

Chapter 5

# Biosafety Monitoring & Enforcement

## Introduction

Monitoring is a process of keeping track of activities so as to determine whether they are on schedule and whether they are meeting the target objective. Evaluation is a process of gathering data to determine the extent to which the project has met the target. There could two types of evaluation namely, the formative evaluation which is undertaken at any time during the implementation of a project and the final evaluation undertaken after the project is completed so as to determine the level of impact. The impact could be noticed in the short or long term.

Inspection is a mechanism to check compliance to regulations and permit conditions. Inspections are usually carried out by government, but there are examples where inspection and reporting is made the responsibility of the applicant, e.g. in Zimbabwe. When planning for an inspectorate there are two choices: create a new group of inspectors that focus on biosafety or train existing inspectors to include biosafety in their job descriptions. The former is an expensive option as the inspectors need to reach legal competence to carry out their function and there is seldom enough work to justify this in the early years of biotechnology implementation. The second option is most used. Many governments have agricultural, health and environmental inspectors and these are trained in biosafety and often twinned with scientists until they feel confident to carry out their biosafety inspection duties on their own.

## Purpose of Monitoring

The purpose of monitoring and evaluation is to gather data concerning the modified organism in order to assess the extent, to which

*Sections of this chapter have been taken from a work book for technical training, Biosafety and Risk Assessment in Agricultural Biotechnology ABSP, Michigan State University, USA*

transgenic have impacted on the environment, and human health. When referring to the environment, the main focus is on trials and release of living organisms such as plants, animals, and microorganisms. Thus, monitoring would determine effects, which could be categorized as severe, moderate, low, negligible or no harm. In the case of plants, monitoring will be undertaken to determine the level of horizontal gene transfer and to develop a monitoring and evaluation prospectus, Monitoring of the genetically modified organisms can be undertaken at different levels. At the initiation of a project it is possible to do an initial monitoring to ensure that all things are organised according to the plan. At several stages during the execution of the project, it is possible to undertake monitoring of the progress. Two types of evaluations can be undertaken, namely at the formative and the final stages. There are two different types of monitoring which can be associated with the release of GM plants:

Monitoring which is required by the government and is intended to confirm any assumptions made in at the risk assessment procedures and voluntary monitoring which is undertaken by the applicant in order to provide further information for his or her own purposes.

## Biosafety Monitoring

Biosafety considerations are important in determining the need for monitoring, identifying appropriate target(s), and justifying the reasons for establishing specified levels of monitoring. One of the important environmental issues for GM crops is whether the GMO or its DNA poses a safety concern should it move into adjacent fields or to related plant species. This out flow of the foreign gene is called gene flow. This issue raises the question to what extent transgene movement can or should be monitored. Furthermore, it necessitates that efficient methods of identifying transgenic plants or transgenes present in non-target species are available.

Current options include use of visual or selectable markers (e.g., b-glucuronidase; antibiotic resistance) or molecular analysis (e.g., PCR, Southern hybridisation). Often the decision as to what to monitor for has depended as much on what is possible to monitor as it has on the identified concern. It is often the case, however, that without knowing what potential problem may arise, it is not possible to know what to monitor. This has presented a monitoring paradox.

## Scales of Monitoring

Monitoring programs, as a part of a field test release or during the pre- or post-marketing stage of product development, fall into three categories: experimentation, surveillance, and tracking. Along with asking and trying to answer different questions at the different stages, the transitions from small scale field testing to test marketing and on to full commercialisation bring with them the need to consider larger geographic sampling area and longer term observation regimes. Further, care must be taken in extrapolating experimental field test results to commercial applications. For example, significant variations in gene flow measurements have been associated with increasing population size. The order of increasing temporal and spatial

scales of monitoring programs mirrors their increasing difficulty to control and implement. Similarly, the magnitude of potential adverse effect and the degree of uncertainty in monitored parameter mirrors the need for increasing the intensity of the monitoring programme.

## Types of Monitoring

Monitoring is used to gather additional scientific data to assist the assessment of risk and decision-making. Monitoring is carried out for specific reasons and at specific times in the development of GMOs. The various types of monitoring are illustrated below.

**Case-specific monitoring**

Serves to confirm any assumption derived from risk assessment regarding potential adverse effects of the GMO or its use on human health or the environment. It deals with the observation of certain adverse effects, i.e. "immediate and direct as well as delayed or indirect effects which have been identified in the environmental risk assessment" relating to individual approvals for placing on the market over a limited period of time.

**General surveillance monitoring**

Used for the long-term observation in Good Manufacturing Practices (GMPs) and covers the observation of adverse effects of the GMO or its use for human health and the environment that were not predicted in the risk assessment for one particular product. To be able to identify these adverse effects, general surveillance should consist of elements based on effect-hypotheses and elements not based on clearly defined hypotheses. If changes in the environment are identified further examination is required. An additional component could be existing observation programmes which could be adapted to the needs of monitoring GMPs. In a first range this could be environment observation programmes as well as programmes in the field of agriculture, food surveys, nature conservation, soil observation and veterinary surveys.

**Voluntary monitoring**

Might include data collection for the further development of a program of release proposals, e.g. by accumulation of data on survival of the GM plant in the environment. It might also mean obtaining data to better understand the probability or impact of risk and thus allow informed relaxation of unnecessary safeguards in future releases.

**Monitoring by applicants**

Monitoring by the applicant is to be done at the field level. It enables the applicant to take measures to ensure that the trial is proceedings as expected and if unexpected problem arise, the applicant should immediately take action and notify the authorities.

Important things to remember are that projects of different types will require their own methods of monitoring and evaluations. The projects could be in the following categories:

a. Institutional laboratories
b. Quarantine and greenhouse
c. Field testing of transgenic plants
d. Small scale field tests of micro-organisms

e. Large scale field releases
f. Release into the market

For the purpose of this resource manual all types of projects are covered starting from laboratory to commercial release. Some of the important things to consider are:

a. Monitoring
   i. develop the monitoring indicators
   ii. develop the target outputs
   iii. develop the performance measures
b. Evaluation
   i. determine at what stage the evaluation will be undertaken , that is, put timelines for both formative and final evaluation.
   ii. provide for the resources which will be needed for both monitoring and evaluation.

Up to now there are no proposed indicators to monitor the effect of transgenic plants in general. Most releases which pose no zero risk to the environment will require appropriate monitoring to ensure that no harm results from the release.

**Experimentation**

Experimentation refers to that exercise that is part of early stage, research and development procedures. In small scale field tests, monitoring might be designed to answer specific questions about product performance or provide basic information on the biology of organisms and their interactions with the environment. With regard to biosafety issues, a monitoring program might be designed to test pre-release evaluations of gene flow or the potential impacts of gene exchange should it occur. Any of these issues may have been raised at the risk assessment stage of an application review. If there are restrictions imposed as a condition for application approval, a monitoring procedure may be proposed to fulfill some or the entire requirement.

When field testing of genetically modified microorganisms first began in the U.S., assumptions regarding monitoring needs led to rather ill conceived and expensive protocols. Perhaps because there was little experience and no experimental evidence to draw upon, unproven methods were often chosen. During the course of the field testing, it was discovered that these monitoring procedures were inadequate (i.e., inappropriately timed, poor detection discrimination, or naively conceived). The result was expensive monitoring schemes that produced little or no usable data. Research and field testing experience have led to the unfortunate conclusion that these early monitoring procedures used would not answer the questions of concern. Experimenters and biosafety authorities must be aware that they will not always know the best monitoring approach a priori. This argues for having a risk assessment/risk management process that balances concerns with the reality of scientific capability.

**Tracking**

Tracking is used primarily to monitor the movement and dispersal of the organisms and their genes. For most crop plants which do not survive well beyond cultivated fields, this has not been of great

concern. For those crop plants that have close relatives in proximity to the cultivated plots, however, there has been concern for outcrossing of the engineered genes. Recent experimental results have shown that such outcrossing does occur (e.g., from mustard to wild mustard and from cultivated sorghum to indigenous species).

Expanding the geographic range or duration of 'sampling' beyond small-scale field testing poses significant difficulties to a comprehensive monitoring program. Assumptions about the best monitoring design and methodologies have to be made based on incomplete or insufficient information despite what is often characterised as long term experience with specific organisms and a full understanding of their growth characteristics. Episodic events at disparate intervals may produce very large differences in monitoring data. For example, the dispersal distance for oilseed rape pollen from commercial fields was measured at >150 m as opposed to <10 m from experimental plots. Recent gene flow studies in Southern Africa include sorghum studies in South Africa and Zimbabwe where pollen dispersal data is needed to help plan effective confinement conditions for future GM sorghum trials. The sorghum gene flow studies are being carried out with non-GM sorghum, using visible phenotypic differences between known varieties. For very low probability events, spatial and temporal expansion of monitoring protocols may be necessary if there is any hope of 'seeing' flow when it does happen.

**Surveillance**

Surveillance here implies post-release observation, often for the survival and dispersal of an organism or for some environmental impact when predetermined sampling regimes are often impractical. The implications of large distances (e.g., kilometres) and long time intervals (e.g., years) to monitor wind driven pollen or seed dispersal, for example, might challenge the most robust budget. Additionally, deciding upon what to look for and devising a meaningful surveillance program may present insurmountable difficulties when there may only be speculation as to what environmental impacts a GMO release might impose. This might result in good faith arguments where responsible investigators suggest 'looking under the lamp post.' In the United States and South Africa, surveillance programs for the occurrence of increased insect pest resistance to the endotoxins of *Bacillus thuringiensis* were required by the national regulators when they granted a permit for the sale of Bt-cotton.

## Monitoring Methodology

It is necessary to establish a common methodology to carry out the environmental risk assessment based on independent scientific advice. It is also important to establish common objectives for the monitoring of GMOs after their deliberate release and or after placing GMO in the market or products of GMOs. Monitoring of potential cumulative long-term effects should be considered as compulsory part of monitoring plan. The objective of a monitoring plan is to:

a. confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental

risk assessment are correct, and;

b. identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

## Critical Pre-trial Data

Obtain data which identify the status quo of the host species or organism in the release environment and determine whether facilities are available with adequate specifications for the required containment. It is necessary to monitor the arrangements for producing the GMO in quantity; transporting it to site and accounting for transported organisms.

## Critical Live-trial Data

Data requirements needed during trial include:

a. potential of gene flow to sexually compatible species
b. efficiency of containment facilities
c. capacity of the organism to survive in the receiving environment
d. products of expression of introduced genes
e. phenotypic and genotypic stability
f. pathogenicity to other organisms
g. potential for other environmental effects, such as release of exudates into the soil
h. potential for harm to humans
i. extent of horizontal gene transfer.

Assess the methods for monitoring the presence of GMOs or transferred genetic material beyond the primary site.

## Critical Post-trial Data

a. Determine whether the trial, or project was properly observed during the implementation
b. Determine whether the aim of the trial was achieved
c. Determine whether there were any adverse effects
d. The survival and dissemination characteristics of the organism were as expected

## Monitoring During Release

Monitoring during release aims to assess the efficacy of any risk management safeguards applied to the release. This should detect whether there is any risk of harm, caused for example by introgression with potential recipients. For example, if the presence of available pollen recipients within the dispersal area is essential to be a risk, their number should be kept below the level at which harm might occur.

The frequency of monitoring should take account of the growth rate and stage of maturity of relevant plants. Monitoring data obtained during and after the release from such voluntary experiments to test survival could help address the uncertainty. A more precise risk assessment could then be made for a subsequent release proposal, and consequently, could allow risk management safeguards to be reduced.

The primary purpose of monitoring during the release is to assess the practical efficacy of adopted safeguards.

The risk assessment should have identified the safeguards (and as a consequence, the management procedures) required to reduce any risks to an acceptable level. The frequency and extent of monitoring during the release should be adequate to ensure that any safeguards applied are effective.

Monitoring can, where appropriate, be carried out during the course of site visits made for other purposes, such as ensuring there is satisfactory agronomic management of the crop. It is essential, however, that sampling regimes are realistic.

It is possible that, despite a thorough risk assessment, unforeseen events will still occur. The monitoring regime may or may not be able to detect whether this is the case. If an unforeseen effect is detected, its significance should be assessed.. If there is a significant adverse impact on the environment, pre-planned emergency control will be required.

## Post Release Monitoring

This is necessary where the risk assessment identifies that continuous presence of the released GM plant or gene presents risk of harm, post-release monitoring will need to concentrate on confirming the removal of the released plants. Where appropriate, monitoring should concentrate on detecting and controlling any volunteer plants arising from the release. In some cases there may be uncertainty regarding the risk of harm from continued presence of an organism, especially over the long term. Post-release monitoring should then be designed to provide data to enable the uncertainty to be resolved. Factors to be taken into account include:

a. Seasonal effects, such as flowing and likely germination times, and
b. Post-trial treatment of the release site
c. Longevity of seed or tubers in soil.

Post-release monitoring of a trial site may give basic data on, for example, the longevity of propagules. In general, where flowering creates a risk of harm, e.g. by gene spread monitoring visits should be planned to coincide with potential flowering times of volunteer plants. If volunteer plants do occur and subsequently flower, the dispersal area should be monitored for potential pollen recipients, or their offspring. Any such plants found should be destroyed. Monitoring information could indicate how long transgenic plants could continue to appear, (and hence indicate the likely duration of post-release monitoring). Estimates of survival times for volunteers should take into account the effects of the volunteer control practices applied to the site. In all cases, the extent and duration of the monitoring should be sufficient to prevent or minimise damage to the environment over the longer term as a consequence of the release. Monitoring should concentrate on ascertaining and demonstrating that the safeguards put into place are effective. Monitoring should concentrate on the release plot, plus the dispersal area identified in the pre-release survey, and relevant species within the area. Methodology used in monitoring may include:

a. Site visit and evaluation missions (teams),
b. Review of reports from the applicant

c. Interviews,
d. Surveillance and inspections.

Many methods can be used to monitor plants released into the field. These vary from simple, traditional methods to the most modem and complex. The following aspects need to be taken into consideration in this respect.

a. The choice of monitoring methods will depend upon the purpose for which the monitoring is done: if the monitoring is done to demonstrate that there is zero or minimal risk of harm to the environment during the execution of the release experiment, then methods of appropriate scope and sensitivity should be used.
b. The validity of any one method, or combination of methods, depends partially upon the ease and accuracy of identification of the introduced plants, and their propagules or pollen.
c. Identification should ideally be by means of easily recognisable phenotypic or genetic characteristics. The choice of monitoring method(s) should be appropriate to the degree of sensitivity of detection required: monitoring methods should be accurate, reliable and operable. There should be a balance between sensitivity and practicality.
d. Ideally, marker characteristics that are cheap and easy to identify would be the most suitable for assessing the spread of the organism or introgression of genetic markers.
e. Direct observation of the trial site forms the basis of all monitoring methods. Regular and methodical inspection of the site, and data recording will often provide much useful monitoring information. The frequency of inspection of the site before, during and after the completion (termination) of the experiment will depend on the estimated risk.
f. For monitoring by direction observation, the released plant should, where possible, be easily and unequivocally identifiable. Any identifying character should be stably inherited and expressed, and clearly different from the equivalent characters displayed by local crops and feral populations of the same species.
g. Direction sampling of the atmosphere (for pollen), or soil (for seeds or vegetative organs) can be used to monitor dispersal. Physical sampling methods are most useful if the pollen or seed are morphologically quite uniform, and distinct form those produced by non-transgenic varieties. For example, a marker that produced a distinctive seed coat colour could be easily detectable.
h. There may be a risk that one or more of the inserted genes can spread to either nearby crop plants, volunteers, or pollen-compatible weedy relatives.

If so, the choice of monitoring method should enable detection of events of this type. Detection of the presence of the inserted gene in a recipient plant may be by means of various biological methods.

g. One such method may assess the presence of a gene by examining potential recipients for signs of the presence of the gene, for example, herbicide tolerance.

i. An example of another method would be if possibly unrelated morphological characteristics of the transgenic plant (such as flower colour, leaf morphology, seed shape and colour) are transmitted to recipients. Such events can be interpreted to presume flow of the inserted gene.

j. Trap plants (of the same species as the plant to be released) can be used to detect the spread of pollen from the experimental plants. Transfer can be inferred from analysis of seeds or progeny of the trap plants. Male-sterile varieties may be particularly useful for this purpose.

k. Other characteristics that may be suitable for monitoring purposes include pest susceptibility; biochemical characteristics or end-products of the gene product (for example, allozyme analysis, carbohydrate analysis), and DNA characteristics, including RFLP mapping and PCR amplification.

## Reporting Requirements

For every introduction of field trial, there is a need to determine when to under take monitoring and when to evaluate the work. This same process would explicitly identify who would undertake the monitoring and evaluation who would receive the reports arising from monitoring & evaluation. Since the applications differ from one to the other, it is not possible to give a specific direction but suffice to say that the reporting of monitoring and evaluation data and information should be to the authorities that will make use of the information. The primary purpose of monitoring during the release is to assess the practical efficacy of adopted safeguards. It is possible that despite a thorough risk assessment, unforeseen events still occur: these should be identified during monitoring Post monitoring release is carried after the release has been completed and the plants harvested. The Government may monitor to ensure that the GMO does not enter the human or animal food chain where authority has not been given. Voluntary monitoring is undertaken by the applicant in order to provide further information. Such information would assist the applicant in developing programmes of release.

## Monitoring Plan Design

The design of the monitoring plan should:

a. be detailed on a case by case basis taking into account the environmental risk assessment.

b. take into account the characteristics of the GMO, the characteristics and scale of its intended use and the range of relevant environmental

conditions where the GMO is expected to be released,

c. incorporate general surveillance for unanticipated adverse effects and, if necessary, (case) specific monitoring focusing on adverse effects identified in the environmental risk assessment,

d. be carried for a sufficient time period to detect immediate and direct as well as, where appropriate, delayed or indirect effects which have been identified in the environmental risk assessment,

e. make use of already established routine surveillance practices such as to how relevant information collected through established routine surveillance practices will be made available to the consent-holder should be provided,

f. facilitate the observation, in a systematic manner, of the release of a GMO in the receiving environment and the interpretation of these observations with respect to safety to human health or the environment,

g. identify who (notifier, users) will carry out the various tasks the monitoring plan requires and who is responsible for ensuring that the monitoring plan is set into place and carried out appropriately, and ensure that there is a route by which the consent holder and the competent authority will be informed on any observed adverse effects on human health and the environment. (Time points and intervals for reports on the results of the monitoring shall be indicated),

i. give consideration to the mechanisms for identifying and confirming any observed adverse effects on human health and environment and enable the consent holder or the competent, where appropriate, to take the measures necessary to protect human health and the environment.

## Suggested Reading

**National Research Council (NRC), 2001**. *Ecological Monitoring of Genetically Modified Craps.* Washington, D.C.: National Academy Press.

**Keeler, K. 1994**. "The Keynote Presentation." In OECD Environment Monographs No. 91: *Compendium of Methods for Monitoring Organisms in the Environment* (pp. 19-23). Paris: Organi- zation for Economic Co-operation and Development.