disabled pathogens, provide isolation distances between infected and healthy plants, and eliminate vectors that could transfer the pathogen;
- for insects: use flight-impaired, sterile strains, conduct experiments at time of year or location where survival of escaped organisms is impossible, or choose organisms that have an obligatory relation with the test plant and no other species in the receiving environment.

Further details, including several proposed genetic modification techniques currently at developmental stages, are provided by the Committee on the Biological Confinement of Genetically Engineered Organisms, 2004.

2.6 CONTAINMENT OF GM ANIMALS

GM ANIMALS
As for GM micro-organisms and GM plants, GM animals require specific considerations regarding the risk assessment and the appropriate containment measures.

For the scope of this document, animals shall be defined as all motile, heterotrophic organisms, including vertebrates, invertebrates (e.g. insects) and other multicellular organisms. The first activity the responsible competent authority should perform in the case of GM animals is to check whether the experimenter, institution or organization has the approval of the local animal ethics/welfare committee for dealing with the animal species and the attempted trait modification. If this approval is not granted, the research should be kept in abeyance.

To date, genetic modification of animals has a much lower importance than genetic modification of plants, especially in the field of agriculture: so far, no GM animal with a proposed use in agriculture has been granted approval for market release and commercialization.

The steps towards successful GM animal containment are the same as those outlined above in sections 2.4 and 2.5 on GM micro-organisms and GM plants. First, a risk assessment is performed to evaluate the potential hazards, both to human health and the environment, of the planned GM animal operation. Subsequently, the GM animal operation is classified into one of four risk classes (biosafety levels), each of which requires a specific set of containment measures to minimize the risk of adverse effects on human health and the environment.

Special attention should be paid to the following points:

- potential disturbing effects of GM animals on ecosystems, especially if the GM animal has selective advantages over naturally-occurring relatives;
- invasiveness of non-indigenous GM species that occupy the niche or prey upon indigenous species;
- altered consummation behaviour of GM animals with effects on plant/animal life in the ecosystem;

» expression of biologically active compounds with possible implications for interacting species or human health (biopharming).

Furthermore, the scale and nature of the activity should be considered, e.g. large-scale production of GM animals, or the use of non-standard equipment and facilities such as breeding GM fish in aquaculture facilities (see also Box 2.3).

The exact parameters and procedures for the risk assessment of GM animals are provided in Annex 6. The detailed containment measures for the four GM animal biosafety levels are listed in Annex 7.

BOX 2.3

CONTAINMENT AND CONFINEMENT OF GM ANIMALS: THE CASE OF GM FISH

So far, the containment and confinement measures discussed have mainly focused on GM plants. The simple reason is that the first GM plant was approved for commercial release well over a decade ago and nowadays a wide variety of GM plants are marketed worldwide, with further varieties in development. For GM animals the situation is different: to date, no transgenic animal with agricultural importance has received market approval. However, research in the area of animal transgenesis is active, and one of the fields considered most promising is the creation of transgenic fish, shellfish or crustaceans for use in aquaculture. Obviously, such research and development processes require containment and confinement measures distinct from measures for GM plants or micro-organisms.

Several lines of transgenic fish, covering several species important in common aquaculture, have been created during the last two decades. In most cases, the genetic modification introduced either genes

for growth hormones, resulting in highly accelerated growth rates, or genes conferring increased cold and freeze resistance.

Furthermore, improved disease resistance is also increasingly targeted (Zbikowska, 2003).

Before receiving market approval, the environmental risks of a transgenic fish line need to be carefully assessed. In common aquaculture, fish are often raised in fish cages or similar installations within the open environment with a relatively high risk of escape.

The perceived major risks associated with such an escape of transgenic fish are:

- advantages and higher competitiveness of transgenic fish over wild fish, either of their own or different species, and subsequent displacement of wild fish species and changes in population structures and biodiversity;
- hybridization with wild fish species, resulting in transgene flow to wild species and effects on genetic diversity.

The assessment of these risks is not straightforward, however they need to be evaluated prior to commercial release (see Hu *et al.*, 2007 for examples of risk assessments, mathematical modelling strategies and use of artificial ecosystems for GM fish risk assessment).

In order to limit the risks associated with transgenic fish, containment and confinement measures need to be implemented. Containment measures could include a variety of physical barriers that limit escape of transgenic fish into the open environment in the first place. Ideally, land-based production systems without access to natural waterbodies should be used. In addition to containment structures, biological confinement measures are considered to have a promising role to play in restricting survival, reproduction and transgene flow in cases where GM fish escape from containment.

Bioconfinement strategies include:

- production of sterile fish through induction of triploidy (presence of three chromosome sets per cell) by temperature, chemical or pressure shock of the fertilized egg;
- combining triploidy with all-female (monosex) lines;
- placing the production site in a region where survival of escaped GM fish is restricted, e.g. due to unsuitable water temperature, salinity, pH or other parameters;
- limiting gene flow by placing the production site in a region where no sexually compatible wild species occur;
- several genetic modification strategies aimed at disrupting or limiting reproduction, survival or essential developmental processes should GM fish escape from confinement.

For a detailed discussion of the individual techniques please refer to Committee on the Biological Confinement of Genetically Engineered Organisms, 2004.

All of the listed techniques have specific strengths and limitations, and to date no single technique has been developed that would confer 100 percent protection from any effects of escaped GM fish on wild fish species or transgene flow. Therefore, further research in this area is being performed, and a combination of multiple physical and biological confinement measures is being considered promising to protect from the ecological risks posed by GM fish.

Market approval and commercial release of GM fish will critically depend on a clarification of these issues and the development of appropriate solutions. The commercial release of GM fish, with its anticipated positive effects on aquaculture, should only be performed if the integrity and diversity of aquatic ecosystems can be guaranteed (FAO, 1995).

2.7 GOOD LABORATORY PRACTICE (GLP)

GOOD LABORATORY PRACTICE
A set of standards to describe how research studies should be planned, performed, recorded, archived and reported.

Effective containment and many testing procedures are based on sound laboratory management practices. Many guidance documents refer to these practices in general terms as **good laboratory practice** ("lower case glp") and more specifically as GLP ("upper case GLP"). The former refers to a set of standards used to accredit testing and calibration laboratories (e.g. ISO/IEC 17025, 2005). The latter refers to the OECD Principles of Good Laboratory Practice (OECD, 1998), which sets the standards for specific test studies. Some countries issue their own versions of the GLP Principles based on the OECD Principles of GLP, incorporated as part of national legislations. Please refer to Annex 3 for a summary of GLPs.

The OECD Principles of GLP describe a "quality system concerned with the organizational process and the conditions under which non-clinical studies are planned, performed, recorded, archived and reported" (OECD definition). It is concerned with assurance of data quality (sufficient, rigorous, reproducible) rather than the technical validity of the studies undertaken.

Data generated under GLP are suitable for product registration, mutual acceptance of data among OECD member countries, and to contribute to protection of human health and the environment.

The GLP Principles describe a set of guidelines for the following: test facility organization and personnel, quality assurance programmes, facilities, apparatus, material and reagents, test systems, test and reference items, Standard Operating Procedures (SOPs), performance of the study, reporting of study results, and storage and retention of records and materials.

GLP compliance monitoring is required for mutual acceptance of data. Periodic inspection of test facilities and/or auditing of studies are conducted for the purpose of verifying adherence to GLP principles. Compliance and monitoring are conducted by international, regional or national accreditation bodies, e.g the International Laboratory Accreditation Cooperation (ILAC), Asia Pacific Laboratory Accreditation Cooperation (APLAC) and Australia's National Association of Testing Authorities (NATA). Different countries may require different proofs of compliance with regard to GLP requirements.

CHAPTER 3

CONFINED FIELD TRIALS

CONFINED FIELD TRIAL
After completing the containment stage GMOs can be evaluated in confined field trials. The aim is to evaluate the characteristics of a GMO in the natural environment, while ensuring that dissemination of the GMO or the transgene(s) to the environment is prevented.

As already pointed out in the introduction, the development of a GMO passes through several stages: from initial research and development in the laboratory and subsequent greenhouse testing, both under containment, to confined field trials in the open environment and finally post-release monitoring after the GMO has been placed on the market.

The aim of a **confined field trial** is to evaluate crops with new genetic and phenotypic traits in the natural environment, while ensuring that dissemination of the plant and the transgene is restricted. Field testing is required to collect information on the agronomic performance and the environmental interactions of newly developed crop lines (both from classical breeding and GM crops). This process is essential to establish a detailed environmental risk assessment (ERA) as well as for the characterization and evaluation of the potential agronomic benefits of the new crop line under local environmental conditions. In the case of GMOs, special attention must be paid to ensure environmental protection

and compliance with basic biosafety regulations while performing the trial. This includes detailed requirements for notification and reporting of the trial, a variety of measures to ensure reproductive isolation of the crop, regular monitoring of the trial site and post-harvest land use restrictions, among others. Thus, the planning, conduct and evaluation of confined field trials require a comprehensive, integrated approach including all aspects of the trial. The detailed procedures and confinement measures that are recommended for the successful performance of a confined field trial are discussed below. The discussion will focus on field trials of transgenic crops because they represent the vast majority of GM organisms (see also Box 2.3).

3.1 CHARACTERISTICS OF CONFINED FIELD TRIALS

Confined field trials represent the first introduction of a newly developed GM crop into the environment, being the intermediate step between research and development under containment and unconfined commercial release. They can be defined as "*a small-scale experiment field trial of a genetically engineered plant species performed under terms and conditions that mitigate impacts on the surrounding environment*" (CropLife International, 2005).

CHARACTERISTICS OF CONFINED FIELD TRIALS
Confined field trials usually share several important characteristics, including: a small size, the goal of collecting a variety of data, detailed notification and reporting requirements, strict measures to ensure confinement of the trial, and strict regulations for all processes and personnel involved in the trial.

As such, a confined field trial has several important characteristics:

- It is an experimental activity performed to collect data on the interaction of the GM crop with the local environment and on its agronomic performance, with the aim of formulating recommendations for its potential benefits and establishing a detailed environmental risk assessment.
- It is a small-scale activity, typically around 1 hectare or less.
- The trial is performed with measures in place that restrict the dissemination of the transgene, e.g. via pollen or seeds, into the environment, that prevent the persistence of the plant or its progeny in the environment, and that restrict plant material from entering human or animal food supplies.

- Access to the site is restricted.
- The trial should be notified to the competent authority, regular monitoring of the site should be performed, and reports of the trial should be prepared.
- Trained and informed staff are required for the correct conduct and surveillance of the trial; SOPs and detailed work plans should be established.

Confined field trials are a prerequisite for the unconfined release of GM plants. When a GMO is approved for commercial release, it is assumed that potential hazards for human health and the environment are not significant, as pointed out in the environmental risk assessment. However, for confined field trials the potential hazards may be unknown and are only evaluated throughout the trial, thus stringent measures must be implemented that minimize the exposure of the environment to potential hazards posed by the tested GMO (minimizing risk by minimizing the exposure component).

Confined field trials serve a variety of purposes: First, the agronomic potential of the newly developed GMO and its traits can be tested in the open environment. This should include the investigation of the expression levels of the transgene(s) throughout different plant tissues and different developmental stages, and the effects of the transgene(s) on plant behaviour and characteristics. Second, field trials can be used to produce sufficient plant material for feeding trials and food safety assessments, or for the scale-up of plant material in preparation for commercial release. Finally, confined field trials are required to collect agronomic and environmental data of the GMO that are essential for the completion of the environmental risk assessment. Data to be collected might include possibilities for transgene transfer, impact on target and non-target organisms, evaluation of the environmental fate of the transgene expression products, and any phenotypic or morphological changes of the GM plant that might impact on the environment or agricultural practices.

3.2 RISK MITIGATION GOALS FOR CONFINED FIELD TRIALS

The compliance with biosafety regulations and the safe conduct of confined field trials with GM plants can be achieved by adhering to three risk mitigation processes:

- preventing the dissemination of transgenes into the environment via pollen or seed (reproductive isolation);
- preventing the persistence of the transgenic plant or its progeny in the environment;
- preventing GM plant material from entering human or livestock food supplies.

RISK MITIGATION GOALS
Three primary risk mitigation goals of confined field trials can be defined: preventing the dissemination of the transgene(s), preventing persistence of the GMO, and preventing GMO material from entering food and feed supplies.

Achieving reproductive isolation of the GM plant and thus limiting gene flow via pollen transfer from the confined site to the environment can be achieved by a variety of measures. A number of factors that affect pollen-mediated gene flow via hybridization and introgression to the same or related species need to be considered: the presence of the same or related species in the environment; in case of presence of a related species, whether the two species are sexually compatible, and whether blooming of the two species takes place at the same time; the presence of pollinating vectors; and the fertility and persistence of the progeny plants.

An investigation of these factors requires that the reproduction characteristics of the (unmodified) GM plant are known in detail, such as time of florescence, whether the plant is self or cross-pollinating, pollen dispersal mechanisms and typical pollen travel distances, pollen viability and sexually compatible species. In this respect, it is highly important to assess if the genetic modification has effects on the reproduction characteristics of the plant, compared with its non-modified counterpart. From an assessment of the above-listed factors, appropriate confinement measures for the field trial can be deduced.

Preventing persistence of the GM plant or its offspring in the environment can be achieved by carefully destroying all GM plant material after termination of the trial. A certain period of post-harvest land use restriction should be implemented in order to detect and destroy any volunteer or progeny plants that may come up on the former trial site.

Preventing GM plant material from entering food and feed supplies is a critical point and can be implemented by a combination of measures. These include controlling the transport of GM plant material to and from the trial site, monitoring storage of seed and GM plant material, monitoring the disposal of GM plant material and the disposition of material retained after harvest, and preventing unauthorized harvest from the trial site. The detailed procedures and practices that are required to comply with the risk mitigation measures described above are discussed below.

3.3 PROCEDURES AND PRACTICES FOR SUCCESSFUL CONFINEMENT OF FIELD TRIALS

In this section, the individual procedures and practices that are required to achieve confinement of a field trial are explained in detail. These include prescriptions regarding the conduct of the trial itself as well as regulations with respect to trial planning, trial reporting and notification and post-trial procedures. In general, the first step to be performed to submit an application for the field trial to the relevant competent authority; usually the competent authority specifies the information that needs to be provided in such an application. After the application has been reviewed and the field trial has been approved, the detailed planning and establishment of the field trial may begin.

3.3.1 Transportation and storage of GM material

Successful confinement starts not only at the trial site, but already at the stage of transportation and storage of GM material. It must be ensured that material is handled, packaged, labelled and stored correctly, and that records of all actions are kept.

TRANSPORTATION AND STORAGE OF GM MATERIAL
Confinement starts not only at the trial site: already during transportation and storage of GMO material specific confinement measures should be adopted.

Prior to importing transgenic plant material into a country, relevant import permits need to be obtained from the relevant competent authority (see also Chapter 6 of this module). Adequate records of all transport processes should be prepared and kept; receipts should be issued upon arrival of the GM plant material at its final destination.

GM plant material should be packaged safely for transport and kept separate from other plant material during transportation. Any accidental release of GM material during transport must be avoided. Different recommendations exist for different plant materials: seed should be packaged in three layers, i.e. a primary, secondary and tertiary container, with each layer being independently sealable and capable of preventing release. The primary container should not allow seeds to become trapped within; examples of suitable containers include plastic bags, plastic bottles or metal cans. Suitable secondary and tertiary containers are metal, plastic, cardboard or wooden boxes or crates. For other plant material, e.g. vegetative plant material or material not capable of propagation, two layers of packaging are considered sufficient (Halsey, 2006). After transport, containers should be thoroughly cleaned or may be disposed of by autoclaving, burning or landfill deposition, verifying that all GM plant material has been removed or has been rendered non-viable.

Containers used for transportation of GM plant material must be clearly labelled, allowing quick establishment of the content identity and contact details of responsible persons.

To this end, the label should include:

- the permit number for import or in-country movement (if applicable);
- details of the GM plant material, i.e. plant species;
- form of GM plant material, i.e. seed, whole plants, tubers, bulbs, etc.;
- amount of GM plant material;
- contact details of responsible persons;
- a standard "do not eat" symbol.

Storage of GM plant material should be performed in a way that prevents its release into the environment, and especially its consumption by humans, livestock or other animals. Storage areas should be cleaned prior to and following the storage of GM plant material. Mixing of GM plant material with conventional plant material during storage must be avoided. An inventory of stored material should be prepared and regularly updated, and GM plant material should be clearly labelled. Access to the storage area should be restricted, and signs should indicate the presence of GM plant material.

In the event of an accidental release of GM plant material during transportation or storage, measures should be taken to stabilize the situation and prevent further releases. The site of the accidental release should be marked, and any actions taken to minimize the impact of the release should be documented. The relevant competent authority needs to be informed of the incident immediately.

3.3.2 Establishing and managing the confined trial site

A variety of management procedures should be implemented before, during and following the termination of a field trial in order to ensure the confinement of the trial. Considerations regarding the choice and maintenance of the trial site, requirements for personnel conducting the trial and treatment of equipment are discussed in this section, whereas management measures regarding reproductive

isolation of the GM plant, post-harvest management and monitoring and recording of the trial are discussed in individual sections below. All management measures should be implemented with the aim of achieving the three goals of confinement: preventing transgene spread, preventing persistence of the plant, and preventing plant material from entering food and feed supplies.

The **selection of a trial site** should be based on various considerations. First, the ecosystem in proximity to the trial site should be considered and be taken into account for the environmental safety assessment. This includes the presence of species sexually compatible with the GM plant in the ecosystem adjacent to the proposed trial site. Furthermore, the possibility of maintaining suitable reproductive isolation distances needs to be assessed. Long-term considerations, especially regarding post-harvest land use restrictions, should also be taken into account. Lastly, the presence of neighbouring third parties that might be affected in the event of an accidental release should be taken into consideration.

SELECTION OF A TRIAL SITE
The selection of a suitable trial site is an important step in the planning process of confined field trials and should be based on a variety of considerations (see text).

Following the choice of a trial site, it should be marked and mapped. It is recommended to mark out the four corners of the site, for example with suitable posts, in order to identify it throughout the growing season and subsequent post-harvest land use restriction periods. Global positioning system (GPS) data, if available, might facilitate the recording of the exact trial site. Signs should be put up indicating the presence of GM plants and prohibiting access to non-authorized persons.

A detailed map of the trial site should be established, incorporating the following information:

- contact details of the responsible trial manager;
- identification and/or permit numbers of the trial, if applicable;
- a descriptive land location, i.e. the city, town or region and specifications of how to reach the site from the nearest town;
- exact trial site dimensions;

- total area planted with GM plants, including guard rows;
- distances to permanent markers or surrounding landmarks (telephone poles, fences, roads);
- closest fields of the same species as the GM plant within 1 km distance from the trial site;
- any adjacent natural ecosystems (natural habitats, waterways, forests, etc.);
- the planting date;
- compass directions, with north at the top of the map.

PERSONNEL
It should be ensured that all personnel working on a confined trial site is familiar with the standard operanting procedures and confinement measures that need to be implemented and adhered to.

It should be ensured that all **personnel** working on the trial site during preparation, conduct and post-harvest management of the trial are aware of the material being handled and of the relevant SOPs in place. During the harvest of GM plant seeds or other material, checks should be conducted to ensure that no material is removed from the trial site entrapped in workers' clothing before exiting the trial site. In addition, suitable safety measures should be implemented that limit access to the trial site to authorized personnel, and restrict access of livestock or large animals. Special attention should be paid to restrict consumption of the GM plant material by humans, livestock or other animals.

Before removing equipment from the trial site, it needs to be cleaned of any remaining GM plant material. Methods considered appropriate include manual cleaning, brushing, compressed air, vacuuming or water. It should be verified that the cleaning procedure was successful, i.e. that all plant material has been removed. Additionally, all personnel working within the trial site should routinely check their shoes and clothing for entrapped plant material before exiting the site.

REPRODUCTIVE ISOLATION MEASURES
Measures for reproductive isolation are a core part of confined field trials: a variety of possible measures exist and are selected depending on the specific crop type being tested.

3.3.3 Reproductive isolation measures

Ensuring reproductive isolation by restricting pollen-mediated gene flow from the GM plant being tested to sexually compatible species and thus confining it to

the trial site is a major aspect of confined field trials. Having detailed knowledge of the plant species concerned, especially its reproduction characteristics, is essential for choosing and implementing the most effective measures that will result in successful reproductive isolation of the GM plant. Detailed information on individual crop species can be obtained from background literature, plant researchers, plant breeders or plant and seed producers. Furthermore, the Organisation for Economic Co-Operation and Development (OECD) has developed a series of consensus documents for major crop species, which are available online (OECD, 1997-2009). The different possibilities for ensuring reproductive isolation, which vary according to the crop species concerned, are discussed in the following paragraphs.

Spatial isolation

One of the most widely applied measures for reproductive isolation is to maintain a minimum isolation distance between the GM plant and sexually compatible relatives. The exact minimum distance that should be maintained is dependent on the individual crop species; examples of isolation distances for some of the most important crop species can be found in Annex 8. Sufficient land to establish the required isolation distances needs to be set aside when first planning the field trial. The land within the isolation distance needs to be kept free of the same or related plant species as the GM plant being tested. If such plants are allowed to flower within the isolation distance, a breach of reproductive isolation is supposed to have occurred.

Temporal isolation

Temporal isolation can be employed when the flowering time of a crop species can be predicted with adequate accuracy. This allows the isolation of two sexually compatible crop species, or of a crop species and related wild relatives, by selecting the planting dates so that there is no overlap between their flowering periods. One species must have completed pollen shed completely before or after pollen

shed of the other species, so that there can be no possibilities for pollen-mediated gene flow. Temporal isolation might be difficult to implement due to the inherent variation of ecosystems and living species, resulting in unpredictable changes in flowering times. Temporal isolation therefore needs to be carefully monitored; if two species accidentally flower at the same time, a breach of isolation has occurred.

Removal of flowers

Reproductive isolation of the GM plant being tested can be achieved by identifying and removing all male flowers prior to anthesis. As with temporal isolation, a strict monitoring scheme must be in place in order to identify and remove all inflorescences in time.

Bagging and tenting

Reproductive isolation of the GM plants being tested can also be achieved by limiting pollen-mediated gene flow by physical means. This includes placing bags that prevent pollen release over all inflorescences of trial plants prior to anthesis, or by placing the entire plant within a pollen tent that prevents release of pollen into the environment. In both cases, flowers/plants must remain covered until the pollen has lost its viability.

Early crop destruction

Should flowering of the GM plant being tested not be required for the purpose of the test, early crop destruction can be employed as a means of reproductive isolation. The trial must be terminated and trial plants destroyed prior to anthesis.

Guard rows

The establishment of guard rows, i.e. planting an uninterrupted perimeter border row of conventional plants around the trial plants, is an effective reproductive isolation measure especially for insect-pollinated plants. The guard row acts as a

pollen-trap, thus limiting pollen movement in the environment. Several factors need to be considered when planning guard rows. The required width of the guard row is species-specific, and should be determined on a case-by-case basis.

The conventional plant variety used for the guard rows should flower at the same time as the trial plant, possess similar growth habits and structure, should be planted at comparable densities as the trial plant and should be managed using similar agronomic practices. There must be no gaps present in the guard row which could create problems such as access of equipment to the trial plants. In case the tested GM plant carries traits for herbicide tolerance, care must be taken that the guard row plants are not killed by herbicide application. Strict monitoring should be performed to verify flowering of the guard row and the trial plants at the same time. For post-harvest restrictions and monitoring, the entire area of trial plants and guard rows needs to be included.

Plant modification methods

Instead of providing passive, physical barriers to limit pollen-mediated gene flow, the transgenic plant itself could be modified in such a way that reproductive isolation is ensured. This could include the use of male sterile plants, cleistogamy, or transplastomic plants (integration of the transgene into the chloroplast genome, which, in many plant species, are maternally inherited). Please refer to Box 2.2 for further details on biological containment/confinement strategies.

In case of a **breach of reproductive isolation** through failure of any of the above-mentioned measures, the competent authority needs to be informed and actions taken to limit the release and dissemination of GM plants or transgenes. These could include complete termination of the trial and destruction of any relevant plants within the isolation distance, or simply stricter requirements for post-harvest land use restrictions and monitoring.

BREACH OF REPRODUCTIVE ISOLATION
In case of failure of reproductive isolation measures, a breach of reproductive isolation has occurred which necessitates special procedures, for example immediate termination of the trial or extended post-harvest monitoring.

3.3.4 Harvest and disposal of GM plant material and post-harvest restrictions

HARVEST AND DISPOSAL OF GM PLANT MATERIAL
Harvest and disposal of GM plant material is a critical step, during which it needs to be ensured that no viable material leaves the trial site and that no viable material remains on the trial site.

The termination and harvest of a confined field trial are critical stages that must be carefully monitored, with special attention to two points: preventing GM plant material from persisting at the trial site, and preventing GM plant material from entering food and feed supplies. The following provisions apply both to normal harvest and termination, as well as to early termination and crop destruction, e.g. as a reproductive isolation measure. The competent authority should be informed of the harvest prior to commencing the harvest procedure.

All personnel working on the harvest site should be instructed on the nature of the material being harvested, and a procedure should be implemented to verify that no GM plant material is accidentally released from the trial site entrapped in workers' clothing.

All equipment required to perform the harvest should be cleaned free of plant material both before entering the trial site, and before removing it from the trial site after harvest. Methods considered appropriate include manual cleaning, brushing, compressed air, vacuuming or water. It should be verified that the cleaning procedure was successful, i.e. that all plant material was removed.

All GM plant material should be disposed of directly at the trial site; if transport of GM plant material is required, it should be secured appropriately during transport to prevent any accidental release. All GM plant material that is not retained for research purposes must be rendered non-viable. Recommended techniques to achieve this are heat, incineration, deep burial, chemical treatment, grinding or crushing, or by cultivation into the soil. Following devitalization, GM plant material can be disposed of by incineration, deep burial or cultivation into the soil. If guard rows are used, the guard row plant material should be treated in the same way as the GM plant material.

If any GM plant material from the trial is to be retained for future research purposes, this should be notified to and receive approval from the competent authority.

Post-harvest restrictions and monitoring

Following harvest and termination of the trial, the trial site is subjected to post-harvest restrictions and monitoring; these restrictions begin with the termination of the trial. The aim of these restrictions is to identify and destroy any volunteer plants arising after the termination of the trial, in order to avoid persistence of the GM plant in the environment, prevent gene flow between the GM plant and sexually compatible relatives, and prevent GM plant material from entering food and feed supplies.

POST-HARVEST RESTRICTIONS AND MONITORING
Following termination and harvest of a confined field trial specific requirements exist for post-harvest land use restrictions and monitoring. Time periods for such measures are dependent on the crop type that was tested.

The exact period of post-harvest land use restrictions and the monitoring intervals are dependent on the GM plant species. During this period, all volunteers as well as sexually compatible related species must be identified and removed prior to anthesis. If a breach of this restriction is encountered, the post-harvest restrictions should be extended. Use of the land is restricted to crop species different from the GM crop species that was tested and preferably showing different morphology and growth habits, in order to easily spot and identify any volunteers. Examples of post-harvest periods and monitoring intervals for selected GM crop species are listed in Annex 8.

Regarding personnel, equipment, and measures for devitalization and disposal of plant material, the same provisions as described above for the harvest procedure should apply.

3.3.5 Monitoring, sampling, accidents, reports and records

MONITORING, SAMPLING, ACCIDENTS, REPORTS AND RECORDS
As for GMO operations under containment, confined field trials of GMOs require defined procedures for notification, reporting, accidents and monitoring, amongst others.

Monitoring

Regular monitoring is an integral part of a confined field trial, with the aim of ensuring reproductive isolation, confinement of the trial, and the collection of data on the characteristics and agronomical performance of the GM plant being

tested. It is recommended to monitor the growth and development of the trial plants at least weekly, starting with planting and ending with the termination of the trial, after which specific post-harvest monitoring provisions apply. Monitoring for specific effects, depending on the individual crop and genetic modification involved, should be included in the monitoring plan.

Sampling

Sampling of GM plant material during different stages of the trial might be required in order to investigate the presence of the transgene in different plant tissues and the expression levels of the recombinant protein, or to perform other compositional analyses. The sampling strategy will vary from case to case, but general recommendations for sampling should be followed. These include avoiding cross-contamination between samples, appropriate sample storage in safe containers and at suitable temperatures (usually frozen), and clear labelling of all samples including all relevant information.

Accidents and breaches of confinement

When an accidental release or breach of confinement occurs, certain steps should be taken in order to minimize the impact of such an incident:

- stabilization of the situation, prevention of further releases;
- prevention of consumption of GM plant material;
- recovery of released GM plant material;
- notification to the competent authority;
- marking and recording the exact site of the incident;
- disposal of GM plant material, if required;
- follow-up monitoring and detection.

All procedures and actions taken during an accident should be recorded and documented.

Reports and records

It is recommended that regular reports on the confined field trial be prepared and evaluated by the competent authority. Reports that could be provided include:

- planting report, with details of trial establishment;
- trial progress report(s);
- harvest report;
- incident and corrective action report, if appropriate;
- unanticipated effects report, stating details of such events;
- experimental report, stating all observation and evaluation methods and outcomes of the trial;
- post-harvest report, after the completion of the post-harvest period.

In addition to evaluating the reports, the competent authority could also regularly inspect the field trial site, in order to verify that all relevant measures and procedures to ensure confinement are in place and implemented.

In addition to the reports, it is recommended that records regarding transportation and storage of GM plant material, confinement measures at the trial site, disposal of GM plant material, reproductive isolation measures, planting and harvest procedures, general monitoring, post-harvest monitoring and any accidental releases and the corrective actions taken, be prepared and kept. Records should adhere to certain standards, i.e. be easily readable, include all relevant information (including date and name of the person doing the recording), be prepared promptly after an event, and should be stored in such a way that they are easily traceable and available for review and control.

In Annex 9, a list providing examples of inspection questions that can be used to verify the correct planning, conducting and recording of confined field trials, and compliance with all relevant points listed above, is supplied.

CHAPTER 4

POST-RELEASE MONITORING OF GMOs

COMMERCIAL RELEASE
The ultimate step for a GMO is commercial release. During this stage, no measures are implemented that limit contact of the GMO with the environment, and the GMO is likely to be released on a large scale and in a variety of different environments.

After completing the research and development phases, passing through confined field trials and receiving approval from the competent authority, a GMO can finally be placed on the market and thus be released into the environment. This is a substantially different process compared with confined field trials. First, in confined field trials the risks posed by the GMO are partially unknown, hence measures are implemented to reduce exposure of the environment to the GMO. During **commercial release**, however, the risks are identified and judged to be negligible or manageable, hence no measures are in place to limit exposure of the environment to the GMO. Second, the scale is different: following commercial release a GM plant is free to be grown on very large areas, implying possible scale-related unanticipated effects on the environment. Furthermore, since GMOs are living organisms, they interact with their environment and are subject to ecological laws and processes, possibly resulting in unpredictable effects and behaviour of the GMO following its release.

4.1 CHARACTERISTICS OF POST-RELEASE MONITORING

In order to assess the impact of the identified risks of a GMO on the environment, identify unanticipated effects and evaluate the agronomic performance of the GMO, post-release monitoring is performed. Monitoring can be defined as "*a procedure that involves the systematic measurement of selected variables and processes that may be affected by a given practice*" (FAO, 2005). With respect to GMOs, the aim of post-release monitoring can be described as "*to identify direct, indirect, immediate,*

delayed, or unforeseeable harmful effects that GMO and their application might cause on the environment and human health." (Wilhelm *et al.*, 2003). The results of such monitoring programmes can be used to formulate additional precautions, influence the maintenance, renewal or withdrawal of an approval for a GMO, and can feed back into the risk assessment procedure. GMO monitoring constitutes an early-warning system, since the detection of adverse effects will allow a fast reaction and the implementation of countermeasures at an early stage (Züghart *et al.*, 2008).

POST-RELEASE MONITORING
In order to assess the impact of the identified risks of a GMO on the environment, identify unanticipated effects and evaluate the agronomic performance of the GMO following its commercial release, post-release monitoring is performed.

The release of a GMO could have impacts on the environment at a variety of levels, from single cells to organisms, populations, communities and ecosystems. Due to the variance inherent to all life and ecosystems, effects of GMOs may be difficult to predict in a spatial and temporal manner; they may appear immediately or only after long time spans, and might impact only on the initial site of release or over wide distances and different ecological compartments. Variation will be observed between farming systems, crop types and the environmental contexts. It is therefore recommended to design monitoring plans for GMOs on a case-by-case basis, taking into account all relevant information regarding the individual GMO and the receiving local environment. The choice and establishment of reliable monitoring indicators, which will allow the detection and quantification of adverse effects caused by the release of the GMO and that are based on specific protection targets, is crucial in this respect.

MONITORING PROGRAMMES
To perform post-release monitoring, a monitoring programme should be developed on a case-by-case basis for each GMO release, taking into account the local receiving environment and the characteristics of the released GMO.

The capacity to implement **monitoring programmes** varies from country to country. Developed countries may have the financial and scientific resources to undertake large-scale, long-term post-release monitoring programmes that form a solid basis for decision-making. However, in developing countries the establishment of monitoring programmes represents a greater challenge, due to possible lack of knowledge concerning hazards and risks, limited opportunities for engagement in public debates, less effective enforcement of environmental protection measures and

limited financial, infrastructural or personnel resources for research and development (FAO, 2005). In such cases, a robust monitoring plan based on limited resources should be established that can nevertheless serve the purpose of post-release monitoring as defined above.

As stated, the reasons for monitoring include the verification and reassessment of the findings from the environmental risk assessment, identification of unforeseen effects, the need to meet environmental protection goals and to ensure the productivity and ecological integrity of farming systems.Therefore, the design of the monitoring programme and the evaluation of data are both dependent on and feed back into the environmental risk assessment. Since a basic understanding of the environmental risk assessment is therefore essential to establish and follow monitoring procedures, a brief introduction to this topic is provided in the following section.

4.2 THE ENVIRONMENTAL RISK ASSESSMENT (ERA)

ERA
The objective of the environmental risk assessment is to evaluate, on a case-by-case basis, the impact of a GMO on human health and the environment. Such an assessment is a prerequisite for developing an effective post-release monitoring programme.

The objective of the environmental risk assessment is to evaluate, on a case-by-case basis, the impact of a GMO on human health and the environment. The outcome is a risk classification of the GMO ranging from negligible to high risk, based on a scientific consideration of the potential of the GMO to cause adverse effects and the likelihood that these adverse effects will occur. Direct, indirect, immediate, delayed as well as potential long-term and cumulative effects, caused by the deliberate release of the GMO, should be taken into account (see Box 4.1). The environmental risk assessment is inherently limited in its scope as only identified potential hazards of the GMO can be assessed. Therefore, monitoring serves two purposes: monitoring of the risks associated with a GMO that were identified in the environmental risk assessment (case-specific monitoring), and monitoring for unanticipated effects that were not identified in the environmental risk assessment (general surveillance; see section 4.4 for further explanations).

BOX 4.1

DIRECT, INDIRECT, IMMEDIATE AND DELAYED EFFECTS OF GMOs (EU, 2002A)

Direct effects are primary effects on human health or the environment that are a result of the GMO itself and which do not occur through a causal chain of events.

Indirect effects are effects on human health or the environment which occur through a causal chain of events, through mechanisms such as secondary interactions between organisms and the environment, transfer of genetic material, or changes in use or management practices. Observations of indirect effects are likely to be delayed.

Immediate effects are effects on human health or the environment which are observed during the period of the release of the GMO; immediate effects may be direct or indirect.

Delayed effects are effects on human health or the environment which may not be observed during the period of the release of the GMO, but become apparent as a direct or indirect effect either at a later stage or after termination of the release.

A consistent, science-based procedure and methodology should be followed to establish the environmental risk assessment. The general objectives, principles and methodologies for the environmental risk assessment, as proposed by the European Union, are exemplarily laid down in Module C. These specifications could serve as a template or guidance for the design of individual, case-specific environmental risk assessments. Further information concerning all aspects of risk analysis procedures and principles can be found in Module C: Risk Analysis.

4.3 ESTABLISHING THE MONITORING PLAN

THE MONITORING PLAN
A monitoring plan, developed for a specific GMO release, should include descriptions of the monitoring strategy, the monitoring methodology, and procedures for reporting of the results and relevant triggers for decision-making.

Before commencing any monitoring activity, a detailed monitoring plan should be developed on a case-by-case basis, taking into account the characteristics and intended use of the individual GMO, the environmental risk assessment and the local receiving environment. The available resources and tools to carry out monitoring, in terms of financing, personnel, methodology and infrastructure, should also be taken into consideration when designing the monitoring plan. An analysis of cost-effectiveness should also be included. The monitoring plan should comprise case-specific monitoring, which focuses on the occurrence and impact of potential adverse effects that were identified in the environmental risk assessment, general surveillance, required to identify the occurrence of unanticipated adverse effects, and monitoring for potential cumulative and long-term effects.

Monitoring programmes should be designed so that their purpose and value are ensured: generating information that directly influences effective management and decision-making. In other words, it is important that the information generated by monitoring programmes is received by decision-makers and everybody with a stake in functioning and productive agricultural ecosystems. If this is achieved, correct decisions regarding the preservation of agricultural production systems, ecosystems and rural livelihoods can be reached. The connection between the results of monitoring and decision-making should be clear; monitoring is pointless if the data generated cannot be used. The early integration of all stakeholders and information of the wider public is essential for this process, to ensure that correct decisions are made and implemented at the farm level (Jepson, 2005). It is recommended that the monitoring plan consist of three key sections (Wilhelm, 2003):

- the monitoring strategy, which is based on the objectives and aims to be achieved, the potential effects likely to be observed, and a description of general approaches and timescales to be followed;
- the monitoring methodology, describing all practical aspects of data collection;

BOX 4.2

EVALUATING THE SOCIO-ECONOMIC IMPACTS OF GM CROPS

The assessment of socio-economic effects of GM crops is not a primary goal of the post-release monitoring process. However, such an assessment, as well as the evaluation of the agronomic performance of a GM crop, can be taken into consideration when designing post-release monitoring programmes. In developing countries especially, an evaluation of the socio-economic effects of a GM crop could be important in order to react quickly should adverse socio-economic effects be found to be associated with the introduction of the GM crop. Several methodologies for the assessment of the socio-economic impact are available (Sonnino *et al.*, 2009) and some of them can be adopted in post-release monitoring of GM crops.

In a short paper on this topic (Sahai, 2005) a few points are highlighted that could be taken into consideration when planning socio-economic evaluations in the context of post-release monitoring, using herbicide-tolerant crops as an example:

- In many developing countries, weeding is a major source of rural employment and generation of income. Herbicide tolerance, being a labour-saving strategy, can have negative social and economic implications. In other instances, where the availability of family labour is a limiting factor, herbicide tolerance can have a positive impact.
- Contrary to monocultures in developed countries, weeds in developing countries might not be recognized as such but instead fulfill useful functions. These include use as food and feed and as medicinal plants.
- Possibilities for growing additional crops on field bunds or for mixed farming, both representing an important source for nutrition and income, would be reduced.

Based on such considerations, monitoring indicators to assess socio-economic effects can be developed. The Sustainable Livelihood Approach offers a comprehensive framework for this kind of evaluation.

- data analysis, reporting and evaluation, describing the data evaluation procedure, procedures for reporting to relevant authorities, stakeholders and the public, and providing feedback into the risk assessment and monitoring process.

When designing a monitoring plan the following key steps, established by an expert consultation held at FAO in 2005, could be used as guidance and for identifying the priorities of the process (FAO, 2005). An evaluation of the socio-economic effects of the GMO could also be included in the monitoring programme (see Box 4.2).

- set monitoring programme goals and immediate objectives;
- consult stakeholders, including farmers and managers, regarding the natural resources to develop the goals and immediate objective;
- identify potential barriers;
- prioritize and develop plans to overcome or minimize potential field barriers or otherwise;
- identify potential risks and benefits;
- use stakeholder and expert knowledge of potential risks/concerns and benefits of GM crops, and ways and indicators to measure these factors;
- develop a testing hypothesis to guide actions and decisions;
- ensure that the hypothesis is simple, robust and can be easily tested in the field;
- identify a limited number of potential indicators;
- ensure that the indicators meet the basic requirements of scientific rigour;
- reflect key elements of the hypothesis tested;
- compare with control sites and/or baseline values prior to GM crop release;
- estimate the status and trends in indicator values;
- determine appropriate trigger values for decision-making and action;
- anticipate the range of decisions and actions if triggers are exceeded;
- prepare a follow-up action plan;
- cultivate a transparent and effective process;

- ensure follow-through continued involvement of stakeholder;
- maintain clarity in analysis and reporting, and identify needs; and
- build linkages with policy development and capacity building.

4.4 THE MONITORING STRATEGY

THE MONITORING STRATEGY
The monitoring strategy should be designed in order to allow evaluation of the findings obtained by the environmental risk assessment as well as potential unforeseen effects, taking into account the intended use of the GMO, the scale of the release and the receiving environment.

The monitoring strategy should be designed in order to allow evaluation of the findings obtained by the environmental risk assessment, taking into account the intended use of the GMO, the scale of the release and the receiving environment. Furthermore, the strategy should be able to identify potential effects that were not foreseen in the ERA, or that were associated with a high degree of uncertainty. The strategy should be capable of detecting such adverse effects at an early stage of manifestation to allow fast implementation of countermeasures. All available background information, including information regarding the GMO and the modification event, data from field trials or data from previous releases, should be taken into consideration when designing the monitoring strategy. Importantly, existing monitoring methodologies and observation programmes (e.g. environmental, agricultural or ecological monitoring programmes, food and veterinary surveys, nature conservation or soil observation programmes) should be included in the post-release monitoring strategy to the extent possible and feasible, in coordination with the parties conducting those programmes. The responsibility for the entire monitoring process needs to be clearly assigned, as well as the responsibilities for individual steps of the monitoring process should they be conducted by different parties.

4.4.1 Case-specific monitoring

Case-specific monitoring is performed to investigate the occurrence and significance of any potential adverse effects on human health and the environment associated with the release of a GMO that were identified in the ERA. Specific

CASE-SPECIFIC MONITORING
Case-specific monitoring is performed to investigate the occurrence and significance of any potential adverse effects on human health and the environment associated with the release of a GMO that were identified in the ERA.

hypotheses regarding the occurrence and impact of potential adverse effects should be formulated based on the ERA and tested by scientific means. This should be achieved by systematically recording relevant indicators at representative geographical locations, e.g. spots where exposure of the environment to the GMO is highest or the environment is most likely to be affected. The selection of monitoring indicators, the monitoring methods and the scale (e.g. in terms of number of areas covered) and time frame of monitoring should be determined on a case-by-case basis, taking into account the inherent nature of the GMO and the transgenic event, the receiving environment and the characteristics (e.g. the scale) of the release (EFSA, 2006a,b). For example, if potential adverse effects of an insect pest-resistant GM crop on non-target insect populations have been identified in the ERA, this crop would be the subject of case-specific monitoring using monitoring indicators that describe the impact of that GM crop on the non-target insect species. A clear, testable hypothesis that could be formulated and subsequently tested in this case could be "A change from conventional (insert crop name) to the GM variety will have significant effects on (insert insect name) population density and mortality of insects feeding on the crop". It should be ensured that not only direct and immediate, but also indirect and delayed effects, as identified in the ERA, are included in the monitoring strategy.

In cases where no potential adverse effects are identified in the ERA, no case-specific monitoring is required and monitoring consists of general surveillance and the observation of only cumulative and long-term effects.

4.4.2 General surveillance

General surveillance can be described as routine observation of the geographic regions where a GMO is released; the process aims at identifying the occurrence and impact of unanticipated adverse effects on human health and the environment associated with the release of a GMO that were not predicted in the ERA. As such, general

surveillance should focus on potential indirect, delayed, cumulative and long-term effects, and be performed over extended time periods and multiple geographic locations. As soon as adverse effects are identified, detailed investigations regarding cause and effect chains clarifying the causal connection to the GMO release should be performed (with an hypothesis-based approach as in case-specific monitoring). General surveillance is adequate for monitoring any GMO in any receiving environment since it is not based on an ERA. The drawback is that no hypotheses that can be tested with directed experimental approaches can be formulated, and thus general surveillance is potentially unlimited in its scope. Since no hypotheses can be tested, it is difficult to choose appropriate monitoring indicators that can indicate the occurrence of an adverse effect. Therefore, it is recommended to focus general surveillance on specific environmental protection targets and the occurrence of environmental damage (Bartsch, 2005; see section 4.5.1).

GENERAL SURVEILLANCE
General surveillance can be described as routine observation of the geographic regions where a GMO is released; the process aims at identifying the occurrence and impact of unanticipated adverse effects on human health and the environment associated with the release of a GMO that were not predicted in the ERA.

For general surveillance, an effect can be defined as an alteration in a parameter that lies beyond the normal variation of the agricultural/ecological system. A good starting point for general surveillance would be an investigation of the receiving environment and the exposure level to the released GMO.

Subsequently, it could be determined whether:

- any unanticipated effects are occurring;
- the observed effects are adverse;
- the adverse effects are caused by the release of the GMO.

This evaluation should also include monitoring for potential adverse effects on human health. Obviously, what constitutes an *adverse* effect needs to be defined: for example, the persistence of a GMO in the environment or transgene flow to other species might not be regarded as adverse effects in themselves. However, if such events are associated with, for example, increased weediness or invasiveness, the effect would be defined as adverse (EFSA, 2006a).

4.4.3 The importance of baselines

BASELINES
The baseline status of the receiving environment, i.e. the environment without influences of the GMO in question, is required as a reference point against which all data collected by monitoring can be compared.

The identification and evaluation of effects caused by the release of a GMO through the implementation of a monitoring programme can only be performed if the baseline status of the receiving environment is known. The baseline is required as a reference point against which all data collected by monitoring can be compared. The identification and evaluation of adverse effects are only possible if baseline data for the chosen monitoring indicators that describe the behaviour of these indicators in a GMO-free system state are available. Ideally, the baseline system should differ from the GMO system only in the presence/absence of the GMO.

To obtain such baseline data, two approaches are possible:

- comparison of the system state before the GMO was released with the system state after the GMO release (subsequent comparison);
- simultaneous comparison of an area not exposed to the GMO with an area exposed to the GMO (time-parallel comparison).

Establishing a baseline by subsequent comparison requires monitoring of the system prior to the GMO release; a time frame of three to five years is recommended. However, subsequent comparison is strongly influenced by the variation inherent to natural systems. For example, an insect population (such as the exemplary non-target insect population described in 4.4.1) might show strong variation from one season to another without being reasonably predictable, which would severely limit the suitability of this insect as a GMO monitoring indicator for subsequent comparison. Therefore, time-parallel comparison provides an essential alternative and is especially useful when environments are highly dynamic (EU, 2002b). Ideally, both baseline assessment strategies should be used to complement one another.

The choice of monitoring indicators used to evaluate the state of the receiving environment in a GMO-free condition depends on the suitability of these indicators to assess, subsequently or in parallel, the GMO-related effects on the environment (see section 4.5.1). Using existing environmental observation programmes could provide valuable baseline data, possibly over many years and different sites, concerning the receiving environment prior to any GMO releases.

4.4.4 Time periods for monitoring

TIME PERIODS FOR MONITORING
Time periods for post-release monitoring should be defined in order to detect not only immediate effects but also delayed effects associated with the release of a GMO.

In order to detect not only immediate effects but also delayed effects associated with the release of a GMO, sufficient time periods should be allowed for monitoring. The probability of a specific effect to occur over time, if such a probability can be assigned, should be taken into account. The duration of the release should also be considered; a long release period might favour the establishment of cumulative effects. Furthermore, the duration of monitoring is not necessarily restricted to the duration of the release, but might well extend over the termination of the release. Characteristics of the individual GMO, e.g. its average lifetime, generation time, lifetime of seed banks and risk for persistence in the environment, should serve as guidance for assigning appropriate monitoring periods. The time period should not be fixed, but be adaptable in response to results obtained by the monitoring procedure (EU, 2002b).

4.4.5 Making use of existing monitoring programmes

As stated in previous sections, existing agricultural, environmental, ecological or other related observation or conservation programmes could be integrated in the monitoring plan to obtain data either on the baseline state of a system or on adverse effects caused by the release of a GMO. For example, in cases where routine agricultural evaluations at the farm level are performed, simple surveys

EXISTING MONITORING PROGRAMMES
Existing agricultural, environmental, ecological or other related observation or conservation programmes could be integrated in the monitoring plan to obtain data either on the baseline state of a system or on adverse effects caused by the release of a GMO.

on the observation of adverse effects associated with GMOs (e.g. dissemination, volunteer plants, etc.) could be included (EU, 2002b). Furthermore, collecting information from growers and seed suppliers, e.g. data on GM seed sales, areas sown and crop management techniques (such as obligations to use refugia as an anti-pest resistance strategy, see Box 4.3) could be useful in establishing a monitoring programme.

However, for many existing programmes relevant data for GMO monitoring is unlikely to be obtained, simply because they have been designed for other purposes and thus the targets as well as the methods for data collection and analysis are not suitable. Furthermore, in developing countries in particular the availability of complementary monitoring programmes is likely to be limited (EFSA, 2006a; FAO, 2005).

If existing monitoring programmes are to be integrated into the post-release GMO monitoring plan, the consistency and reliability of data collection and data quality of these programmes should be ensured. Both the questions of which potential adverse effects of the GMO release will be detected by those programmes and which additional measures are required to detect effects that are not covered should be evaluated. Furthermore, should different programmes be used as data sources, methods to collect, analyse and integrate these data need to be developed (EFSA, 2006a).

4.5 THE MONITORING METHODOLOGY

After the monitoring strategy has been defined, concrete procedures and methodologies determining how the monitoring should be performed can be worked out. This includes the choice of monitoring sites, monitoring indicators and procedures for sampling and data collection.

BOX 4.3

DEVELOPMENT OF PEST RESISTANCES AND REFUGIA

One of the major traits targeted by genetic modification of crops is pest and disease resistance. Frequently, resistance against specific insect pests is achieved by expression of the *Bacillus thuringiensis* (Bt) *cry* genes, also known as Bt endotoxins. However, there are concerns that the widespread release and cultivation of GM crops with pest or disease resistance traits poses a high selection pressure on the pest population and leads to development of a pest population that is no longer susceptible to the GM crop resistance mechanism. Development of such an adapted population of the pest species – also referred to as resistance - would lead to failure of the GM crop pest resistance mechanism and thus failure to protect the crop from the pest.

To avoid this, specific crop management techniques can be employed that minimize the development of pest populations that have overcome the crop resistance mechanism. With regard to Bt crops, the most common resistance management strategy is based on the use of GM crops with a high level of Bt gene expression and the concomitant deployment of a refuge consisting of non-GM, pest-susceptible crops (the high dose/refuge strategy). The basis of this strategy is the assumption that the development of insects that are resistant to Bt endotoxins is conferred by recessive mutations which have only low allele frequency within the insect population. Due to the high level of Bt endotoxin expression in the GM crop, only the very rare insects homozygous for the mutant allele will survive on the GM crops. The deployment of a refuge of non-GM crop close to the GM crop area will ensure that the rare mutant homozygous resistant insects surviving from the GM crop area mate with

non-mutant, susceptible insects from the refuge. Therefore, their offspring will be heterozygous for the mutant allele and thus be susceptible to the GM crop.

Depending on the crop and the local conditions, it is recommended that refuges consist of 20 to 50 percent of the area that is planted with GM crop. Mathematical simulations and experience from the field indicate that deployment of this strategy, possibly embedded in an integrated framework of pest management, can delay the development of resistant pests for several decades (Conner *et al.*, 2003; EPA, 2008).

However, especially in the case of small-scale, resource-poor farmers in developing countries, the deployment of refuges might not be economic, or might be neglected due to lack of knowledge (Sahai, 2005). Therefore, it is recommended that compliance with refuge recommendations and evaluations on the development of resistant insect populations be integrated into post-release monitoring programmes. This could help to ensure that refuge recommendations are being followed and that GM crops expressing pest resistance traits maintain their value.

4.5.1 Selecting monitoring indicators

The identification and selection of indicators/parameters to be monitored is a major and decisive step in the entire monitoring process. A major criterion for the selection of indicators is their potential to indicate changes induced by the GMO release. The selection of monitoring indicators should be performed on a case-by-case basis, based on the characteristics of the GMO and the receiving environment. The conclusions of the ERA of a GMO will be helpful in identifying suitable monitoring indicators. For example, if a GM plant expresses Bt proteins

directed against a specific insect pest, that insect species should be monitored to determine the effect of the Bt toxin expression. However, if potential adverse effects resulting from the Bt toxin expression on a non-target insect population have been identified in the ERA, that non-target insect species should also be monitored to assess the occurrence of adverse effects (see also section 4.4.1 on case-specific monitoring).

General considerations for the choice of monitoring indicators include:

- measurability of the indicator, i.e. the possibilities of collecting reliable data concerning the indicator, and adequacy of the data in terms of statistical power;
- availability of and comparability to baseline data;
- relationship and interaction of the indicator with the GMO, either direct or indirect;
- distribution and abundance of the indicator, preferably widespread and high;
- importance of the indicator for ecosystem processes and functions;
- ability of the indicator to represent protectable items.

A list of possible effects of GMOs on human health and the environment, and thus topics for which suitable indicators should be identified, is provided in Table 4.1.

As pointed out in section 4.4.2 on General Surveillance, it may be difficult to identify suitable indicators for monitoring the occurrence of unforeseen and unanticipated adverse effects. This is simply due to the fact that, since the effects are unforeseen, one cannot predict if such effects will occur at all, and if so, which indicators will be suitable to indicate such effects. Therefore, it has been proposed that general surveillance focus on general environmental protection goals and environmental damage (Bartsch, 2005). In this respect, **environmental damage** can be defined as "*a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly*" (EU, 2002b).

MONITORING INDICATORS
The selection of monitoring indicators is crucial for successful post-release monitoring. A major criterion for the selection of indicators is their potential to indicate changes induced by the GMO release. The selection of monitoring indicators should be performed on a case-by-case basis, based on the characteristics of the GMO and the receiving environment.

ENVIRONMENTAL DAMAGE
One possible approach to identify monitoring indicators is to focus on environmental damage, which can be defined as "a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly".

Damage can manifest itself on environmental protection targets, such as protected or endangered species and natural habitats, water and land including associated biodiversity, ecosystem function and human health, including all services and goods associated with these protection targets. It needs to be assessed if a GMO release negatively and significantly influences any such parameters by collecting reliable data and comparing them with the baseline state of the system. However, care must be taken to verify that any effects are indeed caused by the GMO and not just a variation due to natural causes or within the limits of natural fluctuation. Taken together, environmental protection goals could provide a suitable starting point for defining the indicators and monitoring processes for general surveillance.

Table 4.1 | **Potential impacts of GMOs on human health and the environment for which suitable indicators should be identified in order to assess the occurrence of these effects**

Spread and escape of genetically modified plants into the environment
Volunteers in subsequent crops
Hybridization and introgression with wild relatives and feral crop plants, establishment of hybrids
Effects on non-target flora and fauna in cultivated areas and non-target environments
Secondary infestation of crops and hybrids with bacterial, fungal and viral phytopathogens
Consequences of altered farming practice
Effects of herbicide tolerance technique
Development of crop and weed resistance
Effects on phytophagous invertebrates and their antagonists
Effects on interrelations of the food web
Effects on grain- and plant-feeding mammals and birds
Effects on soil functions
Effects on soil fauna and flora
Horizontal gene transfer on micro-organisms
Effects on water bodies and water organisms
Effects on species biodiversity and habitat diversity
Unexpected gene expression
Unexpected physiological and biochemical plant properties
Effects on human health: toxicity, pathogenicity, allergenicity, nutritional quality

Adapted from: Züghart *et al.*, 2008.

4.5.2 Selecting monitoring sites

Careful choice of monitoring sites is crucial for a successful post-release monitoring programme. The number of areas chosen for monitoring should be sufficient to allow sound statistical analysis of the collected data. Choosing and distributing monitoring sites appropriately enables a carefully designed and systematic monitoring system to be representative for large areas.

SELECTING MONITORING SITES
The choice of monitoring sites is the next important step for any monitoring programme. The number of areas chosen for monitoring should be sufficient to allow sound statistical analysis of the collected data and be representative for larger areas where the GMO is released.

Considerations for the selection of monitoring sites include (Züghart *et al.*, 2008):

- representativeness of sites exposed to GMOs, with special focus on sites under repeated or long-term exposure;
- representativeness of ecological regions containing the chosen monitoring indicators;
- availability of sites already under investigation by complementary monitoring programmes;
- sites facilitating spread or persistence of GMOs due to favourable environmental conditions.

Equally important is the choice of appropriate reference/control sites; such sites must meet minimum requirements regarding representativeness of environmental conditions and comparability to the sites exposed to the GMO to allow meaningful statistical analyses and conclusions to be drawn.

When determining the areas to be monitored the characteristics of the individual GMO (such as its preferred ecological niche, reproduction and growth characteristics, etc.), as well as the ecosystems most likely to be affected by its release, should be carefully considered. If potential adverse effects associated with the release of a GMO are identified and specified in the ERA, the choice of monitoring sites will be straightforward because the areas, and possibly even single parameters, most likely to be affected by the GMO are known. If, however, no specific adverse effects

are identified and general surveillance without concrete testing of hypotheses is performed, the choice of monitoring sites is more difficult. In such cases, the inherent characteristics of the GMO and the occurrence of the selected monitoring indicators at a given site are prime considerations for the determination of suitable monitoring sites. Examples of relevant sites include the fields where a GM crop is grown and the surrounding habitats, i.e. sites that receive the highest exposure to the GMO.

4.5.3 Sampling and data collection methods

SAMPLING AND DATA COLLECTION
The choice of sampling and data collection methods depends on the selected monitoring indicators and monitoring sites. The methodologies used should be scientifically sound and appropriate for the experimental conditions; critical considerations include reproducibility, detection limits, availability of appropriate controls, and specificity and selectivity of each method.

The choice of sampling and data collection methods depends on the selected monitoring indicators and monitoring sites. The methodologies used should be scientifically sound and appropriate for the experimental conditions; critical considerations include reproducibility, detection limits, availability of appropriate controls, and specificity and selectivity of each method. The required sample sizes and sampling frequency required to produce statistically valid results should be defined by statistical means.

Sampling should take into consideration the time and space when potential adverse effects associated with a GMO release are likely to be highest. For example, if a GM crop targets a specific insect pest, sampling should be performed at times when exposure to that insect population is highest. Equally, if a transgenic protein is only expressed in the roots and no other plant parts, sampling should be more focused on soil effects of the GM plant (Layton, 2005). Of course, this does not mean that manifestations of adverse effects at other temporal or spatial points should be neglected.

It is likely that no validated standard methods are available for investigating each monitoring indicator. In such cases, one should adapt available methods to the extent possible and build on the experience of previously performed monitoring

BOX 4.4

MONITORING GM MICRO-ORGANISMS (GMMS)

Genetic modification of micro-organisms is considered to have a promising role to play in obtaining micro-organisms with anticipated usage for bioremediation, protection of plants against pests and diseases or enhancement of symbiosis between plants and beneficial micro-organisms, amongst others. The impact of a GMM release on human health and the environment needs to be carefully assessed and monitored, as for every other GM organism.

However, monitoring GMMs in the environment presents particular difficulties and challenges. In contrast to most GM animals and plants, no direct visual detection of GMMs is possible due to their small size. This requires detection and quantification of GMMs in the environment, and assessment of their potential effects, by laboratory methods. Suitable methods include microscopy, detection of modified DNA via PCR, microarrays and selective plate counting, amongst others (see Module A: Agricultural Biotechnology and Jansson *et al.*, 2000 for detailed introductions to GMO detection and quantification techniques).

Furthermore, specific requirements exist for sampling and statistical analyses. Small amounts of soil may contain billions of bacteria and other micro-organisms representing thousands of different species. This challenges the sensitivity of many available methods for detecting a specific micro-organism, possibly present in only low numbers within the sample. The statistical problems associated with sampling and detection limits are discussed by Heinemann and Traavik (2004), using horizontal gene transfer between GM plants and soil micro-organisms as an example. Further improvement in this area is needed to fully assess the impacts and behaviour of GMMs in the natural environment. An example of a long-term field trial of GMMs is provided by Corich *et al.*, 2007.

programmes as far as possible. Standard ecological sampling and data collection methods should be available and include traps, visual observation and specific sampling techniques e.g. for soil or plant material, possibly in collaboration with subsequent laboratory analyses. Parameters that can be investigated using such techniques include species number, growth rates, biomass, reproduction rates, population increases/decreases and genetic diversity (EU, 2002b). Surveys are an alternative means of data collection, e.g. standardized surveys distributed to farmers that allow the declaration of GMO-related effects and procedures, such as the occurrence of volunteers and persistence of the GMO or changed farming and crop management techniques. In general, it should be specified how, by whom and how often data are collected and collated. The availability of trained personnel to perform sampling and data collection is critical for the entire process.

4.6 DATA ANALYSIS, REPORTING AND REVIEW

DATA EVALUATION
Data analysis should be performed with validated statistical procedures, verifying also the quality of the obtained data. The results of such statistical analyses should allow subsequent decisions to be formulated on a sound scientific basis.

Following the sampling and data collection, the collected data need to be analysed, reported to relevant decision-makers and the public, and fed back into the risk assessment procedure and the design of the monitoring plan.

4.6.1 Data evaluation

The data used for analysis should be of sufficient quality and include relevant baseline data, to allow standard statistical procedures to be applied. Analysis of the data should be performed using validated statistical procedures. The results of such statistical analyses should allow subsequent decisions to be formulated on a sound scientific basis. Furthermore, these analyses should indicate whether the applied sampling and data collection strategies were correct or need to be modified. In cases where adverse effects are identified, it must be clearly distinguished if these effects were caused by the release of the GMO or by other factors. If this is uncertain, further assessments should be performed to clarify this issue.

The results obtained by the data evaluation procedure should be usable in decision-making processes. These include decisions concerning the validity of the ERA and risk management, decisions on renewal or withdrawal of the approval for market release of the GMO, and decisions on countermeasures against adverse effects. As already mentioned, the connection between the results obtained by monitoring and the resulting options and triggers for decision-making need to be verified before commencing any monitoring activity.

4.6.2 Data reporting and data storage

DATA REPORTING
The ability to base decisions on the monitoring data is inherently linked to the reporting of the data. It needs to be ensured that data are communicated to all relevant stakeholders with an interest in agriculture and ecosystem function, relevant decision makers and the general public.

The ability to base decisions on the monitoring data is inherently linked to the reporting of the data. It needs to be ensured that data are communicated to all relevant stakeholders with an interest in agriculture and ecosystem function, relevant decision-makers and the general public. The availability of competent personnel who are capable of translating scientific research findings obtained by the monitoring procedure into a common language is important in this respect. Transparency of the entire monitoring process and subsequent decision-making processes need to be ensured. Methods for communicating and publishing monitoring results could include (EU, 2002b):

- information sheets distributed to users and stakeholders;
- presentation and exchange of information with stakeholders during workshops;
- publication of information in relevant media, e.g. scientific journals;
- archiving of information by the company responsible for the GMO or the responsible competent authority;
- availability of information online, e.g. on company Web sites or Web sites of the responsible competent authority.

In addition, a national database comprising all information obtained from post-release monitoring could be established. Such a database could be used for centralized collection of data, providing processed information to stakeholders,

decision-makers and the general public, and facilitate the exchange of data (Wilhelm *et al.*, 2003). The database could also contain background information on the monitoring programmes. However, the establishment, maintenance and administration of such a database will require a certain amount of financial and personnel input.

4.6.3 Review of the monitoring plan

Following the first monitoring period, the obtained data should be used to review and analyse the monitoring programme itself. Following such an analysis, necessary adjustments or upgrades on the monitoring programme, the monitoring goals and the methodology can be performed. The effectiveness and efficiency of data collection and measurements should be evaluated, including the statistical methods used for data evaluation. Furthermore, it should be verified that the employed measures are effective at addressing the questions and goals of the monitoring programme. If models have been used for predictive purposes and the formulation of hypotheses to be tested, these models should be evaluated and compared with the collected data. Progress and new developments in methods for data collection and measurement should also be incorporated when revising and updating a monitoring programme. In addition to the monitoring plan, the ERA for a given GMO should also be revised and updated using the information generated by the monitoring programme.

4.7 CRITICAL CONSIDERATIONS AND PROBLEMS

A basic goal of monitoring is to create knowledge necessary for the protection of agrosystems, rural livelihoods, human and animal health, and environmental and ecological integrity. Monitoring should be a goal-oriented process, with the aim of identifying and quantifying the effects that a GMO release has on selected agro- and ecosystem parameters; it is not a broad environmental research programme.

Monitoring should address the priorities of all stakeholders concerned with the process; the connection between the results obtained through monitoring and their impact on subsequent decision-making should be clearly defined. This requires precise formulation of goals and questions to be investigated, careful planning of the process, early and continous involvement of stakeholders, and the definition of triggers for decision-making (FAO, 2005).

A major challenge for monitoring is the large variation between agro-ecosystems, individual crop types and their interaction with the environment. Therefore, monitoring programmes need to be designed with regard to the local context and the individual GMO in order to obtain significant and valuable results. Furthermore, even clear effects might be difficult to quantify due to the complexity of agro-ecosystems, and agriculture in itself generates strong ecological signals. Therefore, care must be taken to design monitoring programme so that effects can be detected above the ecological "noise" produced by agriculture, and that a clear cause can be assigned to such effects – i.e. if they are caused by the GMO or not (Jepson, 2005). The careful choice of monitoring indicators and the availability of long-term baseline data and negative controls are critical in this respect.

Another point that needs to be taken into consideration when planning a post-release monitoring programme is the availability of financial resources, infrastructure and trained personnel. The scale of the monitoring programme should be adapted to the available resources, and the costs of monitoring should be in relation to the potential value of the GMO and the consequences of potential adverse effects. Maintaining a correct balance between sound science and practicability in terms of cost and other resources should be aimed at (Bartsch, 2005). An efficient coordination and splitting of tasks between all parties involved in the monitoring process is recommended in order to render the process as effective as possible. Harmonizing and standardizing GMO monitoring procedures and criteria and establishing good monitoring practices will be helpful

REVIEW OF THE MONITORING PLAN

Following the first monitoring period, the obtained data should be used to review and analyse the monitoring programme itself. Following such an analysis, necessary adjustments or upgrades on the monitoring programme, the monitoring goals and the methodology can be performed.

in this respect, for example by systematic and consecutive documentation of monitoring programmes and the establishment of methodological handbooks (Wilhelm *et al.*, 2003).

A summary of recommendations and guidance for scientists, the international community, policy- and decision-makers and international organizations concerning all aspects of monitoring can be found in FAO, 2005. This publication also contains two monitoring programme design templates addressing all relevant points and including relevant case examples, one for developed countries with sufficient knowledge and resources to carry out detailed monitoring programmes, and one for countries with limited experience, information and resources available.

CHAPTER 5

GMO TRACEABILITY AND LABELLING - A NEED FOR COMMERIAL MONITORING

Traceability can be defined as the ability to trace GMOs and products derived from GMOs throughout all stages of the placing on the market, i.e. through all production and distribution chains and networks. Traceability and correct labelling of approved GMOs and products derived from them need to be ensured at all stages of commercial release and placing on the market. Such requirements for traceability of GMOs and correct labelling will ensure that products can be easily withdrawn from the market in case unforeseen adverse effects on human health or the environment are found. Furthermore, traceability will allow targeted monitoring for potential effects of the GMO, and facilitate the implementation of risk management measures (EU, 2003b).

TRACEABILITY
Traceability can be defined as the ability to trace GMOs and products derived from GMOs throughout all stages of the placing on the market, i.e. through all production and distribution chains and networks.

Another important aspect of efficient traceability and labelling systems is the provision of correct and accurate information to every person involved in the trade and marketing of GMOs, and especially to the final consumer. Detailed, complete and reliable information regarding GMOs and derived products will allow consumers to make informed and free product choices.

The more complex the production chain network is, the more difficult it becomes to trace individual products or components of products. Tracing becomes even more difficult in production chain networks with extensive product branching, or with continuous rather than batch production methods. At present, GMO traceability and labelling systems are not being adequately implemented and monitored. Monitoring is only practised for certain *Identity Preservation* systems, representing only a very small proportion (< 1 percent) of the total market. Detailed, clear and feasible provisions and instructions should be given for implementing and monitoring GMO traceability and labelling systems. Steps towards effective and reliable traceability and labelling systems could include:

- the assignment of a simple numeric or alphanumeric code (Unique Identifier) to each single GMO, allowing fast identification of the GMO and retrieval of specific information about that GMO;
- clear and reliable transmission of information, from each stage of market placing or production chain to the next, that the material contains or consists of GMOs; provision of the unique identifier, if available;
- for processed products, an indication of each of the ingredients which is produced from GMOs;
- for pre-packaged products available to the final consumer, a clear notification that the product contains or consists of GMOs should be placed on the label;
- for non pre-packaged products available to the final consumer, a clear notification that the product contains or consists of GMOs should appear in connection with the display of the product.

THRESHOLD VALUES
For labelling purposes, it is recommended that threshold values for the presence of GMO material in food or other products be defined, which, in case they are exceeded, require appropriate labelling of the product.

In many cases, traces of GMO material in processed products may be adventitious or technically unavoidable due to the production and processing processes. In such cases, no traceability and labelling requirements should come into force. However, defined **threshold values** for the presence of adventitious or technically unavoidable GMO material in products should be set. Compliance with such threshold

values should be regularly controlled by adequate GMO detection and quantification techniques (see Module A: Agricultural Biotechnology). If the set threshold value is exceeded, the presence of the GMO material needs to be indicated on the label of the product. Furthermore, only the adventitious or technically unavoidable presence of approved GMOs should be tolerated; material from GMOs that have not received approval for commercial release and placing on the market must not be contained in any products placed on the market and available to consumers.

It is recommended that the responsible competent authority for traceability and labelling requirements regularly perform inspections and controls to check for compliance with traceability and labelling requirements. Several testing methods to detect and quantify GMO material in different samples, both raw material and processed products, exist and should be employed for such inspections and controls (see Module A: Agricultural Biotechnology). Furthermore, it is recommended that information on all stages and transactions performed during placing on the market and processing of a product containing GMO material be recorded and kept for an appropriate time period (e.g. five years in EU legislation) by the person performing such operations. Compliance with such information holding requirements could also be verified by the responsible competent authority.

A detailed discussion on traceability and labelling, focusing on the legal background and relevant international legislative documents, can be found in Module E: Legal Aspects.

CHAPTER 6

MONITORING GMO IMPORTS AND TRANSBOUNDARY MOVEMENTS

MONITORING GMO IMPORTS
Monitoring and controlling imports of GMOs or derived material is an important aspect associated with the commercial release of a GMO.

Worldwide plant quarantine is a legal enforcement measure aimed at preventing pests and pathogens from spreading or, in case these have already found entry and have established in a restricted area, preventing these from multiplying further. The same procedure should be extended to imported GMOs or GMO products from a foreign source which are destined for release within the importing country. There is a need for controlled testing of GMO material in a containment facility prior to release into the environment in order to identify and avoid its potential risks to human health and the environment. Therefore, monitoring the import, the quarantine procedure and post-quarantine handling/movement of the GMOs is crucial to regulate and implement proper application and deployment of GMOs and prevent any form of unintended biosafety regulation violations or oversight.

6.1 IMPORT PROCEDURES AND INFORMATION REQUIREMENTS

The information that should be collected and collated by the exporter prior to any export of GMO or GMO material, and which should be carefully checked by the importer, is listed in Annex 10. The individual steps that should be followed during the export/import procedure by the importing country are delineated and explained in detail below:

a. Collection and verification of adequate information on the nature of the transgene and its expression characteristics in the host organism

The importing institution/organization should be fully aware of the nature of the transgene, its source of origin (bacterial, animal/insect, plant), hazards/risks associated with it and the final expression product(s) of the transgene in the specific host organism.

b. Receive clearance from the GMO regulatory authority in the GMO receiving (importing) country

The statutory GMO regulatory authority is required to clear the import proposal of the GMO or GMO material after assessing:

- the purpose behind the import;
- the product(s) of the transgene with reference to the targeted ecological area;
- detection methodologies employed and validated for detecting the presence of the transgene in the GMO or derived material;
- all information regarding the toxicity/allergenicity/other effects of the transgene product;
- characteristics of transgene expression in the host organism;
- biochemical/physiological consequences or output of the transgene product(s) in the host organism;
- altered characteristics of the host organism due to transgene expression;
- research/commercial permit that the GMO or GMO material has been granted in the exporting country;
- any intellectual property rights regulations connected with the transgene, the GMO or GMO material restricting use in the importing country;
- potential utility and benefits of the transgene and the resulting GMO.

c. Award of the import permit and authorized import accompanied by a phytosanitary certification

For efficient monitoring of imports, it is recommended that a single competent authority (with multiple terminals in the case of large countries) be authorized

to award an import licence. Specific attention should be paid to the existence of earlier imports of the material and the concomitant assignment of an accession number/unique identifier (see below). If there are multiple agencies authorized to award import licences, documentation of the incoming material and assignment of accession numbers can become unsystematic and redundant, thus making monitoring of imports a difficult as well as expensive task. During the import process of the GMO or GMO material, it should be accompanied by the original import permit and the phytosanitary certificate (in the case of plants) from the country of export.

d. Documentation of the national accession number/unique identifier after entry into the importing country

ACCESSION NUMBER/UNIQUE IDENTIFIER
It is recommended that a specific accession number or unique identifier be assigned to each GMO in order to facilitate traceability and tracking of all operations performed with that GMO.

A data bank of all imports should be maintained with complete documentation regarding the material being imported. This will facilitate the evaluation of material in quarantine facilities if similar material has a history of import and quarantine processing. The potential risks can be directly associated with the foreign transgene and the host organism. Assigning a specific **accession number/unique identifier** to every GMO or GMO material entry has to be done carefully in order to prevent any duplication in case the material was already imported earlier. The accession number/unique identifier of the material should be stated as reference for every utilization, deployment or processing of the specific GMO material in the country of import. This will allow fast retrieval of relevant information about the GMO at every stage of GMO usage and by every person involved in any GMO operation (see EU, 2004 as an example).

e. Quarantine processing

Once the GMO or GMO material has received an accession number/unique identifier by the importing country, the material is passed through quarantine filters and procedures. Recommendations of the GMO regulatory authority on the GMO or GMO material that were made while granting the clearance of the import proposal should be taken into consideration for planning and conducting the quarantine procedures.

After passing through the routine quarantine processes the GMO should be kept under containment for a specified time period, depending on the individual GMO (for plants, one reproductive cycle, i.e. one growing season is recommended). During the contained growth, the GMO and derived material are subjected to:

- detection of the transgene that it is documented to be carrying and analysis of the expression characteristics;
- testing for any non-target trait expression of unusual or hazardous nature including pathological indications;
- testing of harvested seed for genetic use restriction technologies (GURT);
- analysis for phytosanitory aspects, i.e. if the GMO and derived material are harbouring any diseases or presents any other relevant phytosanitary hazard (in case of plants).

Following such careful experimental analyses of the imported GMO and verification of the characteristics and specifications of the GMO provided by the exporter, the GMO and derived material should be approved for release in the importing country. However, if any of the provided information is found to be incorrect or any other deviations regarding the characteristics of the GMO are detected, approval should not be granted. In such a case, it is recommended that clarification of the issue be requested from the exporter, and that the impact of the identified deviations of the GMO be analysed further. Specifically, the impact of any detected deviations on the risk assessment of the GMO, i.e. if they represent any form of risk in the context of the importing country and the conditions of the anticipated release, should be carefully assessed.

f. Recording and sample storage of imported GMOs

It is recommended that a "gene bank" of imported GMO material be developed, i.e. a facility to store references of GMO material that has been imported for prolonged periods of time. The samples should be maintained both as viable material and as isolated DNA containing the transgene as extracted from the imported material.

6.2 POST-QUARANTINE HANDLING AND MONITORING OF THE GMO

The competent authority responsible for GMO monitoring should review the import procedure both at the site and time of import as well as during quarantine and post-quarantine processing to ensure compliance with relevant legislation and procedure recommendations. The indicators for monitoring those procedures could be, amongst others:

- the permit for legal entry of the imported material;
- the accompanying phytosanitary certification from the source (exporting) country;
- detection of the transgene in the GMO material imported during quarantine;
- evaluation of the imported GMO material under containment for the recommended time period, including progeny analysis of the imported material and presence of marker genes the material is known to possess;
- correct handling of the GMO material and checking the biosafety level it is grouped in, for work within the quarantine containment facility;
- documentation and maintenance of the DNA from the imported material, with reference to the transgene detected and storage as national referral sample; these reference samples can also be important as standards for comparison in the post-release monitoring process of the imported GMO material.

6.3 FURTHER RECOMMENDATIONS FOR GMO TRANSBOUNDARY MOVEMENT

Efficient supervision and control of transboundary movements of GMOs is recommended in order to limit the potential risks associated with the release of GMOs and allow consumers to make free and informed choices regarding GMOs and derived material. In this respect, the establishment of legal frameworks regulating import, export and transboundary movement of GMOs and derived materials is

recommended. Information plays a critical role in those processes; efficient coordination and sharing of all relevant information concerning a GMO between exporting and importing parties are required in order to allow the parties to make informed decisions on any import/export processes (see Annex 10). Ensuring this is especially important in developing countries, where institutional and/or human capacities to evaluate import/export processes might be limited. In addition to providing and exchanging information prior to import/export activities, relevant information documents should also accompany GMOs and GMO derived material during the import/export and transboundary movement processes; the list provided in Annex 10 can also be used as guidance in this respect.

One international document that extensively addresses the issue of transboundary movement and related problems of GMOs is the Cartagena Protocol on Biosafety (CPB) (CBD, 2000). Please refer to Module E: Legal Aspects for a detailed introduction to the topic.

In the event of an unintentional release in a state of a GMO that has potential adverse effects on human health and the environment, and this release leads to unintentional transboundary movement of the GMO to neighbouring states, the responsible national competent authority should take appropriate measures. Such measures include providing information to the public, affected or potentially affected states, the Biosafety Clearing House created under the CPB and relevant international organizations. Providing detailed information about the GMO and the details of the unintentional release will allow fast and appropriate responses and the implementation of measures to limit the risks posed by the GMO (EU, 2003a).

GMO TRANSBOUNDARY MOVEMENT

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ANNEX 1

RISK ASSESSMENT PARAMETERS AND PROCEDURES FOR GMMS

The following parameters should be taken into account during the risk classification procedure for a GMM operation and result in a classification of the operation into one of the four risk classes. Special attention should be paid to the following harmful effects (EU, 1998):

- diseases to humans including allergenic or toxic effects;
- diseases to plants and animals;
- deleterious effects due to the impossibility of treating a disease or providing an effective prophylaxis;
- deleterious effects due to establishment or dissemination in the environment;
- deleterious effects due to the natural transfer of inserted genetic material to other organisms.

The assessment should be based on the following key points (EU, 1998):

- the identification of any potentially harmful effects, in particular those associated with:
  - the recipient micro-organism;
  - the genetic material inserted (originating from the donor organism);
  - the vector;
  - the donor micro-organism (as long as the donor micro-organism is used during the operation);
  - the resulting GMM;

- the characteristics of the activity;
- the severity of the potentially harmful effects;
- the likelihood of the potentially harmful effects being realized.

The detailed list of parameters recommended for the assessment is provided below (extracted from EU, 1990), structured into thematic groups A to D:

A. Characteristics of the donor, recipient or (where appropriate) parental organism(s)
B. Characteristics of the modified micro-organism
C. Health considerations
D. Environmental considerations

A. Characteristics of the donor, recipient or (where appropriate) parental organism(s)

- name and designation;
- degree of relatedness;
- sources of the organism(s);
- information on reproductive cycles (sexual/asexual) of the parental organism(s) or, where applicable, of the recipient micro-organism;
- history of prior genetic manipulations;
- stability of parental or of recipient organism in terms of relevant genetic traits;
- nature of pathogenicity and virulence, infectivity, toxicity and vectors of disease transmission;
- nature of indigenous vectors;
- DNA sequences;
- frequency of mobilization;
- specificity;
- presence of genes which confer resistance;
- host range;
- other potentially significant physiological traits;
- stability of these traits;

- natural habitat and geographic distribution; climatic characteristics of original habitats;
- 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 or symbiotic properties);
- ability to form survival structures (such as spores or sclerotia).

B. Characteristics of the modified micro-organism

- the description of the modification including the method for introducing the vector insert into the recipient organism or the method used for achieving the genetic modification involved;
- the function of the genetic manipulation and/or of the new nucleic acid;
- 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;
- stability of the micro-organism in terms of genetic traits;
- frequency of mobilization of inserted vector and/or genetic transfer capability;
- rate and level of expression of the new genetic material; method and sensitivity of measurement;
- activity of the expressed protein.

C. Health considerations

- toxic or allergenic effects of non-viable organisms and/or their metabolic products;
- product hazards;
- comparison of the modified micro-organism with the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
- capacity for colonization;
- if the micro-organism is pathogenic to humans who are immunocompetent:

a) diseases caused and mechanism of pathogenicity including invasiveness and virulence;
b) communicability;
c) infective dose;
d) host range, possibility of alteration;
e) possibility of survival outside of human host;
f) presence of vectors or means of dissemination;
g) biological stability;
h) antibiotic resistance patterns;
i) allergenicity;
j) availability of appropriate therapies.

D. Environmental considerations

- factors affecting survival, multiplication and dissemination of the modified micro-organism in the environment;
- available techniques for detection, identification and monitoring of the modified micro-organism;
- available techniques for detecting transfer of the new genetic material to other organisms;
- known and predicted habitats of the modified micro-organism;
- description of ecosystems into which the micro-organism could be accidentally disseminated;
- anticipated mechanism and result of interaction between the modified micro-organism and the organisms or micro-organisms which might be exposed in case of release into the environment;
- known or predicted effects on plants and animals such as pathogenicity, infectivity, toxicity, virulence, vector of pathogen, allergenicity, colonization;
- known or predicted involvement in biogeochemical processes;
- availability of methods for decontamination of the area in case of release into the environment.

Ultimately, careful evaluation of these parameters and, possibly, additional consultation of relevant background literature and risk classification manuals (e.g. WHO, 2004; NIH, 2009) should allow the risk classification of the GMM operation. This risk classification then allows the appropriate containment level and the containment structures that are required to guarantee safe working procedures and protection of human health and the environment to be determined.

The ultimate assignment of a containment level could be further influenced by the following considerations:

- 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);
- the characteristics of the activity (e.g. its scale; nature);
- any non-standard operations (e.g. the inoculation of animals with GMMs; equipment likely to generate aerosols).

An assessment of the above points could lead to a change in the level of risk assigned to the GMM operation, and similarly to the containment level required for that operation (lowering, increment or no effect).

ANNEX 2

CONTAINMENT MEASURES (BIOSAFETY LEVELS) FOR LABORATORIES PERFORMING GMM OPERATIONS

The following table was adapted from Health and Safety Executive, 2007. Different requirements exist for large-scale operations involving GMMs, operations involving GMMs and animals, and operations involving GMMs and plants; please refer to the Health and Safety Executive publication or similar publications (e.g. NIH, 2009; WHO, 2004) for detailed lists of the relevant containment requirements. Detailed annotations of how to comply with the individual points in the table are also included in those publications.

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
Laboratory suite isolation	not required	not required	required	required
Laboratory suitable for fumigation	not required	not required	required	required
EQUIPMENT				
Surface impervious to water and resistance to acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	required for bench	required for bench	required for bench and floor	required for bench, floor, ceiling and walls

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
Entry to laboratory via airlock	not required	not required	required where and to extent the risk assessment shows it is required	required
Negative pressure relative to the pressure of the immediate surroundings	not required	required where and to extent the risk assessment shows it is required	required	required
Extract and input air from the laboratory should be HEPA filtered	not required	not required	HEPA filters required for extract air	HEPA filters required for input and extract air
Microbiological safety cabinet/enclosure	not required	required where and to extent the risk assessment shows it is required	required and all procedures with infective materials required to be contained within a cabinet/ enclosure	Class III cabinet required
Autoclave	required on site	required in the building	required in the laboratory suite	double ended autoclave required in laboratory
SYSTEM OF WORK				
Access restricted to authorized personnel only	not required	required	required	required via airlock key procedure
Specific measures to control aerosol dissemination	not required	required so as to minimize	required so as to prevent	required so as to prevent
Shower	not required	not required	required where and to extent the risk assessment shows it is required	required

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
Protective clothing	suitable protective clothing required	suitable protective clothing required	suitable protective clothing required; footwear required and to extent the risk assessment shows it is required	complete change of clothing and footwear required before entry and exit
Gloves	not required	required where and to extent the risk assessment shows it is required	required	required
Efficient control of disease vectors (e.g. for rodents and insects) which could disseminate the GMM	required where and to extent the risk assessment shows it is required	required	required	required
Specified disinfection procedures in place	required where and to extent the risk assessment shows it is required	required	required	required
WASTE				
Inactivation of GMMs in effluent from hand washing sinks and showers and similar effluents	not required	not required	required and to extent the risk assessment shows it is required	required
Inactivation of GMMs in contaminated material	required by validated means	required by validated means	required by validated means, with waste inactivated in the laboratory suite	required by validated means, with waste inactivated within the laboratory

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
OTHER MEASURES				
Laboratory to contain its own equipment	not required	not required	required so far as is reasonably practicable	required
An observation window or alternative is to be present so that occupants can be seen	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required	required
Safe storage of GMMs	required where and to extent the risk assessment shows it is required	required	required	secure storage required
Written records of staff training	not required	required where and to extent the risk assessment shows it is required	required	required

ANNEX 3

GOOD LABORATORY PRACTICE

The following points should be considered for every operation with GMOs and within containment facilities (EU, 1998; see also WHO, 2004):

- to keep workplace and environmental exposure to any GMM to the lowest practicable level;
- to exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary;
- to test adequately and maintain control measures and equipment;
- to test, when necessary, for the presence of viable process organisms outside the primary physical containment;
- to provide appropriate training of personnel;
- to establish biological safety committees or subcommittees, if required;
- to formulate and implement local codes of practice for the safety of personnel, as required;
- where appropriate to display biohazard signs;
- to provide washing and decontamination facilities for personnel;
- to keep adequate records;
- to prohibit eating, drinking, smoking, applying cosmetics or the storing of food for human consumption in the work area;

- to prohibit mouth pipetting;
- to provide written standard operating procedures, where appropriate, to ensure safety;
- to have effective disinfectants and specified disinfection procedures available in case of spillage of GMMs;
- to provide safe storage for contaminated laboratory equipment and materials, when appropriate.

In addition to these principles, the appropriate containment measures for the risk class of the operation should be in place in order to asssure protection of human health and the environment.

The containment measures applied shall be periodically reviewed by the user to take into account new scientific or technical knowledge relative to risk management and treatment and disposal of wastes.

ANNEX 4

RISK ASSESSMENT PARAMETERS AND PROCEDURES FOR GM PLANTS

The following parameters should be taken into account during the risk classification procedure for a GM plant operation and result in a classification of the operation into one of the four risk classes. The containment measures associated with each of the four risk classes (also referred to as biosafety levels) should be sufficient to control all potential harmful effects of the organisms assigned to a risk class and provide sufficient protection for human health and the environment. The risk assessment procedure can be divided into two parts: a risk assessment for the environment, and a risk assessment for human health. The risk assessment should also take into account the nature of the work, for example, large-scale operations, non-standard operations or non-standard growth facilities (tanks or fermenters for algae, cages for GM trees, etc).

Risk assessment for the environment:

Potential hazards to be considered include:

- the ability of the GM plant to survive, establish and disseminate in the receiving environment;
- hazards associated with the inserted transgene;
- the potential for transfer of the transgene between the GM plant and other organisms;
- phenotypic and genetic stability of the genetic modification.

In detail, the points to be evaluated include (adapted from Health and Safety Executive, 2007):

- the ability of the GM plant to survive and reproduce in the receiving environment;
- the ability of the GM plant to establish, i.e. to colonize habitats and compete with native species (invasiveness);
- enhanced competitiveness of the GM plant compared with other plant species or the unmodified species (weediness);
- the ability of the GM plant to form survival structures (e.g. seeds) and the distance over which they are distributed;
- the ability of a GM plant to cause harm even if it is unable to survive, e.g. by gene transfer;
- the potential of a GM plant to cause adverse effects on organisms in the receiving environment due to the expression of the transgene (nature of the transgene and expressed proteins);
- the ability to cause harm to plants, e.g. by root exudates;
- the ability to cause harm to animals, e.g. by toxic or allergenic expression products;
- the ability to cause harm to beneficial mirco-organisms in the soil or water, e.g. by expression of anti-fungal proteins;
- the ability to cause harm to non-target organisms, e.g. expressing pest-resistance traits that affect a broad range of non-target organisms;
- the possibility of virus transencapsidation, if the transgene codes for a viral coat protein;
- the possibility of recombination between the mRNA of the transgene with the RNA genome of a plant virus;
- the possibility of synergistic effects, e.g. between an infecting virus and an expressed viral coat protein;
- the properties of the transgene product in combination with the expression characteristics, i.e. the temporal and spatial expression profile of toxic or allergenic transgene products;

- verify the genetic and phenotypic stability of the transgene over several generations, e.g. investigate the amount of gene silencing;
- evaluate the possibilities for transgene transfer between the GM plant and other organisms;
- evaluate the possibilities for pollen transfer and outcrossing with related, compatible species;
- special attention should be paid to novel genes, e.g. transgenes coding for biologically active compounds (biopharming).

Risk assessment for human health:

- Nature of the transgene/the expressed proteins: toxic or allergenic effects on humans? Expression of biologically active compounds, e.g. vaccines or other pharmaceutical compounds (biopharming)?
- Possible routes of exposure to transgenic plant material; indirect (e.g. pollen via air), direct contact or ingestion required to obtain adverse effects?

Following the evaluation of these factors, the likelihood of identified potential hazards being realized should be assessed. This can be a difficult process, however several indicators might facilitate this evaluation. For example, specific parameters, obtained by laboratory testing, can be assigned to many processes, such as typical frequencies for hybridization, pollen dispersal ranges, survival rates of the non-modified parent organism in the receiving environment, etc. Characteristics of the receiving environment that either support or restrict potential adverse effects are especially important in this evaluation. A final assessment should classify the likelihood of adverse effects being realized from "negligible" to "high".

Following the assessment of likelihood, the severity of the potential consequences of each hazard should be assessed, again using a classification from "negligible" to "high". Combining the likelihood of a hazard with its consequences yields the

final risk classification (see Module C: Risk Analysis). A precautionary approach should be applied to both the assessment of likelihood and the consequences: when the level of knowledge is insufficient to establish a classification with certainty, a higher level should be employed. The final risk level then defines the containment measures that are required to reduce the risks to "low or effectively zero" (Health and Safety Executive, 2007); the containment measures for the four plant risk classes are provided in Annex 5.

ANNEX 5

CONTAINMENT MEASURES (BIOSAFETY LEVELS) FOR GREENHOUSE ACTIVITIES WITH GM PLANTS

The following containment measures relating to the four biosafety levels for plants (BL1-P to BL4-P) were extracted from the NIH Guidelines (NIH, 2009). Please refer to this or similar publications (e.g. WHO, 2004) for detailed descriptions of the individual containment measures and background information. Where research involving both plants and micro-organisms is performed, the containment measures for GMMs should also be taken into consideration (Annex 2).

In addition to the containment measures listed below, the standards of good laboratory practice (Annex 3) should be followed at all times.

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
GREENHOUSE ACCESS:				
Limited or restricted	Yes	Yes	Yes	Yes
Access managed by responsible individual	/	/	/	Yes, access through secure, locked doors
Warning of potential hazards prior to entering	/	/	/	Yes
Entrance only through clothing change and shower room	/	/	/	Yes, shower each time greenhouse is left
Training prior to access	Yes	Yes	Yes	Yes

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
RECORDS:				
Record of current experiments	Yes	Yes	Yes	Yes
Record of all organisms that are brought into or removed from the greenhouse	/	Yes	Yes	Yes, plus of all materials
Reporting of any accident involving release of GMOs	/	Yes	Yes	Yes
Record of persons entering/ exiting the greenhouse	/	/	/	Yes
DECONTAMINATION AND INACTIVATION:				
GMOs rendered biologically inactive before disposal	Yes	Yes	Yes, autoclaving recommended	Yes, by autoclaving
Decontamination of run-off water	/	Recommended	Yes	Yes
Decontamination of equipment	/	/	Yes	Yes
CONTROL OF UNDESIRED SPECIES:				
Programme to control undesired species	Yes	Yes	Yes	Yes, chemical control
Anthropods and motile macro-organisms kept in cages; precautions to minimize escape	Yes	Yes	Yes	Yes
CONCURRENT EXPERIMENTS CONDUCTED:				
Experiments with a lower biosafety level can be conducted concurrently	Yes	Yes	Yes	Yes
GREENHOUSE DESIGN:				
Greenhouse floor	Gravel or other porous material	Impervious material. Gravel under benches and soil beds acceptable.	Impervious material with collection of run-off water	Walls, roof and floor form sealed, resistant internal shell
Windows and wall/roof openings	May be open for ventilation	May be open for ventilation	Closed and sealed	Closed and sealed
Glazing	/	/	Resistant to breakage	Resistant to breakage
Screens	Recommended	Required	/	/
Greenhouse isolation and entry	/	/	Closed self-contained structure, self-closing locking doors	Closed, self-contained structure, self-closing locking doors

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
Fencing and security	/	/	Yes	Yes
Internal walls, ceilings and floors	/	/	Resistant to penetration	Resistant to penetration
Benchtop material	/	/	Impervious, resistant surfaces	Impervious, resistant surfaces
Hand washing sink/shower	/	/	Sink, automatically operated	Shower
Changing rooms	/	/	/	Yes, outer and inner and shower
Airlock	/	/	/	Yes, for material passage
AUTOCLAVES:				
An autoclave should be available	/	Yes	Yes	Yes, double-door
Air ventilation systems:				
Minimize entrance of anthropods	/	Yes	/	/
Individual supply and exhaust systems	/	/	Yes	Yes
Negative pressure	/	/	Yes	Yes
HEPA filtering of exhaust air	/	/	Yes	Yes
HEPA filtering of ventilation lines	/	/	Yes, on vacuum lines	Yes
SIGNS:				
Signs indicating that a restricted experiment is in progress	/	Yes	Yes	Yes
Signs indicating the presence of organisms with potential for environmental damage	/	Yes, if applicable	Yes, if applicable	Yes, if applicable
Sign indicating risks to human health (biohazard sign)	/	Yes, if applicable	Yes, if applicable	Yes, if applicable
TRANSFER OF MATERIALS:				
Transfer of viable organisms to/from the facility	/	Transfer in a closed, non-breakable container	Transfer in a sealed secondary container	Transfer in a sealed secondary container

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
Transfer of materials and supplies	/	/	/	Transfer through autoclave airlock or fumigation chamber
PROTECTIVE CLOTHING:				
Disposable clothing should be worn in the greenhouse	/	/	Yes, if considered necessary	Yes, may be disposable
Exchange of street clothing to complete laboratory clothing	/	/	/	Yes
Protective clothing removed before exiting the greenhouse and decontaminated	/	/	Yes	Yes, by autoclaving
GREENHOUSE PRACTICES MANUAL:				
A greenhouse practices manual should be prepared and adopted	/	Yes	Yes	Yes
OTHER:				
Hand wash upon exiting the greenhouse	/	/	Yes	/
Shower upon exit	/	/	/	Yes
Procedures performed to minimize creation of aerosols/splashes	/	/	Yes	Yes

ANNEX 6

RISK ASSESSMENT PARAMETERS AND PROCEDURES FOR GM ANIMALS

The risk assessment process for GM animals is essentially the same as already described for GM micro-organisms and GM plants in Annexes 1 and 4, respectively. Again, the risk assessment procedure can be divided in a risk assessment for the environment and a risk assessment for human health. Points to evaluate include:

Risk assessment for the environment:

- ability of the GM animal to survive in the receiving environment;
- adverse effects if the GM animal cannot establish, but is able to survive in the short term;
- interactions of the GM animal in the receiving environment, e.g. displacement of or competition with native species, prey upon native species (including plants) and physical damage, including all direct and indirect implications for ecosystem function;
- effects of the genetic modification on the animal's survivability and niche range (e.g. increased tolerance to environmental conditions or increased fecundity);
- feasibility of recovering escaped individuals;
- expression of biologically active compounds (biopharming) and effects on interacting species;
- potential of the GM animal to act as a novel animal disease vector or reservoir;

- transfer of transgenes to other species in the receiving environment; presence of sexually compatible species;
- the nature of the transgene with regard to possible transgene transfer: if it confers a selective advantage or disadvantage;
- transgene stability and possible transgene loss with subsequent effects.

Risk assessment for human health:

- nature of the transgene and expressed protein: possible toxic or allergenic effects, bioactive compounds;
- GM animals acting as vectors or reservoirs for human diseases due to the genetic modification;
- altered behaviour of the GM animal, e.g. enhanced aggressiveness;
- general risk for human health arising from animal handling that might be influenced by the genetic modification, e.g. bites, scratches, zoonotic infections or allergenic reactions.

For further discussion of the individual points, please refer to Health and Safety Executive, 2007.

Following the hazard identification procedure, an assessment of the likelihood of these hazards being realized, as well as an assessment of the consequences in case the hazards are realized, is performed. This allows the establishment of a final risk classification and the grouping of the GM animal operation into one of four risk classes (biosafety levels). The characteristics of the receiving environment as well as the scale and nature of the GM animal operation are critical parameters in these assessments and require special consideration.

The containment measures for biosafety levels 1 to 4 for GM animals, which are required to reduce the risks to human health and the environment to low or effectively zero, are listed below in Annex 7.

ANNEX 7

CONTAINMENT MEASURES (BIOSAFETY LEVELS) FOR GM ANIMALS

In addition to these general biosafety requirements (extracted from NIH, 2009; please refer to that publication for details) special recommendations concerning the housing of specific groups of organisms (large and small mammals, aquatic animals, insects, etc.) exist. Details can be found in relevant guidance documents, see for example Health and Safety Executive, 2007; WHO, 2004.

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
ANIMAL FACILITY:				
Animals contained in enclosed structure (animal room)	Yes	Yes	Yes	Yes
Interior walls, floors and ceilings impervious and resistant	/	Yes	Yes	Yes
Windows	/	Fitted with fly screens	Closed, sealed, breakage resistant	Closed, sealed, breakage resistant
Autoclave available	/	Yes	Yes	Yes, or incinerator
Self-closing doors	/	/	Yes	Yes
Anthropod-proof structure	/	Yes	Yes	Yes
Double barrier between containment area and environment	/	/	Yes	Yes, animal area separated from all other areas

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
Necropsy room	/	/	/	Yes
Decontamination of waste and run-off water	/	/	Yes	Yes, by heat or chemical methods
Directional airflow (inwards)	/	/	Yes	Yes
Double HEPA filtering of exhaust air	/	/	Single filter, if required	Yes
Exhaust air incinerator	/	/	/	Yes, as alternative to double HEPA filtering
Floor drains with deep traps	/	/	/	Yes
Hand washing sink	/	/	/	Yes, automatically operated
Restraining devices for animals	/	/	/	Yes
Supply water system with backflow preventer	/	/	/	Yes
All utilities, liquid and gas services with backflow preventer	/	/	/	Yes
Ventilation lines with HEPA filters	/	/	/	Yes
ANIMAL FACILITY ACCESS:				
Individuals under 16 years not permitted	/	/	/	Yes
Containment area locked	Yes	Yes	Yes	Yes
Containment area patrolled or monitored	Yes	Yes	Yes	Yes
Containment building patrolled, with locking access	/	Yes	Yes	Yes
Restricted access, warning of potential hazards	Yes	Yes	Yes	Yes
Entrance/exit through clothing change/shower rooms	/	/	/	Yes

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
All closures closed when experiment in progress	/	/	Yes	Yes
DECONTAMINATION AND INACTIVATION:				
All wastes decontaminated	/	Yes	Yes	Yes
Work surfaces and equipment decontaminated after work	/	/	Yes	Yes
Removal of material	/	/	Special requirements	Only after autoclaving
Chemical disinfectant shower for ventilated suits	/	/	/	Yes, if such suits are required
Needles and syringes placed in puncture-resistant containers	/	Yes, and decontaminated	Yes, and decontaminated	Yes, and decontaminated
SIGNS:				
Biohazard sign if special provisions (e.g. vaccination) required for entry	/	Yes	Yes	Yes
PROTECTIVE CLOTHING:				
Complete change of street clothing to laboratory clothing	/	No, but laboratory coats and gloves required	Yes, special care to minimize skin contamination	Yes, entry/exit only through change and shower rooms
Decontamination of clothing	/	/	Yes	Yes
Ventilated positive pressure suit	/	/	/	If appropriate
Respiratory protection	/	/	Yes	Yes
Records:				
Records of animal use and disposal	/	/	Yes	Yes
Records of incidents and accidents	/	Yes	Yes	Yes
Record of baseline serum samples	/	Yes, if appropriate	Yes, if appropriate	Yes
Record of personnel entry/exit	/	/	/	Yes

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
TRANSFER OF MATERIALS:				
Decontamination of material before removal	/	Yes	Yes	Yes, by autoclaving or gaseous/vapour methods
Material container for transport	/	Primary and secondary container required	Primary and secondary container required	Primary and secondary container required
Entry of materials and supplies	/	/	/	Through double-door autoclave or airlock
OTHER:				
Mark all GM neonates within 72 hours after birth	Yes	Yes	Yes	Yes
Eating, drinking, smoking and applying cosmetics not permitted	/	Yes	Yes	Yes
Hand wash before exiting containment area	/	Yes	Yes, or showering	Showering required
Concurrent conduct of experiments with a lower BL	Yes	Yes	Yes	Yes
Animal areas cleaned daily	/	/	Yes	Yes
Minimize creation of aerosols	/	/	Yes	Yes
Separate male and female animals	Yes	Yes	Yes	Yes
Life support system for ventilated suits with alarms and backup air tanks	/	/	/	Yes, if such suits are required
Specifications for needles and syringes	/	Yes	Yes	Yes
Quarantine, isolation and medical care facility for personnel	/	/	/	Yes
Preparation and adoption of a biosafety manual	/	Yes	Yes	Yes
Vacuum lines protected with HEPA filters	/	/	Yes	Yes
Appropriate steps to prevent horizontal transmission	/	Yes	Yes	Yes

ANNEX 8

MINIMUM ISOLATION DISTANCES AND MONITORING FREQUENCY FOR CONFINED FIELD TRIALS

The following table, stating minimum isolation distances and monitoring frequencies for selected GM crops in confined field trials, was adapted from Adair and Irwin, 2008.

Table | **Minimum isolation distances, periods of post-harvest land use restriction, and minimum monitoring frequency for confined research field trials**

CROP	MINIMUM ISOLATION DISTANCE	PERIOD OF POST-HARVEST LAND USE RESTRICTION	MONITORING FREQUENCY	
			Trial period	Post-harvest period
Agrostis palustris Huds. (creeping bentgrass)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Beta vulgaris L. (sugar beet)	3 m and harvest before flowering	2 years	weekly	every 2 weeks
Brassica carinata A. Braun (Ethiopian mustard)	200 m from other *Brassica* spp. 50 m from weedy relatives	3 years	weekly	every 2 weeks
Brassica juncea L. (brown mustard)	200 m from other *Brassica* spp. 50 m from weedy relatives	5 years	weekly	every 2 weeks
Brassica napus L. (Argentine rape canola)	200 m from other *Brassica* spp. 50 m from weedy relatives	3 years	weekly	every 2 weeks
Brassica rapa L. (Polish rape canola)	400 m from other *Brassica rapa* 200 m from other *Brassica* spp. 50 m from weedy relatives	5 years	weekly	every 2 weeks
Capsicum annuum (pepper)	20 m	1 year	every 2 weeks	every 2 weeks
Carthamus tinctorius L. (safflower)	400 m	2 years	weekly	every 2 weeks

CROP	MINIMUM ISOLATION DISTANCE	PERIOD OF POST-HARVEST LAND USE RESTRICTION	MONITORING FREQUENCY	
			Trial period	Post-harvest period
Cucurbita pepo L. (squash)	650 m	1 year	weekly	every 2 weeks
Glycine max (L.) Merr. (soybean)	10 m	1 year	every 2 weeks	every 2 weeks
Helianthus annuus L. (sunflower)			weekly	every 2 weeks
Hordeum vulgare L. (barley)	10 m	2 years	every 2 weeks	every 2 weeks
Lens culinaris Medik (lentil)	10 m	1 year	every 2 weeks	every 2 weeks
Linum usitatissimum L. (flax)	10 m	2 years	weekly	weekly
Lolium perenne L. (perennial grass)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Lycopersicon esculentum Mill. (tomato)	20 m	1 year	weekly	every 2 weeks
Medicago sativa L. (alfalfa)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Nicotiana tabacum (tobacco)	400 m	1 year		
Phalaris canariensis L. (canary seed)	10 m	2 years	every 2 weeks	every 2 weeks
Picea spp. (spruce)	removal of seeds and pollen cones	2 years minimum	monthly, twice a week during cone formation	monthly
Pisum sativum L. (pea)	10 m	1 year	every 2 weeks	every 2 weeks
Populus spp. (poplar)	removal of inflorescences	3 years minimum	monthly, twice a week during flowering and budburst	monthly
Sinapis alba L. (white mustard)	400 m from other *S. alba* 50 m from other *Brassica* spp. and weedy relatives	5 years	weekly	every 2 weeks
Solanum tuberosum L. (potato)	one blank row (~ 1 metre)	2 years	weekly	every 2 weeks
Trifolium repens L. (white clover)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Triticum aestivum L. (wheat)	30 m	2 years	every 2 weeks	every 2 weeks
Vitis spp. (grapevine)	bagging of flowers	3 years minimum	monthly, weekly at pollen shed	monthly
Zea mays L. (corn)	200 m	1 year	weekly	every 2 weeks

ANNEX 9

EXAMPLES OF INSPECTION QUESTIONS/MONITORING INDICATORS FOR CONFINED FIELD TRIALS

The following points can be used as a checklist to verify the compliance of a confined field trial with basic confinement and biosafety requirements as described in the main text. They could be used either by trial managers (permit holders) themselves to verify if their management of a confined field trial is correct, or by the competent authorities to check if confined field trials are being performed according to the issued release permit. The list only provides examples and thus is not exhaustive and should be adapted, and possibly extended, according to the local conditions and requirements of a field trial on a case-by-case basis. Adapted from Gosh, 2002; Department of Biotechnology, 2006; APHIS, 2008.

- Were the competent authorities informed of the trial? Was a correct application handed in and a release permit issued?
- Do the shipping and packing containers used for this field trial meet the specifications in the release permit?
- Were packing and shipping materials used for this field trial cleaned out and disposed of to meet the release permit?
- Were transport and storage containers employed so as to fully contain the GMO material at the field trial location?
- Are seed bags, packages, pots or other containers used for the GMO material clearly and durably marked so that each individual GMO can be distinguished and identified by the permit holder throughout the field trial process?

- Was an up-to-date map of the field trial site prepared and supplied to the competent authority?
- Conduct of the trial: Is the trial being conducted according to the approved field design with the replications and plot size mentioned (with acreage at or below the area indicated in the release permit)?
- Isolation: Is the isolation distance around the experimental area maintained with no related species or varieties of the same species in the area?
- If border rows are present in the field trial site, are they grown to meet permit conditions?
- If flower removal was used to control reproduction, was the technique employed successfully and recorded?
- If flower bagging was used to control reproduction, was the technique employed successfully and recorded?
- If temporal isolation (flowering time) was used to control reproduction, was the technique employed successfully and recorded?
- Is the design and management of the outermost boundary of the field site(s) sufficient to assure segregation and confinement during all field operations and growth stages?
- Are photographs clearly documenting the isolation of the crop right through planting to harvesting and post harvest management of crop debris?
- Does the permit holder have monitoring and removal records for sexually compatible plants within the isolation area of the field trial?
- Are measures being taken to minimize or prevent expected human or animal incursions onto the field trial?
- Toxicity/allergenicity data: Is the evaluation of the impact of the transgene product for its likelihood of causing any allergies or toxicity based on the guidelines in use?
- Safe storage of harvested seed and salvaging any spill in the field: Is sufficient care taken to harvest as much seed as possible and no seed is spilled and left behind? What measures were taken?

- Were operations to dispose and devitalize the GMO material (including field trial borders) fully employed?
- Do records show that equipment used in this field trial meets the specifications for the frequency and type of cleaning required in the release permit?
- Maintenance of field data: Were entire experimental data maintained and recorded and supplied to the competent authority?
- Do descriptions or records demonstrate that the permit holder is monitoring for deleterious/negative effects expressed by the regulated crop on itself, other plants, non-target species, or the environment?
- Are all the safety guidelines with respect to the personnel working with the experimenters taken care of?
- Were any accidents encountered? How was the emergency attended to by the competent authorities? Were any accidents and the countermeasures taken clearly documented and reported?
- Was a logbook recording the entries of all persons into the trial site correctly maintained?
- Was any unknown pest, insect or pathogen harmful or otherwise noted on the transgenic crop? If so, was it brought to the notice of the competent authority? What was the action taken after the observation?

ANNEX 10

RECOMMENDATIONS FOR INFORMATION THAT SHOULD BE PROVIDED BY THE EXPORTING PARTY FOR TRANSBOUNDARY MOVEMENTS AND IMPORT OF GMOs OR GMO-DERIVED MATERIAL

The following list provides indications on information that should be collected and collated by the exporting party prior to any GMO export. The information should be made available to the importing party before commencing any intentional transboundary movements. This list shall serve as a guideline, and may be extended or modified in adaptation to national requirements and the specific context and local conditions for import/export. Adapted from EU, 2003a.

- Name, address and contact details of the exporter.
- Name, address and contact details of the importer.
- Name and identity of the GMO, as well as the domestic classification, if any, of the biosafety level of the GMO in the state of export.
- Intended date or dates of the transboundary movement, if known.
- Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety.

- Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate.
- Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety.
- Description of the nucleic acid or the modification introduced, the technique used, and the resulting characteristics of the GMO.
- Intended use of the GMO or products thereof, namely, processed materials that are of GMO origin, containing detectable novel combinations of replicable genetic material obtained through techniques listed in Box 2.1.
- Quantity or volume of the GMO to be transferred.
- A previous and existing risk assessment report.
- Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate.
- Regulatory status of the GMO within the state of export (for example, whether it is prohibited in the state of export, whether there are other restrictions, or whether it has been approved for general release) and, if the GMO is banned in the state of export, the reason or reasons for the ban.
- Result and purpose of any notification by the exporter to other states regarding the GMO to be transferred.
- A declaration that the above-mentioned information is factually correct.

ANNEX 11

SUMMARY OF INFORMATION RECOMMENDED TO BE COLLECTED AND COLLATED PRIOR TO THE COMMERCIAL RELEASE OF A GMO[1]

I. GENERAL INFORMATION

A. Name and address of the notifier (company or institute)

B. Name, qualifications and experience of the responsible scientist(s)

C. Title of the project

D. Designation and specification of the GMO and/or derived products

E. Where applicable, a detailed description of the method of production and manufacturing

F. Where appropriate, the conditions for placing on the market the food(s) or feed(s) produced from it, including specific conditions for use and handling

II. INFORMATION RELATING TO THE GMO

A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):

» scientific name;

1 Adapted from: EU, 2001; EFSA, 2006a

- taxonomy (family genus, species, subspecies, cultivar);
- other names (usual name, strain name, etc.);
- phenotypic and genetic markers;
- degree of relatedness between donor and recipient or between parental organisms;
- description of identification and detection techniques;
- sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
- description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts;
- organisms with which transfer of genetic material is known to occur under natural conditions;
- verification of the genetic stability of the organisms and factors affecting it;
- pathological, ecological and physiological traits:
  - classification of hazard according to the existing European Union's rules concerning the protection of human health and/or the environment;
  - generation time in natural ecosystems, sexual and asexual reproductive cycle; specific factors affecting reproduction, if any
  - information on survival, including seasonability and the ability to form survival structures;
  - pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism; possible activation of latent viruses (proviruses); ability to colonize other organisms;
  - antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;
  - involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.
- Sexual compatibility with other cultivated or wild species;

- Other potential interactions of the GMO with organisms in the ecosystem where it is usually grown, or elsewhere;
- Dissemination:
  - ways and extent (for example, an estimation of how viable pollen and/or seeds decline with distance) of dissemination;
  - specific factors affecting dissemination, if any.
- Nature of indigenous vectors:
  - sequence;
  - frequency of mobilization;
  - specificity;
  - presence of genes which confer resistance.
- History of previous genetic modifications.

B. Characteristics of the vector

- nature and source of the vector;
- sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO;
- frequency of mobilization of inserted vector and/or genetic transfer capabilities and methods of determination;
- information on the degree to which the vector is limited to the DNA required to perform the intended function.

C. Characteristics of the modified organism

- Information relating to the genetic modification:
  - methods used for the modification;
  - methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;
  - description of the insert and/or vector construction;
  - purity of the insert from any unknown sequence and information on the

degree to which the inserted sequence is limited to the DNA required to perform the intended function;

- methods and criteria used for selection;
- sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question, with particular reference to any known harmful sequence;
- location(s) of the insert(s) in the cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination;
- in case of deletion, size and function of the deleted region(s).

- Information on the final GMO:
  - description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;
  - structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;
  - stability of the organism in terms of genetic traits;
  - rate and level of expression of the new genetic material; method and sensitivity of measurement;
  - parts of the organism where the insert is expressed (for example roots, stem, pollen, etc.);
  - activity of the expressed protein(s);
  - description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;
  - sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
  - information on how the genetically modified plant differs from the recipient plant in:
  - mode(s) and/or rate of reproduction;

- dissemination;
- survivability.
- history of previous releases or uses of the GMO;
- considerations for human health and animal health, as well as plant health:
- toxic or allergenic effects of the GMOs and/or their metabolic products;
- comparison of the modified organism with the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
- capacity for colonization;
- if the organism is pathogenic to humans who are immunocompetent:
- diseases caused and mechanism of pathogenicity, including invasiveness and virulence
- communicability
- infective dose
- host range, possibility of alteration,
- possibility of survival outside of human host
- presence of vectors or means of dissemination
- biological stability
- antibiotic resistance patterns,
- allergenicity,
- availability of appropriate therapies.

» (v) other product hazards.

III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A. Information on the release

» description of the proposed deliberate release, including the purpose(s) and foreseen products;

» foreseen dates of the release and time planning of the experiment, including frequency and duration of releases;

- methods for preparing and managing the release site, prior to, during and post-release, including cultivation practices and harvesting methods;
- size of the site;
- method(s) to be used for the release;
- quantities of GMOs to be released;
- disturbance on the site (type and method of cultivation, mining, irrigation, or other activities);
- worker protection measures taken during the release;
- post-release treatment of the site;
- techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment;
- information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

B. Information on the environment (both on the site and in the wider environment):

- geographical location and grid reference of the site(s) (in case of notifications under part C the site(s) of release will be the foreseen areas of use of the product);
- physical or biological proximity to humans and other significant biota;
- proximity to significant biotopes, protected areas, or drinking water supplies;
- climatic characteristics of the region(s) likely to be affected;
- geographical, geological and pedological characteristics;
- flora and fauna, including crops, livestock and migratory species;
- description of target and non-target ecosystems likely to be affected;
- a comparison of the natural habitat of the recipient organism with the proposed site(s) of release;
- any known planned developments or changes in land use in the region which could influence the environmental impact of the release;
- presence of sexually compatible wild relatives or cultivated species.

IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT

A. Characteristics affecting survival, multiplication and dissemination

- biological features which affect survival, multiplication and dispersal;
- known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.);
- sensitivity to specific agents.

B. Interactions with the environment

- predicted habitat of the GMOs;
- studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses;
- genetic transfer capability
  - post-release transfer of genetic material from GMOs into organisms in affected ecosystems;
  - post-release transfer of genetic material from indigenous organisms to the GMOs;
- likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism;
- measures employed to ensure and to verify genetic stability; description of genetic traits which may prevent or minimize dispersal of genetic material; methods to verify genetic stability;
- routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.;
- description of ecosystems into which the GMOs could be disseminated;
- potential for excessive population increase in the environment;

- competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s);
- identification and description of the target organisms, if applicable;
- anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable;
- identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction;
- likelihood of post-release shifts in biological interactions or in host range;
- known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens;
- known or predicted involvement in biogeochemical processes and other effects on the abiotic environment;
- other potential interactions with the environment.

V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A. Monitoring techniques

- methods for tracing the GMOs, and for monitoring their effects;
- specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques;
- techniques for detecting transfer of the donated genetic material to other organisms;
- duration and frequency of the monitoring.

B. Control of the release

- methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of release or the designated area for use;

- methods and procedures to protect the site from intrusion by unauthorized individuals;
- methods and procedures to prevent other organisms from entering the site;
- description of methods for post-release treatment of the site.

C. Waste treatment

- type of waste generated;
- expected amount of waste;
- description of treatment envisaged.

D. Emergency response plans

- methods and procedures for controlling the GMOs in case of unexpected spread;
- methods for decontamination of the areas affected, for example eradication of the GMOs;
- methods for disposal or sanitation of plants, animals, soil, etc. that were exposed during or after the spread;
- methods for the isolation of the area affected by the spread;
- plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

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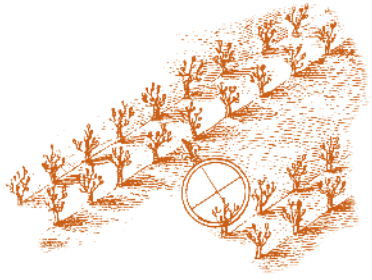

Design and layout: Pietro Bartoleschi and Arianna Guida (Studio Bartoleschi)
Cover illustrations elaborated from “l’Encyclopédie Diderot et d’Alembert”
Printed in Italy on ecological paper, Forest Stewardship Council (FSC) certified, May 2011

Biosafety Resource Book

MODULE D

TEST AND POST-RELEASE MONITORING OF GMOs

addresses the use and monitoring of GMOs under containment, confinement and limited field trials, as well as the monitoring of commercially released GMOs. It also covers surveillance and emergency planning.

For additional information please consult
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or contact
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