BIOSAFETY GUIDELINES

ENVIRONMENTAL RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS IN MALAYSIA

DEPARTMENT OF BIOSAFETY

Ministry of Natural Resources and Environment Malaysia

Level 1, Podium 2, Wisma Sumber Asli
No. 25, Persiaran Perdana
Precinct 4, Federal Government Administrative Centre
62574 Putrajaya
MALAYSIA.

T: +603 8886 1580 / 1579
F: +603-8890 4935
E: biosafety@nre.gov.my
W: http://www.biosafety.nre.gov.my

ISBN No: 978-967-10117-4-4

The Biosafety Act 2007, Biosafety (Approval and Notification) Regulations 2010, Guidelines and Forms may be downloaded from the Malaysian Biosafety Clearing House Website at http://www.biosafety.nre.gov.my

Any future regulations, guidelines and related documents will be posted to this website.

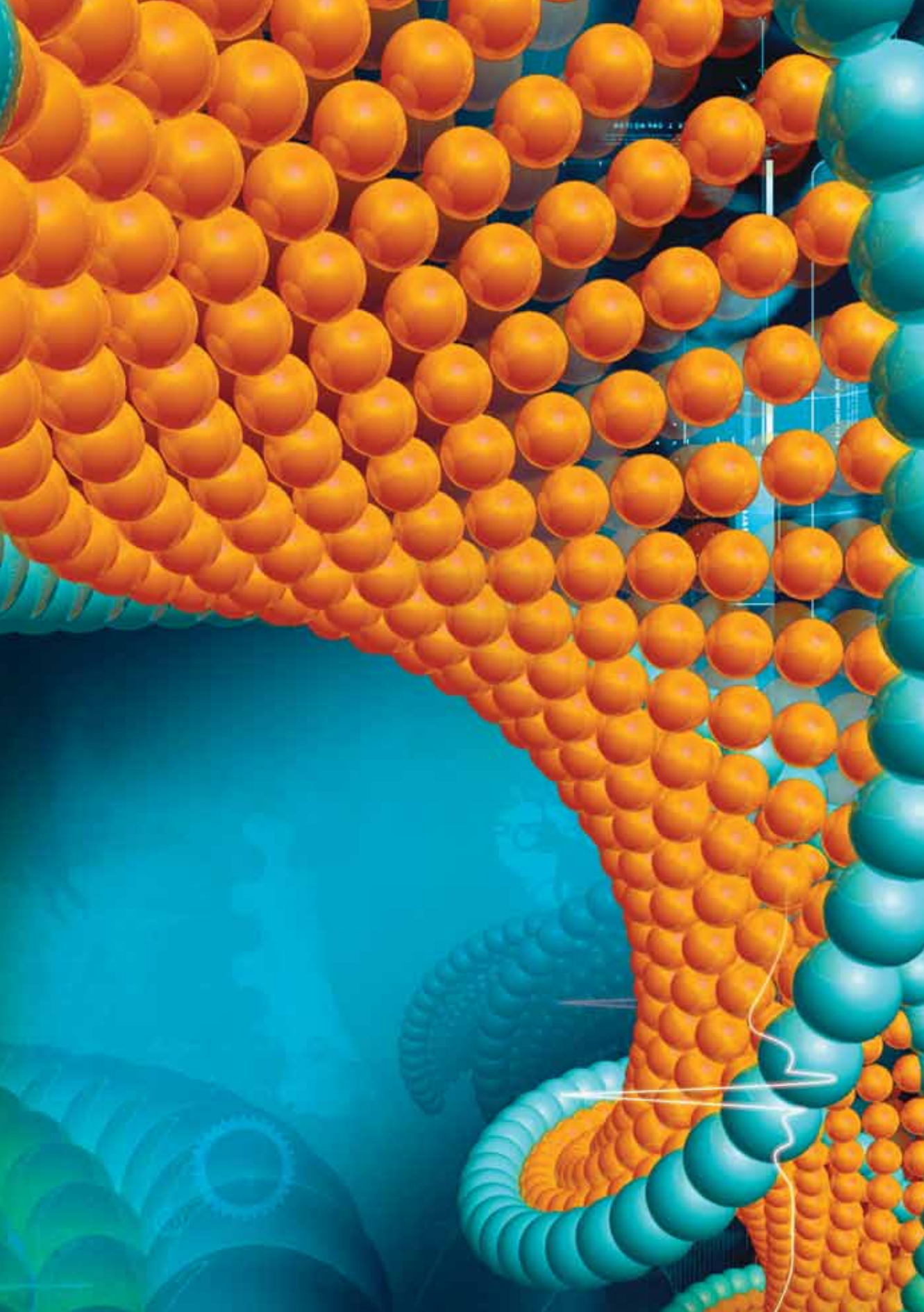
ACKNOWLEDGEMENT

We wish to thank and acknowledge Dr Tan Swee Lian, a member of Genetic Modification Advisory Committee (GMAC) for her effort and time in drafting this Guideline. Our thanks also go to all others who generously contributed to this Guideline.

CONTENTS

LIST OF TABLES

LIST OF FIGURES



CHAPTER 1

INTRODUCTION

This document provides guidelines for the Environmental Risk Assessment (ERA) of Genetically Modified (GM) plants in Malaysia. It covers ERA of applications for the cultivation of GM plants, as well as for the import of food and feed containing or consisting of GM plants, or produced from GM plants. It also includes a chapter on ERA of plant-associated GM microorganisms.

These guidelines do not consider issues related to traceability, labelling or co-existence. Neither do they cover socio-economic and ethical issues, focusing primarily on potential environmental risks arising from GM plants.

The guidelines cover ERA of deliberate releases into the environment of GM plants for experimental purposes. While a formal ERA may not be necessary in applications for the contained use of GM plants, the guidelines nevertheless provide a framework which helps in the decision-making as to whether such an application should be approved or rejected.

Applications for the release, testing or importation should be made using the appropriate forms, NBB/A/ER/10/FORM A or NBB/A/ER/10/FORM C, which are included as Appendices 1 and 2, respectively. These can be downloaded from the website of the Malaysian Biosafety Clearing House of the Department of Biosafety, under the Ministry of Natural Resources and Environment (www.biosafety.nre.gov.my).

1.1 What are Genetically Modified Plants?

Genetic manipulation (or genetic modification / genetic engineering) is a technology by which a gene or genes are taken from one organism (the donor), or are synthesized *de novo*[1], possibly modified, and then inserted into another organism (the recipient) in an attempt to transfer a desired trait or character (definition by Tzotzos *et al*., 2009).

[1] anew or afresh

This technology is also known as genetic engineering, recombinant DNA technology or bioengineering. The process of obtaining the desired gene/s from a donor and inserting it/them into a recipient is called genetic transformation, and the recipient organism is now known as the transformant. In the case of plants, the transformant is selected and identified to carry the desired gene/s, grown to a whole plant, and enters the conventional backcrossing programme.

The use of genetic modification has also permitted the study of interactions between microorganisms and their host plants. This includes investigations into the mechanisms of pathogenesis, symbiosis and mutualism, and the elucidation of plant gene functions. GM plant viruses, in particular, have been exploited for both research and biotechnology applications. While genetic transformation is only possible in a restricted number of plant species, plant viruses can be used to inoculate a wide range of plant species. For example, GM plant viruses can be used in the study of plant functional genetics by exploiting post-transcriptional gene silencing. Inoculation of a virus vector carrying a copy of the gene to be silenced triggers plant RNA-mediated defence mechanisms that counter viral threats resulting in the silencing of both the vectored gene and the cellular equivalent. This system has been dubbed virus-induced gene silencing (VIGS). GM plant viruses have also been heavily exploited for biotechnological purposes. They have been used to transform plants for the purposes of metabolic engineering and the expression of foreign genes, such as antigens for vaccine production and novel therapeutic products.

Examples of crop plants which have been successfully propagated through genetic modification and are widely grown today include the following:

1. Roundup Ready® soybean that is tolerant to Roundup (the brand name for glyphosate), a systemic broad-spectrum herbicide produced by the same company which developed this soybean variety. The donor of the C4 EPSP gene is a strain of *Agrobacterium*. Other crops which carry this glyphosate-tolerant gene include Roundup Ready® cotton, Roundup Ready® corn and Roundup Ready® canola.
2. Bt corn carries a gene which produces a protein called the Bt delta endotoxin that kills Lepidopteran larvae, particularly that of the European corn borer. The donor of the gene is a naturally occurring soil bacterium, *Bacillus thuringiensis*. Growers of Bt corn can therefore refrain from spraying insecticides to control the corn borer. There is also Bt cotton which is resistant to the cotton boll worm.
3. There have also been commercial successes with virus-resistant GM papaya (using a coat protein gene from the ringspot virus itself) and squash (having a coat protein gene from the cucumber mosaic virus),

and blue carnations (inserted with blue genes from petunia and snapdragon).

1.2 What are Risk Analysis and Risk Assessment?

Risk analysis can be broadly defined as an integrated process consisting of three major components: risk assessment, risk management and risk communication. The individual components are distinct, but are linked to achieve a well-functioning risk analysis process that forms the basis for decision-making on any operation or dealing of genetically modified organisms (GMO).

In the case of biosafety, risk analysis involves a scientific process to estimate the risks to human life and health, as well as the impact on the environment, associated with the use of a particular GMO or its products. The prevention, reduction or elimination of these risks requires methods of risk management that are normally implemented as actions conforming to particular regulations. Risk assessment and risk management have to be implemented along with risk communication, which involves all interested parties and allows for an iterative process of risk analyses.

Risk assessment is thus a tool for decision-making in relation to any number of activities which may have an impact on the environment – from construction to land use management to the importation of exotic species. While the potential impact of such activities may vary greatly, the methodology for risk assessment is fundamentally the same. First, potential risks or hazards are identified, and then the likelihood and consequence of these hazards are characterized. Thus, the product of risk assessment is an estimate of the likelihood and magnitude of the harms or adverse effects that may result from an activity. Good risk assessment provides relevant and useful information to the decision-maker in a clear and comprehensible form. The rationale used in risk characterization must be unambiguous, and any uncertainties are explained.

Risk assessment is important in the process of risk analysis given that if a particular risk is not identified, the steps taken to reduce it cannot be formulated in the risk management process. Risk assessment relies on a solid scientific base. Each case has to be dealt with individually and a separate evaluation has to be undertaken for each phase of obtaining, researching, testing, producing and releasing into the environment of GMO on a large or small scale. The risk analysis process when applied to a large variety of genes and gene combinations becomes very complex, because it can result in a vast range of effects and interactions. In this sense, evaluation of possible impacts over the long term presents many difficulties. Moreover, the results of risk assessment from small-scale tests cannot be extrapolated to the large scale.

1.3 Definitions

Environmental risk assessment is the process of identifying significant risks to the environment, estimating the level of risk, and determining those risks that require measures to reduce the level of risk (USEPA, 1998).

A *hazard* is anything, including a situation or state that may cause harm, without considering its probability or the consequences.

Harm is a negative outcome of effect of an action or event; in other words, an adverse effect.

A *consequence* is the result of an undesired event, such as harm to health, life or the environment.

A *risk* expresses a combination of the probability of an undesired event, and the scope of the consequences. Risk is used to compare various events in terms of having the highest or lowest risk. Risks can be classified qualitatively or quantitatively, if they are to be ranked. A common quantitative expression for risk is the consequence (expressed in a particular unit, e.g. number of deaths, or financial loss) multiplied by the probability. Such an expression of risk is also called the *expected loss*.

Risk can be defined thus:

$$\text{Risk} = f(\text{Hazard, Exposure})$$

Exposure refers to the contact or occurrence of a potential hazard with an environmental entity of value.

What follows is an example of the importance of considering the probability of exposure in ERA:

The Tale of the Monarch butterfly

In a study by Losey et al. (1999), caterpillars of the Monarch butterfly showed a lower survival rate and slower development when fed on milkweed leaves coated with pollen from Bt corn compared to when fed on milkweed leaves alone, or on milkweed leaves coated with pollen from non-GM corn. It was inferred that planting Bt corn will decimate the Monarch butterfly populations in USA. In fact, this conclusion was flawed because while Bt corn pollen presents a hazard to Monarch caterpillars (to be expected as the Cry proteins expressed in Bt corn target Lepidopteran pests), these caterpillars were not exposed to levels of Bt pollen sufficient to cause adverse effects:

- *The density of Bt pollen on milkweeds within the corn field*

ranges from 78-229 grains/cm^2, and quickly drops to 17-28 grains/cm^2 at a distance of 1-3 m from the corn field, and Bt pollen is practically absent (0-2 grains/cm^2) at a distance of 7-9 m.

- *The no effect level is established at 150 grains/cm^2.*
- *Less than 1% of the Monarch population is present within Bt corn fields during pollen shed.*

Thus, there is a need to understand exposure when assessing risk. Hazard alone does not constitute a risk.

Thus, a *postulated risk* is the potential harm that may manifest from a plausible exposure scenario.

While it is difficult to generalize on potential risks arising from a GM plant – because different types of introduced traits pose different types of risks arising from the phenotype or from the use of the product, Table 1 shows some indicative risks.

A risk hypothesis is a tentative explanation taken to be true for the purpose of argument or investigation. This should not be confused with a scientific hypothesis which is a specific, testable postulate which will be part of the analytical phase of ERA.

Table 1. Indicative direct and indirect risks arising from the GM phenotype, the introduced trait or changes in agricultural practice (after Tzotzos *et al.*, 2009)

Risk source	Potential risk	Mechanism
GM phenotype	Evolution of increased weediness (direct)	Sexual transfer of crop alleles to wild relatives; seed dispersal
GM phenotype	Loss of biodiversity in the wild (indirect)	Extinction by hybridization. Indirectly, from the intensification of agriculture
GM trait	Harm to non-target organisms (direct)	Toxicity. Starvation through reduction of food resources
GM trait	Evolution of resistance in the targeted pathogen, pest or weed population (direct)	Selection pressure from transgene products (e.g. Bt toxin) or application of agricultural input (e.g. herbicide)
Change in agricultural practice	Loss of agricultural biodiversity (indirect)	Increased use of chemical inputs

1.4 Why is there a Need for Environmental Risk Assessment?

The purpose of ERA is to assess if the introduction of a GM plant into the environment would have adverse effects on human and animal health or the environment. ERA of a GM plant involves generating, collecting and assessing information on the GM plant to determine its potential adverse impact relative to its non-GM counterpart (or comparator), thus, assessing its comparative safety.

The underlying assumption of comparative assessment for a GM plant is that the biology of a traditionally cultivated plant, from which the GM version was derived, is well known. This employs the concept of familiarity (*Figure 1*) developed by the Organization of Economic Cooperation and Development (OECD, 1993). In ERA, it is appropriate to draw on previous knowledge and experience, and to use a suitable comparator to highlight the differences associated with the GM plant in the receiving environment/s. ERA of GM plants which contain events combined by conventional breeding (stacked events) may also involve comparisons with GM events as well as appropriate comparators.

ERA should be carried out in a scientifically sound and transparent manner. It should include any relevant data (e.g. research data, scientific publications, monitoring reports) obtained prior to and/or during the risk assessment process. The purpose of performed studies, data and their interpretation, as well as the assumptions made during ERA, should be clearly described. In addition, the use of models could provide further information useful for ERA. The final risk evaluation should result in qualitative and if possible quantitative conclusions on the risks which provide information to risk managers and allow for sound decision-making. Any uncertainties associated with the identified risks should also be outlined.

ERA should be carried out on a case-by-case basis, meaning that the required information may vary according to the species of GM plants concerned, the introduced genes, their intended use/s and the potential receiving environment/s, taking into account specific cultivation requirements and the presence of other GM plants in the environment.

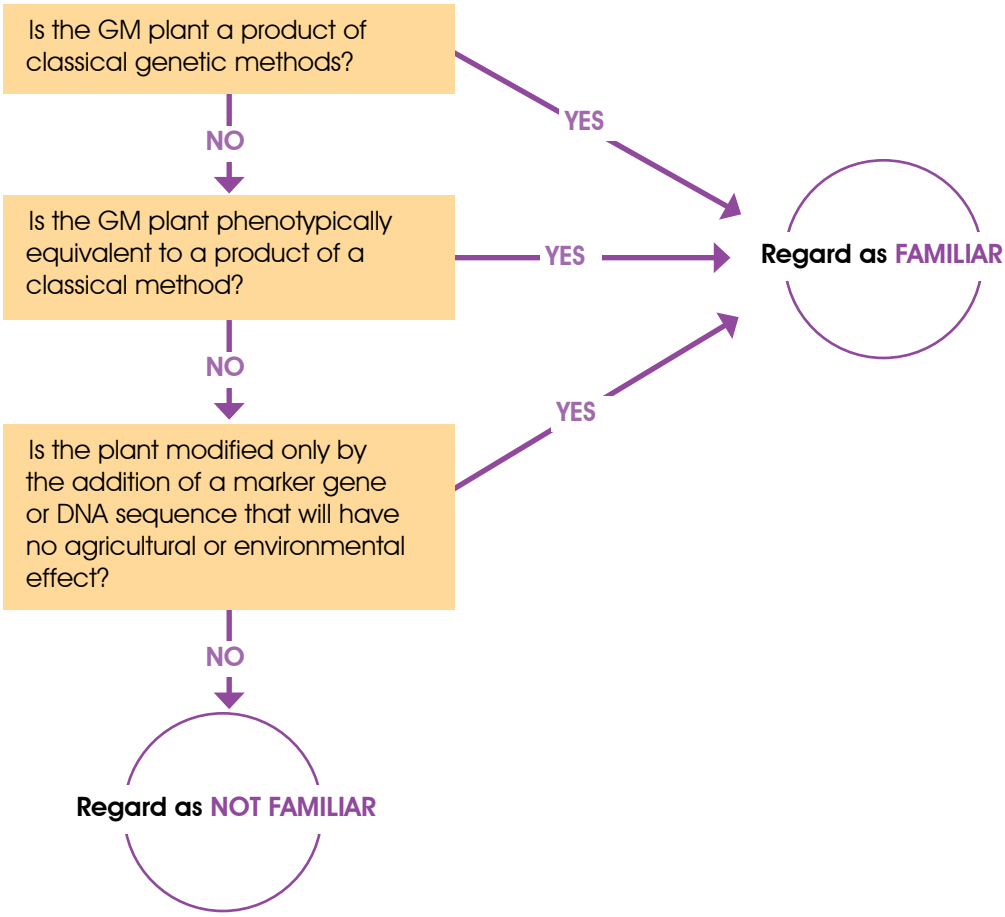


***Figure 1**. A familiarity assessment framework*
(From: FAO's Biosafety Resource Book)

ENVIRONMENTAL RISK ASSESSMENT

CHAPTER 2

2.1 Comparative Assessment as a General Principle for the Risk Assessment of GM Plants

The risk assessment strategy for GM plants uses appropriate methods to compare the GM plant and its derived products with their appropriate comparator (i.e. the non-GM counterpart). The comparative safety assessment is adopted to identify similarities and differences caused by either intended or unintended effects.

Comparative safety assessment includes description of the host and donor(s) organisms, molecular characterisation, the agronomic and phenotypic comparison of the GM plant in question, as well as its compositional analysis (OECD, 1993). In addition, the comparative safety assessment within ERA uses information on the interactions of the GM plant with its receiving environment/s.

Any type of genetic modification results in intended effects, but may also result in unintended effects. ERA focuses on the identification and characterisation of both effects with respect to possible adverse impacts on human and animal health, and on the environment. Effects can be direct and indirect, immediate and delayed, including cumulative long-term effects.

Intended effects are those that are designed to occur and which fulfill the original objectives of the genetic modification. Alterations to the phenotype may be identified through a comparative analysis of growth performance, yield, pest and disease resistance, *etc*. Intended alterations in the composition of a GM plant, compared to its appropriate comparator, may be identified by the presence and levels of single compounds.

Unintended effects of genetic modification are those which are consistent (non-transient) differences between the GM plant and its appropriate comparator, which go beyond the primary intended effect/s of introducing the transgene/s. As these unintended effects are *event-*

specific, the applicant must supply data on the specific intended event. Data that may reveal such effects are those related to:

1. **Molecular characterisation**: A starting point in the identification of potential unintended effects is analysis of the DNA construct and insertion site to establish whether the insertion is likely to have potential effects other than the intent of the original genetic modification (e.g. unintended effect/s could be due to a loss in function of an endogenous gene at the insertion site).
2. **Compositional analysis**: Unintended effects may be detected through a comparison of the compositional characteristics of the GM plant with its appropriate comparator (e.g. unintended effect/s could potentially be linked to metabolic disruptions).
3. **Agronomic and phenotypic characterisation**: Unintended effects may also be detected through a comparison of the phenotypic and agronomic characteristics of the GM plant with its appropriate comparator (e.g. unintended effects could be linked to morphological and phenotypical changes).
4. **GM plant-environment interactions**: Unintended effects may be detected through comparisons of biotic and abiotic interactions of the GM plant and its appropriate comparator with components of their receiving environment/s. *In planta* data are the fundamental source of information (e.g. unintended effects could be linked to changes in the interaction of the GM plant on the functionality of non-target organism communities).

Statistically significant differences between the GM plant and its appropriate comparator, which are not due to the intended modification, may indicate the occurrence of unintended effects, and should be assessed specifically with respect to their biological relevance and potentially hazardous environmental implications. The outcome of the comparative safety assessment allows for the determination of those "identified" characteristics that need to be assessed for their potential adverse effects in the environment, regardless of whether they were intended or unintended, and will thus further structure ERA.

The level and routes of environmental exposure to the GM plants are taken into account (e.g. in relation to the scope of the application: cultivation in the country vs. import and processing). Comparisons should be made between the GM plant and its appropriate comparator, wherever applicable, by growing them in relevant production systems and in commercial production environments.

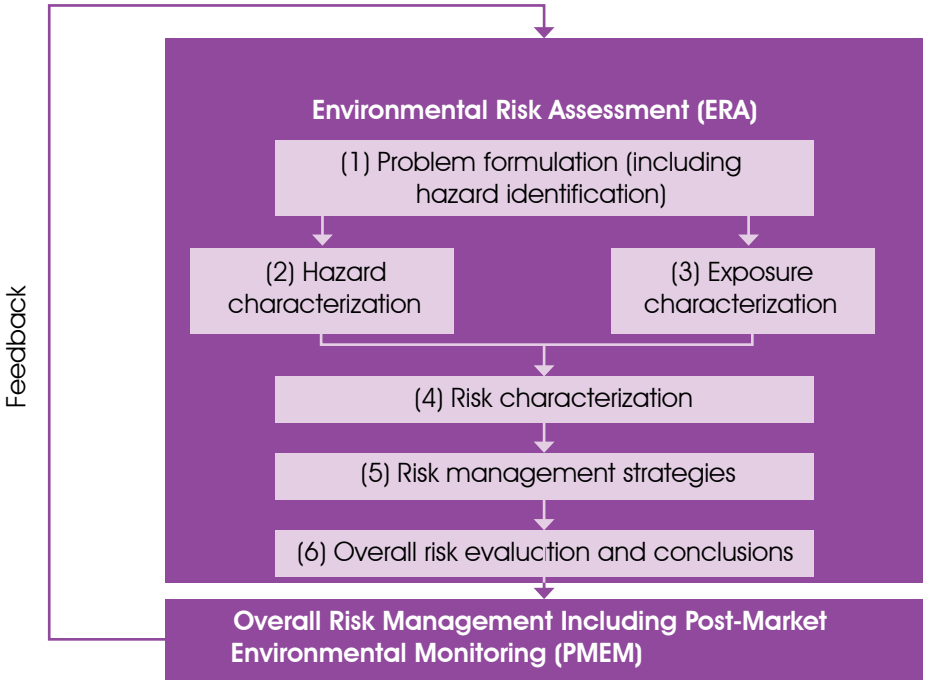
2.2 Objectives of the Different Steps of ERA

The objective of ERA is, on a case-by-case basis, to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate

or delayed (including cumulative long-term effects) on the receiving environment/s where the GM plant will be released. The European Food Safety Authority (EFSA, 2010) proposed that ERA follows six steps (*Figure 2*):

1. Problem formulation including hazard identification
2. Hazard characterisation
3. Exposure characterisation
4. Risk characterisation
5. Risk management strategies
6. Overall risk evaluation and conclusions

While ERA is conducted starting from 1 and moving towards 6, steps 2 and 3 can, however, be carried out in parallel.



Source: EFSA (2010)

***Figure 2**. Six steps in an environmental risk assessment*

The Centre for Environmental Risk Assessment (CERA) of the International Life Sciences Institute Research Foundation adopts the process flow for risk assessment proposed by Wolf *et al*. (2010), as shown in *Figure 3*.

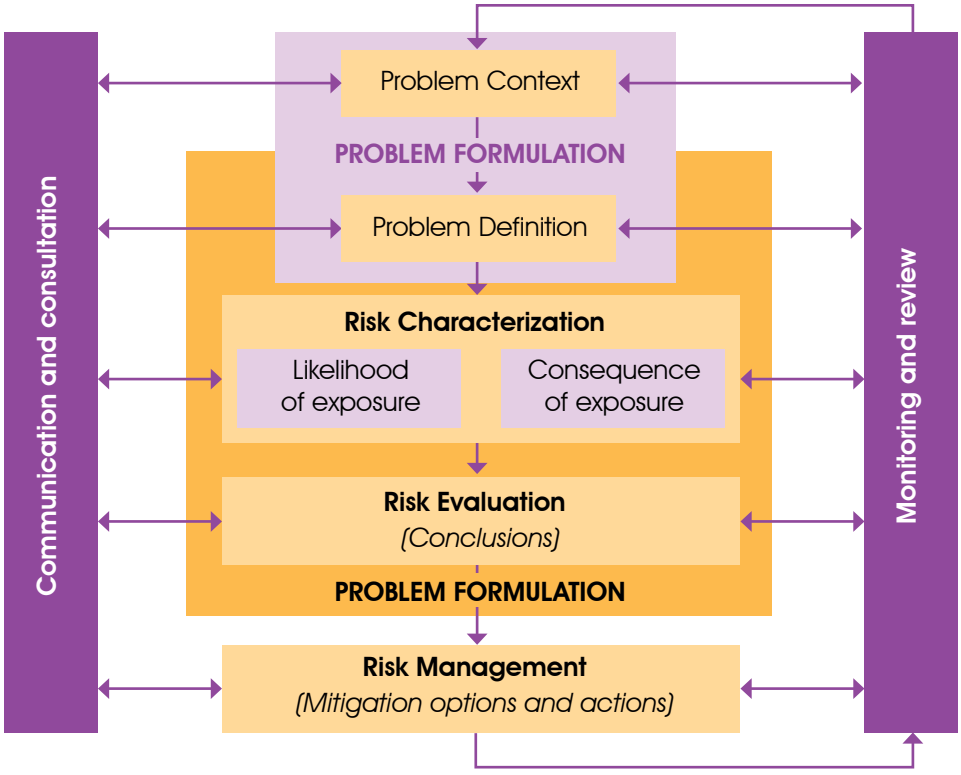


***Figure 3**. Process flow for risk assessment – an alternative (after Wolt* et al.*, 2010)*

Nevertheless, for the purposes of this book, the EFSA six-step ERA is adopted.

2.2.1 Step 1: Problem formulation, including hazard identification

The risk assessment begins with problem formulation in which all important questions for the risk characterisation are identified. Problem formulation helps to make the risk assessment process transparent by explicitly stating the assumptions underlying the risk assessment.

Problem formulation includes the identification of those characteristics of the GM plant capable of causing potential adverse effects to the environment (*viz*. the hazards), the nature of these effects, and the pathways of exposure through which the GM plant may adversely affect the environment. Problem formulation also defines the assessment endpoints and sets specific hypotheses to guide in the generation and evaluation of data in the next risk assessment steps (i.e. hazard and exposure characterisation). In this process, both existing scientific knowledge and knowledge gaps (such as scientific uncertainties) are considered.

Problem formulation starts with the identification of hazards arising from the GM plant and its use. A comparison of the characteristics of the GM plant with those of its appropriate comparator enables the identification of differences in the GM plant that may lead to harm. These differences are identified in the problem formulation process in order to focus ERA on the potential environmental consequences arising from these differences. While some differences may be deemed irrelevant to the assessment, others will need to be assessed for their potential to cause harm.

After identifying the hazards and potential adverse effects that warrant further consideration, problem formulation then considers the available information on exposure through which the GM plant may interact with the environment. Depending upon the intended uses of a GM plant (such as import, processing, food, feed and/or cultivation), the pathways and levels of exposure of the GM plant to the environment will vary. In the case where the use of GM plant does not include cultivation, problem formulation will consider exposure by the following ways:

(i) *via* the accidental release into the environment of propagules, such as seeds, of the GM plant during transportation and processing which can potentially lead to sporadic feral GM plants, and

(ii) indirect exposure, e.g., through manure and faeces from the gastrointestinal tracts mainly of animals that fed on the GM plant, and/or

(iii) organic GM plant matter either imported as a fertilizer or soil amendment or derived from other GM bioproducts of industrial processes.

In the case where the GM plant use includes cultivation, problem formulation will consider exposure resulting from the expected cultivation of the GM plant in the receiving environment/s.

A crucial step in problem formulation is to identify the aspects of the environment that need to be protected from harm according to environmental protection goals set out by legislation. As protection goals are general concepts, they should be translated into measurable assessment endpoints. Defining assessment endpoints is necessary to focus the risk assessment on assessable or measurable aspects of the environment – a natural resource (e.g. natural enemies) or natural resource service (e.g. biological control functions of pest populations performed by natural enemies) that could be adversely affected by the GM plant, and that require protection from harm.

Subsequently, within the problem formulation, the identified potential adverse effects need to be linked to the assessment endpoints in

order to derive testable hypotheses that allow for the quantitative evaluation of the harm posed to those assessment endpoints. The hypotheses are of importance as they will further guide in the setting up of a methodological approach on how to evaluate the magnitude of harm. Through hypothesis, assessment endpoints are translated into quantitatively measurable endpoints, termed measurement endpoints (such as measurements of mortality, reproduction, abundance). A measurement endpoint can be regarded as an indicator of change in the assessment endpoint, and constitutes measures of hazard and exposure.

Finally, for each measurement endpoint, the level of environmental protection to be preserved is expressed through the setting of 'limits of concern' which may take one of two forms. For studies in an environment that is controlled, the limits of concern will usually be trigger values which, if exceeded, will either lead to conclusions on risks, or the need for further assessment in receiving environment. For field studies, the limits of concern will reflect more directly the minimum effect that is considered to potentially lead to harm. If these limits are exceeded, then detailed quantitative modelling of exposure may be required to scale up effects at the field level both temporally and spatially. Limits of concern can be defined by, for example, data from literature, modelling, existing knowledge and policy goals.

The information considered in problem formulation can take many forms, including published scientific literature, scientific and expert opinions, and/or research data. Available data from analyses performed to characterise the GM plant, including molecular, compositional, agronomic/phenotypic analysis and plant-environment interactions, shall also address the occurrence of unintended effects.

Data generated outside Malaysia from experiences with the GM plant might be used by the applicant only if its relevance for the local environment/s is justified.

Problem formulation should be on a case-specific basis:

(1) To identify characteristics of the GM plant and, where appropriate, the associated production and management systems capable of causing potential adverse effects to the environment;

(2) To identify the potential adverse effects linked to those harmful characteristics;

(3) To identify exposure pathways through which the GM plant may adversely affect the environment;

(4) To define assessment endpoints being representative of the aspects of the environment that need to be protected from harm according to protection goals set out by Malaysian legislation and

national policies, and to describe criteria used for the selection of assessment endpoints (e.g. relevance, practicality);

(5) To define measurement endpoints that can be used to assess the potential harm to the assessment endpoints defined;

(6) To formulate testable hypotheses that are clearly phrased and easily transferable to data to be generated or evaluated;

(7) To set the limits of concern for each measurement endpoint; and

(8) To consider knowledge gaps (such as scientific uncertainties).

2.2.1.1 Examples of protection goals with generalized and specific assessment endpoints (CERA, 2011)

1. Protection of agricultural production and agro-ecosystems
    a. Prevention of the introduction of weeds
        i. Weediness of the GM plants
        ii. Weediness of sexually compatible relatives
    b. Prevention of the introduction of pests
        i. Pest potential of the GM plants
    c. Prevention of impacts to non-target organisms including human beings
        i. Populations of beneficial organisms (e.g. pollinators)
        ii. Health status of humans with relevant environmental exposure (e.g. farm workers)
2. Protection of biodiversity and environmental safety
    a. Prevention of serious and irreversible harm to threatened and endangered species
        i. Populations of threatened and endangered species
        ii. Quality of critical habitats
    b. Prevention of the introduction of toxins or hazardous substances
        i. Toxicity of introduced substances

Specific examples

A. Commercial introduction of an herbicide-tolerant *Brassica napus* BN 1995 into Canada (CERA, 2011)

To consider the potential for the introduction of a new weed (the GM plant itself)

The risk hypothesis will be: BN 1995 will be costly to control or will decrease yields in subsequent crops as a new problematic weed.

To determine the likelihood of this risk being realized, the hypothesis is elaborated into a *conceptual model* – either a simple statement, a

Scenario	Risk hypothesis
Cultivation of transgenic TuMV OSR ↓ Hybridization between transgenic OSR and wild species ↓ Transgene increases VR of wild species ↓ Wild species infected by TuMV in the field ↓ Infected transgenic wild species plants produce more seed than ↓ infected non-transgenic plants Increased abundance of wild species reduces abundance of valued species	No hybridization Wild species immune to TuMV TuMV does not infect wild species in the field TuMV infection does not produce more seed Wild species do not increase in abundance

diagram, or a listed series of events – describing the necessary steps to inflict harm:

1. BN 1995, its seed or progeny remain in a field after harvest
2. BN 1995 or its progeny survive intervening weed management measures and grow concurrently with a subsequent crop
3. The growth of BN 1995 causes loss of yield, or threatens to cause loss of yield
4. Additional management costs are incurred to control BN 1995
5. BN 1995 or its progeny persist in the field despite these measures and continue to necessitate additional management (i.e. it establishes as a problematic weed).

The conceptual model is a tool to enable the identification of information and methodologies which might be useful for risk assessment. Then, in establishing that any one of the steps is impossible or unlikely will lead to the conclusion of minimal risk. If all of the steps are possible (or probable), then the assessment would need to present an estimate of the likelihood and consequence of the risk.

B. Commercial release of a transgenic virus-resistant oilseed rape (OSR) (Raymond and Cooper, 2005)

Again, if it can be established that any one of the risk hypotheses holds true, then low or little risk can be concluded.

2.2.2 Step 2: Hazard characterization

Hazard characterisation is defined as the qualitative and/or quantitative evaluation of environmental harm associated with the hazard as set out in one or more hypotheses derived from problem formulation.

The magnitude of each potential adverse environmental effect should, if possible, be expressed in quantitative rather than qualitative terms. Ordered categorical descriptions such as "*major*", "*intermediate*", "*minor*" or "*marginal*", where the ordering is from '*major*' at one end to '*marginal*' at the other, may be used to place an identified hazard on a scale of severity. If at all possible, these terms should themselves be defined in quantitative terms, as precisely as possible. If the expression of magnitude is not made in quantitative term, but solely using the "ordered categorical description", a justification for this categorisation is necessary and should be provided.

Examples of consequences:

Major consequences: Significant changes in the numbers of one or more species of other organisms, including endangered and beneficial species, in the short or long term. Such changes might include a reduction in or complete eradication of a species leading to a negative effect on the functioning of the ecosystem and/or other related ecosystems. Such changes would probably not be readily reversible and any recovery of the ecosystem that did take place would probably be slow;

Intermediate consequences: Significant changes in population densities of other organisms, but not a change which could result in the total eradication of a species or any significant effect on endangered or beneficial species. Transient and substantial changes in populations might be included if likely to be reversible. There could be long-term effects, provided there are no serious negative effects on the functioning of the ecosystem;

Minor consequences: Non-significant changes in population densities of other organisms, which do not result in the total eradication of any population or species of other organisms, and have no negative effects on functioning of the ecosystem. The only organisms that might be affected would be non-endangered, non-beneficial species in the short or long term;

Marginal consequences: No significant changes had been caused in any of the populations in the environment or in any ecosystems.

2.2.3 Step 3: Exposure characterization

This step evaluates the exposure, *i.e.* likelihood of adverse effects occurring, and estimates the exposure quantitatively.

For each hazard identified and characterised, it may not be possible to estimate the exposure (likelihood) precisely. Likelihood of exposure can be expressed either qualitatively using an ordered categorical description (such as "*highly likely*", "*likely*", "*unlikely*" or "*highly unlikely*"), or quantitatively as a relative measure of probability (from 0 to 1, where 0 represents impossibility and 1 certainty). However, if qualitative terms are used to express such likelihoods, then the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication should be given of the range, within a numeric scale of 0 to 1, to which the term is intended to refer. For example, "The likelihood of exposure of a non-target lepidopteran species to Bt toxin (Cry1Ab protein) in field margins is estimated to be moderate", where 'moderate' in this context means "within the range 0.1 to 0.4".

2.2.4 Step 4: Risk characterization

Risk is characterised by combining the magnitude of the consequences of a hazard and the likelihood that the consequences occur. It is a quantitative or semi-quantitative estimate of the probability of occurrence and severity of harmful effect/s based on problem formulation, hazard and exposure characterisation. It is important that the values obtained for each measurement endpoint are related to the limits of concern to test whether the observed effect falls within those limits and, thereby, to aid in the assessment of the biological relevance of the observed effect.

On the basis of the conclusions reached in steps 2 and 3, an estimate of the risk of adverse effects should be made for each hazard identified in step 1. If a hazard has more than one adverse effect, the magnitude and likelihood of each individual adverse effect should be assessed. Where precise quantitative evaluation of risk is not possible, terms should be defined where possible. The evaluation for each risk should consider:

- The magnitude of the consequences of the hazard ("*major*", "*intermediate*", "*minor*" or "*marginal*", with an explanation of what is meant by these terms);
- The likelihood of the consequences related to hazard occurring ("*highly likely*", "*likely*", "*unlikely*" or "*highly unlikely*", with quantified definitions of the terms, using ranges of probability) in the receiving environment/s;
- The risk characterised by combining the magnitude of the consequence of the hazard and its likelihood (*Table 2*).

The uncertainty for each identified risk should be described where relevant, possibly including documentation relating to:

- Assumptions and extrapolations made at the various levels in ERA;
- Different scientific assessments;

Table 2. Risk determination matrix

		Likelihood of hazard			
		Highly likely	**Likely**	**Unlikely**	**Highly unlikely**
Consequence of hazard	**Major**	High	High	Moderate	Moderate
	Intermediate	High	Moderate	Moderate	Low
	Minor	Moderate	Low	Low	Negligible
	Marginal	Low	Low	Negligible	Negligible

- Specified uncertainties;
- Conclusions that can be derived from the data.

The risk characterisation should indicate whether the problem formulation (including hazard identification), hazard characterisation and exposure characterisation are complete.

The conceptual model is a tool to enable the identification of information and methodologies which might be useful for risk assessment. Then, in establishing that any one of the steps is impossible or unlikely will lead to the conclusion of minimal risk. If all of the steps are possible (or probable), then the assessment would need to present an estimate of the likelihood and consequence of the risk.

2.2.5 Step 5: Risk management strategies

When risk characterisation (step 4) identifies risks, then the applicant should propose measures to manage them. These risk management strategies should aim to reduce the identified risks associated with the GM plant to a level of no concern, and should consider defined areas of uncertainty. The applicant should describe risk management in terms of ways to reduce hazard and/or exposure, and the consequent reduction in risk should be quantified (when possible). Where the applicant has identified risk management characteristics (e.g. reduced fertility) in the GM plant which can reduce these risks, then the reliability and efficacy of these characteristics should be assessed. In addition, if the applicant places restrictions or conditions on the release of a GM plant in order to reduce risks, then the efficacy and reliability of these measures should be assessed.

The applicant should also state the measures to be put in place post-commercialisation in order to monitor and verify the efficacy of the risk management measures, and to allow changes in risk management strategies in case circumstances change, or when new data become available which require changes to the risk management.

2.2.6 Step 6: Overall risk evaluation and conclusions

An evaluation of the overall risk of the GM plant/s should be made taking into account the results of ERA and associated levels of uncertainty, the weight of evidence, and the risk management strategies proposed (step 5) in the receiving environment/s.

The overall risk evaluation should result in informed qualitative and, if possible, quantitative guidance to risk managers. The applicant should explain clearly what assumptions have been made during its own ERA, and what is the nature and magnitude of uncertainties associated with the risk/s. When risks are identified in the overall risk evaluation, the applicant should indicate why certain levels of risk might be acceptable.

The overall risk evaluation, including risk management strategies, may give indications for the requirement of specific activities within post-market environmental monitoring (PMEM) of GM plants. ERA and environmental monitoring are closely linked. ERA provides the basis for the monitoring plans, which focus on detecting any adverse effects on human health and the environment in the receiving environment/s. PMEM may provide data on the long-term, potentially adverse effects of GM plants. Monitoring results may confirm the assumptions of ERA or may lead to its re-evaluation.

ERA is an iterative process. If new information on the GM plant and its effects on human health or the environment becomes available, ERA may need to be re-addressed:

(1) to determine whether the risk characterisation has changed; and

(2) to determine whether it is necessary to amend the risk management.

GENERAL CONSIDERATIONS IN ERA

CHAPTER 3

3.1 Selecting Comparators

3.1.1 Single events

Where feasible and appropriate, similarities and differences in the interactions between the GM plant and the environment due to genetic modification and induced changes in management should be estimated in relation to a conventional counterpart

In the case of vegetatively propagated crops, the conventional counterpart shall, in principle, be the near-isogenic variety used to generate the transgenic line.

In the case of crops that reproduce sexually, the conventional counterpart shall have a genetic background comparable to that of the GM plant. As many crops used to produce food and feed are developed by back-crossing, the conventional counterpart would be the recurrent parent which has a genetic background that is as close as possible to the GM plant. On a case-by-case basis, and if there is explicit justification, the applicant may instead consider the use of a non-GM variety with agronomic properties as similar to the GM plant as possible, as the appropriate comparator for ERA. In all cases, information on the breeding scheme (pedigree) in relation to both the GM plant and all chosen comparator/s, and justification for the selected use of all chosen comparator/s shall be provided.

For certain assessment issues, such as the effects of management, cultivation and harvesting, the applicant should consider the inclusion of an additional comparator/s. It is imperative to consider the use of different current management techniques that can help to place any effects of the genetic modification into context, particularly concerning the agronomic management of both the GM plant and the chosen comparator/s.

For example, for insect-resistant GM plants, equivalence with a conventional counterpart is highly unlikely, if the latter is

> *managed without the pest control that would be typically applied to conventional non-GM plants. Hence, for such crops a conventional counterpart managed without pest control, and the same conventional counterpart managed with pest control measures that are typically applied in the area are recommended. The management techniques applied shall be compatible with the principles of good agricultural practice and recommended Integrated Pest Management strategy.*

When more than one management technique is employed, the principal comparison for inferences regarding environmental harm should be representative management techniques, rather than 'untreated' regimes which may be agronomically less realistic. In some circumstances, it may be advantageous for ERA to include an *additional* comparator with a closer genetic background to the GM plant than the conventional counterpart (such as a negative segregant). In all cases where an additional comparator is used, the motivation and choice shall be justified explicitly.

It is recognised that appropriate management is site- and year-specific; thus, management should follow standard farming practices and clearly document any deviations. The applicant must provide detailed management records and carry out independent agronomic audits by trained personnel to give sufficient confidence that the management practices are appropriate. Any additional treatments and/or comparators should be fully integrated within the experimental design, randomised and replicated in the same way as the GM plant and its conventional counterpart.

Furthermore, although the term 'comparator' applies to the plant, ERA must account for the production system as a whole. The production system includes the following scales:

- spatially – the landscape and region as well as the field;
- agronomically – the cropping system (including rotation) and the crop/s as well as the plant;
- temporally – the long-term, rotational and yearly effects as well as the seasonal.

So for ERA, the impacts of management, cultivation and harvesting must be considered at larger temporal and spatial scales than those that apply to the relatively small-scale experiments. For ERA, upscaling, modelling, simulation and analysis of production systems will typically be required, in addition to analysis of the smaller scale experiments referred to in this guide, to provide parameter values for such modelling. Allowance shall be made that a range of management options are possible in production systems using GM or conventional plants, and a range of comparisons might therefore be necessary.

ERA of the effects of persistence and invasiveness requires a wide variety of information from specific experiments which tend to be case-specific, and of a research-driven nature rather than of a routine nature. The effects studied include: reproduction, germination, seed persistence, invasiveness and hybridisation. Selection of the comparator should therefore be done on a case-by-case basis.

> *In the case of herbicide-tolerant GM plants incorporating a single event, at least three test materials are recommended:*
>
> - *the GM plant exposed to the intended herbicide and associated weed management,*
> - *the conventional counterpart treated with current weed management regimes, and*
> - *the GM plant treated with the same weed management.*
>
> *Such a comparison allows for the assessment of whether the expected agricultural practices influence the expression of the studied assessment endpoints.*

If no extra comparator is employed, it may still be necessary to consider the use of some form of positive control (Perry *et al.*, 2009) to demonstrate *post-hoc* that the study was capable of detecting the desired effects (for example, that there was a sufficient population density of organisms available in the experimental area to be sampled). If the positive control is external to the experiment (for example on a single unrandomised plot), then data from the control may not enter the statistical analysis in any form.

In this ERA guide, the term 'GM plant' refers to the specific GM event for which approval is requested. However, in practice, commercially available GM varieties are often produced from crosses of this event with other varieties. The applicant should discuss the potential risks arising from the genetic background of the varieties which might subsequently include the GM event, and how these might alter the conclusions of the risk assessment. On a case-by-case basis, depending on the nature of the event, and according to the scope of the application, data may be required on the safety of the event when present in different genetic backgrounds.

3.1.2 Stacked events

Stacked events combined by conventional crossing pose particular challenges for ERA. The comparators should be selected to establish whether the combination of events raises safety concerns with regard to stability and/or interactions. In addition, ERA should consider to what extent the combination of events results in changes in management systems which could lead to additional environmental impacts compared

to the single events. For stacked events, a conventional counterpart, if available, should be used as the comparator.

In an *n*-event stack, there are, in addition to the stacked events themselves, the negative segregant and the *n* single events, a further $2^n - n - 2$ different possible sub-combinations of events. For example, for 5-stacked events, there are a further 25 possible sub-combinations, and therefore a multiplicity of interactions that might give rise to potential risks. For ERA, field trials for comparative analysis will normally comprise the stacked event under assessment and its conventional counterpart. Selection of the comparators for ERA must take into account the need for relatively large plots, consequently a relatively restricted ability to increase replication, and, crucially, the consequent need to restrict the number of treatments compared to a minimum (often of two). It is acknowledged that if concerns over stability and/or interactions are indicated by such initial experiments, then further more detailed experimentation encompassing a greater number of treatments may be required. Whilst the most relevant study for ERA is the observed potential adverse effect itself, rather than the potential interaction that is the cause, it may well be useful to identify the source of the interaction.

It is very unlikely that any scientific rationale could justify the absence of experimental data for ERA, because there would need to be considerable evidence from previous risk assessments to rule out *ab initio*[2] interactions between the events and biota, even if the proteins themselves could be shown not to interact. Furthermore, for cultivation, it should be stressed that it is essential to consider management as well.

As comparators should be selected to establish whether the combination of events raises safety concerns relating to stability and/or interactions, protein expression levels associated with single events from only historic data, i.e. not obtained from concurrent data in trials of the higher stacked events, may not be acceptable for ERA of that higher stacked events and/or its sub-combinations. To assess interactions between events that could impact on protein expression levels, any set of events which have all been risk-assessed, and which contain between them all the events present in the stacked events, should be included as comparators.

In case a conventional counterpart is not available, different comparator/s may be appropriate, depending upon the issue/s under consideration.

To evaluate the impact on non-target organisms, the effects of management, cultivation and harvest, as well as bio-geochemical processes, the conventional counterpart can be substituted, on a case by

[2] "from the beginning"

case basis, by either a non-GM line derived from the breeding scheme used to develop the GM plant, or by a non-GM line with agronomic properties as similar as possible to the stacked events. The applicant must justify the choice explicitly in such cases.

The same applies for substituting the conventional counterpart when evaluating the effect of persistence and invasiveness. As assessment of the effects of persistence and invasiveness requires information from specific experiments which tend to be of a case-specific and research-driven nature, the selection of the appropriate comparator should be done on a case-by-case basis according to the effect studied. The applicant must justify the choice explicitly in such cases.

The applicant should consider whether the use of extra comparators, such as negative segregants or the parental lines, may be appropriate.

For herbicide-tolerant GM plants from stacked events, GM plants treated with conventional herbicides are not required in field trials for ERA, because the primary concern of these trials is to provide data to establish that the combination of events does not raise any additional safety concerns over protein and trait expression compared to the single events. However, if these initial trials identify unintended effects that raise safety concerns, then further, more detailed experimentation is required which includes additional comparator/s. However, on a case-by-case basis and, particularly, when assessing the effects of changes in management, it may be necessary to include GM plants treated with conventional herbicides as an additional comparator.

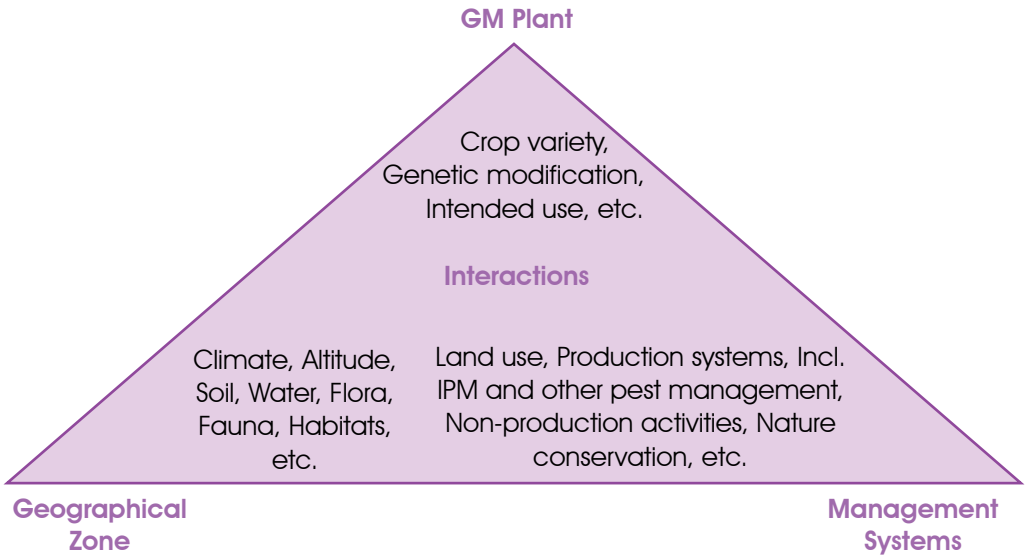
3.2 Receiving Environment/s

The receiving environment/s is the environment into which the GM plant/s will be released and into which the transgene/s may spread. The receiving environment/s is characterised by three components (*see Figure 4*):

- The GM plant (e.g. plant species, genetic modification/s and intended use/s);
- The Geographical Zones (e.g. the climate, altitude, soil, water, flora, fauna, habitats);
- The Management Systems (e.g. land use and production systems, other cultivated GM plants, cultivation practices, integrated and other pest management, non-production activities and nature conservation activities).

Land use, and production systems are considered because these systems can differ significantly between geographical regions (e.g. irrigated maize versus non-irrigated). Moreover, in a specific region,

the cultivation of GM plants for different purposes may have specific risk assessment implications (e.g. green maize harvested early for biogas or silage compared with grain maize harvested at maturity).



Source: EFSA (2010)

***Figure 4**. The receiving environment/s made up of three components that can interact*

The three components listed above result in biotic and abiotic interactions that shall be considered by the applicant when establishing representative scenarios considering the receiving environment/s for carrying out ERA of the GM plant (*Figure 4* and *Table 3*). A broad range of environments in terms of fauna and flora, climatic conditions, habitat composition and ecosystem functions, and human interventions may occur. Accordingly, the GM plants will potentially interact with these differing environments.

To support a case-by-case ERA (depending on the types of the GM plants and trait/s concerned, their intended use/s, and the potential receiving environment/s), it may be useful to classify regional data, to reflect aspects of the receiving environment/s relevant to the GM plant. These include botanical data on the occurrence of compatible relatives of GM plants in different agricultural, semi-natural and natural habitats, or effects of production systems on the interactions between the GM plant and the environment.

Relevant baseline/s of the receiving environment/s, including production systems, indigenous biota and their interactions, should be established to identify any potentially (harmful) characteristics of the GM plant. Relevant baselines refer to current production systems for

Table 3. Selection process of relevant receiving environment/s (after EFSA, 2010)

Step 1 Plant	Consider the present distribution range of the plant species
Step 2 Plant x trait	Revise current cultivation areas and their production systems according to the nature of the trait: • Add potential future cultivation areas • Where relevant, consider changes in production systems • According to the nature of the trait, concentrate on those areas and production systems in which the GM plant is most likely to be grown
Step 3 Plant x trait x environmental issue of concern	Select appropriate receiving environment/s for each environmental issue of concern identified in the problem formulation, taking into consideration assessment endpoints

which generally published literature is available. These baselines serve as a point of reference against which future changes can be compared. The baselines will depend to a considerable extent on the receiving environment/s, including biotic and abiotic factors (e.g. natural preserved habitats, agricultural farmland or disturbed land).

Both the plant and the transgenic trait/s determine where the GM plant will most likely be grown (see *Table 3*). Some GM plants (e.g. cotton, rice) can realistically be cultivated in some geographical zones only, while others, like maize, may be cultivated more widely. Transgenic traits such as biotic (e.g. pest resistance) and abiotic (e.g. drought and salt) stress tolerance will also determine where GM plants are likely to be grown. Therefore, all these elements should be taken into account when defining the receiving environment/s (e.g. considering geographical zones) for ERA of each GM plant.

The applicant shall take into account the potential risk implications of the presence of any other GM plants that have been placed on the market in the same receiving environments, including interactions between the specific cultivation characteristics (e.g. use of plant protection products) associated with the different GM plants. In addition, the applicant shall consider likely and/or predicted trends and changes to receiving environments, and how these might interact with the GM plants.

There are many climatic, ecological, agricultural and political ways of defining geographical regions or zones. The variety of the methods and criteria used to define these zones reflects the diversity and multivariate nature of the characteristics of the potential receiving

environments of a GM plant. In some cases, such methods may assist the applicant to select study sites. However, the applicant shall also consider selecting sites where the exposure and impacts are expected to be highest, and where it is anticipated that if effects exist they will be detected. The applicant shall explain why the results of their studies in certain receiving environment/s are considered representative for other receiving environment/s.

The applicant shall initially consider representative scenarios for the GM plants, including a worst-case scenario where the exposure and impact are expected to be the highest. The receiving environment/s is characterised by the GM plant, the geographical zones and the management systems (including production systems) (*Figure 4*). Cultivation areas may cover one or more geographical regions or zones. The applicant may use the step-wise approach in *Table 3* to select appropriate receiving environment/s for ERA,

For the set of selected receiving environment/s identified in step 3 of *Table 3*, the applicant shall describe:

- The characteristics of these receiving environments where the plant is likely to be distributed, specifically considering the transgenic trait/s (e.g. that might induce farmers to adopt it);
- The representative management systems (e.g. use of the plant, crop rotation, other GM plants, production systems, cultivation techniques);
- The range of relevant biotic and abiotic interactions (e.g. the interactions between plants and target organisms (TO) and/or non-target organisms (NTO)) likely to occur in the receiving environment/s taking into consideration the range of natural environmental conditions, protection goals and production systems. Where appropriate, the presence of cross-compatible wild/weedy relatives nearby, the ability of the GM plant to form feral populations and hence the potential impacts on the receiving environment should be considered.

Based on the criteria listed above, the applicant shall provide evidence that data generated are representative of the range of receiving environment/s where the crop will be grown, e.g. for the selection of field trial sites.

3.3 General Statistical Principles

This section refers to data collected from experiments in which specific hypotheses are tested. When such experiments are conducted in the field, they are termed 'trials'. This section, however, does not apply to data obtained from surveys or observational data.

For ERA, the applicant shall list explicitly *in words* all the questions that each study – be it a field trial, a trial in semi-field conditions or a laboratory study – was designed to address. In addition, each of these questions shall be re-stated *in formal terms*, in the form of the precise null hypothesis that was tested to answer the question. This shall apply equally to those studies that seek confirmatory data on unintended effects when some evidence already exists, as to those that take an ecotoxicological approach with a specific null hypothesis. For field trials, the applicant shall provide a clear and explicit statement concerning the minimum levels of abundance acceptable for each taxa sampled, below which results would lack credibility. The applicant shall supply justification for the values chosen. In mathematical modelling for the assessment of long-term or large-scale effects, the applicant shall state explicitly all assumptions made, and provide justification for each. The principles underlying the statistical tests of difference and equivalence (EFSA, 2009b) described below are to provide information with quantified uncertainty that may be used by biologists in risk characterisation of those endpoints for which differences or lack of equivalence are found. In order to place differences or lack of equivalence into context, allowance must be made for the distinction between statistical and biological significance. The two approaches are complementary: statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be relevant on safety grounds (see limits of concern below). For risk assessment, it is not the function of statistical analysis to provide results that lead automatically to a particular decision; instead, the case-by-case approach shall remain paramount.

ERA is often hampered by the difficulty of conducting experiments with sufficient statistical power. The use of meta-analysis (Marvier et al., 2007) is an option for the applicant to consider, but is not mandatory. It may be useful to quantify studies that may not all have the power to be individually significant, in the statistical sense, and also to provide an overview of broad patterns when individual studies appear to contradict each other.

The comparative analysis referred to above shall involve two approaches:

(1) a proof of difference, to verify whether the GM plant is different from its conventional counterpart/s, and might therefore be considered a potential risk depending on the type of the identified difference, extent and pattern of exposure; and

(2) a proof of equivalence to verify whether the GM plant is equivalent or not to its conventional counterpart/s (Perry *et al*., 2009) within bounds defined by so-called '*limits of concern*'.

For each measurement endpoint, the level of environmental protection to be preserved is expressed, directly or indirectly, through the setting of 'limits of concern' which may take one of two forms. For lower-tier studies, the limits of concern will usually be trigger values which, if exceeded, will usually lead to further studies at higher tiers. Then the relationship of the limits of concern to environmental protection goals is indirect. For higher tier studies, especially field studies, the limits of concern shall reflect more directly the minimum ecological effects (in positive and negative directions) that are deemed biologically relevant. For field studies, at least one of the limits of concern shall represent the minimum effect that is considered by the applicant to potentially lead to environmental harm. If this limit is exceeded, then detailed quantitative modelling of exposure may be required to scale up adverse effects at the field level both temporally (to seasons, generations, rotations) and spatially (to farms, landscapes, regions and ecosystems) (EFSA, 2008). Baseline data can be used to define the limits of concern.

> *Purely as a guide, for laboratory studies, a multiplicative effect size of 20% is often taken as the trigger value for further, higher-tier studies. Similarly, for semi-field testing, a trigger value of 30% has been used. For field studies, several studies, both in the USA and in the EU (Heard et al., 2003), have adopted 50% as a limit of concern. By contrast, the effect size threshold for classification set by the International Union for Conservation of Nature (IUCN) for butterflies is a reduction in population size of at least 30% over three generations (but here 'population' is defined at a larger than field scale).*

Note that, unless there is explicit justification, the limits of concern for lower-tier studies shall usually be less than those for higher-tier studies, because it makes no sense for the results from laboratory studies to exclude from further study effects that might be manifest in the field. Whatever the limits of concern adopted, the applicant shall state their value and justify the choice explicitly, for each measurement endpoint. For field studies, it will usually be the lower limit (which might correspond for example to a decrease in the abundance of a particular species in the presence of the GM plant relative to that for the conventional counterpart) that will be defined as the threshold effect deemed to be of just sufficient magnitude to cause environmental harm. Notwithstanding this general approach, it is acknowledged that the multiplicity and diversity of questions that might be posed in ERA may demand alternative statistical approaches, on a case-by-case basis.

All test materials, the GM plant and conventional counterpart/s, whether in the field, in semi-field conditions or in the laboratory, shall be fully randomised to the experimental units. Other aspects of experimental design are addressed below.

Whether analysis is of field, semi-field or laboratory data, results shall be presented in a clear format, using standardised scientific units. The applicant shall provide the raw data and the programming code used for the statistical analysis in an editable form. Other aspects of reporting and analysis are addressed below.

3.3.1 Testing for difference and equivalence

In testing for a difference, the null hypothesis will state that there is no difference between the GM plant and its conventional counterpart, against the alternative hypothesis that a difference exists. In testing for equivalence, the null hypothesis will be that there is lack of equivalence, in the sense that the difference between the GM plant and its conventional counterpart is at least as great as a specified minimum size, against the alternative hypothesis that there is no difference or a smaller difference than the specified minimum between the GM plant and its conventional counterpart. Rejection of the null hypothesis (*i.e.* a finding that the difference is no greater than this minimum size) is required in order to conclude that the GM plant and the conventional counterpart are unambiguously equivalent for the measurement endpoint considered. The two approaches are complementary: statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be relevant from the viewpoint of environmental harm. For studies that use extra comparators, the analysis shall encompass separate difference tests (between the GM plant and each of its different comparators) and separate equivalence tests (between the GM plant and each of its different comparators), and these shall be reported similarly. Further discussion of the principles of equivalence testing, with practical examples, is given in EFSA (2009b).

3.3.2 Specification of the effect size and the limits of concern

Problem formulation and risk characterisation make up the major parts of the risk assessment dossier. Notwithstanding the well-known distinction between biological relevance and statistical significance (Perry, 1986), risk characterisation cannot be done without relating effects to potential harm. Therefore, it is essential to specify for each variable studied a minimum effect size which is considered to potentially have a relevant impact on the receiving environment/s. Based on such effect sizes, power analyses aid transparency and may engender public confidence that risk to the consumer is well-defined and low (Marvier, 2002); these require specification of the magnitude of the effect size that the study is designed to detect. Good scientific studies are planned carefully enough for the researchers to have a reasonable idea of the size of effect that the study is capable of detecting. For all these reasons, for each study, whether in the field, in semi-field conditions or in the laboratory, the applicant shall state explicitly the size of the effect that

it is desired to detect in the study, for each measured endpoint. The effect size may be asymmetric, and in particular may be set as zero in one direction to yield a non-inferiority form of the equivalence test (Laster and Johnson, 2003). The magnitude of the effect size that the study is designed to detect will generally be greater for trials designed to provide confirmatory field data for the assessment of unintended effects on non-target organisms than for specific hypotheses. The effect size will often be placed on the multiplicative scale; however, the natural scale or some other scales are admissible alternatives, on a case-by-case basis. In principle, where more than one comparator is used different effect sizes may be specified for the different comparators; however, this is unlikely to be necessary in practice. The applicant shall provide a full justification for all effect sizes chosen.

The applicant shall state explicitly how the chosen effect size/s relates to the limits of concern through the minimum relevant ecological effect that is deemed biologically relevant. Usually, these quantities will be identical; hence, the applicant shall justify cases where this is not so. The applicant shall state explicitly the limits of concern that were used for each equivalence test. If justified appropriately, more than one pair of limits of concern may be set for each measurement endpoint, and an equivalence test shall then be performed for each pair of limits.

3.3.3 Power analysis

For each study, be it a field trial, a trial in semi-field conditions or a laboratory study, the applicant shall ensure that the design is such that the test of difference has sufficient statistical power to provide reasonable evidence (Perry *et al*., 2009). Statistical power is the probability of detecting an effect of a given size, when such a real effect exists. In medical science, a level of 80% is usually considered to be an acceptable level for statistical power, but it is recognised that for ecological GM field trials the restriction on the land available for experimentation combined with unavoidable environmental heterogeneity usually necessitates some compromise between the replication required for high power and the experimental resources available (Perry *et al*., 2003). Notwithstanding, optimal experimental design shall be directed to attain as high a power as possible.

For each study, the applicant shall provide an analysis that estimates the power for each test of difference on each measurement endpoint, based on the stated effect size and assuming a 5% type I error rate. The analysis shall be done at the planning stage of the study. The power analysis shall use only information verifiable as available prior to the study; under no circumstances shall data from the study itself be used. For field trials, because each field trial at a site on a particular occasion shall have sufficient replication to be able to yield a stand-alone analysis if required (see below), this power analysis shall relate to a single site,

not to the entire set of trials. For situations where many species are sampled such as in field trials, the power analysis is required only for those species of prime importance and those expected to be the most abundant.

3.3.4 Experimental environment

The first decision in conducting a study is whether the questions asked are best answered by data produced in the laboratory, mesocosm, semi-field, field or region.

The effect of plant-environment interactions can be studied starting from studies that encompass a range of environmental scales. For this, hazards are evaluated within environments that progress from worst-case scenario conditions with laboratory experiments, up to ecological field trials with relatively large plots.

The laboratory environment is favoured for studies where it is important to control and define closely the conditions for tested organisms. As environmental variability and interfering factors which can mask potential effects are minimised, laboratory studies yield results of relatively high precision. The laboratory environment is used particularly for the identification of acute and direct impacts of GM products and metabolites on individuals. In particular dose-response relationships may be well described. It also provides the possibility to study indirect and multi-trophic effects at small scales. Trait x environment interactions may be studied in the laboratory, but only to a limited extent. The laboratory is often used as an initial environment in the tiered approach, particularly for tier 1 studies. In a laboratory study, decisions must be made as to whether the test materials should be of synthetic or *in planta* form.

Semi-field trials are manipulative test systems that are designed to control the inherent variability of the environment. They usually incorporate some form of protected environment or containment, such as field cages or screen houses, designed both to isolate the organisms under test and exclude unwanted biotic (e.g. predators) or non-biotic (e.g. rainfall) factors. Semi-field trials allow exposure to ambient weather and light conditions. The larger cages may result in more natural behavioural interactions between the organisms and plants tested. The semi-field environment is not subject to large variations in the ecology of habitats, and any variability due to different receiving environments is suppressed. Semi-field trials may have greater sensitivity than less-controlled open field trials, and it may be that lower levels of statistically significant differences may therefore be detected. Examples include studies on possible indirect effects on non-target pollinators using bees in screen house trials. Mesocosms are experimental ecosystems that can be used to perform tests under realistic semi-field conditions. Examples

include studies of bio-geochemical cycles using residue decomposition, although litterbag experiments within field trials provide a more realistic alternative.

Field trials allow for the study of indirect and multi-trophic effects on a larger scale, including in some cases the population level. Trait x environment interactions may be tested validly. Although they must, by definition, suffer from less ability to control environmental conditions and therefore produce results subject to greater environmental variability, they provide the only way in which relevant lower-tiered results may be validated under natural conditions. They allow experimental tests of parameters of importance in ecosystem functioning (such as the predation and/or parasitism rate of a species, the decomposition rate of plant residues, etc.) and the estimation of overall ecosystem functions (such as pollination, natural pest control, etc.). Another advantage of field trials is that genotype x environment interactions may be studied in the receiving environment/s.

Field surveys are scientifically designed studies without a hypothesis and where there is no experimental imposition of treatments. However, data are collected in the receiving environment/s. For example, these may provide appropriate data relevant to the identification of unintended effects on non-target organisms and to changes in plant fitness.

The importance of field trials in ERA of GM plants is widely accepted. One crucial aspect is the increase in ecological realism that can be achieved as the scale of tests move up from laboratory through mesocosm to semi-field, field and region. For example, when any organism is in contact with a GM plant within a multi-trophic context, identification of the impacts on ecological functioning is facilitated by an increase of scale of the experimental arena.

Field studies (semi-field, field trials and field surveys) for environmental effects of GM plants is of special importance because there are organisms for which particular ecological or behavioural tests in the laboratory fail to encompass realistic conditions (for example in some studies of species that are highly mobile, such as adult butterflies or bees; or species for which rearing methods are inadequate). Field testing allows a wide range of arthropod characteristics to be assessed (such as species number, life stages, exposure to abiotic and biotic stress, complexity of trophic[3] interactions) that cannot easily be reproduced in laboratory settings. Conversely, laboratory studies may incorporate controlled conditions that are impossible to reproduce in the field, which may prevent the identification of causal relationships. Attention shall therefore be paid to the differences in inferences that may be

[3] The trophic level of an organism is the position it occupies in a food chain.

drawn between standardised tests and field testing. For example, due to the lack of well-defined standards, the number of standard laboratory tests on necrotrophic decomposers is very limited, and, in particular, some bio-geochemical processes cannot be investigated in artificial environments, such as pot experiments. Therefore, field trials may be essential to produce results in such cases.

3.3.5 Experimental design

Experimental designs for laboratory experiments shall conform to accepted international standards and protocols such as those published, for example, by OECD or similar organisations specialising in ecotoxicology.

For field trials, the principle shall be followed that each field trial at a site on a particular occasion shall have sufficient replication to be able to yield a stand-alone analysis if required, although the main analysis shall derive inferences from averages over the complete set of field trials at all sites and years. The level of within-site replication shall be informed by the power analysis referred to above.

Notwithstanding this, it is most unlikely that less than three replicates per site would provide an adequate design. A completely randomized or randomized block experimental design is usually appropriate; appropriate extensions to these designs are discussed by Perry *et al.* (2009). The applicant shall justify explicitly why the different sites selected for the trials are considered to be representative of the range of receiving environments where the crop will be grown, reflecting relevant meteorological, ecological, soil and agronomic conditions. The choice of plant varieties shall be appropriate for the chosen sites, and shall also be justified explicitly. Within each site the GM plant and its conventional counterpart/s and any additional test material, where appropriate, shall be identical for all replicates. Environmental variation is manifest at two scales: site-to-site and year-to-year. The primary concern is not environmental variation *per se*, but whether potential differences between the test materials vary across environmental conditions (*i.e.* statistical interactions between test material and environmental factors, often referred to as genotype by environment (GxE) interactions). Hence, in addition to within-field replication, there is a need to replicate over sites and years to achieve representativeness across geography and climate. Unless explicit appropriate justification is given by the applicant, each field trial shall be replicated over at least two years, within each of which there shall be replication over at least three sites. In the case that sites cover a very restricted geographic range, further replication of trials, over more than two years, may be required. The use of available data from different continents may be informative, but the applicant must justify explicitly why the sites within these continents are representative of the range of the receiving environments where the

GM plant will be grown, reflecting relevant meteorological, ecological, soil and agronomic conditions.

However, these explicit requirements above for replication to achieve representativeness do not apply to confirmatory field data for the assessment of unintended effects, e.g. on non-target organisms, when some evidence already exists, or to the great variety of field trials designed to provide data for a wide range of purposes, to assess aspects of potential persistence and invasiveness. Many experimental designs used for research purposes are available in the literature as a guide for the very specific requirements for such trials. Data concerning phenotypic and agronomic characteristics of plants are often derived from the same trials designed to supply data for compositional analysis; statistical guidance (EFSA, 2009b) is already available for compositional analyses, and the requirements above do not apply to them. However, for some non-food and non-feed applications for cultivation, such as potato modified to enhance the content of the amylopectin component of starch, compositional trials may not be conducted. Then, the experimental design of phenotypic and agronomic trials shall follow the guidelines in this section.

For non-target organisms, plant performance and data on environmental measurement endpoints (*e.g*. agronomic characteristics, including herbivore interactions with the plant, responses to specific environmental exposure) may provide indications concerning the likelihood or otherwise of unintended effects. This may, for example, include evidence for unchanged ecosystem functions. Under the weight of evidence approach, data from field trials may be used to provide such confirmatory data to underpin conclusions that unintended effects are unlikely. While the requirement for statistical power for these field trials shall be carried out as outlined earlier, the requirements for representativeness may be relaxed. Hence, as long as there is explicit justification, under these circumstances, there is no requirement for a minimum number of sites and/or years.

Experimental units (field plots) that are of the spatial scale of a whole or half-field are probably of most use in post-commercialisation studies, for monitoring or mitigation. For pre-commercialisation experimentation, smaller plots, where variation may be controlled and defined treatments imposed more easily, are more appropriate for experimental units (Perry *et al*., 2009). It is recommended to separate plots within sites, often by strips of bare soil of specified width, and to sample from the centre of the plots to avoid border effects. Unless the experiment is set up specifically to study residual effects from one season to the next, or to study long-term effects, it is recommended not to utilise exactly the same plots for over more than one year at a particular site (Perry *et al*., 2009).

When it is desirable to assess several different GM plants for one crop species (e.g. *Zea mays*), the generation of data for the comparative assessment of these different GM varieties may be produced simultaneously, at the same site and within the same field trial, by the placing the different GM plants and their appropriate conventional counterparts in the same randomized block. This is subject to two conditions which shall be strictly met:

(1) each of the appropriate counterpart/s shall always occur together with its particular GM plant in the same block;

(2) all the different GM plants and their counterpart/s shall be fully randomized within each block.

For further details on the use of partially balanced incomplete block designs see EFSA (2009b).

In general, it is easier to impose controlled conditions in semi-field trials, and these are not subject to environmental variability to the same extent as are field trials. However, if semi-field trials do not control conditions, then the need to test in different environments (at different sites and/or in different years) shall be considered.

For some GM perennial plants (*e.g.* trees), the plants themselves may be more appropriate experimental units than are field plots. Care should be taken to choose an experimental design that does not suffer unduly from loss of plants during the trial. Whilst it is largely unnecessary to control for positional variation, plant-to-plant variability should be minimised when selecting experimental material.

3.3.6 Analysis and reporting

It is recommended that the applicant prepares an experimental design protocol and a statistical analysis protocol for each study (refer to Perry *et al.*, 2009 for a suggested checklist). It is recommended that the experimental design protocol comprises full information on the study, and includes but is not restricted to:

(1) a list of the measurement endpoints, and why they were included;

(2) a description of and justification for the experimental design;

(3) a description of the experimental units, including dimensions;

(4) the blocking structure of the experimental units, in terms of the factors that represent it, their levels and whether the factors are nested or crossed;

(5) the sampling regime, within and between experimental units, and through time;

(6) any repeated measurements made in the study;

(7) the test materials and the justification for their inclusion;

(8) the treatment structure of the study, in terms of the factors that represent it and their levels;

(9) a list of the interactions, if any, that are of interest, and why they are of interest; and

(10) a description of how the treatment factors listed will be randomized to the experimental units specified in the blocking structure above.

It is recommended that the statistical analysis protocol comprises full information on the analysis, and includes but is not restricted to:

(1) a description of the generic form of the analysis, and why it was chosen;

(2) the criteria for identifying outliers;

(3) a description of the likely transformations planned, with reasons;

(4) justification for any distributional assumptions;

(5) the scale on which the effects in the experiment are assumed to be additive; and

(6) justification for any other assumptions made in the analysis.

For field trials, the protocols shall also include:

(1) details of the management of the fields before sowing, including the cropping system and rotation;

(2) the dates of sowing;

(3) the soil types;

(4) insecticide and herbicide use, and use of any other plant protection products or techniques;

(5) climatic and other cultivation/ environmental conditions during growth, and where appropriate during harvest;

(6) relevant details of the field margins and neighbouring fields;

(7) brief descriptions of pest and disease infestations.

When many measurement endpoints have been included in a study (e.g. where the endpoints represent several NTO species), the results of all endpoints for which sufficient records have been obtained shall be reported, not just those deemed to be of particular biological or statistical interest. Data transformation may be necessary to ensure normality and to provide an appropriate scale on which statistical effects are additive. As is routine in ecological applications, for many measurement endpoint response variables, a logarithmic transformation (or a generalized

linear model with a logarithmic link function) may be appropriate. In such cases, any difference between the GM plant and any other test material is interpreted as a ratio on the natural scale. However, for other measurement endpoints, the logarithmic transformation may not be optimal and the natural scale or another scale may be more suitable.

Allowance must be made for possible correlations between repeated measurements from the same experimental units. This is especially important where: (1) sampling is repeated over several occasions during a season; and (2) the GM plant is a perennial.

Analyses will involve a test for difference and a test for equivalence. Specifically, for a particular measurement endpoint, the mean difference/s between the GM plant and its conventional counterpart/s is computed and a 90% confidence interval constructed around it, as in Perry *et al.* (2009). The means, these confidence limits and all equivalence limits shall be displayed on a graph/s where the values are plotted relative to a zero baseline defined by the mean of the GM plant test materials. The line of zero difference on the logarithmic scale corresponds to a multiplicative factor of unity on the natural scale. The horizontal axis shall be labelled with values that specify the change on the natural scale. In the case of logarithmic transformation, changes of 2x and ½x will appear equally spaced on either side of the line of zero difference.

Both the difference test and the equivalence test may be implemented using the well-known correspondence between hypothesis testing and the construction of confidence intervals. In the case of equivalence testing, the approach used shall follow the two one-sided tests (TOST) methodology (*e.g.* Schuirmann, 1987) by rejecting the null hypothesis when the entire confidence interval falls between the equivalence limits. The choice of the 90% confidence interval corresponds to the customary 95% level for statistical testing of equivalence. As the confidence interval graph is used also for the test of difference, each difference test will have a 90% confidence level. Although 1 in 10 of these tests is expected to yield a significant result by chance alone, the applicant shall report and discuss all significant differences observed between the GM plant, its conventional counterpart and, where applicable, any other test material, focussing on their biological relevance within the context of risk characterisation. Regarding the simultaneous tests of difference and equivalence, each outcome from the graph shall be categorised and the respective appropriate conclusion shall be drawn, exactly as described in EFSA (2009b).

3.3.7 Statistical analysis of field trials

The main analysis shall address all field trials simultaneously and shall be based on the full dataset from all sites. Accordingly, the form of the

equivalence test shall be that termed 'average equivalence' in the drug testing literature (Wellek, 2002). The use of a statistical mixed model is an important feature of analysis for food-feed assessments because of the need to estimate the natural variation of the commercial varieties. However, as stated above, for ERA it is recommended that equivalence limits are set explicitly. Therefore, the use of commercial varieties for this purpose is not necessary, although it might be appropriate for other biological reasons. Hence it is not recommended that statistical mixed models be required forms of analysis, as they are for food-feed assessments (Perry *et al*., 2009). Indeed, it is recommended to use simple statistical models; effects due to environmental factors such as seasons and sites may be represented by fixed factors if desired. The applicant shall ensure that each analysis has the potential to identify any interactions between sites and years and the test materials. For each measurement endpoint studied, the applicant shall make an explicit statement concerning the presence or absence of any such interactions. If interactions are found, the possible reasons for their existence and the implications for the inferences drawn from the trials shall be discussed. The applicant shall also provide a table or graph giving, for each site and year and for each (transformed) measurement endpoint, the means and standard errors of means of the GM plant and its conventional counterpart/s, and any other test material, where applicable.

Diversity indices are not recommended for general risk assessment in pre-commercialisation studies, because it is most unlikely that studies will yield sufficient samples of individuals to characterise indices adequately or that a sufficient degree of ecological background information will exist to give confidence that biodiversity can be represented adequately as a single number. By contrast, multivariate approaches may be useful, especially for summarising data and for analysing principal response curves (Perry *et al.*, 2009).

Particular recommendations apply for the very wide range of possible studies of persistence and invasiveness, and the related estimation of selective advantage and disadvantage.

3.4 Uncertainties

Risk can be defined as the product of the magnitude of the consequences of the hazard and the likelihood of the adverse effect. Both the effect and the likelihood are measured with uncertainty.

ERA has to take into account uncertainty at various levels. Uncertainties may arise from problem formulation, limitations in the data (*e.g*. limited exposure data), gaps in the effect database, model choice, the limitation of the test systems and measurement endpoints selected, inadequacy of study designs and the uncertainties in extrapolating between species (EFSA, 2009a). Scientific uncertainty may also arise from differing

interpretations of existing data, publication bias or lack of some relevant data. Uncertainty may relate to qualitative or quantitative elements of the analysis. The level of knowledge or data for a baseline is reflected by the level of uncertainty, which shall be discussed by the applicant. The applicant shall in addition assess the degree of uncertainty within ERA in comparison with the current uncertainties displayed in the scientific literature.

Although it may be impossible to identify all the uncertainties, the assessment shall include a description of the types of uncertainties encountered and considered during the different risk assessment steps. Their relative importance and their influence on the assessment outcome shall be described. Any uncertainties inherent in the different steps of ERA (steps 1 to 5) shall be highlighted and quantified as far as possible. Distinction shall be made between uncertainties that reflect natural variations in ecological and biological parameters (including variations in susceptibility in populations or varieties), and possible differences in responses between species. Estimation of uncertainties in experimental data shall be handled by proper statistical analysis, while quantification of uncertainties in assumptions (*e.g.* extrapolation from environmental laboratory studies to complex ecosystems) may be more difficult. The absence of data essential for the environmental risk assessment shall be indicated, and the quality of existing data shall be discussed.

It should be clear from the discussion how this body of information has been taken into account when the final risk characterisation is determined. Risk characterisation may be qualitative and, if possible, quantitative depending on the issue to be addressed and the available data. The terms for the expression of risks and associated uncertainties shall be as precise as possible. For example, expressions like '*no/negligible/acceptable/significant risk*' need, where possible, further numerical quantification in terms of probability of exposure and/or occurrence of adverse effects.

It is recognised that ERA is only as good as our state of scientific knowledge at the time it was conducted. Thus, ERA is required to identify areas of uncertainty or risk which relate to areas outside current knowledge and the limited scope of ERA. These include such factors as the impact of the large-scale exposure of different environments when GM plants are commercialised, the impact of exposure over long periods of time and cumulative long-term effects. When uncertainty factors are used, an explanation of their basis and a justification of their appropriateness need to be provided, or a reference to documents where that information may be found shall be included. When point estimates are used for uncertain quantities, justification for the values chosen and assessment of their influence on the assessment shall be included.

Predicting impacts of GM plants on complex ecosystems which are continually in flux is difficult and largely based on experiences with other introductions and an understanding of the robustness of ecosystems. It is recognised that ERA is limited by the nature, scale and location of experimental releases, which biospheres have been studied and the length of time the studies were conducted. Probabilistic methods could be used to determine ranges of plausible values rather than single values or point estimates, which are subsequently combined in order to quantify the uncertainty in the end result. These methods could provide a powerful tool to quantify uncertainties associated with any steps in ERA. When such probabilistic approaches are used, the outcome of ERA should be characterised by reporting a distribution of the risk estimates. However, the use of quantitative methods does not remove the need for a qualitative evaluation of the remaining uncertainties.

Scientific knowledge from the literature and experience gained from growing GM plants encompassed in PMEM following past applications and approvals may also guide the risk assessment process. Notwithstanding the requirement to fully assess all possible risks based on reliable data, this is but one example of the responsibility on the applicant to continually update ERA in the light of new knowledge.

3.5 Long-Term Effects (Including Techniques for Their Assessment)

A general requirement of ERA is that an analysis of the cumulative long-term effects relevant to the release and placement in the market is to be carried out. Predicting and assessing (adverse) long-term effects requires information about the GM plant and the receiving environment/s, both in terms of the baseline conditions in the receiving environment/s and temporal changes in these conditions independently of the GM plant and following GM plant introduction. The rate and degree by which the baseline is likely to change independently of the GM plant (*e.g*. as a result of new crops and agronomy) will vary among production systems. The consideration of long-term effects in ERA should address effects that might arise up to a minimum of 10 years after the start of cultivation for annual plants, *i.e*. corresponding to the time frame of the consent authorisation, but possibly longer for perennial species, and should in all cases cover the time period over which progeny of the GM plant might persist and appear as volunteers or ferals. Thus, the analysis should be conducted case-by-case, and the applicant should fully justify their approach.

3.5.1 Categories of long-term effects

Long-term effects might result from a diversity of primary causes and secondary interactions, which make it difficult to generalise on methods of investigation. Such effects can, however, be considered in two broad categories:

Category I: Long-term or chronic exposure to a particular GM plant or practice resulting in a delayed response by organisms or their progeny.

In some instances a response occurs immediately, but is not detected by the measuring tools or the particular indicators employed. For example, exposure over time may affect a species or community by suppressing certain functional forms in relation to others, or acting on natural mutations that exist at very low frequency such as occurs when pests develop resistance to a pesticide.

Category II: Effects occurring as the result of an inevitable increase in spatial and temporal complexity, determined by the number of possible interactions that a GM plant would have with the biota and the physical and chemical environment as it is grown more widely throughout the landscape and in more extended sequences of cropping.

There may not necessarily be a chronic or delayed effect as in Category I; rather, the effect occurs in certain contexts that are outside those experienced in the initial testing, or that have arisen as entirely new contexts due to global environmental change, or the adoption of new forms of management. The latter may indeed arise as a downstream effect of the introduction of the GM plant cultivation itself, if this causes a change in the sequence or range of plants grown in the production system.

An estimate of whether long-term effects of both categories are expected to occur and how PMEM should be followed after commercialisation should be given in every application. Based on the characteristics of the GM plant, ERA should consider these long-term effects by referring to existing examples, long-term datasets, and in some instances modelling, as indicated below. The analysis and conclusions should be presented in the form of a desk study based on the interpretation of existing information.

3.5.2 Techniques and information required to assess long-term effects

Some effects of Category I might already have been investigated within constrained experimental systems maintained over several generations of the GM plant/trait combination under study. While some potential long-terms effects might be revealed by such studies, questions will still remain, as to how much the constrained system restricts the range of possible reactions or encourages untypical reactions, such as caused by a reduced choice in the foraging range and food available to invertebrates that are kept for months or years in controlled environment chambers or restricted to intensely managed field plots. Information from such studies might be useful for defining the primary mechanisms by which the GM plant might interact with other

organisms and their abiotic environment, but would not be sufficient alone as a basis for assessment of long-term effects in an agricultural or ecological context.

Category II, by definition, cannot be investigated through an initial experimental phase of testing, even at the scale of the field plot, half-field or paired field, none of which can provide the range of complexity experienced after full commercial release. For example, the unpredicted increase in grass weeds compared with broadleaf weeds in GM herbicide-tolerant crop trials on winter oilseed rape, and the consequent reduction of the arable food web, were probably a combination of the timing of herbicide application, the local climate and the local weed profile – a context that had not been, and could not be, examined before large scale, multi-site testing. Category II effects can only be investigated by reference to existing examples and case histories that provide evidence of rates and magnitudes of environmental impact due to change in agricultural (e.g. pesticides, crop type) or external (e.g. extreme weather) factors, including GM cultivation where data are available.

Despite these uncertainties, there is now a great deal of information in the published literature, and in accessible reports and databases, on long-term ecological and environmental effects due to agricultural change. The applicant should conduct appropriate desk-based studies to assess long-term environmental effects of the GM plant in relation to both categories of long-term effects. It is not the intention here to give precise instruction to the applicant on which data, processes and indicators should be considered, because they will vary case-by-case. However, examples of the type of information that could be used in assessment are as follows:

- Experience of cultivating the GM plant or long-term environmental exposure to GM cultivation in other regions;
- Experience from cultivation of similar plants (GM and non-GM);
- Long-term ecological or environmental datasets applicable to the receiving environment/s; e.g. government statistics on cropped areas, pesticide usage, nutrient inputs, agrochemicals in water; ecological surveys showing change in the range or abundance of organisms;
- The results of major field experiments on GM plants that have examined effects or GM events similar to those of the GM plant under assessment; e.g. the field trials elsewhere of GM herbicide-tolerant crops; and long-term field exposure studies to Bt maize;
- Quantitative examples of the degree to which previous agricultural change, even if not involving GM plants, has affected ecological and environmental indicators; *e.g*. response of plants and animals

to change in pesticide usage, and to expansion or contraction of cropped area;

- The results of meta-analyses drawing together data from different sources (*e.g*. Marvier *et al*., 2007);
- The use of models of ecological processes to explore or test scenarios: mathematical models of ecological processes are unlikely to be considered justification on their own, but may be used to argue or interpret data or to demonstrate that possibilities have been explored; descriptions would be necessary of the model, its verification using existing data, the input variables, etc;
- Foreknowledge of relevant change in the production system and wider environment that can be expected in the years following release; an example is the withdrawal of pesticides from commercial usage.

CHAPTER

4

RISK ASSESSMENT OF GM PLANTS CONTAINING STACKED TRANSFORMATION EVENTS

4.1 Introduction

In these guidelines, the term *stacked transformation event* or *stacked events* will refer to a GM plant derived from conventional crossing of GM plants consisting of one or more events. ERA of stacked events should include a comparative safety assessment, and follow the six steps of ERA (*Figure 2*).

ERA of the single events is a pre-requisite for the risk assessment of stacked events. ERA of stacked events shall start when the risk assessment of each single event is finalised. In case single events cannot exist separately, an alternative rationale for the risk assessment approach should be provided by the applicant.

For GM plants containing stacked events, the primary concern in risk assessment is to establish if the combination of events might result in interaction/s that would raise safety concerns compared with the single events, or, in case of stacked events containing three or more events combined by conventional crossing (defined as *higher staked events*), compared to already assessed sub-combinations (defined as *lower stacked events*). ERA of higher stacked events shall cover all sub-combinations of these events.

For applications for import and processing: ERA of higher stacked events shall cover all sub-combinations of these events as independent stacked events.

For applications for cultivation of the higher stacked events only: The applicant should consider the full range of environmental issues of concern, including change in management of the higher stacked events compared to lower stacked events or single events already risk assessed. In addition, ERA of higher stacked events shall consider all other sub-combinations of these events that may occur by natural segregation (e.g. volunteers).

For applications for cultivation of the higher stacked events and specified sub-combinations (cultivation stack-n, stack-n-1, stack-n-2,

etc.): The applicant shall consider the full range of environmental issues of concern. In particular, the applicant shall describe fully the management of each of the cultivated sub-combinations individually and assess their environmental impacts. In addition, ERA of higher stacked events shall consider all other sub-combinations of these events that may occur by natural segregation (e.g. volunteers).

The applicant shall provide a scientific rationale justifying the range and extent of information used to support the risk assessment of sub-combinations.

ERA of stacked events shall mainly focus on the characterisation and potential consequences of issues related to:

- stability of the inserts;
- expression of the events;
- potential synergistic, additive or antagonistic effects resulting from the combination of the events;
- changes in management (if applicable).

The appropriate comparator for stacked events should be selected in accordance with the requirements defined earlier, with the applicant justifying the choice of all comparators.

4.2 Specific Considerations for Stacked Events

While areas of risk described in the subsequent chapters should be considered on a case-by-case basis, some specific considerations for stacked events are listed below.

4.2.1 Persistence and invasiveness, including plant-to-plant gene flow

For stacked events, the applicant should consider, during the problem formulation phase, whether the combination of events may lead to enhanced persistence or invasiveness that is more than that expected from the simple product of the single traits. Additional field data may be required if changes are observed in the phenotype or growth characteristics (*e.g*. such as behaviour, fitness, reproduction, survivability or dissemination).

4.2.2 Interactions of stacked events with target organisms

For stacked events, potential synergistic, additive or antagonistic effects of different biocidal substances should be taken into consideration.

For example, in cucumbers, replication of the tomato aspermy virus (TAV) is restricted to the leaves unless the plant is also infected with

the cucumber mosaic virus (CMV), or where there is expression of the CMV coat protein.

In order to confirm the absence of these potential effects, the potential impact on target organisms should be assessed. In addition, consequences of any interaction on the development of resistance in target organisms should also be assessed and considered when developing risk management strategies.

4.2.3 Interactions of stacked events with non-target organisms (NTO)

For stacked events not expressing biocidal compounds, if scientific knowledge does not indicate the possibility of synergistic, additive or antagonistic interactions between these compounds that may affect NTO, then no specific testing is necessary.

Stacked events expressing more biocidial compounds that the single events, may have different adverse effects on NTO than the single events due to synergistic, additive or antagonistic effects. The applicant shall perform studies (or provide existing data) with combined administration of proteins when the genetic modification results in the expression of two or more proteins in the GM plant. *In planta* tests with the stacked events shall be included in tier 1 studies. Testing should follow the same approach as described in Chapter 9.

4.2.4 Impacts of the specific cultivation, management and harvesting techniques

The applicant is requested to describe the specific cultivation, management and harvesting techniques of the GM plant containing stacked events, as well as of each of the cultivated sub-combinations covered by the application, to and assess their potential environmental impacts with respect to the appropriate comparator. In this evaluation, any differences in the specific cultivation, management and harvesting techniques between: (1) the stacked events; (2) the single events contained in the stacked events; (3) the conventional counterparts, if available; and (4) each of the cultivated sub-combination of stacked events shall be explicitly stated and assessed with regard to their environmental impacts.

4.2.5 Post-market environmental monitoring plan (PMEM)

The general principles of the PMEM as described in Chapter 13 are appropriate for applications concerning stacked events. Case-specific monitoring should take into account the results of ERA, plus any monitoring already proposed or established for single events previously assessed. Consideration should be given to any additional environmental

exposure or other effect due to the combination of events identified in ERA. General surveillance should proceed as for any other GM plant and take account of any general surveillance plans already proposed or established for single events previously assessed.

CHAPTER

5

SPECIFIC AREAS OF RISK TO BE ADDRESSED IN ERA

Environmental risks can be grouped into seven specific areas of risk. For each specific area of risk, the applicant is requested to provide information in a clear and concise way, following systematically the first five steps of ERA as described below and in Chapter 2. To reiterate:

- Step 1: Problem formulation
- Step 2: Hazard characterisation
- Step 3: Exposure characterisation
- Step 4: Risk characterisation
- Step 5: Risk management strategies
- Step 6: Conclusions

For each specific area of risk (progressing from steps 1 to 5), the applicant should conclude by summarising the assessment, the assumptions taken, the available information and identified gaps, the data produced, the estimated uncertainty, the characterisation of the risk/s, and the need, or not, for risk management strategies.

At step 6, the applicant is requested to consider the overall evaluation performed and to provide overall conclusions and recommendations of ERA. The overall conclusions and recommendations should provide the framework for the risk management strategies including PMEM and, therefore, a link to Chapter13 should be made.

What follows are detailed guidelines on how to carry out ERA for each specific area of risk, running through the six steps given above.

CHAPTER 6

PERSISTENCE AND INVASIVENESS, INCLUDING PLANT-TO-PLANT GENE FLOW

6.1 Step 1: Problem formulation

Some environmental concerns about GM plants relate to the potential persistence or invasiveness of the plant itself, or of its compatible relatives, as a result of vertical gene flow within either agricultural or other production systems, or semi-natural and natural habitats. The potential adverse effects are of two main types.

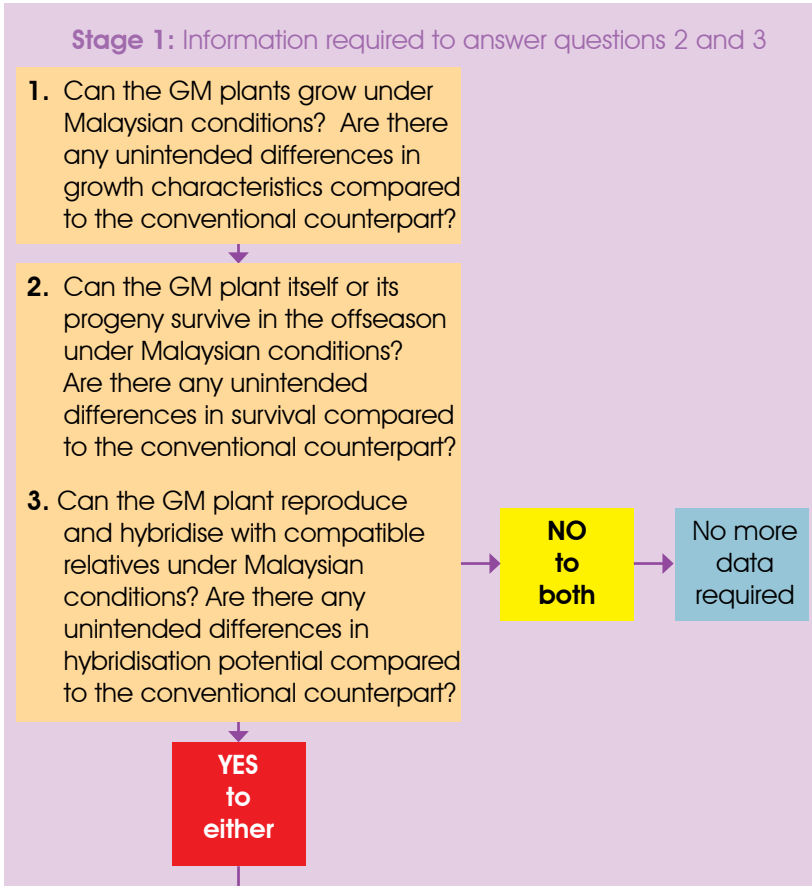
1. Enhanced fitness[4] of the GM plant or of transgenic (introgressed) wild relatives within production systems may make them more persistent, exacerbating weed problems that may need to be controlled by more complex weed control strategies, which themselves might cause environmental harm.
2. Enhanced fitness of transgenic feral plants, or of transgenic (introgressed) wild relatives, in semi-natural or natural habitats may reduce the diversity/abundance of valued flora and fauna. For example, native plant species may be displaced, which in turn might affect species that use those plants as food, shelter, etc. Alternatively, depending on which plant and which transgenes are involved, gene flow to wild relatives may decrease the fitness of hybrid offspring. If rates of gene flow are high, this may cause wild relatives to decline locally, or to become extinct (*e.g.* swarm effect, outbreeding depression).

Therefore, problem formulation should focus on the potential of a GM plant to be more persistent or invasive than its conventional counterparts, and on the potential for gene flow to compatible relatives whose hybrid offspring may become more weedy or invasive, or may

[4] Fitness is defined as the number of seeds (or propagules) produced per seed sown, and includes the whole life cycle of the plant. Components of fitness, such as fecundity, may be measured. Thus, enhanced fitness would be defined as a characteristic of an individual or subpopulation of individuals that consistently produces more offspring to the subsequent generation. Variations in fitness due to biotic and abiotic conditions are referred to as genotype x environment interactions.

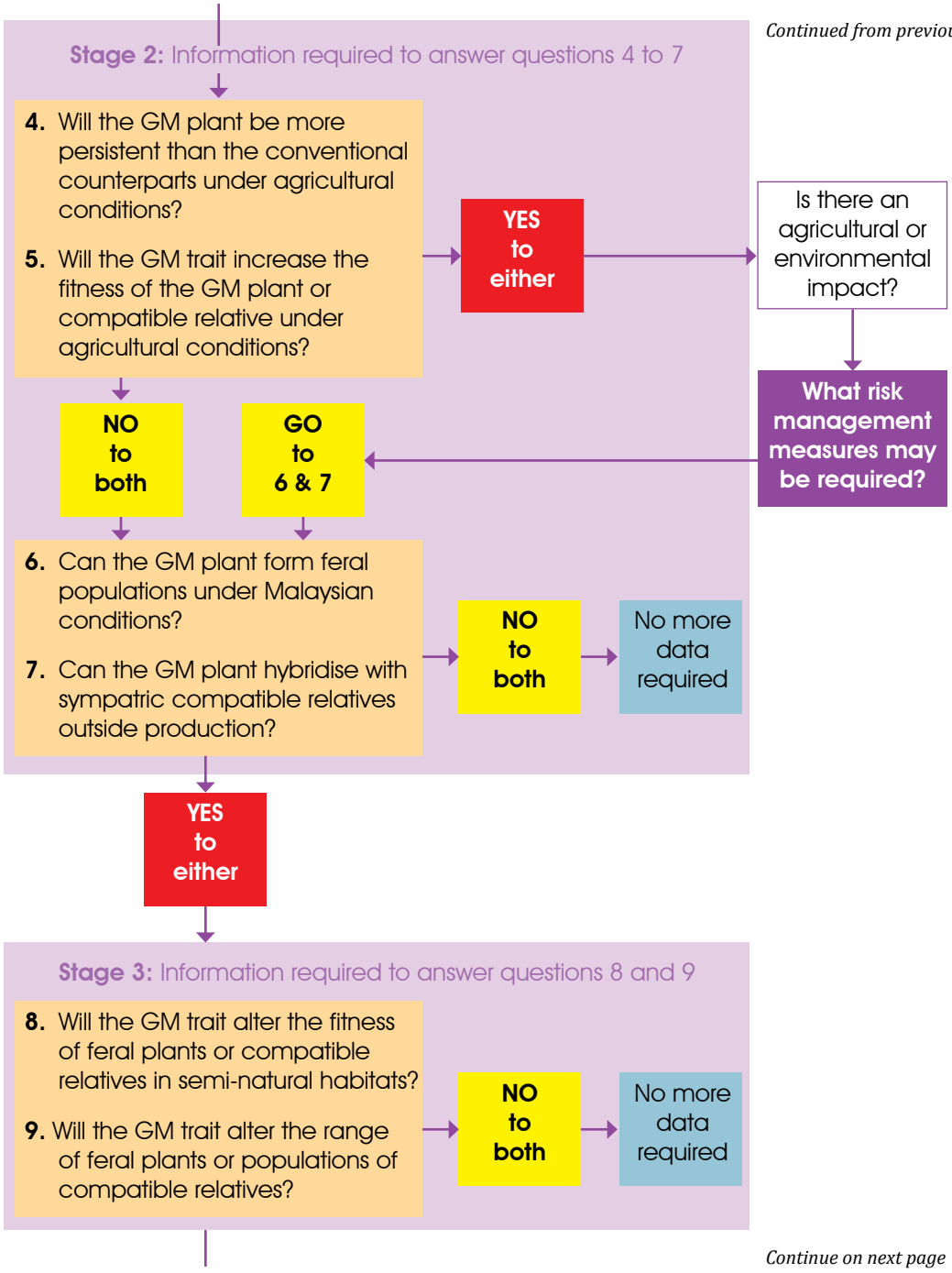
suffer from outbreeding depression. To cover all relevant receiving environments of the GM plant and its compatible relatives, problem formulation should address not only the conditions of the production system under which the GM plant will be grown, but also relevant semi-natural and natural habitats. It should also consider viable GM plant seeds or propagules spilled during import, transportation, storage, handling and processing that can lead to feral plants that colonize and invade disturbed, semi-natural and natural habitats

A staged approach describing how the presence of an introduced trait may exacerbate weed problems in a production system, or cause environmental harm within the wider environment is proposed as outlined in *Figure 5*. The purpose of the staged approach is to ensure that relevant case-specific information is supplied to test hypotheses formulated in the problem formulation process, and that information



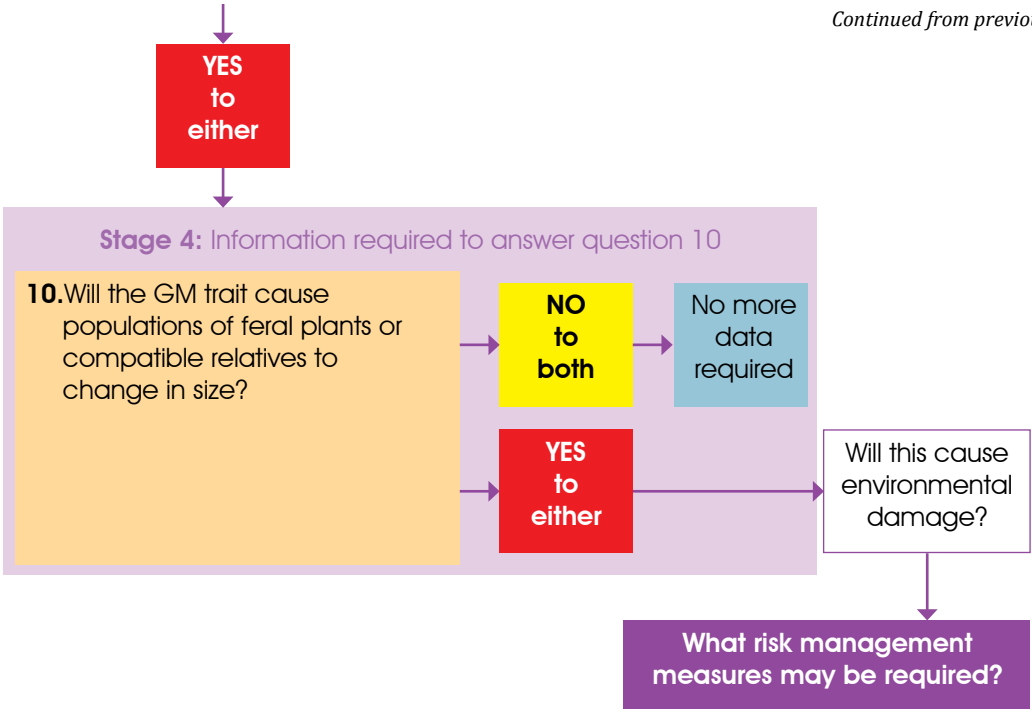
Continue on next page

Continued from previous page



Continue on next page

Continued from previous page



The applicant should provide answers to all the questions within any of the boxes as they proceed stage by stage.

Source: EFSA (2010)

***Figure 5**. Questions defining the different stages of information required to test formulated hypotheses concerning the persistence and invasiveness of a GM plant itself, or any of its introgressed relatives, as a result of vertical gene flow*

requirements remain proportionate to the potential risk. Questions 1 to 10 in *Figure 5* outline the issues to be addressed to estimate the likelihood of occurrence of adverse effects in disturbed, semi-natural and natural environments. These questions are divided into different stages. Whether information is required for all stages or only for specific stages will depend upon the trait/s, plant species, the intended use, receiving environments under consideration, and the conclusions drawn from lower stages.

Information required for testing the hypotheses formulated in the problem formulation process can be *species-*, *trait-* or *event-specific*. This information can be extracted from data generated by the applicant, from the scientific literature, or from any other relevant sources. Some GM plants with the same traits or similar events may have been grown for a number of years on a large scale outside Malaysia; hence, field-generated data on fitness, persistence or invasiveness are available. If the applicant uses data from outside the country, they should justify

why these data are relevant for the range of receiving environments where the plant will be grown in Malaysia.

Species-specific background information is required at the outset, describing the biology of the parental species including reproductive biology, survival, dispersal and cultivation characteristics in different environments. In addition, sexual compatibility with other cultivated or wild plants occurring in Malaysia, and the biology and ecology of these relatives should also be considered.

Stage 1 consists of providing *event-specific* information that enables the GM plant to be characterised, identifying intended and potential unintended differences between it and its conventional counterparts. Information provided should be used to establish whether (1) the GM plant can grow, reproduce and persistent in the offseason under Malaysian conditions, and, if so, (2) how its growth and reproduction characteristics compare to its conventional counterpart. It is possible that GM traits may move to wild relatives through hybridisation within one growing season – consequently, it is important that the hybridisation potential described in the background information is considered before concluding on stage 1 information requirements. It should thus be considered whether sexual compatibility with any relative species is altered because this may result in differences in the rate of gene flow and the establishment of transgenes in other species.

For plants that can reproduce in Malaysia, **stage 2** should explore whether the GM trait will enhance the potential for the GM plant to contribute to volunteer populations and persist in production systems, and, if so, assess the potential environmental consequences. Stage 2 will also establish whether the GM plant will be capable of forming feral populations outside production systems, or whether the transgene can be transmitted to any relatives independently of the existence of volunteers or ferals. Together, these considerations allow for an assessment of whether the transgene is likely to remain confined to production systems.

If feral populations are likely, and/or if hybridisation is plausible, then **stage 3** requires information to establish if GM traits will alter the fitness of feral plants, or of transgenic (introgressed) wild relatives. As feral plants, or transgenic (introgressed) wild relatives may exhibit fitness differences across a wider range of environmental settings, stage 3 also consists of providing information that enables the assessment of the ability of these plants to occupy larger ecological niches than their conventional counterparts. It is possible that certain GM traits may enable the GM plant to expand its geographical range, and to grow in new areas close to wild relatives from which it was previously isolated, so the potential for this should be considered.

Finally, if altered fitness or the ability to occupy new niches is demonstrated, **stage 4** information is needed to establish whether this will allow populations to increase and invade new communities, or, alternatively, if this will lead populations of wild relatives to decline or become extinct. In both cases, the potential environmental consequences should be assessed.

Trait-specific information will be appropriate to address questions of changed fitness in stages 2 to 4, provided that potential unintended effects, resulting from the transformation process, have been shown not to alter the fitness of the GM plant compared to its conventional counterpart in stage 1.

In considering the questions in *Figure 5*, the mechanisms and routes by which plants are exposed to the introduced trait should be taken into account. For GM plant applications for cultivation, the principle route will be through the sowing of seeds/ propagules in fields, and the consequent movement of pollen and distribution of seed or propagules to other fields and the wider environment. For GM plant applications for food and feed uses, import and processing, ERA on persistence and invasiveness is concerned mainly with the environmental consequences of accidental release of viable GM seeds or propagating material during import, transportation, storage, handling and processing. Therefore, ERA needs to consider the scale of environmental exposure, and if this could ultimately lead to GM plants being established in receiving environments. In the latter case, the risk assessment described above and in *Figure 5* is applicable

6.2 Step 2: Hazard characterization

Step 2 of ERA consists of characterising any hazards, identified during the problem formulation process, which might lead to adverse effects as a consequence of altered persistence and invasiveness at the production site or in the wider environment.

6.2.1 Background information requirements

All GM plant applications, including those for import and processing of viable propagating plant material, should provide general background information describing the parental species. *Species-specific* information on the following characteristics should be given in order to summarise existing knowledge of that species.

a) *Reproductive biology*. The reproductive biology of the parental species, including their mode/s of reproduction, dissemination and survivability are important, as plants have different reproduction strategies. As genetic material can move spatially and temporally *via* the transfer of pollen, seeds, or vegetative propagules, this description should consider relevant avenues and vectors for

gene flow, together with factors that affect the probability of these processes.

b) *Characteristics associated with weediness and invasiveness*. Characteristics associated with weediness or invasiveness have been bred out of many crops during domestication, although the degree of domestication varies by crop. While most crops share a similar suite of domestication characteristics, some species may still contain weedy or invasive characteristics (such as seed dormancy, discontinuous germination, rapid seedling growth, phenotypic plasticity, asynchronous flowering, propagule shattering, seed dispersal mechanisms and strong competitive ability). It is therefore considered useful to describe the characteristics of the parental plant species that may favour weediness or invasiveness. In this respect, the history of cultivation of the parental species can be examined for confirmatory evidence of whether these plants have become a weed or invasive elsewhere. Historic data from a region may be a valuable indicator of the potential for persistence or invasiveness of the GM plant itself.

c) *Factors limiting persistence and invasiveness*. Many abiotic and biotic factors limit the ability of plants to form self-sustaining populations under either cultivated or uncultivated conditions. It is therefore relevant to describe factors that may restrict or limit the niche of the plant to certain habitats, or that may control its population size, according to the current state of knowledge.

d) *Hybridisation and introgression potential with any sympatric compatible relatives*. Sexual compatibility with other cultivated or wild plants occurring in Malaysia is to be considered in general terms. The potential for a plant to hybridise with a wild relative is highly dependent on their sexual compatibility and relatedness. Some level of genetic and structural relatedness between genomes of both species is needed to produce viable and fertile plant x wild relative hybrids that stably express the transgene. Also, both species must occur in their respective distribution range of viable pollen, which requires at least partial overlap in flowering in time and space, and common pollinators (if insect-pollinated). For the stabilisation of the transgene into the genome of the recipient (introgression), genes must be transmitted through successive backcross generations or selfing. Therefore, the risk characterisation should consider features such as the proximity and flowering synchrony of wild relatives, and the viability, fertility, genetic compatibility and fitness of hybrid and backcross plants.

6.2.2 Stage 1 information requirements

All GM plant applications, including those for import and processing of viable propagating plant material, should provide information to answer

all questions in stage 1 of *Figure 5*. The purpose of this information is to answer whether the GM plant and its progeny can grow, survive in the offseason, reproduce and hybridise under Malaysian conditions, and if so, how the phenotypic growth and reproduction characteristics compare to those of the conventional counterparts. Stage 1 information should include whether there are any unintended differences between the GM plant and its conventional counterpart in growth, reproduction or hybridisation. To answer these questions, *event-specific* information on the following characteristics should be collated and assessed, and compared with those of the conventional counterparts.

a) *Seed germination characteristics*. Growth chamber experiments or information collected during field trials enable the assessment of seed germination characteristics of the GM plant under various conditions. The comparison of germination characteristics between the GM plant and its conventional counterpart might identify potential unintended changes, resulting from the transformation process, in the GM plant that will require further analysis.

b) *Phenotype under agronomic conditions.* The general phenotypic and agronomic characteristics of the GM plant should be assessed in multi-location field trials representative of the different environments where the GM plant may be grown in order to establish intended or potential unintended differences between the GM plant and its conventional counterpart. Characteristics under consideration include plant establishment and vigour, time to flowering and maturity, growth, plant height and dry matter production, seed and yield characteristics, need for a dry spell to induce flowering, attractiveness to pollinators, and pollen shed, viability, compatibility and morphology.

   In addition to plant growth, development and reproduction observations, any visually observable response to naturally occurring insects, diseases and/or abiotic stressors (such as heat, drought, and excess of water) should be recorded during the growing season, as these observations provide indications of biotic and abiotic stress responses and thus susceptibility/ adaptation to stresses.

   The comparison of phenotypic and agronomic characteristics between the GM plant and its conventional counterpart might identify potential unintended changes, resulting from the transformation process, in the GM plant that require further analysis.

c) *Reproductive biology*. When considering the potential impact of gene transfer from GM plants, it is important to assess whether the GM plant has any capacity for gene transfer that is different from its conventional counterpart. The gene/s inserted may modify the potential for plant-to-plant gene transfer due to altered flower biology (e.g. altered flowering period), attractiveness to pollinators,

fertility, or changed pollen viability and compatibility.

d) *Seed persistence leading to volunteer occurrence*. Measurements or observations such as volunteer number in subsequent crops/ plantations indicate the potential for seeds and vegetative propagules from a GM plant to give rise to volunteer populations. Post-harvest field inspection data in which volunteer numbers are reported can serve as an information source and provide indications on the survival potential of the GM plant seeds. Seed burial experiments can also give indications of changes in dormancy and seed persistence.

6.2.3 Stage 2 information requirements

Stage 2 information will be required for plants that could survive in some parts of Malaysia under production system (e.g. agricultural) conditions, and/or transmit genes to compatible relatives that occur between croppings. The risk assessment should consider whether the GM trait (or unexpected phenotypic trait)[5] could cause the plant to become a more serious weed within the production site. In GM plants with more than a single event (e.g. stacked events), the applicant should consider whether the combination of events may lead to enhanced persistence or invasiveness that is more than the simple product of the single traits.

Data on the relative persistence and fitness of the GM plant under production conditions may be available in the scientific literature, or new data may be required in the form of:

(1) monitoring of existing GM plants in comparable climatic conditions;

(2) manipulative field experiments comparing GM and conventional plant fitness under a range of environmental conditions representative of Malaysian production receiving environments; and/or

(3) population models using as parameters appropriate field data to explore the long-term persistence of GM traits in relevant crop rotations.

The most direct way to measure fitness is by conducting experiments in production sites in representative regions over a minimum of two years. Relative fitness is dependent upon the environmental context. Glasshouse, growth chamber and microcosm experiments can reveal differences under specific, possibly ideal conditions, and such experiments can be more highly replicated and therefore more powerful than field experiments. However, observed differences in controlled conditions do not necessarily translate into field conditions and may

[5] From this point on, the term 'GM trait' will include any event-specific unintended trait identified in stage 1.

require further data or population modelling to allow a complete and confident interpretation.

Persistence or enhanced fitness of volunteers or hybrids should be considered in the context of typical crop rotations. For example, herbicide-tolerant *Brassica napus* may be used as a break crop one year in four, and could transmit herbicide tolerance genes to weedy *Brassica rapa*. The presence of herbicide tolerant *B. rapa* in years 2-4 may be relatively inconsequential as this weed, and crop volunteers, may be controlled by alternative herbicides. However, persistence of transgenic weedy *B. rapa* x *B. napus* hybrids in year 5 could have consequences for the following *B. napus* crop.

Crops vary considerably in their ability to form feral populations and this has been extensively recorded in the scientific literature. If the conventional crop forms feral populations, then this will allow the GM trait to persist outside production systems, and the consequences of this will need to be assessed (stage 3). Similarly, there is extensive literature available on the sexual compatibility of crops with their wild relatives. The assessment should also consider whether the GM trait has the potential to move beyond production sites through hybridisation and introgression into wild relatives. If the GM trait is unlikely to move beyond production sites via either of these routes, then the characterisation should stop at stage 2.

6.2.4 Stage 3 information requirements

Stage 3 information will be required for plants that can form feral populations in semi-natural habitats, or for which there are sexually compatible wild relatives that are likely to be recipients of transgenes. The risk assessment will need to evaluate whether feral plants, or compatible relatives containing the GM trait, will exhibit changed fitness in semi-natural habitats. If fitness is enhanced, populations may increase; if fitness is reduced, outbreeding depression may occur. The potential for changes in fitness may be estimated through:

(1) observations from regions growing the GM plant;

(2) manipulative field experiments;

(3) greenhouse, microcosm or growth chamber experiments with additional field data and/or models to aid interpretation; or

(4) knowledge of the ecology of feral crops and wild relatives and the phenotypic consequences of the presence of the GM trait.

Fitness will vary depending upon the environmental context (including anthropogenic influences like mowing), particularly upon the presence of inter- and intra-specific competitors, the presence of herbivores and pathogens, and the abiotic conditions. The variation in fitness according

to biotic and abiotic conditions is often referred to as a genotype x environment interaction. It is therefore important that an appropriate range of environmental conditions be considered.

Detailed knowledge in the ecology of feral crops and wild relatives and the phenotypic consequences of carrying the GM trait may lead to the conclusion that the GM trait is extremely unlikely to confer a fitness advantage in semi-natural habitats. This may be supported by information from other events of the same GM trait. For example, it is unlikely that herbicide-tolerant genes will influence fitness except in the presence of the herbicide. There is now a body of evidence to support this conclusion.

However, in some cases, the existing evidence may be insufficient to draw firm conclusions, and further experiments may be required. The most direct way to measure relative fitness is via manipulative field trials in a range of suitable habitats and over a minimum of two years. In designing such experiments, the field sites should be representative of the receiving environments. The timescale should be sufficient to ensure a range of abiotic conditions are experienced by the experimental plants. The number of seasons should also be sufficient to ensure that a range of biotic pressures (pathogen and herbivore pressure, for example) are experienced, although this may also be enhanced by experimental treatments. Treatments should always include disturbance, in which perennial vegetation is removed before experimental seed is sown, as many crops are not strong competitors with species in semi-natural habitats, but may be able to exploit disturbed areas. Other treatments should be guided by the GM trait being considered. For example, enhancing the densities of herbivores (insect pests) within limits not infrequently experienced in the field could simulate years of high herbivores. This would allow the hypothesis to be tested that insect-resistant GM crops may have enhanced fitness under these conditions. The experimental design should allow the treatment-by-disturbance interaction to be tested. Fitness advantages in response to certain selection pressures may only be manifest under disturbed or undisturbed conditions. Plot size should be sufficient to allow the subsequent generation to be monitored, following seed dispersal and recruitment. The parameters measured should include survival in the soil seed bank as well as survival and fecundity of adult plants, to allow the lifetime fitness to be estimated.

Greenhouse, microcosm or growth chamber experiments can be used to manipulate the relevant ecological factors to determine the potential impact on the fitness of feral plants or wild relatives. However, the detection of fitness differences from controlled greenhouse experiments requires further information for accurate interpretation. For example, the frequency and intensity of herbivore (insect) and pathogen attack

under field conditions would be needed to interpret the consequences of the possession of herbivore or pathogen resistance traits in the field. Furthermore, competition is likely to modulate the rate at which individual plants recover from herbivore or pathogen attack, and so possession of resistance genes may be more valuable when competition is high. Population models, based on parameters for greenhouse and/or field data, can be adopted to explore the conditions under which GM plants may invade and establish. This allows worse-case scenarios to be explored, and the consequences of any uncertainty in parameter estimates to be explicitly defined.

A form of outbreeding depression may occur if (1) there are high rates of hybridisation with a wild relative, and if (2) the GM trait decreases hybrid fitness. The methods outlined above, specifically manipulative field experiments and/or parameterised population models, could be used to estimate the conditions under which this is likely to occur.

For some GM traits, for example some of the stress tolerance genes, it is possible that the GM plant, or any introgressed compatible relative would be able to grow beyond the geographical range of the conventional crop. The methods outlined above, particularly manipulative field experiments, knowledge of the ecology of the feral plant and its compatible relatives, microcosm experiments and modelling approaches, are tools that can address this issue.

For those crops for which no significant changes in fitness can be detected, or are thought likely, for either GM plants or their compatible relatives, then exposure characterisation should stop at stage 3. However, if fitness differences are detected, then further assessment is required to interpret the potential consequences (stage 4).

6.2.5 Stage 4 information requirements

Stage 4 information would be required for those GM crops for which the presence of the GM trait in either the feral crop plant or a compatible relative causes an alteration in fitness, or increases the range of habitats in which the plant may survive and reproduce.

Enhanced fitness may or may not result in population increase of the transgenic plant compared to its appropriate comparator, depending upon the factors limiting or regulating the population. A combination of field experiments, growth chamber data, population models and knowledge of the ecology of the potential recipients of the GM trait would then be required to interpret the potential consequences of enhanced fitness.

Detailed knowledge of the ecology of the feral crops and compatible relatives including knowledge of the habitats in which these relatives have established populations, and the factors that limit and regulate

populations will facilitate an interpretation of the likely impact of a GM plant. For example, if specific herbivores (insects) are known to have an impact on the fecundity of a particular plant species, and these herbivores are susceptible to insect resistance GM traits, then introgression of those insect-resistant GM traits could lead to ecological release – but only when those plant populations are seed-limited.

Manipulative field experiments may be required to determine if a plant species is seed- or microsite-limited. For example, seed addition experiments, in which seeds are added as a supplement to undisturbed habitats, followed by monitoring of subsequent generations (and appropriate controls) can determine the degree to which a species may be seed-limited, and may be carried out with conventional counterparts. A reasoned argument may then be presented to assess whether the GM plant would be expected to behave in a similar manner, and whether enhanced fecundity would alter dynamics. Similar experiments may be used to deduce other limiting or regulating factors

Population models (e.g. stochastic models), parameterised with field data, may be required to interpret the long-term impacts of GM trait presence on field populations. For example, it is likely that more than one biotic or abiotic factor is influential in determining population levels of a plant species over a number of seasons. Parameterised models may allow the impact of the presence of a GM trait to be modelled over several seasons, in which putatively important biotic factors (such as herbivores and pathogens) fluctuate in abundance. The range of conditions under which population increase may occur could then be estimated, in order to determine the occurrence and extent of environmental damage.

Finally, the consequences of an increase in abundance or increased range of the transgenic species or of outbreeding depression could be the decline or even extinction of desirable species, or another form of habitat alteration that is undesirable.

6.3 Step 3: Exposure characterization

An exposure characterisation should be conducted for any hazards identified in the ten questions and four stages of *Figure 5*. Exposure characterisation should be carried out for all applications, including those for import and processing of viable propagating plant material.

6.4 Step 4: Risk characterization

The answers to the questions posed in *Figure 5* lead to the characterisation of possible risks – that of an adverse effect in the production area, in which the GM trait causes the plant and/or its wild relatives to become a more persistent weed in subsequent croppings; and that in the wider environment, where the presence of the GM trait affects plant populations and species, leading to, for example, a decline

in biodiversity. The applicant should characterise these risks, e.g. by determining whether any expected change falls within the range defined as being acceptable during problem formulation.

6.5 Step 5: Application of risk management strategies

If ERA identifies risks related to persistence and invasiveness, strategies to manage these risks may be required and should be defined by the applicant. These strategies might focus on reducing transgene movement by lowering sexual fertility, or be directed at controlling the progeny of GM plants resulting from gene flow. If measures for controlling volunteers, ferals or wild relatives are proposed, the associated impacts should be considered, i.e. impacts of specific cultivation, management and harvesting techniques. The applicant should evaluate the efficacy and reliability of any risk mitigation measures, and draw conclusions on the final level of risk resulting from their application. Remaining identified risks and risk management measures should be considered when formulating post-market environmental monitoring plans.

6.6 Conclusions

The risk assessment should draw conclusions on:

(1) the impact of the GM plant and/or hybridising relatives in the production system, particularly through increased weediness and more intense weed control;

(2) the impact of the GM plant and/or hybridising relatives in semi-natural and natural habitats, through a change in invasiveness or reduction of biodiversity or ecological function;

(3) why any anticipated harm may be considered acceptable; and

(4) what risk management measures may be required to mitigate any harm.

PLANT TO MICROORGANISM GENE TRANSFER

CHAPTER 7

In the context of cultivation and use, recombinant DNA will be released from GM plants into the environment, e.g. into soil, or inside the gut of animals feeding on plant material. Therefore, it is necessary to consider the likelihood of gene transfer into microorganisms and its stabilisation, *e.g.* by integration into their genomes. Horizontal gene transfer (HGT) is here defined as any process by which an organism incorporates genetic material from another organism without being the offspring of that organism. The evaluation of the impact from this HGT includes analysis of the transfer of recombinant plant DNA to initially receiving microorganisms and potential transfer to other organisms (microorganisms, plants) and the potential consequences of such a gene transfer for human and animal health and the environment. Although the extent of environmental exposure is likely to differ between applications for import and processing and for cultivation, the issues to be considered in ERA are expected to be similar.

7.1 Step 1: Problem formulation

Microorganisms, especially bacteria, are capable of exchanging genetic material directly between each other and even across species boundaries using different mechanisms, *i.e.* conjugation, transduction or transformation. HGT can be initiated by the uptake of cell-free DNA from the environment, which may also include DNA derived from GM plants. After initial HGT from plant to microorganism, the horizontally transferred genes may be further spread to other microorganisms.

Although HGT from plant to microorganisms is regarded as a rare event under natural conditions, there may be consequences for human and animal health and the environment, and therefore they should be considered in ERA. This ERA will depend on the potentially acquired character and the prevalence of similar traits in microbial communities. The problem formulation also needs to consider the routes of exposure in the receiving environment/s as well as the assessment endpoints being representative of the aspects or parts of the environment/s that need to be protected from adverse effects.

Therefore the problem formulation should focus on:

- Detailed molecular characterisation of the DNA sequences inserted in the plant, including information on the potential of the promoter elements that could drive expression in microorganisms;
- Presence of antibiotic resistance marker genes;
- Presence of inserted plant DNA sequences showing similarities with DNA sequences from relevant microbial recipients enhancing the probability of recombination and subsequent stabilisation, or mobile elements[6];
- Presence of recipient microorganisms for transgenic DNA in the receiving environment/s;
- Selective conditions (including co-selection) enhancing the probability of dissemination and maintenance of the genetic material from GM plants in natural microbial communities (*e.g.* the presence of antibiotics in the receiving environment/s;
- Persistence of GM plant material after harvesting, until degradation of the material has occurred;
- Potential for long-term establishment of the genetic material from GM plants in natural microbial communities;
- Ecological or human and animal health consequences of a potential HGT from GM plant to microorganisms[7].

7.2 Step 2: Hazard characterization

If a hazard has been identified in step 1 of ERA, the hazard should be further characterised (*e.g.* the potential spread of antibiotic resistance genes and potentially reduced efficiency of antibiotic treatment). Hazard characterisation should consider information on the prevalence and distribution of genes (similar to the transgene/s in natural environment/s) and try to establish potential consequences (*e.g.* for a gene or trait that is already widespread in the environment).

7.3 Step 3: Exposure characterization

Exposure characterisation should consider the sub-cellular location and copy number of the recombinant DNA, the environmental routes of exposure of the GM plant and the recombinant DNA, and the stability of the DNA in the relevant environment/s. After GM plant degradation,

---

[6] Mobile genetic elements present in the vicinity of the insertion site could enhance the potential for gene transfer.

[7] For example, the contribution of antibiotic resistance marker genes to the development and dissemination of antibiotic resistance in pathogenic microorganisms of clinical importance should be evaluated.

cell-free DNA may persist in the environment for up to weeks or even years, being influenced by a number of biotic and abiotic factors.

It is recognised that the experimental acquisition of data on DNA exposure levels in complex microbial communities is severely limited by methodology constraints under natural conditions. In most cases, the frequency of HGT will be below the detection threshold of particular experiments. Other limitations are related to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable genes to a defined source. In the light of such technical limitations, however, the applicant is requested to provide an exposure characterisation (of the hazards characterised under step 2), considering the various routes of exposure in the receiving environment/s:

- Plant production (*e.g*. DNA from GM plants might be released into the environment during cultivation and after harvest as a result of degradation of plant material and might persist in the field and move to aquatic environment/s);
- Food and feed chain (*e.g.* GM plant intended for food and feed use is often subject to a variety of processing and storage regimes, and might be stable or degrade during processing and storage as in silage);
- Gastro-intestinal system (*e.g.* DNA of GM plant might be consumed as food and feed and might be in contact with microorganisms, mainly bacteria, present in the gastrointestinal tract, and subsequent routes of environmental exposure. These exposure scenarios should include both vertebrates and invertebrates that feed on plants or processed plants and plant ingredients above or below ground, pollinators and humans).

7.4 Step 4: Risk characterization

It is important to focus the risk characterisation on potential impacts on indigenous microbial communities that occur in the various receiving environment/s (as outlined above in step 3). Environmental microbial communities may include certain human or animal pathogens (*e.g. Pseudomonas aeruginosa*, some *Enterobacteriaceae*), or non-pathogenic bacteria, which could serve as first recipients of genes derived from GM plants, and the transgenes could be then transferred to other microorganisms including pathogens. Any risk identified should be characterised by estimating the probability of occurrence, any positive selection conferred by the horizontally transferred trait and the magnitude of the consequences of the adverse effect/s.

7.5 Step 5: Application of risk management strategies

Based on the outcome of the risk characterisation, the applicant may

need to determine and evaluate targeted risk management strategies. Potential strategies may be related to the avoidance of conditions which allow for positive selective pressure.

7.6 Conclusions

A conclusion is required to be made of the overall risk, *i.e.* a clear rationale on the potential for plant to microorganism gene transfer and its consequences, taking into account any risk management strategies. The potential impact (consequences) of such an event should be evaluated also for indirect effects on bio-geochemical cycles, in particular in the light of possible long-term maintenance of the genetic material from GM plants in natural microbial communities.

INTERACTION OF THE GM PLANT WITH TARGET ORGANISMS

CHAPTER 8

Target organisms (TO) are organisms on which specifically designed characteristics of a GM plant are intended to act, and are generally pests or pathogens of the plant. These target organisms should be defined by the applicant. All other organisms should be considered as non-target organisms. Due to the levels of exposure, resistance development is only relevant for applications with the scope of cultivating GM plants, and not for applications restricted to import and processing of GM plants and their products.

8.1 Step 1: Problem formulation

The focus in the problem formulation for herbivore- (pest-) or pathogen-resistant plants is to determine the likelihood that TO will develop resistance, and to design strategies to delay or prevent the occurrence of resistance, or to prevent undesired changes in the interaction between TO and GM plants. Resistance is defined as the occurrence of a phenotype of an individual of TO that can survive on the GM plant and produce viable offspring. In case of herbivore or insect resistance, the development of resistance in target pests is considered an environmental as well as an agronomic concern. Adverse effects from resistance development may compromise other pest control products, can destabilise pest control strategies, and may lead to increased pesticide use. As a consequence, it might lead to changes in cultivation management, and might result in an increased environmental impact.

8.1.1 Insect resistance development

Various strategies are being used to make plants resistant to pests. Currently, most insect pest-resistant plants express insecticidal substances (*e.g.* Bt proteins). The potential future design of GM plants may use other mechanisms, *e.g.* expression of repellent substances, anti-feedants, morphological changes or altered volatiles to influence the host-finding process.

However, a potential hazard is the development of resistance to toxic substances in pests, which is already a well-known phenomenon in

plant protection using chemical pesticides, and it is likely that resistance to GM plants expressing certain pesticidal toxins can also occur.

> *For example, laboratory studies have shown the widespread potential for the development of resistance in the European corn borer (Ostrinia nubilalis) to different Cry proteins, and two instances of field-evolved resistance to Bt maize were recently reported in the scientific literature: Busseola fusca in South Africa on maize MON810 (van Rensburg, 2007, Kruger et al., 2009) and Spodoptera frugiperda on Vry1F expressing maize in Puerto Rico (USA) (Moar, 2008, Tabashnik et al., 2008, Storer, 2010).*

Therefore, applicants should consider in problem formulation the potential for resistance development.

8.1.2 Plant pathogen interaction

Various strategies are used to make plants resistant or tolerant to plant pathogens; these include:

(1) expressing proteins, peptides or antimicrobial compounds that are directly toxic to pathogens or influence their growth *in situ*;

(2) producing products that destroy or neutralise a component of the pathogen,

(3) expressing gene products releasing signals that can regulate plant defence;

(4) expressing resistance gene products involved in hypersensitive response and interaction with avirulence; or

(5) expressing recombinant antibodies that inactivate pathogens or pathogen proteins.

However, plant pathogens have the potential to develop resistance to a wide range of plant defence systems – which may be identified as a potential hazard. Potential mechanisms for evolving resistance could be based on (1) phenotypic effects such as complementation, heterologous encapsidation and synergy, or (2) genotypic changes in the plant pathogen leading to the development of new virulence determinants (*e.g.* for viruses). Co-evolution may result in adaptive functional modifications of an enzyme active site. Hence, there is an expectation that pathogens will evolve resistance to GM plant resistance traits. The applicant should consider the mechanisms used to protect plants and their interactions with pathogens. The resistance mechanisms that evolve in pathogens should be considered, taking into account their genetic control and heritability. Linkages to pathogen virulence and selective advantage should also be considered in the assessment of the potential for resistance development.

Furthermore, the possibility of development of new pathogen strains with resistance to the transgenic trait is an additional hazard in relation to plant pathogen.

8.2 Step 2: Hazard characterization

It is important to identify the target organism (TO) of the GM plant in the receiving environment/s where the GM plant is likely to be grown. The potential of these target species to develop resistance to GM plants should be evaluated based on their history of developing resistance to conventional pesticides and resistant host plants. Data should be provided by the applicant to characterise the potential of resistance development depending on TO and the genetic modification including:

- Data on biology, life cycle, ecology and/or behaviour of TO. Data on resistance mechanisms that develop in TO and their genetic control, heritability and linkages to virulence, fitness and selective advantage. In most cases, these data can be sourced from literature or from the experience of breeders and plant protection services;
- Distribution of TO and its resistant populations in Malaysian environments;
- Host range of TO;
- Information on the population genetics, and epidemiology of susceptible and resistant TOs;
- Frequency of resistant individuals or resistance alleles. Related data can be found in available scientific literature (*e.g.* for Cry1Ab and ECB, or could be generated, *e.g.* for insects by F_1 or F_2 screening or by other screening methods. Data generated outside Malaysia with the GM plant itself, or other plant species might be used by the applicant, only if its relevance for Malaysian environment/s has been justified;
- Mode of action of the active GM plant product towards TO and GM plant characteristics related to this trait;
- Data on baseline susceptibility of TO to transgenic products either from the literature elaborated for *Ostrinia nubilalis* and *Sesamia nonagroides*, or from laboratory tests according to published testing protocols.

In some cases, the data might be obtained from literature, but in other cases, data sets might be incomplete. Therefore the applicant should consider various scenarios, including a worst-case scenario, to estimate the potential of resistance development in Malaysia.

8.3 Step 3: Exposure characterization

By definition, TO are exposed to the GM plant. Data characterising the exposure of TO should include:

- Expression level of the transgenic products in the plant tissues consumed by TO;
- Estimation of the levels of intake of the transgenic product/s by various developmental stages of TO;
- Influence of the expression level and its variability on the interaction between GM plant and TO;
- Proportion of population of TO exposed to the GM plant in the receiving environment/s;
- Baseline frequency of resistant individuals or resistance/virulence alleles. Relevant data can be found in available scientific literature (e.g. for cry1Ab and ECB, or could be generated, e.g. for insects by F_1 or F_2 screening or other screening methods. Data from outside Malaysia could be considered if they can be shown to be relevant to local conditions;
- Deployment of other GM plants expressing similar trait/s.

8.4 Step 4: Risk characterization

After assessing all data, the risk should be characterised for:

a) evolving resistance, or

b) developing undesired changes in the interaction between the target plant pathogens and plants in the receiving environment/s.

8.5 Step 5: Risk management strategies

Based on the outcome of the risk characterisation, the applicant should propose resistance management strategies. The applicant should evaluate the effectiveness of targeted risk management strategies which could minimise undesired interactions between GM plants and target organisms[8] in the local receiving environment/s. The applicant should indicate the efficacy, reliability and expected reductions in risk associated with the strategies. In addition, the risk of resistance may change when taking into account newly available information or changes in production systems. Therefore, management measures

[8] In the context of lepidopteran pest species and Bt maize, the high dose refuge strategy is a good example of the introduction of a successful risk management strategy. The consequent use of refuge areas prolonged the expected development of resistance for most lepidopteran pest species so far. However, the possible resistance management strategies are dependent on the biology of the target organism, the genetic transformation and interactions between the target organism and the GM plant and the receiving environment. In cases where the high dose refuge strategy failed, other management options are possible (*e.g.* Bt cotton [Tabashnik *et al.*, 2009]). One such strategy is the additional use of insecticides, or the use of pyramiding in GM plants producing more than one Bt protein.

need to be able to respond to these changes, and appropriate resistance monitoring measures are likely to be required as part of case-specific monitoring within PMEM (*see* Chapter 13).

8.6 Conclusions

A conclusion is required of the overall risk considering resistance development of TO or undesired changes in the interaction between the GM plants and TO. The risk characterisation and conclusions will determine the resistance management measures and requirements for the PMEM plan.

CHAPTER

9

INTERACTIONS OF THE GM PLANT WITH NON-TARGET ORGANISMS

ERA should consider the possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of GM plants with non-target organisms (NTO). ERA as described in these guidelines should address the potential environmental impact on population levels of herbivores, natural enemies, symbionts (where applicable), parasites and pathogens.

9.1 Step 1: Problem formulation

9.1.1 Environmental concerns and hazard identification

One environmental concern is that GM plants may have an adverse effect on biodiversity and its functioning at several levels, through interactions with populations of other species associated with or sympatric with the GM plant, and referred to as non-target organisms (NTO). Biodiversity is interpreted broadly and covers both species richness and agro-eco functions providing ecosystem services. As the environment (including biodiversity) is to be protected from harm according to protection goals set out by Malaysian legislation, the protection of species richness and ecological functions should be considered in ERA.

Specifically when considering NTO, the receiving environment consists of the managed terrestrial ecosystem (*e.g.* agro-ecosystem) including the GM cultivated fields, orchards and plantations and their margins, and the wider environment (*e.g.* other adjacent GM or non-GM cultivated fields and non-cultivated habitats) and, where relevant, aquatic ecosystems.

In a human-managed context, sustainable land use (*e.g.* for agriculture and forestry) is considered a primary environmental protection goal. For the benefit of sustainable production, the scope is to maintain a certain level of biodiversity, providing essential ecological services, including pollination, biological control of pests and diseases, nutrient fixing and cycling, decomposition of plant materials, maintenance of soil quality and fertility, and structural stability. Therefore, the criterion of functional biodiversity is deemed important in this context,

because preserving functional biodiversity may guarantee the quality of the production systems (*e.g.* agro-ecosystems) and ensure their sustainability.

The applicant shall consider whether a GM plant and its use will directly and/or indirectly (*e.g.* through food web interactions, scale of adoption) cause potential harm to species guilds involved in ecosystem functions. Problem formulation starts with the identification of potential hazards through a comparison of the GM plant with its conventional counterpart. The different features of the GM plant are considered the novel stressor because environmental impacts can be a consequence of changes to the GM plant and to its management, as well as the effects of the introduced traits.

These differences are initially assessed theoretically in the problem formulation process in order to identify the potential environmental consequences of these differences. While some differences may be deemed irrelevant to the assessment, others will need to be practically evaluated for their potential to cause harm.

9.1.2 Definition of assessment endpoints

Protection goals are general concepts; therefore, they need to be translated into measurable assessment endpoints. The assessment endpoint is an explicit expression of the environmental value that is to be protected. This necessitates defining (a) *species* and (b) *ecosystem functions* that could be adversely affected by the GM plant, and that require protection from harm.

In any ecosystem, there is usually a high number of NTO species that may be exposed to GM plants. Considering that not each of these species can be tested, a representative subset of NTO species (referred to as '*focal species*') shall be selected, on a case-by-case basis, for consideration in the risk assessment of each GM plant. To lead the applicant to a decision on which focal NTO species are to be used as assessment endpoints, species selection shall be performed according to the four steps outlined in *Figure 6*.

Step 1 - Identification of functional groups

As a first step in species selection, it is necessary to identify the ecosystem functions and services (including maintenance of herbivores (including insects) as part of a food web, pollination, regulation of arthropod pest populations by natural enemies, and decomposition of plant material) provided by the production system (*e.g.* agro-ecosystem) and the functional groups of species involved, in the environment/s where the GM plant is likely to be grown.

Step 2 - Categorisation of NTO species from identified functional groups

In the second step, the main species linked to the functional groups identified in the previous step should be listed, considering the GM plant and the organisms associated with it in its receiving environment/s. An indicative list detailing the ecological role for common invertebrates in agro-ecosystems is provided in *Table 4*. Some taxonomically related species and/or life stages of the same species may have different ecological roles (*e.g.* different feeding habits), and this aspect should be considered.

Step 3 - Ranking species based on the ecological criteria

From the list built in step 2 of species selection, the applicant shall prioritise NTO species from each relevant functional group.

The main criteria to be considered in this prioritisation process are:

- Species exposed to the GM plant under field conditions, specifically considering the life stages present during the period of exposure;
- Known sensitivity of the species to the product/s expressed in the GM plant;
- Linkage to the production system (*e.g.* agro-ecosystem), and presence of an alternative food source;
- Abundance;
- Interactions with target species (trophic and plant-mediated);
- Species vulnerability (*i.e.* are certain populations already threatened and thus more vulnerable to additional pressures?);
- Relevance to adjacent habitats, including natural and semi-natural habitats.

Step 4 - Final selection of focal species

Based on the considerations addressed in the previous steps of species selection, a restricted number of focal species needs to be selected from each functional group. At this stage, some practical criteria may be considered in the final selection of focal species. It may be that, among the prioritised species, some can be tested more effectively under laboratory conditions, or are more likely to be available in sufficient numbers in the field to give statistically meaningful results. Legal constraints may limit testing of certain NTO (e.g. protected species), so this aspect may also influence the final choice of focal species.

It is expected that, at the end of the selection process, the applicant has selected at least one focal species from each relevant functional group

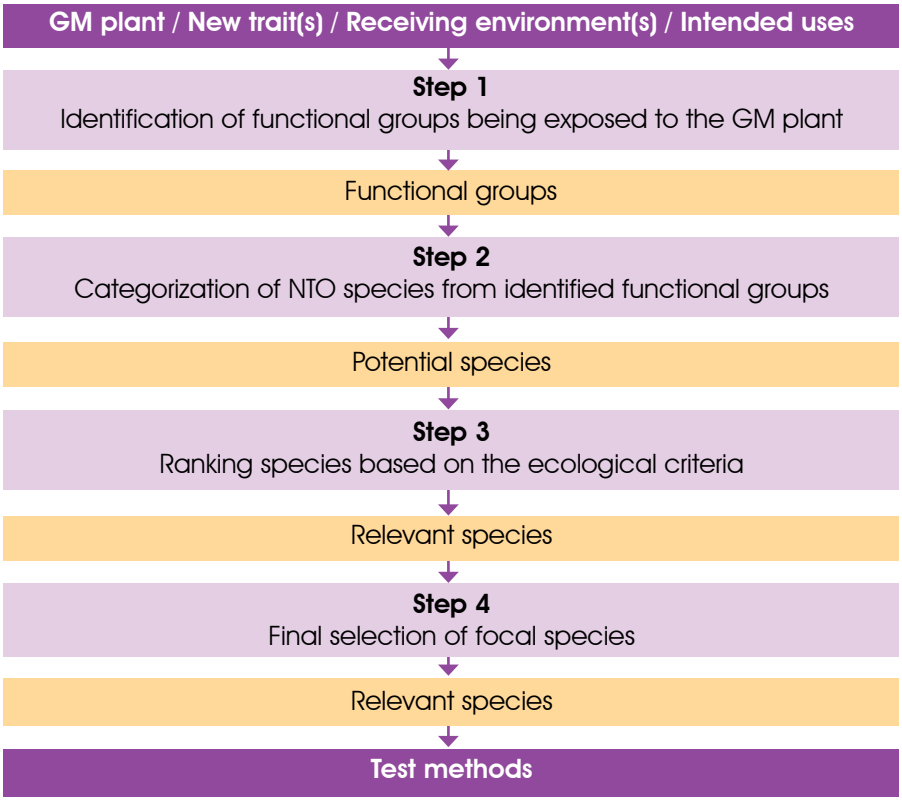


Figure 6. *Four steps for selecting focal NTO species to be tested (after EFSA, 2010)*

identified in the problem formulation for further consideration in ERA. Different possible sources of exposure for each focal species (in the most relevant developmental stages) to be tested should be considered in the focal species selection process.

For field trials, the estimation of ecosystem functions and services could complement or replace data on focal species. Ecological functions (such as pollination, biological control, soil functions[9]) depend on the number of species, their abundance and different types of assemblages. In a particular assemblage, the abundance of any species naturally fluctuates, and the decline of a certain population might be compensated by another species within the same guild without adversely affecting functionality. For example, the overall predation rate of a guild of predatory species could be selected as an assessment endpoint in field trials. Likewise, evaluating the earthworm community as a whole might provide data that are more ecologically relevant than measuring the effects on a single (focal) earthworm species.

[9] To cover soil respiration, biomass decomposition and nutrient dynamics.

Table 4. Examples of functional groups (exposure through trophic interactions)

Functional group		Examples of taxonomic groups
Herbivores		Phloem-feeders: aphids (*Hemiptera: Aphididae*), leafhoppers (*e.g. Hemiptera: Cicadellidae*), certain *Heteroptera*
		Cell-content feeders: thrips (*Thysanoptera: Thripidae*), spider mites (*Acarina*) and *Nematoda* (*Tylenchida: Meloidogynidae*)
		Chewers: leaf beetles (*Coleoptera: Chrysomelidae*), *Leptidoptera* larvae, *Diptera* larvae, grasshoppers (*Orthoptera, Ensifera*), gastropods (*Mollusca, Gastropoda*)
Natural enemies	Predators	Beetles: *Coleoptera* (*e.g. Coccinellidae, Carabidae, Staphilinidae*)
		Predators: *Heteroptera* (*e.g. Nabidae, Anthocoridae*), *Diptera* (*e.g. Syrphidae*)
		Lacewings: *Neuroptera* (*e.g. Chrysopidae, Hemerobidae*)
		Thrips: *Thysanoptera* (*e.g. Aeolothrips*)
		Spiders: *Araneae* and *Opiliones*
		Mites: *Acarina* (*e.g. Phytoseiidae*)
		Nematoda (*e.g. Mononchus* sp.)
	Parasitoids	*Hymenoptera* (*e.g. Ichneumonidae, Braconidae, Aphelinidae*)
	Parasites and Pathogens	*Bacteria, fungi, viruses*
	Entomo-pathogenic organisms	*Nematoda (e.g. Heterorhabditidae, Steinernematidae),* pathogenic microorganisms
Pollinators		Solitary and social bees (*Hymenoptera: Apidae*), hover flies (*Diptera: Syrphidae*), *Coleoptera* (*e.g. Melyridae, Curculionidae, Scarabaeidae*)
Decomposers		*Diptera* larvae (*e.g. Phoridae, Sciaridae*), *Nematoda* (*e.g. Rhabditidae, Dorylaimidae*), springtails (*Collembola*), mites (*Acarina*), earthworms (*Haplotaxida: Lumbricidae*), *Isopoda*, microorganisms
Plant symbionts		Rhizobacteria, mycorrhiza

Source: EFSA (2010)

9.1.3 Considering the exposure patterns to NTOs

The overlap of the life cycle and developmental stages of the focal species and the phenology of the GM plants needs to be evaluated. Exposure may also happen after the transgene has moved via dispersal of pollen and grain/seed in and away from the cultivation site of the GM plant (*e.g.* pollen deposited on leaves of host plants for non-target Lepidoptera and Coleoptera). Moreover, gene flow via outcrossing may result in gene expression in related species, and result in additional levels of exposure to other NTO species.

The level of exposure of NTO to the GM plant will depend on the intended uses of a GM plant:

- In cases where the application does not include cultivation in Malaysia, direct environmental exposure of NTO to the GM plant is via the accidental release into the environment of seeds or propagules of the GM plant during transportation and processing. This may result in the sporadic occurrence of feral GM plants, and therefore exposure of NTO populations is likely to be negligible. ERA will then focus on the indirect exposure to products of the GM plant (*e.g.* through manure and faeces from the animals fed with the GM plant; and other by-products of industrial processes);
- In cases where the application includes cultivation in Malaysia, the level of environmental exposure is estimated on a case-by-case basis depending upon several factors. These include the biological and ecological characteristics of the GM plant and its transgene/s, the range of expected scales and frequencies of GM plant use, the receiving environment/s where the GM plant is likely to be cultivated, and the interactions among these factors.

If gene flow to cross-compatible wild/weedy relatives and feral plants inside or outside the areas of cultivation is likely to occur, then exposure of NTO to these GM plants and their products over life cycles and seasons should be assessed.

9.1.4 Definition of measurement endpoints

Through the formulated hypotheses, assessment endpoints are made operational into quantitatively measurable endpoints, termed measurement endpoints. Indicators of change, that will be recorded as part of the comparative risk assessment, need to be defined and established by the applicant through the measurement endpoints. These measurement endpoints should constitute measures to characterise both exposure and/or hazard, and shall be selected when there is an univocal interpretation of the biological data, *i.e.* how to relate the results to the assessment endpoint.

An alteration in plant metabolism could substantially affect components

of the life history of organisms associated with these plants, and consequently alter the growth of NTO populations. Both lethal and sub-lethal effects are relevant in the assessment of a possible hazard for a given NTO species. Testing for sub-lethal effects is important because it can also give indications of possible long-term effects. An appropriate measurement endpoint for NTO testing is relative fitness (or some component of relative fitness), which is the relative lifetime survival and reproduction of the exposed versus unexposed non-target species. It is therefore required that NTO tests consider both toxic effects (short-term mortality, longevity) and sub-lethal effects. The latter can be assessed through growth pattern, development rate, reproduction parameters (*e.g.* number and size of offspring, percentage of egg hatch, sex ratio of progeny, age of sexual maturity), and, when appropriate, behavioural characteristics (*e.g.* searching efficiency, predation rates, food choice).

In field conditions, the abundance and species diversity of certain groups of NTO at a relevant life-stage are typical measurement endpoints. The choice of specific measurement endpoints shall be done according to the problem formulation on a case-by-case basis.

Long-term effects on NTO populations or functional guilds are an important element of ERA, meaning that, in the context of NTO testing, reproduction parameters and testing over multiple generations are considered as appropriate endpoints. In addition, modelling and/or post-market environmental monitoring can also be suitable methods for addressing potential long-term effects.

Measures of hazard: Measures of hazard represent the measurable change of the measurement endpoint/s in response to the GM plant and/or its products to which it is exposed. Measures of hazard may be an acute lethal concentration resulting in the death of, e.g. 50% of the organisms tested, or the effective response concentration for chronic effects measured, or altered reproduction (*e.g.* fecundity), growth, development and behaviour in a receptor population. These measurements can be expressed as the *effective concentration* affecting an NTO x percentage of individuals (ECx). In addition, it is necessary to consider reproduction parameters (*e.g*. number and size of offspring, percentage of egg hatch, age of sexual maturity), growth pattern, development rate and behavioural characteristics (*e.g.* searching efficiency, predation rates, food choice) which may also be appropriate measures of hazard for long-term effects. At a population level, an important predictor is the *intrinsic rate of increase* (rm) that integrates measures of survivorship and fecundity. Moreover, the calculation of the *instantaneous rate of increase* (ri) allows a good estimate of rm for the study of insect populations at lower tiers.

Measures of exposure: Measures of exposure shall describe the contact or co-occurrence of the GM plant with the valued entity, and can be

expressed as *predicted* (or *estimated*) *environmental concentrations* (PEC or EEC). The description of the novel attribute of the GM plant (*e.g.* transgenic protein) in terms of the route, frequency, duration, and intensity of exposure for the change relative to the valued entity is considered relevant information. Both plant and NTO features assume an important role here; for instance, overlapping of the NTO biology (*e.g.* life cycle stages) with the spatio-temporal concentration of the transgenic product/s is to be considered to quantify exposure. If a non-target species is not directly exposed to the transgene and/or its product/s from the plant, but indirectly via other target or non-target species, these pathways of exposure need to be evaluated as well.

9.1.5 Hypotheses testing and tiered approach

A case study approach describing how the GM plant may adversely affect NTO or their ecological functions is proposed in *Table 5*. Based on plant x trait x NTO interactions, five possible cases are foreseen. On the one hand, GM plants may express new proteins/metabolites that have:

Table 5. Identified cases and hypotheses testing

	GM plants expressing new proteins/ metabolites with:			**GM plants with intentionally altered composition**	
	Toxic properties	Non-toxic properties	Unknown toxicity	Alteration of metabolic pathways known to affect NTO-plant relationships	No alteration of metabolic pathways known to affect NTO-plant relationships
	Ia	**Ib**	**Ic**	**IIa**	**IIb**
Possible effects of the transformation process	Intended and unintended	Unintended	Intended and unintended	Intended and unintended	Unintended
Could specific hypotheses be defined?	Yes	No	Yes	Yes	No

Source: EFSA (2010)

(Ia) toxic properties;

(Ib) non-toxic properties; or

(Ic) unknown toxicity.

On the other hand, GM plants may have an altered composition, in which metabolic pathways known to affect NTO-plant relationships (*e.g.* glucosinolates in *Brassicaceae*, alkaloids in *Solanaceae*, lignin in trees) are:

(IIa) altered, or

(IIb) not altered.

In all of those five cases, the metabolism and/or the composition of the GM plants may in addition be unintentionally altered as a consequence of the genetic modification in a way that could affect NTO-plant relationships ('unintended effects'). The presence of unintended effects in GM plants can be due to different reasons (*e.g.* pleiotropic effects), and this has been well documented in the scientific literature.

Only in some of the five identified cases (*i.e.* Ia, Ic and IIa) can a **specific hypothesis** be formulated to assess plausible intended effects (*e.g.* a GM plant intentionally altered to produce biologically active compounds may produce the same effects on non-target species).

To test these hypotheses and thus assess possible adverse effects on NTO, relevant data need to be supplied and considered by the applicant.

For the two remaining classes of GM plants (Ib and IIb), only the absence of possible unintended effects on NTO needs to be demonstrated according to the principle described below.

9.1.6 Specific hypothesis-driven investigation

For the case studies Ia, Ic, and IIa, specific hypotheses can be formulated and assessed (*e.g.* the new metabolite can be toxic to some non-target species, or the change in the metabolic pathway will possibly influence the plant's interactions with other organisms on various trophic levels) according to the flow chart illustrated in *Figure 7*.

Based on specific hypotheses, NTO risk assessment can be performed in a tiered manner; whereby, hazards are evaluated within different tiers that progress from worst-case scenario conditions under highly controlled laboratory environments to more realistic conditions in the field. Three main tiers can be used, which comprise experimental tests under controlled conditions (*e.g.* laboratory tests under tier 1a and 1b, and semi-field[10] tests under tier 2), and field tests (tier 3). Within a tier, all relevant data shall be gathered to assess whether there is sufficient information to conclude on the risk at that tier. In case no reliable risk conclusions can be drawn, further data might be needed. The decision to

[10] Outdoor tests carried out in some containment that controls variability, with manipulation treatments on relatively small experimental units (*e.g.* caged plants, screen houses)

move between tiers needs to be driven by trigger values. These values shall be set for the species under consideration taking into account the intrinsic toxicity (*e.g.* estimated by effective concentration (ECx) of the newly expressed products and the expected concentration in the plant), and the sensitivity of the various NTO developmental stages.

Based on the experience with Cry toxins, tier 1 tests generally seem to represent useful predictors for results at higher tier tests, provided that designs include all ecologically relevant ways of exposure. When laboratory studies are performed, both *in vitro* and *in planta* tests (tiers 1a and 1b) should be done to reach a reliable risk conclusion after tier 1. Tier 1a testing is of crucial importance for ERA if no or little data on the metabolites expressed by similar GM traits are available (*e.g. Table 5*: case Ic). Tier 1a tests require purified metabolites in the same form as expressed in the GM plant. Tier 1b complements the results obtained with purified metabolites as they give indications on possible interactions between plant compounds and reflect realistic exposure conditions through bioavailability. In fact, it has been demonstrated that laboratory studies incorporating tri-trophic interactions of Cry1-expressing plants, herbivores and parasitoids were better correlated with the decreased field abundance of parasitoids than were direct exposure assays. Where purified metabolites are not available, only tier 1b studies shall be conducted using GM plant material that guarantees exposure to both transgene products and the plant.

Likewise, it is possible that for some NTO focal species, no reliable protocols for performing such experiments exist. In this case, the applicant may perform this type of test on some focal species only. In all justified cases where testing on a lower tier is not appropriate (*e.g.* test organisms cannot be reared in the laboratory), the applicant can perform tests at the next tier.

The diet regime for each focal species (in the most relevant developmental stages) to be tested must reflect the different possible sources of exposure in nature.

Some impacts on multi-trophic interactions and ecosystem functions may not be observed in tier 1 tests. Higher tier testing may therefore be needed on a case-by-case basis before decisions on the level of risks can be made. In particular, field testing is essential to investigate trait *versus* environment interactions when laboratory tests give reason to assume a possible adverse effect.

The NTO testing phase can be finalized when sufficient information is compiled to reject the tested hypotheses. The applicant, who concludes that further tests are not required, based on available information, is required to explain the rationale for this conclusion. If at any tier adverse effects are detected, a hazard characterisation is required to determine

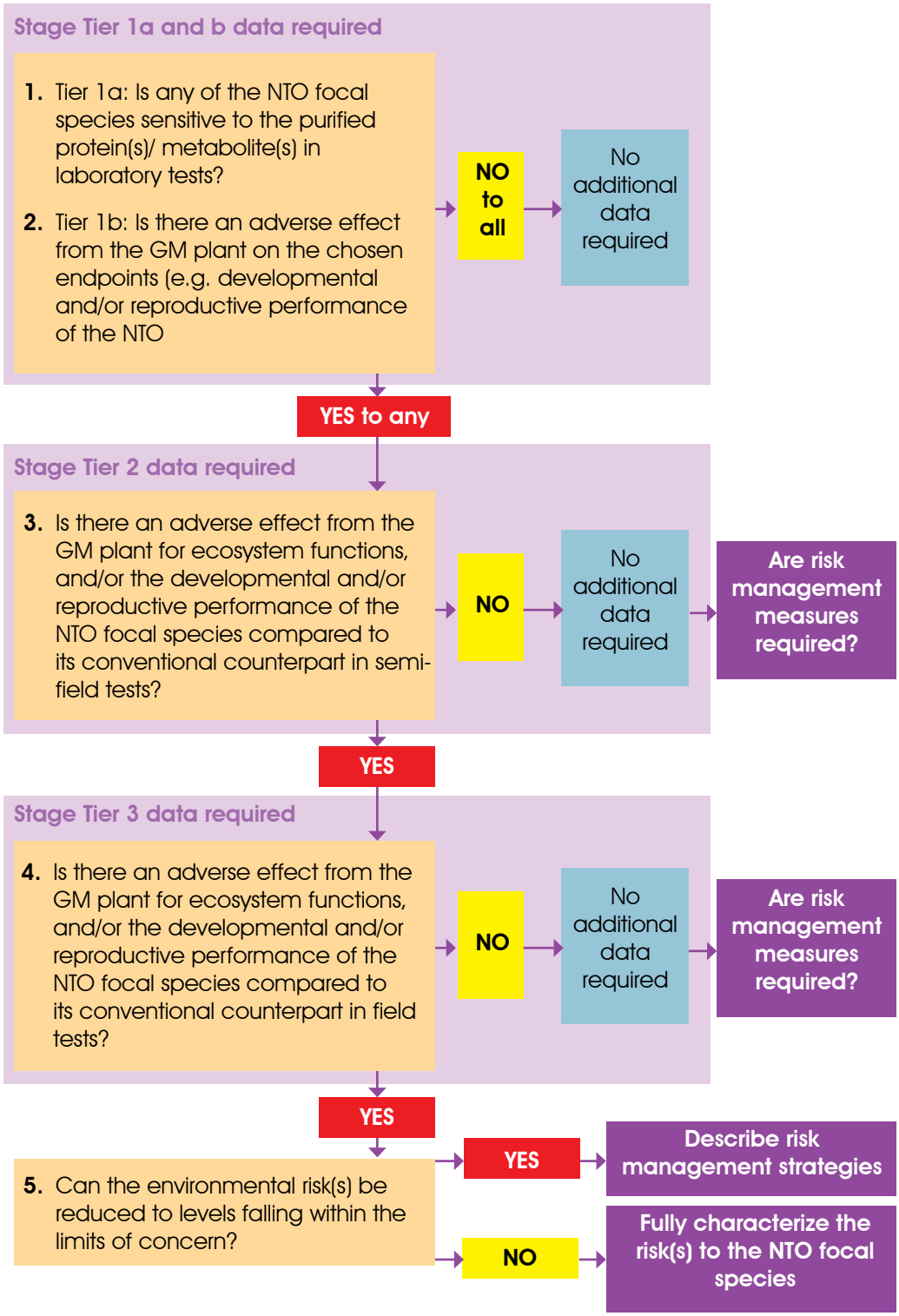


***Figure 7**. Decision tree for carrying out a specific hypothesis-driven investigation (after EFSA 2010)*
The applicant shall provide answers to all the questions within the grey boxes.

the biological relevance of these effects. Also, the use of more NTO species in the same functional group might help to clarify how common these adverse effects might be for the specific agro-ecosystem. In some cases, it might be necessary to go back to the problem formulation phase to redefine a hypothesis, and to design additional experiments to generate the data needed.

The Environment Protection Agency (EPA), USA, typically carries out the following Tier I subchronic tests using the purified novel protein in an artificial diet:

- Avian oral toxicity test on an upland game bird
- Freshwater fish oral toxicity test
- Freshwater invertebrate test on *Daphnia* or aquatic insect species
- Honey bee test for larval and adult bee toxicity (representing pollinators)
- Test on Insect predators and parasites, e.g. green lacewing larvae, ladybird beetle, and a parasitic wasp
- Non-target soil insect and/or other invertebrate, typically including *Collembola* and an earthworm species.

Where Tier I tests are not possible, or show adverse effect on NTO at field use rates, then testing of additional species and/or at a higher tier level is required. Tier II involves testing with the GM plant tissues alone, or in an artificial diet. In Tier III, tests are carried out to determine chronic, reproduction, lifecycle and population effects, *e.g.* chronic broiler study. Finally, in Tier IV, simulated or actual field testing is carried out to determine if there is a noticeable change in the wildlife population under field use conditions.

9.1.7 Data requirement for the evaluation of possible unintended effects

GM plants may have unintended adverse effects on biodiversity through interactions with populations of other species associated or sympatric with the GM plant. It is important that species richness and ecological functions, especially considering guilds that provide ecosystem services, are not disrupted to the extent that populations decline and/or vital functions are impaired. Unintended impacts of GM plants on species richness and ecological functions shall be considered in ERA.

Problem formulation thus seeks to collect all available information to decrease uncertainty of unintended effects to an acceptable level. The evidence to exclude the likelihood of unintended effects on NTO can come from numerous sources, including data already collected for other parts of the risk assessment, collating all the appropriate information

from these data sources to provide a weight-of-evidence approach. Data sources relative to plant-environment interactions are always necessary to support the possible exclusion of unintended effects. The sources of data should be properly justified.

The applicant is requested to consider all the information available from these different data sources and to ensure that some field-generated data are included. The use of field-generated data from outside Malaysia may be informative in this context, but the applicant must justify why these data are relevant to the ecological functionality of receiving environments in Malaysia where the GM plant will be grown. As unintended effects are to a large extent event-specific, data from other events or from similar events in other plant species will carry little weight in supporting an application.

9.2 Step 2: Hazard characterization

Once specific measurement endpoints are chosen, appropriate methods and criteria of measurement should be selected and described. This includes information on the studies to be conducted, the appropriate tier for analysis, and the design of experimental protocols with a definition of the appropriate statistical power.

9.2.1 Laboratory studies

Two kinds of methodologies are relevant for laboratory studies. First, existing conventional eco-toxicology methodologies and standardized methods can be used and adapted in order to assess the sensitivity of NTO to different levels of exposure to the GM plant-produced proteins. The methodologies must be adapted to fulfil the measurement endpoint requirements. Secondly, an *in planta* experimental protocol is required in which the GM plant x NTO interactions are evaluated at exposure levels likely to occur in the field. For in planta studies, the testing scheme should ensure that the food used is ecologically relevant for the chosen NTO life stage to be tested (*e.g.* mimicking the trophic interactions existing in nature), and that the specimens are exposed to the expected concentration throughout the study duration.

In addition to the above examples, several tier 1 studies that have been published in scientific literature can be considered by the applicant.

All laboratory tests shall satisfy the following requirements:

- The endpoint and the species are unequivocally identified;
- The rationale for the selection of the species and endpoint is given;
- Variability is sufficiently low for precise effect level estimation;
- Exposure to known quantities of testing material is maintained throughout the study;

- The experiment is conducted for a time span adequate to reliably estimate measurement endpoints.

When reproduction is an endpoint, the following requirements shall also be fulfilled:

- The processes of the reproductive biology must be included in the testing phase;
- The life-history must be known, e.g. age at maturation, duration of egg development, and instars subjected to exposure;
- Optimization of conditions for growth and reproduction must be provided by the test substrate and food supply.

The applicant can develop their own protocols for particular NTO species that are considered in ERA. In this case, it is requested that, among others, the following aspects of the experimental protocols are correctly addressed:

- The organisms used during tests shall be healthy and of similar age;
- The biological performance of the organisms used as controls shall be within acceptable limits (control mortality less than, for example, 20% depending on the testing system and organism);
- Environmental conditions in growth chambers, mesocosms and greenhouses shall be described explicitly and justified;
- Plant material shall be checked for transgene expression;
- Direct and indirect exposure pathways shall be clearly identified in the experimental setup.

When designing experiments with natural enemies, the following additional requirements shall be considered:

- The suitability of the artificial diet or surrogate host/prey species vs. natural food (*e.g.* some species do not grow well or do not reproduce when reared on artificial diet);
- Host/prey herbivores have to be properly exposed (possibly from hatching) to the right treatments;
- A uniform supply of prey/host quality, age, etc;
- The availability of additional food sources for species with mixed feeding habits (*e.g.* availability of pollen, honey or sugar solution, possibility for sucking from plants, etc.);
- The availability of an appropriate oviposition surface for predators;
- The provision of particular micro-habitats (*e.g.* providing additional sources of water-saturated surfaces).

For tier 1a studies, it is assumed that the test substance can be dosed

and conventional testing approaches of chemicals can be followed. The sensitivity of the endpoint must be presented as EC10 and EC50 with confidence intervals. Laboratory practices (*e.g.* environmental conditions, specimen handling) should be carried out according to standardised and published testing procedures. Limitations of some laboratory protocols should be considered when designing tests and when drawing conclusions from test results. When novel or non-standardised testing procedures are used, it must be demonstrated that the method is appropriate, reproducible, reliable and of correct sensitivity.

The *in planta* testing required for tier 1b needs particular considerations concerning modifications of the standard procedures to accommodate plant material. NTO in tier 1b tests could be exposed to plant material through whole plants, plant parts (*e.g.* leaves, pollen) or ground plant material in diets or soil.

For *in planta* tests where feeding is an important route of exposure, it will not normally be possible to produce doses of the GM product that exceed the concentrations in plant tissues. Thus, the normal level will act as the maximal exposure concentration in a test. Doses lower than the maximal dose can be made by dilution with a near-isogenic non-GM variety, and EC10 and EC50 effect levels may be obtained. Different levels of exposure can also be achieved by mixing levels of GM plant material into the test substrates, *e.g.* soil, and a true dose-response relationship can be established delivering EC10 and EC50 effect levels. Appropriate controls for the effects of these diet regimes can be made by making similar mixtures with near isogenic non-GM materials.

In order to provide an optimal nutrition in soil eco-toxicological tests, a food source may be added. The amount of additional food source may need to be adjusted in order to ensure worst-case exposure.

When the aim is to demonstrate equivalence of the GM plant to the appropriate comparator, the standard tests should include the appropriate comparator as a negative control at an exposure level identical to the GM plant, as well as a positive chemical control to prove the functionality of the experimental setup, as advised in pesticide testing guidelines.

9.2.2 Field trials

Experimental complexity and variability increase from tier 1 (*e.g.* toxicological studies), to bi- and tritrophic studies with plant parts, bi- and tritrophic studies with whole plants, to field assemblage studies. Laboratory testing provides the best way to control and manipulate experimental conditions (environmental factors, set-up) and to limit complexity and variability. In contrast, field tests allow for the evaluation of trait x environment interactions, but they exhibit the

highest experimental complexity, and provide the lowest ability to control experimental conditions due to large natural variability.

The objectives of field trials are:

- To identify and study exposure routes (including trophic relationships) and confirm observed effects in lower tier experiments;
- To discover potential unintended effects not anticipated in lower tier tests;
- To provide feedback for further testing hypotheses;
- To study food chain and indirect effects;
- To determine effects of scale on NTO populations, including effects on generations and other spatial/temporal interactions;
- To study effects of interactions between several NTO species in natural environment/s.

Field testing for NTO is of special importance for certain species that cannot be tested in the laboratory (*e.g.* rearing methods and experience are not available). Field testing provides a very broad range of arthropods in terms of species number, life stages, exposure to abiotic and biotic stresses, complexity of trophic interactions, etc. that cannot be reproduced in the laboratory. Hence, attention should be paid to the trade-off between standardised laboratory tests in lower tiers and the testing of NTO species in field experiments. Moreover, field studies offer the opportunity to estimate the functioning of whole ecological functions in natural conditions.

The design and analysis of field trials should be performed according to the criteria explained earlier.

9.3 Step 3: Exposure characterization

A major factor in evaluating the likelihood or probability of adverse effects occurring in NTO is the characteristics of the environment into which the GM plant is intended to be released, and the manner of release. Several ecological characteristics specific to the crop trait-receiving environment interactions need to be taken into account to characterise NTO exposure.

The introduction of a GM plant into a productive system will indeed introduce two new stressors, the transgene and its products and the GM organism itself. In addition to this, new management practices may be associated with the cultivation of the GM plant. If hazards are identified (step 1) and hazard characterisation gives sufficient evidence for potential environmental damage (step 2), an exposure characterisation is conducted (step 3) to determine whether and to what degree the NTO

species come into contact with the GM plant and the transgene product. This assessment requires information on the phenotypic pattern of transgene expression in the various parts of the plant over the growing season. This exposure can be bitrophic via exposure to the GM plant (or plant parts, e.g. pollen), or can occur in higher trophic level organisms exposed to prey or host feeding on the GM plant. Organisms at higher trophic levels can be exposed in different ways to the plant and/or its products; therefore, direct, indirect or mixed exposure models need to be evaluated according to NTO and the GM plant characteristics. For example, a carnivore in an agro-ecosystem carrying GM plants will be faced with the presence in its diet of the transgene product and/or its metabolites, combined with the constitutive compounds of the prey/host species, and the combination of both might interfere with the normal development of the natural enemy.

Based on the specific biological characteristics, the likelihood of exposure needs to be estimated. For this purpose, the highest mean protein expression level in any plant tissue is often taken as the worst-case expected environmental concentration (EEC) in regulatory risk assessments. The *maximum hazard dose* (MHD) can be established as follows:

Maximum hazard dose (MHD) = EEC x MOE

where EEC = expected environmental concentration

MOE = *margin of exposure*

$$\text{MOE} = \frac{\text{NOAEL}}{\text{EED}}$$

where NOAEL = *no observed adverse effect level*

EED = *estimated exposure dose*

9.4 Step 4: Risk characterization

Based on the conclusions reached in steps 2 and 3, the applicant should estimate each identified risk that a GM plant will cause to NTO considering the magnitude of the effects detected and the likelihood of their occurrence. The applicant should summarise the outcomes of ERA considering intended and unintended effects as outlined in step 1. Hence, the applicant should draw conclusions on the risk for intended and unintended effects on NTO taking into account focal species as well as the overall functionality of the agro-ecosystem. The applicant should provide an assessment of the range of effects likely to occur in different receiving environments based on the collected data and other relevant information.

Considering receiving environment-plant trait combinations, the applicant is also required to characterise the risks (a) in the production site of the GM plant, and (b) outside the production site in different habitats (*e.g.* adjacent crops and other non-crop habitats) where relevant exposure to sensitive NTO may occur. Quantification of risks and their relative uncertainties shall be provided in relation to each selected assessment endpoint, and upscaling of data from laboratory, semi-field and field trials to eco-systems considering the expected adoption rate of GM plants. The conclusions of risk characterisation should assess the consequences of each identified risk to NTO, and the applicant should propose appropriate risk management measures where levels of risk exceed threshold levels.

9.5 Step 5: Risk management strategies

In situations where risk due to the GM plant and/or its product/s on NTO and on related ecosystem services has been identified and characterised, the applicant should propose appropriate risk management strategies. These strategies should be designed, under assumptions of high exposure scenarios, to reduce the risk to a level considered acceptable (criteria defining this acceptability should be explicitly discussed). The implementation of measures should fit common principles, *e.g.* the principles of good agricultural practice and Integrated Pest Management (IPM) that are being encouraged in Malaysia.

These mitigation measures may include measures to reduce exposure in order to reduce risk to NTO and ecosystem services.

> *Examples include the planting of non-Bt plants as border rows, or, where feasible, detasselling of GM maize plants in border rows in order to limit Bt maize pollen dispersal outside the maize field.*

Also, the establishment and maintenance of habitats (ecological compensation areas) that provide refuge, feeding source, etc. for NTO populations over a larger area and longer time might also be considered.

The applicant should also consider the implications of introducing the GM plant on present cultivation and farming practices. The applicant should describe how the GM plant will be introduced in tandem with IPM and farming systems so that the current pest management strategies and practices continue to contribute to the sustainability of pest management. These practices that should be in line with general IPM principles may cover crop rotation and crop varieties, the use of pesticides with different modes of action in order to maintain and support natural regulating mechanisms, including beneficial NTO.

These mitigation measures and strategies should be devised in the light of long-term management and maintenance of NTO and ecosystem services in rural eco-systems.

9.6 Conclusions

The applicant should draw conclusions on the risk of intended and unintended effects on NTO taking into account focal species and considering all relevant ecosystem services. The applicant should provide an assessment of the range of effects likely to occur in relevant local receiving environments based on the collected data and other relevant information. The applicant is also required to characterise the risks (1) in the production site of the GM plant, and (2) outside the production site in different habitats considering relevant exposure routes. Quantification of risks and their relative uncertainties shall be provided in relation to each selected assessment endpoint in comparison to relevant baselines. The consequences of these risks for all relevant protection goals, including the overall functionality of the ecosystems, IPM and the sustainability of production systems, should be considered.

The conclusions of risk characterisation should assess the consequences of each identified risk to NTO, and the applicant should propose appropriate risk management measures where the levels of risk exceed acceptable threshold levels.

CHAPTER 10

IMPACTS OF SPECIFIC CULTIVATION, MANAGEMENT AND HARVESTING TECHNIQUES

A GM plant for cultivation will be introduced into various receiving environment/s, and will be managed according to the requirements of the plant and the production systems into which it is introduced. It is necessary to assess the environmental impact of the specific management and production systems (*e.g*. agriculture, forest tree or others) associated with the GM plant, including how the plant will be cultivated, managed, harvested and processed.

The introduction of GM plants for cultivation may require specific management practices and cultivation techniques, and these may lead to additional changes in management and production systems. In Malaysia, current agricultural management and production systems are diverse (intensive, integrated, organic, etc.), and already cover a wide range of management practices and cultivation techniques which, in addition, are continuously evolving under external drivers (*e.g.* regulation on pesticides, agricultural policies, market requirements or agricultural innovations). Changes in management practices and cultivation techniques due to the introduction of GM plants and their potential environmental impacts shall therefore be seen in the context of this already existing and evolving range of current management and production systems and their environmental impacts. Here, ERA shall aim at comparing the range of different systems likely to occur in the practical management of GM crops, with the continuously evolving management in non-GM systems, using scenario analysis (see below). The comparative environmental impacts of different management systems will vary according to the receiving environment/s, intensity of crop production, rotational systems and a range of other factors. Thus, ERA shall consider under what circumstances the specific GM management and production systems adopted may lead to greater, similar or lower adverse environmental effects than the current systems they are likely to replace.

Due to the high diversity of management and production systems across multiple receiving environments, ERA is based on a scenario analysis which shall consider scenarios representative of the diversity of situations that may occur, and assess their potential implications.

The assessment of potential consequences is carried out by reviewing scientific literature of both peer-reviewed and technical publications, performing meta-analyses, conducting field experiments, studying commercial uses in other countries, and/or modelling studies.

The cultivation of GM plants in other countries where imports come from could change management practices and cultivation techniques in those countries and, in turn, may have adverse environmental impacts. However, for GM plants to be imported and processed and not intended for cultivation in Malaysia, there is no need for ERA for altered cultivation, management and harvesting techniques; only the assessment of the consequences of accidental loss and spillage of the GM plant need be considered. Nevertheless, experience in cultivation in other countries can provide useful information relevant to the management practices and cultivation techniques of the GM plant in Malaysia.

Stacking of GM events by conventional crossing may lead to additional and/or specific management practices and cultivation techniques not necessarily associated with the single events, which, in turn, may affect the environment. Therefore, GM plants with stacked events shall be fully risk assessed for the potential impacts of their cultivation, management and harvesting techniques. The applicant shall therefore describe the specific cultivation, management and harvesting techniques of the GM plant containing stacked events, and of each of the cultivated sub-combinations covered by the application, taking into consideration the various receiving environments, and shall assess their potential environmental impacts with respect to the parental lines or conventional counterparts. In addition, ERA of a GM plant containing three or more single events combined by conventional crossing shall include a consideration of the management of all other sub-combinations of these events that may occur by natural segregation (*e.g*. volunteers) (see Section 4.2.4).

Effects of management practices and cultivation techniques are more related to the GM trait than to the specific transformation event. Nevertheless, whenever studies based on other events expressing the same trait are used, the applicant shall provide evidence that the assessment of environmental impacts of management techniques of the GM plant can be derived from such studies, as variation among transformation events (*e.g.* expression level) may alter the conclusions of ERA.

The applicant shall also consider potential adventitious stacked events (resulting from outcrosses between GM plants and existing GM volunteers or neighbouring GM crops, or from co-mingling of seeds of different GM events) and the environmental impact of their cultivation and management.

10.1 Step 1: Problem formulation

The production system is defined by the specific use of the GM plant, the context in which the GM plant is grown, its cultivation (including crop rotation), management and harvesting, and the crop type in which the transgenic trait/s has been introduced.

> *For example, grain maize, forage maize and sweet corn have different production systems with different environmental impacts in similar receiving environment/s. All may receive the same GM event but the subsequent changes in management and production systems, and, consequently, the resulting environmental impacts may differ.*

Similarly, GM plants introduced for amenity, forestry, land reclamation and other uses may also possess traits or other characteristics which require different management practices and cultivation techniques, and the impact of these must also be assessed. Consequently, the problem formulation shall take into consideration receiving environment/s, which include the various agricultural production systems where the GM plant might be grown, and any potential subsequent changes in the cultivation, management, harvesting and processing techniques associated with the GM plant compared to its conventional counterpart.

Examples of GM plants that can cause significant changes in production systems and, in turn, affect the environment, are provided below:

> *GM herbicide-tolerant (GM HT) plants will change herbicide regimes (e.g. type of herbicides and timing of application) and may induce additional weed control changes to minimize weed shifts and manage weeds that have evolved resistance to the broad-spectrum herbicide. In addition, crop rotations and cultivation of other plants in a rotation may change in response to enhanced weed control or to the presence of GM HT plant volunteers. Additional environmental harm and greater adverse effects on biodiversity may result from these altered weed control systems (fewer weeds and/or weed shift).*
>
> *GM HT plants facilitate the adoption of minimum tillage or no-till cultivation techniques which may lead to beneficial or detrimental environmental effects. Potential changes in soil tillage resulting from adoption of such GM plants are likely to affect soil structure, moisture retention, greenhouse gas emissions and the overall energy balance. These changes may also have impacts on soil biodiversity or flora, and their importance may be higher than the direct effect of the GM plant. In addition, for some GM HT plants (e.g. soybean) management practices might have an effect on nitrogen-fixing symbiotic partners and might therefore induce*

a change in nitrogen fertilizer use, and subsequently affect bio-geochemical cycles functioning.

GM insect-resistant (GM IR) plants will reduce the use of some insecticides and may cause changes in crop rotations in response to reduced pest pressure. GM IR plants may require the establishment of non-IR refuges with specific cultivation requirements. Efficient control of target pests by the adoption of GM IR plants may result in situations where the niche of the suppressed target pests will be occupied by other herbivores. This may lead to changes in pest management which can have further environmental implications.

GM drought-tolerant or salt-tolerant plants could change irrigation regimes and other management practices and cultivation techniques as well as expand the receiving environment into which the GM plant might be grown; depending on the crops which might be substituted. This may lead to additional impacts on biodiversity.

GM plants with a high potential for gene flow may require specific management techniques to minimize flowering or seeding (e.g. coppicing or "topping" of trees). More generally, the adoption of pest-, disease- and herbicide-tolerant GM plants will alter the requirements for IPM in these plants and in other plants in rotation or proximity, and may affect the spatial organisation of cropping systems (e.g. to reduce selection pressure on weeds, mitigate insect resistance).

GM plants with characteristics which enable them to compete with other plant species. For example, they may have superior symbioses with soil-borne microorganisms, or inherent allelopathic mechanisms, enabling the GM plants to grow more easily in the presence of native plants. Introduced genes which impart a competitive advantage may enable the GM plant to occupy a wider niche than the unmodified plant, e.g. due to increased environment tolerance or resistance to herbicides or insect pests.

Stacked events, combining various GM events (e.g. several HT traits combined with several IR traits) may lead to changes in crop management and/or allow changes in cropping systems causing novel impacts compared to those anticipated from the combination of traits. For example, a GM plant containing both IR and HT traits may result in changes in weed control practices that affect host plant densities for non-target insect species, and, therefore, alter the potential mortality of such species. Stacking several HT traits into one variety will allow the use of novel combinations of herbicide treatments which may lead to additional alteration of biodiversity.

An assessment is required of the possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the different receiving environment/s in which the GM plant may be grown when these techniques are different from those used for non-GM plants. This shall include the impact on bio-geochemical processes, as well as on biodiversity in the receiving environment/s.

Current agricultural production systems are diverse and their environmental impacts display huge variability. Changes in management practices and cultivation techniques due to the introduction of GM plants and their potential environmental impacts shall therefore be seen in the context of the already existing and evolving range of current management and production systems and of their environmental impacts. ERA shall:

- describe the potential range of GM-based management and production systems likely to occur across the receiving environments, and how they differ from current management systems;
- identify the potential adverse environmental impacts associated with these systems;
- assess to what extent the environmental impacts overlap those of the range of non-GM systems;
- determine which conditions (receiving environments, management and production systems) are related to potentially higher adverse effects than current systems;
- assess to what extent the range of GM management and production systems would meet the assessment endpoints identified elsewhere in these guidelines.

When addressing these steps, the applicant shall discuss to what extent their conclusions depend on the deployment scale of the GM plant (see scenario analysis below).

The problem formulation shall first identify, through relevant assessment endpoints, the aspects of the environment/s that need to be protected from adverse effects due to changes in cultivation, management and harvesting techniques (biodiversity, water and air quality, etc.).

Second, the problem formulation shall consider potential changes in the receiving environment/s and management and production systems (*e.g.* crop rotations and cropping systems, rate of adoption of the GM plant, introduction of other GM crops, pest pressure evolution) which are foreseeable in the near future (*e.g.* consequences of the implementation of IPM by 2014 under the framework of EU Directive 2009/128/CE on Sustainable Use of Pesticides)

Third, the problem formulation shall identify the potential adverse effects that may result from the changes in management and production systems in a range of different environments, taking account of anticipated future changes in agriculture associated with other drivers (*e.g.* market forces, legislation, etc.).

10.2 Step 2: Hazard characterization

Based on the hazards identified in step 1, the applicant is requested to further characterise hazards associated with the change in specific cultivation, management and harvesting techniques in the receiving environment/s. In assessing the information on the receiving environment/s, the applicant shall identify the various representative management and production systems (*e.g.* use of the plant, crop rotation, cultivation techniques and crop type) in which the GM plant may be introduced, and then consider how the GM plant is likely to alter the existing management and production systems, taking into consideration both direct and indirect effects as illustrated in the problem formulation chapter. The applicant shall also consider via relevant hypotheses, potential changes to the receiving environment/s within the timeframe of the authorisation.

For each of the representative management and production system, the applicant shall identify the possible adverse effects due to changes in management practices and cultivation techniques. Introduction of the GM plant may induce changes in applications of plant protection products (*e.g.* pesticides and/or biocontrol agents), rotations and other plant management measures. These changes may result from the characteristics of the GM plant itself, or from the implementation of management measures aiming to mitigate potential adverse effects (*e.g.* insect resistance or weed resistance).

Information is required for all foreseen potential changes in management practices and cultivation techniques, and an assessment shall be made of likely adverse environmental impacts of these changes. In addition, the impact of the GM plant on the cultivation of other plants (*e.g.* change of weed control regimes in subsequent crops) shall be considered, and the consequences of any changes in the management practices and cultivation techniques of these plants shall also be risk assessed.

The application of risk management measures (*e.g.* to limit gene flow to weeds, feral plants and crop volunteers) may result in new cultivation, management and harvesting techniques, and the consequences of these for the environment shall be assessed.

Where cultivation and management techniques of GM plants and their associated production and management systems have different effects on species, or both increase and decrease biodiversity throughout

cropping seasons or rotations, then an assessment shall be made of any overall long-term harmful effects of these changes on biodiversity. Examples of indicators of such changes can be changes in weed seed bank populations or higher species in the food web.

Longer-term and indirect effects due to changes in cultivation, management and harvesting techniques might be difficult to evaluate through small-scale and short-term field experiments. The applicant is requested to analyse this information and any information on potential environmental impacts of the management and production systems in those countries where the GM plant has been or is currently grown.

In addition, as far as they have been validated, models may be used to complement the applicant's statements. The applicant may provide simulations, carried out under representative receiving environment/s and various GM adoption scenarios, to assess to what extent the changes in management and production systems may have adverse effects on the environment.

10.3 Step 3: Exposure characterization

The applicant shall assess the magnitude of changes in cultivation, management and harvesting techniques for each selected representative receiving environment/s, and also consider whether the changes in practices are likely to change the range of environments in which the GM plant is cultivated. Changes in management practices and cultivation techniques in Malaysia cannot always be anticipated, but data on cultivation of GM plants outside the country can provide some indications. Applicants shall consider various scenarios which might occur in representative receiving environment/s and assess, via scenario analysis, the consequences in relation to different levels of adoption of GM plants (in term of exposure). Due to the diversity of management practices and cultivation techniques across the country, the applicant shall consider possible scenarios by combining selected receiving environments and representative management and production systems.

At least three kinds of scenarios shall be considered:

- A ''field level' or 'substitution' scenario which describes the foreseen introduction of GM plants and their recommended management practices and cultivation techniques into the most common current management and production systems and receiving environments (*e.g.* at field level, over a rotation, where applicable, or a crop season). This scenario considers the substitution of the non-GM plant (and its specific cultivation techniques) by the GM plant and its specific management without any other changes in other management practices (only direct effects, field and its immediate surroundings considered here).

- A 'landscape level' or 'typical' scenario which considers the likely rate of adoption of the GM plant in production systems, the indirect effects in management which are foreseen as well as the upscaling effects (*e.g.* at field, farm and landscape level, over a rotation, where applicable, or a crop season). In this scenario, management systems are adapted to take advantage of the GM plant (indirect changes in cultivation techniques occur, management of other crops may be affected). The likely uptake at the landscape level is considered and mitigation measures are adopted.
- A "worst-case" scenario which describes the effects of repeated, large-scale, and intensive management of production systems on receiving environments, where additional impacts are likely to occur (*e.g.* at field, farm and landscape level, over a rotation – where applicable – or a crop season). This scenario considers the effect of large-scale cultivation of the GM plant with its adapted management practices (temporal and spatial scales) and of high selection pressure factors.

These scenarios shall be elaborated upon by considering the factors which may drive the environmental effects in terms of exposure (crop area, GM adoption rate) and hazard (selection pressure, etc.).

- Whenever relevant, a fourth scenario shall consider the potential adoption of other GM plants, the potential changes in the management and production systems which may result from adoption of such other GM plants within the receiving environments, as well as their potential additional adverse environmental effects.

The applicant shall justify that the selected scenarios cover the range of receiving environments and management and production systems which may occur.

As far as they have been validated, models may be used to support that scenario analysis and complement the applicant's statements on exposure characterisation, *e.g.* exposure assessment models, or gene flow models.

10.4 Step 4: Risk characterization

The applicant shall characterise the identified risks related to changes in management and production systems. The scenario approach, covering representative situations that may be encountered, shall indicate the circumstances that may lead to specific GM management practices causing greater, similar or lower adverse environmental effects than the current management and production systems they are likely to replace. Even if the scenario analysis can cover representative situations, it may be difficult to predict the whole range of impacts that the changes in management practices and cultivation techniques may have. The

conclusions for risk characterisation shall take into account the consequences of this unpredictability of management and relate them to proposed mitigation measures to ensure that adverse environmental impact is maintained at or below current levels found in comparable non-GM management and production systems.

In addition, as far as validated, models may be used to complement the applicant's statements and clarify uncertainties. The applicant may provide simulations, carried out under representative receiving environment/s, and various GM adoption scenarios, to assess the level of risk.

10.5 Step 5: Risk management strategies

In situations where ERA concludes that changes in management and production systems may cause adverse environmental impacts compared with the comparable non-GM management and production systems, the applicant shall present and assess risk management strategies to mitigate adverse effects.

The efficacy of each proposed management strategy in the relevant receiving environment/s shall be presented and discussed by the applicant.

The applicant shall assess to what extent the proposed management strategies or options do not induce more harm than non-GM management and production systems, and are consistent with the environmental protection goals.

Validated models, *e.g.* models used for assessing the efficacy of the high dose/refuge strategy for Bt crops, may be used to complement the applicant's statements. The applicant may provide simulations, carried out under representative receiving environment/s and GM adoption scenarios, to assess to what extent the proposed risk management strategies may prevent adverse effects on the environment. This would help with the establishment of monitoring schemes because their design may depend on adoption scenarios and other factors

10.6 Conclusions

The applicant shall draw conclusions on the overall risk considering immediate and delayed effects on the environment, both in-field and wider, resulting from potential direct and indirect effects of changes in management and cultivation practices. The applicant shall also consider effects of further potential changes in the receiving environment/s and farming systems.

Where specific risks associated with the cultivation of a GM plant are identified during ERA, risk management strategies shall be proposed to

mitigate these risks, and the applicant shall indicate how these measures will be introduced and enforced. Furthermore, monitoring is required either to confirm any assumptions regarding the occurrence of adverse effects or to verify the efficacy of mitigation measures.

EFFECTS ON BIO-GEOCHEMICAL PROCESSES

CHAPTER 11

11.1 Step 1: Problem formulation

Bio-geochemical processes underlie the movement, transformation and storage of energy, water, carbon, nitrogen and other elements in ecosystems. Bio-geochemical processes include the uptake of carbon dioxide from the atmosphere by plants, the degradation of plant material, the formation of soil organic matter, the evaporation of water from fields, and the transformation of nitrogenous compounds. Bio-geochemical processes can build soil fertility, but they may also bring about mobilization and loss of materials, *e.g*. in the form of greenhouse gases (CO_2, CH_4, N_2O). Therefore, the applicant should assess whether GM plants and their associated management have potential adverse effects on bio-geochemical processes compared to the effects of a range of current production systems. Problem formulation should cover principally two scales:

- the *production site*, e.g. a field, in which the GM plant is grown; and
- the *wider environment* with which the field interacts through exchanges of energy, elements and materials.

Indirect impacts due to altered cultivation, management and harvesting techniques could affect both of these scales and should be considered by reference to the previous Chapter.

The production site comprises the soil, plants, animals and microorganisms within the area in which the GM plant is to be grown (*e.g.* an agricultural field). Soil organisms are the main drivers of bio-geochemical processes in the production site, determining soil structure, nutrient cycling, immobilization and mobilization of nutrients, degradation of soil organic matter (SOM) and emission of greenhouse gases. Soil fertility is a key parameter of soil quality, and is to a large extent a result of previous generations of plants and microorganisms acting on and mediating the bio-geochemical processes. As plant-associated (*e.g.* rhizosphere) and soil microbial communities perform the vital biotransformations for sustainable soil fertility, any negative impact/s on these organisms should be carefully evaluated on a case-

by-case basis with particular reference to the characteristics of the introduced trait and the consequences of the genetic modification or alteration of the GM plant.

> *For example, a GM plant expressing a novel anti-fungal compound could kill soil fungi or mycorrhizas if it escaped and became established. Or, a GM plant secreting high levels of antibiotics could harm beneficial soil bacteria.*

The wider environment comprises land, water and air outside the production site, with which the GM plant and its management might interact. An assessment of the impacts on the wider environment should take account of the import and export of materials (such as fertilisers, fuel, seed, pesticides, carbon amendments, plant matter), and losses to the atmosphere and water as a result of human activities (*e.g.* agriculture). When taking account of the import of materials, the manufacture and procurement of fertilisers (organic and inorganic) are included, and not only their application or turnover at the production site.

Admittedly, information is limited on many aspects of bio-geochemical processes. Accordingly, the level of detail required in ERA will depend upon the characteristics of the plant and the transgenic trait, and the scope of the application. Problem formulation should start with a desk study comparing the cultivation system used for the GM plant with current production systems. The desk study would refer to available data and apply published methods of assessing, for instance, greenhouse gas emissions, erosion, soil degradation, and the potential to pollute watercourses, backed by more specific experimental data, if available.

With respect to bio-geochemical processes in the production site, the evaluation should address the potential impact of GM plants through factors such as:

1. Release of recombinant gene products or GM specific metabolites into the plant-soil system, which may directly influence soil fertility, nutrient transformations and food webs;
2. Altered movement of other compounds from roots to soil, which may directly influence soil fertility, nutrient transformations and soil food webs;
3. Altered plant litter that decomposes differently from that of non-GM plants due to either the presence of specific compounds (*e.g.* toxic metabolites), or altered concentration of substances resistant to decomposition;
4. Altered uptake and recycling of plant nutrients within the plant-soil system (including the fixation of atmospheric nitrogen).

With regard to bio-geochemical processes in the wider environment, the evaluation should address the potential impact of GM plants and the associated production (*e.g*. agricultural) management on:

5. Losses from production sites/systems to air or water, e.g. greenhouse gas emissions, including those that result from operations and processes that are essential to plant production but which occur outside the production site (*e.g.* manufacture and transport of fertiliser);
6. The capacity of production (*e.g.* agricultural) systems to store water, carbon, nitrogen, phosphorus and other elements essential for plant growth and ecosystem functioning.

Any indications in the desk study that the GM plant and its management have potential effects on bio-geochemical processes should receive detailed attention in the following steps.

11.2 Step 2: Hazard characterization

Step 2 consists of characterising any hazards identified during consideration of the problems in step 1 that might lead to adverse effects on bio-geochemical processes in the production site or in the wider environment.

Hazards to be considered might result from an intended change in the plant (*e.g.* change in plant-nutrient relations), or an ancillary change related to the GM plant or its method of cultivation. For example, if plant compositional analysis indicates a substantial change in the C/N ratio of plant structures, or the lignin composition of plant litter, then the potential effects of these changes on bio-geochemical processes should be evaluated. Similarly, with respect to wider bio-geochemical processes, if the GM plant and its cultivation are likely to alter fertiliser inputs, tillage or the timing of cultural operations in the receiving environment/s, then effects of these on the wider bio-geochemical processes should be evaluated.

Many of the potential impacts, particularly those with respect to wider bio-geochemical processes, may result from the interaction of the GM plant and its management with general agricultural practices in the receiving environments. Indeed, variables such as greenhouse gas emissions, pollution of water and reduced carbon sequestration will be strongly affected by a general change in the production system (*e.g*. agricultural), for example in the extent to which inversion tillage is practised, and the type and origin of fertiliser. The aim of step 2 is to assess whether the hazards identified in step 1 would have additional adverse effects relative to the current production practice. The applicant should make reference to Chapter 10 if changes in cultivation (*e.g.* soil tillage) associated with the GM trait are likely to have a major effect on bio-geochemical processes.

11.3 Step 3: Exposure characterization

An assessment is required of the likelihood that bio-geochemical processes in the receiving environments will be exposed to any hazards arising from the GM plant and its cultivation. Exposure in this instance should be considered in terms of the GM plant and its management affecting bio-geochemical processes both in the production site and in the wider environment, as previously defined. The degree of exposure is likely to be high at the production site, *e.g.* exposure to the plant-soil matrix, because it is the intention to grow the GM plant within that matrix. However, the degree to which the wider environment is exposed to a hazard is more likely to depend also on the local context.

For example, if a GM plant and its management are considered in step 2 to cause a potential hazard through an adverse change in production practice (*e.g.* increased use of mineral nitrogen fertiliser), but that change is not likely to occur in a particular receiving environment because of soil type, climate, local fertiliser practice or any other reason, then the exposure may be low or zero in that receiving environment.

In most cases, there will be little or no exposure of bio-geochemical processes to imported GM plants and their products. However, ERA should consider whether there will be exposure to products of a GM plant through manure or organic plant matter, either imported as a fertiliser or soil amendment, derived from the faeces of animals that are fed an imported GM plant or plant product, or derived from other bio-products of industrial processes.

11.4 Step 4: Risk characterization

Risk characterisation should aim to establish the degree of risk from the characterisation of the hazard/s in step 2 and exposure in step 3. Risk characterisation should be carried out for both the production site and the wider environment (as defined) by considering the six potential impacts listed in step 1.

Risk characterisation for bio-geochemical processes could initially compare existing data from current production systems (*e.g.* fertiliser and pesticide applications, frequency and depth of tillage) with the practice expected during the growing of the GM plant, possibly supported by data from GM field trials. For example, if growing the GM plant is unlikely to change the current input of nitrogenous fertiliser, then the risk characterisation should be able to consider the consequences of this without further field experiment.

However, the choice of the comparator needs to be considered carefully and justified. It is accepted that:

(a) most methods and materials used in current production (e.g. agricultural) cause losses from and reduced storage capacity of the production system,

(b) there may be several types of production system operating in a receiving environment, and

(c) the systems may change over time (*e.g.* due to phasing out of pesticides).

Therefore, risk characterisation should ideally make reference to existing information and experiments from a range of production systems, including optimised systems if present. The characterisation should demonstrate that the GM plant and its management do not have more adverse effects on bio-geochemical cycles than any present system, and assess whether they will contribute to more sustainable or optimised production. Such comparisons can be conducted initially as part of the desk study, referred to in step 1. If any factors are identified that are likely to alter the bio-geochemical; processes, then experimental work may be needed to substantiate the risk characterisation.

11.5 Step 5: Risk management strategies

Based on the outcome of the risk characterisation, the applicant should determine and evaluate targeted risk management strategies (altered production practices, effects on human and animal health) which could minimize undesired impacts of the GM plant on bio-geochemical processes. As bio-geochemical processes are influenced by many operations in farming, it may be possible to compensate for the negative effects associated with the release of the GM plant by modifying other operations in the production system. The assessment should consider the general scope for such modification in the production systems of the receiving environment/s.

11.6 Conclusions

A conclusion is required of the overall risk of the GM plant on bio-geochemical processes in both the production site and the wider environment. The applicant should also consider any long-term effects of adverse changes in bio-geochemical processes, and should address indirect effects on bio-geochemical processes as a consequence of altered production practices related to the GM plant in the Chapter 10. The risk characterisation and conclusions will determine the requirements for the post-market environmental monitoring plan.

CHAPTER 12

EFFECTS ON HUMAN AND ANIMAL HEALTH

12.1 Step 1: Problem formulation

GM technology is now widely used in the production of such crops as corn, soybean, rice, canola, tomato, brinjal and papaya. All these crops are used as human food, or as animal feed (*e.g.* grain corn, soybean cake).

In the framework of GMO risk assessment, an assessment is required to determine whether the GM plant and its products present a new hazard for human and animal health. Thus, the risk assessment should consider:

- the nature of the introduced protein/s and its potential effects on humans and animals, and
- whether the phenotype of the GM plant has been significantly altered during transformation in ways that could affect human health.

In particular, if a potential hazard has been identified, the risk to persons working with the GM plant, coming into contact with it, or exposed to products such as pollen or dust from processed plants, should also be assessed. This assessment is particularly required for GM plants which are not destined for human or animal consumption, and where impacts on human health may not have been so apparent or meticulously studied.

For GM plant applications for food and feed purposes, where relevant, the toxicity and allergenicity of GM plants and derived food and feed should be assessed.

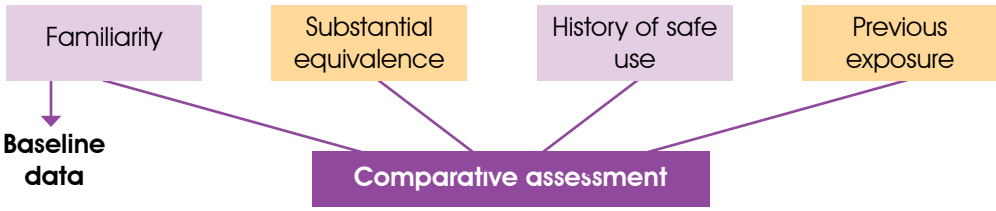
Exposure to the GM crop is also assessed based on how the crop will be used in the *food supply*. In ERA, the GM crop and food products derived from it are compared to a non-GM comparator or non-GM foodstuffs to show substantial equivalence[11] in terms of safety.

[11] The concept of substantial equivalence was first raised by OECD in 1993, and further developed by various organizations. It is analogous to the familiarity concept but focuses on GMOs used as food or feed. Thus, an existing organism

The applicant should follow the six-step approach as outlined in Chapter 2 on a case-by-case basis. A conclusion is required of the overall risk on human and animal health.

There are well-characterized model systems, such as animal and *in vitro* studies, which are routinely used in assessing the risk of a GM plant and products. Such testing provides very conservative estimates of potential risks, which is important because only a little or no risk can be accepted when considering human health, and because most testing cannot be carried out directly on humans. While the objective of risk assessment is not to demonstrate "zero risk", in the case of human food and animal feed, it is important to show that GM food/feed is as safe as the non-GM equivalent.

Taking the comparative approach mentioned earlier, a comparison is made of the new GM crop with its conventional counterpart; and this involves looking at the following:



To identify differences which *could result in adverse effects*, the GM crop or product/s is assessed against its non-GM counterpart grown under the same regime/s and environment/s for information on the following:

- Molecular characterization
- Compositional analyses
- Agronomic comparison

All these will provide the weight of evidence on whether or not the GM crop is accepted to be as safe as the conventional crop.

12.2 Step 2: Hazard characterization

While a number of potential hazards from a GM food can be identified based on known adverse effects related to the introduced protein/s, there may also be effects arising from unintended changes that occur during the process of introducing the novel protein/s. Testing is therefore required to assess whether significant unintended changes have taken place.

---

used as food/feed with a history of safe use can serve an appropriate comparator when assessing GM food/feed. Any identified significant differences would then trigger additional tests.

12.2.1 Potential Toxicity of the Novel Protein

Toxins are naturally produced by plants as a defence mechanism against pests and diseases, and also under various stress conditions. Domestication and conventional plant breeding has succeeded in selecting for reduced level of many of such toxins. Even then, when ingested in large quantities or over time, some of these toxins in modern crop varieties still cause problems (*Table 6*), remembering also that people differ in their tolerance to foods generally considered non-toxic[12]. It should also be noted that most of the toxins are removed by food preparation and cooking techniques.

The three possible toxicity hazards that need to be assessed in GM plants are:

- Is the introduced protein/s toxic?
  - Check amino acid sequence for similarity between that of the introduced protein and those of known toxins, and test the novel protein in model systems.
- Has genetic modification activated a natural toxic through a pleiotropic effect?
  - Assess by standard toxicity tests.
- Does the gene encode biologically active, pharmaceutical (*e.g.* antigens from human or animal pathogens expressed as part of a vaccine development programme) or industrial products not normally found in plants?
  - Assess for chronic toxicity if there is inadvertent transfer of such a gene to edible species.

To assess potential toxicity, the following procedure is to be adopted:

(i) Toxicity of the source of the novel protein, *i.e.* the origin of the transgene/s should be assessed for no history of toxicity to humans and animals, ideally with a history of safe consumption.

Example: Bacillus thuringiensis used as an insecticidal protein has been consumed safely by humans for about 50 years through its use in bio-pesticide sprays.

(ii) Bio-informatic comparisons with known toxins, i.e. comparing the sequence of the novel protein to databases containing known toxins and allergens. Software packages for sequence comparisons are available in the public domain:

---

[12] An example of food intolerance: lactose intolerance due to insufficient production of lactase in the sufferer. Compounds known to result in food intolerance can also be assessed by compositional analysis.

Table 6. Some examples of naturally occurring toxins in crop plants (after Tzotzos *et al.*, 2009)

Crop	Natural toxin
Beans (especially kidney bean)	Lecitins
Cassava	Cyanogenic glycosides, producing hydrogen cyanide
Fruit seeds (apple, pear and kernel of apricot and peach)	Amygdalin; can be converted to hydrogen cyanide
Parsnip and sweetpotato produced in response to insect and	Furocoumarins and ipomeamarone, fungal attack
Potato	Solanin, a glycoalkaloid, produced in shoots and green potatoes
Rhubarb	Oxalic acid
Zucchini to insect damage	Cucurbitacins, produced in response

- FASTA (fasta.bioch.virginia.edu)
- SWISSPROT (*www.expasy.ch/sprot*)

(iii) Acute toxicity testing. Where proteins are found to be toxic, they are acutely toxic. Acute toxicity tests are carried on highly sensitive (and physiologically similar) surrogate species *in lieu* of humans and domesticated animals, e.g. acute mouse gavage. Relatively high levels of the novel protein or GM product can be tested this way, while compounds known to be toxic to humans can be included as controls. The tests will establish the no adverse effect level (NOAEL) and compare this with the expected exposure level (EEC) to the GM product that could occur through known consumption patterns, with a wide margin of safety achieved by using higher concentrations of the novel protein.

12.2.2 Potential Allergenicity of the Novel Protein or GM Product

Allergens can cause an immune reaction when ingested (e.g. foods), inhaled (e.g. pollen and house mites) or when in contact with the skin (e.g. certain metals). All food allergies are caused by proteins (*Table 7*).

To assess for potential allergenicity, the following are determined:

(i) Allergenicity of the source of the novel protein, i.e. the source of the novel protein should have no history of allergenicity.

Table 7. Common food allergens (after Tzotzoes *et al.*, 2009)

Food	Natural toxin
Milk	Caseins, ß-lactoglobin
Egg	Ovomucoid, ovalbumin, ovotransferrin
Fish	Flesh proteins (parvalbumins)
Shellfish	Flesh and shell proteins
Groundnuts (plus some other legumes)	Storage proteins
Treenuts (e.g. walnut, Brazil nut)	Storage proteins
Soybean	Storage proteins
Wheat (and some other cereals)	Gluten, other storage proteins

A more comprehensive list is available at: http://www.eatwell.gov.uk/healthissues/foodintolerance/foodintolerancetypes/soyaallergy/

(ii) Bio-informatic comparisons with known allergens, i.e. comparing the molecular structure of the novel protein to the various databases on structures of known allergenic proteins (e.g. www.allergenonline.com, www.allermatch.org). Similarity of both small (at least 6-8 amino acids, 100% identical) and large (80 amino acids, at least 35% identical) segments of the linear sequence of the amino acids of a protein is considered significant.

(iii) *In vitro* digestibility and processing stability. Degradation of the novel protein under simulated conditions of food processing and digestion is important for assessing potential allergenicity because known food allergens are resistant to digestion. The allergic reaction develops when the undigested protein pass to the intestines where it comes to contact with immune cells in the wall. Alternatively, if the new protein resembles another allergen, allergic reactions will develop upon immediate contact. Stability is one of the factors making it more likely that a protein is an allergen.

Digestion is commonly tested in the laboratory using *in vitro* methods which simulate digestion in the stomach, *i.e.* placing the protein in an acid solution and adding pepsin, an enzyme produced in the stomach that degrades proteins. Most proteins are degraded within seconds whereas known allergens may remain stable for one hour or more. Another *in vitro* method

mimics digestion in the intestine using neutral conditions and other protein-degrading enzymes typically occurring in the intestines.

It should be remembered that allergens causing "oral allergy syndrome" (*e.g.* apple allergy) may not be stable under digestive conditions. This type of allergy occurs when the protein comes into contact with tissues in the mouth.

(iv) The reaction of antisera from allergic patients with the novel protein. This is tested when the initial analyses of sequence and digestibility of the novel protein are inconclusive. Binding tests with antisera of allergic patients will help determine if the tested protein will cause a reaction by the immune system of such patients.

(v) Clinical testing (*e.g.* skin prick test) with allergic patients of the novel protein or the whole genetically modified product. This is carried out when a positive reaction is evoked by testing with antisera, or if suitable antisera are not available. Food challenges using allergic patients in double-blind testing may be considered, but may be dangerous and is generally not recommended for ethical reasons. A GM product or novel protein showing allergenic traits as revealed by steps (i) to (iv) should not be approved by the regulators.

(vi) Animal testing of the novel protein or the whole genetically modified product, *e.g.* the Brown Norway rat is a IgE-hyperresponder, meaning it has a high tendency of producing IgE in its immune sera against allergens with which it comes into contact – as in its feed.

The Codex Alimentarius guidelines for food safety assessment may be used to test for potential allergenicity (Codex Alimentarius, 2003). The allergenicity of a novel protein as a risk component of a GM plant can be assessed as suggested in *Figure 8*. Two case studies follow where the development of GM crops had to be terminated due to unforeseen allergenicity which showed up during testing.

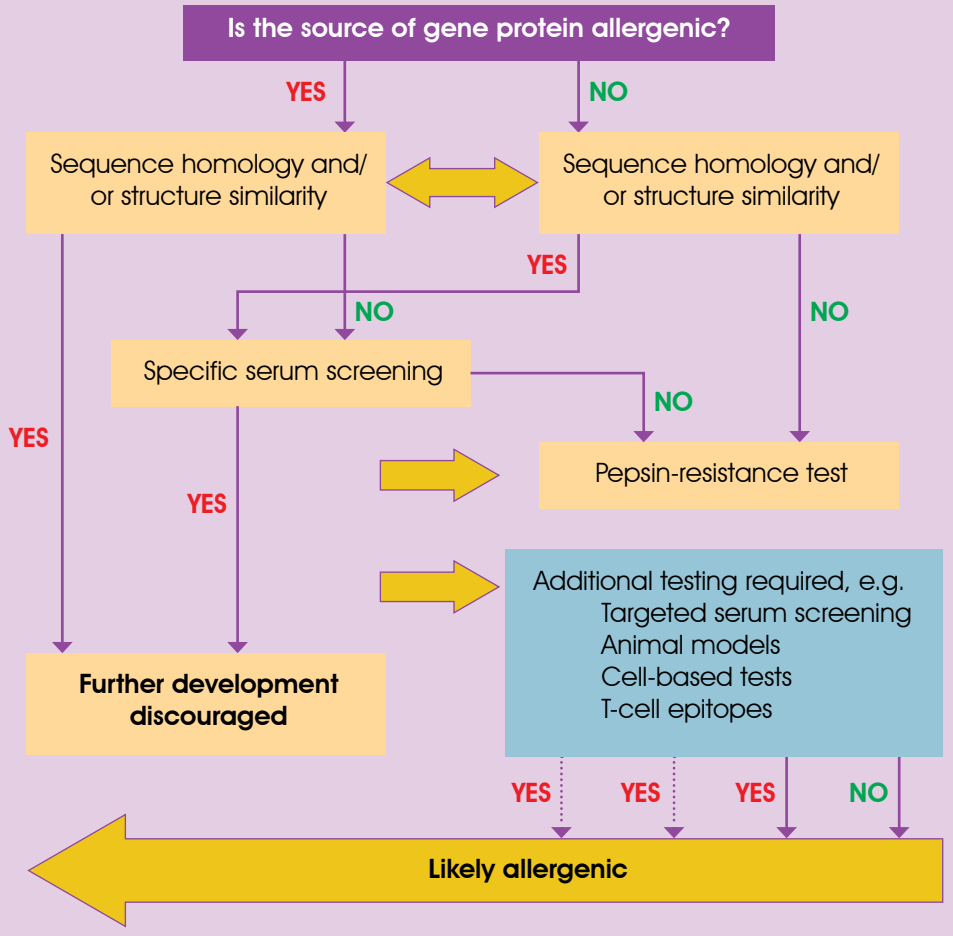
Case study on GM soybean with protein from Brazil nut.

An amino acid often limiting in animal feed is methionine, which is frequently added to the feed to make up for this lack. A soybean was genetically modified with a protein high in methionine from Brazil nut. However, Brazil nut is a known allergen, and in clinical testing, patients allergic to Brazil nut also reacted to the new GM soybean, but not to conventional soybean. Thus, the allergenicity of Brazil nut had been transferred together with the protein to the GM soybean, and further work on the new variety was halted.

Case study on pea and anti-pest protein from bean

Pea is susceptible to the pea weevil which causes substantial yield loss. An α-amylase inhibitor gene was transferred into pea from *Phaseolus vulgaris* to inhibit the digestion of starch by α-amylase in the weevil to starve it to death. Subsequent allergy testing on mice showed that the inserted gene product was allergenic although it was not so in the original bean donor. Small changes resulting in different post-translational glycosylation in the gene product in pea probably led to its allergenicity.

Figure 8. Flow chart for assessing allergenicity of a novel protein in a GM plant.



Note: **YES** *indicates potential allergenicity, with fainter shades of red indicate less potential*
(after Davies, 2005)

12.2.3 Possible Changes in Nutritional Value and Other Unintended Effects

Whether the nutritional composition of the product of a GM plant has changed can be determined in two ways:

- Using the compositional analyses to compare the macronutrients (fats, proteins and carbohydrates), micronutrients (vitamins and minerals) and anti-nutritional factors in the GM plant/product and its non-GM counterpart.

  A useful database providing compositional data of various crops is hosted by the International Life Sciences Institute (ILSI) at: www.cropcomposition.org (data on crop, location, nutritional component, year). There are also reports by OECD at: http://www.oecd.org/find Document/0,3770,en_2649_34385_1 _119666_1_1_1,00.html, covering crops such as papaya, sweetpotato, cassava, grain sorghum, sunflower, tomato, mushroom (*Agaricus bisporus*), rice, maize and soybean.

  It may be necessary to analyse specific plant parts because of different uses to which they are put, *e.g.* whole maize plant for silage (animal feed), kernels for both human and animal consumption, and baby corn used as a vegetable (human consumption). Analyses should be by standard methods (*e.g.* Association of Official Analytical Chemists), following good laboratory practice. The comparator used should be a near isogenic line (*e.g.* parental lines) as far as possible, with commercially planted varieties used as controls.

  When compositional changes do occur, it is important to distinguish whether the differences are relevant to safety, *i.e.* whether they cause adverse effects.

- Additional feeding studies on target animal species (*e.g*. rapidly growing broiler chickens and lactating dairy cows) to evaluate the use of the GM plant/product in feeds on bodyweight gain or milk yield, again compared to the non-GM counterpart.

  Other means of assessing if a GM product has unintended changes are by examining:

- DNA insertion site. This is to ensure that the inserted gene does not interfere to a significant degree with any normal gene, especially housekeeping genes and sequences that regulate gene function.
- Function and substrate specificity of introduced enzymes to determine if other reactions are possible other than the intended ones.
- Phenotypic changes, such as appearance and development, which make the GM plant clearly different from its compactor.

- Profiling, using such tools as micro-arrays for messenger RNAs (genomics); two-dimensional gel-electrophoresis of proteins (proteomics); and liquid chromatography coupled to nuclear magnetic resonance for chemical compounds (metabolic).

12.2.4 Antibiotic Resistance Markers

Antibiotic resistance in pathogens has arisen from the overuse of antibiotics in treating human diseases and the use of low doses in animal feeds to promote growth. There is now a major concern that antibiotic resistance genes will get transferred from GM organisms to pathogenic micro-organisms, conferring the latter with antibiotic resistance. While there is no evidence (because most DNA in food and feed is degraded when passing through the alimentary canal) to show this has happened, this is still considered a risk. Hence, resistance markers for antibiotics widely used on humans and animals have been phased out, through the following classification:

- **Group 1**: Limited use, having antibiotic resistance genes which are ubiquitous in nature and their corresponding antibiotics are seldom or never used in medicine, *e.g.* nptII gene (for kanamycin resistance).
- **Group 2**: Not to be used in commercial GM plants. While such plants may be tested in field trials, they may not be grown for agricultural production, because these genes confer resistance to antibiotics used in human and veterinary medicine to treat specific infections. An example is the ampicillin resistance gene.
- **Group 3**: Not allowed, because these marker genes confer resistance to antibiotics of high value in human medicine, and the effectiveness of the said antibiotics should not be compromised. An example is the nptIII gene (for amikacin resistance).

12.3 Step 3: Exposure characterization

When assessing exposure, the following factors must be taken into consideration:

1. Use of the GM crop

Is it to be used directly as food, or after processing into a food product, or as feed for animals? The uses usually do not differ from the conventional uses of the non-GM counterpart. Thus, information on the latter can be used to assess the exposure. Nevertheless, it should be remembered that there can be differences in use (and in exposure) in subgroups of a population (*e.g.* infants) which may be particularly sensitive to certain products, and also differences in digestive systems of ruminant and non-ruminant livestock species, affecting GM protein uptake.

2. Amount of novel protein in the GM product

The amount of novel protein consumed depends on the level of expression of the protein in the target tissues (*i.e.* those parts of the GM plant used for food or feed). The expression level may vary according to environment, so it is important to determine the level in multiple locations, as well as in all the plant tissues which will be used for food or feed, and at different stages of growth. The highest measured expression levels in the relevant tissue are then used to ensure that the estimates have a large margin of safety. Processing can also alter the amount of the novel protein/s as it can be selectively enhanced or reduced to almost zero (*e.g.* in vegetable oils or sugar).

12.4 Step 4: Assessment of stacked traits

There is a need to look out for possible interactions between the proteins of two parental traits, and also for unintended effects caused by interactions between two parental genomes. First, the individual novel proteins and events are assessed for risk. If no significant risk is found, the stacked product is then compared to the individual proteins and events to determine whether the single-gene risk assessments are applicable to the stacked trait product. This requires characterization of the proteins produced in both cases, measurement of the expression levels of the novel proteins, and characterizing the gene insertions. If both are comparable, and if the introduced proteins are not expected to interact, then the single-gene assessments can be used with only limited additional characterization of the stacked gene product. Such a situation is more likely when the stacked product is a result of conventional breeding between single-gene events, and where the traits involved work through very different mechanisms (*e.g.* stacking an insecticidal trait with a herbicidal tolerance trait).

In contrast, if the stacked trait product is a result of a new transformation event, or the traits are expected to interact in complex ways (*e.g.* two traits involving the same plant metabolic pathway), then such GM plants should be assessed for food and feed safety as though they are new transformed lines.

12.5 Step 5: Management strategies and monitoring

For any potential risks identified in the earlier steps, there must be risk management strategies designed to reduce or preclude them. It is best to build in such strategies into the product development process. For example, early stage bio-informatic analyses will ensure that the novel protein/s produced in the new GM product is not related to a toxin or allergen.

For a GM product already approved for commercial use, risk management strategies can limit the product with likely risks to human and animal health from going into parts of the food or feed supply by channelling. However, channelling requires rigorous and resource-intensive enforcement, and may still not be 100% effective. It would therefore be better to have a management strategy that really precludes exposure to the GM product.

In the case of the possibility of unintended adverse effects, monitoring of human populations (just like is done for novel technologies such as the mobile phone) can be carried out. However, because it is not clear what are the endpoints and baseline to use for comparison on unexpected effects, monitoring might indeed have limited value. Some organizations are already monitoring the safety of food produced by conventional means; thus, the same system may be used to monitor potential risks from GM food.

12.6 Overall Risk Evaluation and Conclusion

On the basis of ERA performed under the chapters for the various effects, the weight-of-evidence and the conclusions reached in each chapter, the applicant is requested to perform an overall evaluation of the risk/s of the GM plant in the receiving environment/s. The overall evaluation of the risk/s of the GM plant should take into account the risk characterisation (step 2 to step 4) and any risk management strategies proposed (step 5).

The overall risk evaluation should be expressed in a form of a summary, in a concise way, of the overall risk/s from deliberate release or placing on the market of the GM plant, including the overall uncertainties. The quality of existing data and information should be discussed, an explanation on how the body of information has been taken into account and the potential uncertainties. The overall risk evaluation should result in an informed qualitative, and if possible quantitative, guidance to risk managers. The applicant should explain clearly what assumptions have been made during ERA, and what is the nature and magnitude of uncertainties associated with establishing the risk/s.

The applicant should provide a summary of the overall risk evaluation in a way that conclusions can be drawn up for post-market environmental monitoring (PMEM).

POST-MARKET ENVIRONMENTAL MONITORING PLAN

CHAPTER 13

13.1 Introduction

The applicant is to implement, if appropriate, a GM plant monitoring plan for environmental monitoring to identify any direct or indirect, immediate and/or delayed, adverse effects of GM plant, its products and their management on human health or the environment, after the GM plant has been placed on the market. This requirement also applies to GM plant or product (food/feed) that an applicant intends to import into Malaysia.

The extent of the market release should be taken into account. Thus, the monitoring plan should be targeted rather than considering every possible environmental aspect. Applications for use only as food/feed or ingredients (for example, imported into but not cultivated in Malaysia) will thus not normally be required to describe a detailed environmental monitoring plan if the applicant has clearly shown that environmental exposure is absent, or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment.

Monitoring can be defined as the systematic measurement of variables and processes over time, and assumes that there are specific reasons to collect such data. For example, it may be needed to ensure that certain standards or conditions are being met, or to examine potential changes with respect to certain baselines. Against this background, it is essential to identify the type of effects or variables to be monitored, an appropriate time-period for measurements and, equally importantly, the tools and systems to measure them. Monitoring results, however, may lead to adjustments of certain parts of the original monitoring plan, or may be important in the development of further research.

13.2 Interplay between Environmental Risk Assessment and Monitoring

13.2.1 Monitoring of effects: Foreseen and unforeseen

Environmental monitoring of a GM plant has two objectives:

(1) to study any possible adverse effects of the GM plant identified in the formal risk assessment procedure, and

(2) to identify the occurrence of adverse unforeseen effects of the GM plant or its use which were not anticipated in ERA.

Where there is scientific evidence of a potential adverse effect linked to the genetic modification, then case-specific monitoring should be carried out after placement in the market, in order to confirm the assumptions of ERA. Consequently, case-specific monitoring is not obligatory and is only required to verify the risk assessment, whereas a general surveillance plan must be part of the application. The applicant who proposes that there is no need for case-specific monitoring is encouraged to provide arguments in support of this. These arguments should relate to the assumptions the applicant has made in ERA as well as to the lack of any identified adverse effects in steps 1 to 5 (ref. Chapter 5, page 52).

13.2.2 Monitoring framework

General surveillance should include long-term monitoring, to allow for unexpected effects that may occur after longer periods of environmental exposure.

The environmental monitoring plan should describe in detail the monitoring strategy, methodology, analysis, reporting and review. In this respect, GM plant-based parameters will depend on the particular GM plant, trait and environment combination. Key parameters to be observed may refer to species/ecosystem biodiversity, soil functionality, sustainable agriculture, or plant health. Indicators should be measurable, appropriate, adequate in terms of statistical power, and comparable with existing baseline data. Background and baseline environmental data, e.g. soil parameters, climatic conditions, general crop management data (such as fertilisers, plant protection and crop rotations) and previous crop history, should be collected, where appropriate, to permit the assessment of the relevant parameters.

13.2.3 Case-specific GM plant monitoring

The main objective of case-specific monitoring is to determine the significance of any adverse effects identified in the risk assessment.

Case-specific monitoring should be targeted at those environmental factors most likely to be adversely affected by the GM plant which were identified in ERA. The scientific approach should be designed to test the specific hypothesis of expected adverse effects derived from ERA. The design of the monitoring programme should also reflect the levels of exposure in different geographical regions and other specific site influences. Such monitoring may be carried out at a limited number of sites ('local monitoring'), where exposure is greatest and intensive

recording and data collection can take place. This would be particularly appropriate when it is envisaged that there will be a phased or gradual introduction of the GM plant into a limited number of regions in the country. The scale of the monitoring should be increased as the area and range of the GM plant expands, and the plant is grown in more regions. The monitoring should consist of the systematic recording of relevant parameters at representative locations where there is significant and repeated growing of the GM plant. This might also be defined according to the extent of the cultivation of the GM plant, the occurrence of targeted pest species or particular climatic/eco-regions. The methods selected, the duration of the monitoring, the extent or number of areas and the parameters to be monitored will be determined on a case-by-case basis. Whilst the planning and execution of case-specific monitoring is the applicant's responsibility, it may be appropriate for the applicant to involve public institutions to contribute to the agreed work.

13.3 General Surveillance for Unanticipated Adverse Effects

The objective of general surveillance is to identify the occurrence of unanticipated adverse effects of the GM plant or its use on human health or the environment that were not covered in ERA. General surveillance applies where no adverse effect has been identified in ERA, but is always required in order to detect unanticipated adverse effects. Monitoring of potential adverse cumulative long-term effects and areas of uncertainty identified in ERA are important objectives of monitoring which should be considered initially within case-specific monitoring. When there is a negligible degree of uncertainty in ERA then no case-specific monitoring is necessary. However, general surveillance is always required for monitoring any unanticipated adverse effects.

An effect can be defined as an alteration that results in values that fall outside the normal range, given the variation due to the constant changes in agricultural practices, rural environment and associated biota in the country. Major challenges in general surveillance are determining whether:

- an unusual effect has been observed,
- the effect is adverse, and
- the adverse effect is associated with the GM plant or its cultivation.

The use of a range of monitoring systems to supply data, and the ability to compare data from these different sources will help to indicate whether an effect is unusual and adverse. The identification of an adverse effect which is potentially linked to specific GM plants would trigger the need for a specific study to evaluate harm and to determine cause.

The overriding objective is to protect the environment, including biodiversity, water and soil. One important task within general surveillance is to link monitoring to these environmental protection goals. Environmental damage is defined as a measurable adverse change in a natural resource or a measurable impairment of a natural resource service which may occur directly or indirectly.

Within a broader concept of environmental issues, unanticipated adverse effects on human health have also to be addressed in the monitoring plan presented by the applicant. The scope of monitoring for unanticipated adverse effects on human health is defined as monitoring for unanticipated adverse effects that may result from handling of the GM plant. It might prove very difficult to design monitoring (including general surveillance) for unanticipated adverse effects on human health. However, knowing that the release of GM plants needs to be considered in the context of their interaction with other environmental components, monitoring for health effects could be considered in conjunction with human population screening methods currently used by public health organisations (for assessing such elements as incidences of allergic reactions) and as part of suggested plant production and farm questionnaires.

13.3.1 Approach and principles of general surveillance

Applications concerning food/feed uses and import for processing require scientific information on possible environmental effects associated with the cultivation of the plant to be included as supplementary background information. The extent of general surveillance for these GM plants will depend on the level of environmental exposure. Therefore, general surveillance plans for applications to import/process and plans for applications to cultivate GM plants are differentiated.

Approach and principles for GM plants intended for import and processing only

General surveillance plans as part of an application for import and processing will need to take account of the modified characteristics specific to the GM plant in question, its intended use and the receiving environment/s. The extent of the general surveillance plan will depend on the level of environmental exposure, the establishment, persistence and spread of the GM plant, and should require scientific information on possible environmental effects associated with the cultivation of the plant. The applicant has to show that environmental exposure will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment.

In the case of non-viable GM material (*e.g.* derived products not

containing any living GMOs), the applicant does not have to provide any environmental monitoring plan (including general surveillance).

In the case of imported GM products containing viable propagating material, general surveillance plans should consider that if substantial loss, spillage and establishment is possible, appropriate management systems should be in place to restrict environmental exposure.

Approach and principles for GM plants intended for cultivation

General surveillance plans as part of applications for cultivation will need to take into account the full environmental effects of the GM plant including its cultivation.

General surveillance is a general overseeing of the geographical regions where GM plants are grown without having any specific hypothesis on adverse effects on human health or the environment. As general surveillance is not hypothesis-driven, it is not conducted using directed experimental approaches. However, robust scientific methodology should be applied wherever possible to evaluate empirical knowledge. This especially refers to defining sample size, sampling and recording methods, in order to produce statistically valid data for determining causes and effects.

Existing surveillance systems should be used where practical (*e.g.* routine farm recording systems) and any 'unusual' effect, not occurring in similar situations within conventional cropping, should be recorded (*e.g.* effects on soil).

The establishment, persistence and spread of a GM plant are not environmental hazards in themselves. Similarly, dispersal of pollen and seeds and gene flow *per se* are not environmental hazards, and thus the focus of general surveillance should be on recording any unanticipated consequences of the cultivation of the GM plant, such as unforeseen weediness, invasiveness or changes in plant population dynamics or populations of biota associated with the GM plants. However, an unanticipated adverse effect is most likely to occur where the level of environmental exposure is highest. Thus, an evaluation of how and where the GM plant will be grown and the associated environmental exposure is considered a good starting point in any general surveillance plan.

General surveillance of the impact of GM plant should:

- be applicable, in a proportionate and cost-effective manner, for monitoring the GM plant in a range of representative environments, reflecting the range and distribution of farming and environments exposed to the GM plants and its cultivation. If unusual effects on

human health or the environment are reported, more focussed in-depth studies should be carried out to determine cause and relationship with the GM plants. These additional studies would be case-specific monitoring studies as they would require an experimental approach to confirm the specific hypothesis that an observed effect is associated with the GM plant;

- complement available general environmental monitoring. The higher the ecological integration and scale (from the individual to a population, from single farm to regions), the more difficult it is to distinguish potential effects of the GM plants from other factors. Initially, general surveillance should focus on each event individually. Additionally, when several GM plants have been commercialised, the interactions between these GM plants and their management may need to be considered, where appropriate.

13.3.2 Main elements of general surveillance

The applicant should:

- define the methods and approaches that will be used to conduct general surveillance of regions where the GM plant occurs,
- refer to introduction, stewardship and exploitation plans for the GM plant, and
- make proposals for the time period, area covered, and the frequency of monitoring.

Existing monitoring systems

The applicant shall develop plans for the introduction, marketing, management and stewardship of the GM plant. The applicant should include these into the monitoring plan, where appropriate, as they will contain some data of relevance to the implementation of the monitoring plan.

General surveillance should, when compatible, make use of established routine surveillance practices such as monitoring of agricultural plants, variety/seed registration, plant protection, plant health and soil surveys as well as ecological monitoring and environmental observations.

Many of the existing monitoring systems and networks collecting environmental data are unlikely to always provide data of relevance that may be used in monitoring the impacts of GM plants. The design of the existing monitoring programmes, the targets (*e.g.* birds, plant protection, etc.), the time, frequency and scale of data collection, sampling, analysis and reporting methods may not suit the monitoring of GM plants because they have been designed for other purposes. Thus, the applicant may not consider existing networks to be sufficiently useful sources of information for monitoring. There may be a need

for additional environmental surveys and to amend the monitoring objectives of existing monitoring systems.

Existing monitoring systems can be of variable quality and consistency; thus, it is important that the consistency and reliability of surveys utilised in general surveillance is evaluated in order to ensure long-term coherence and reliability of data collection and data quality. In addition, as environmental surveys will differ between networks, methods for integrating data from different origins should be evaluated.

Knowing the limitations of existing monitoring systems, it is important for the applicant to describe the processes and criteria that will be used for selecting and evaluating existing monitoring systems for supplying data related to the unanticipated adverse effects of GM plants in the general surveillance.

Specifically the applicant should:

- describe which observations could be monitored through existing monitoring schemes,
- identify the type of existing monitoring systems that would be appropriate for this in the areas where the GM plant will be grown (*e.g.* monitoring of agricultural cultivars and plant protection surveys),
- describe the criteria and generic approach used to evaluate existing monitoring networks, and how appropriate networks will be selected,
- describe how arrangements for collecting, collating and analysing data will be made,
- identify which category of additional surveys could be required to contribute to the general surveillance (*e.g.* public institutions, farm associations) in selected areas,
- describe how formal agreements, procedures and communication will be established with third parties before commercial market introduction, although detailed arrangements may not have been agreed upon at the time of the application.

The responsibility for each step in the monitoring plan should be clearly assigned by the applicant. Where third parties are employed or contracted to conduct monitoring studies, the nature of their involvement should be detailed.

Use of GM plant-focused monitoring systems

In addition to using existing monitoring systems, the applicant is encouraged to develop new and more focused monitoring systems, especially at the production level. Questionnaires, directed at farms

where GM plants are grown, are considered a useful method in collecting firsthand data on the performance and impact of a GM plant, and for comparing it with conventional plants. Experience from other established surveillance and monitoring systems (*e.g.* the approach used for consumer and pharmaceutical surveillance systems) could be used in designing the questionnaires. Special emphasis should be given to the statistical design of such questionnaires. Issues related to human health (*e.g.* due to exposure and handling of GM plants) may also be integrated into the farm questionnaires.

As appropriate, the applicant should:

- inform growers, seed suppliers or other stakeholders about the GM plant and the need to supply data on seed sales, areas sown, plant management, etc.
- be pro-active in developing reporting systems so that farmers (or their agents and advisors) intending to purchase GM seeds will be fully informed about the GM plant, the importance of the monitoring programme and in reporting unanticipated effects during and after the cultivation of the GM plant,
- describe the number of farmers/growers involved, the area covered, the reporting methods and the suitability of the data collected for statistical analysis,
- establish independent audits to ensure the independence and integrity of all monitoring data,
- indicate the likely frequency of inspections.

Farm questionnaires should:

- be designed to ensure the statistical validity and representativeness of the collected data, including the proportion of fields growing the GM plant in a region, and the number of questionnaires required to achieve statistical power in the data collected,
- be designed to generate data on the agronomic management of GM plants, as well as data on the impacts on farming systems and the farm environment,
- use a field or group of fields growing the GM plant as the basic unit for monitoring,
- observe the field/fields in subsequent years for any unusual residual effects,
- be user-friendly but also information-rich,
- be constructed to encourage independent and objective responses from farmers, land managers and others involved with the GM plant or its products.

Questionnaires adapted to agronomists or other stakeholders working on the farms growing the GM plants may also be useful sources of information. Focused questionnaires and interviews are generally accepted by respondents. Professional interviewers may be an additional help.

Farm questionnaires should be distributed, completed and collated annually via an arranged reporting system (*e.g.* farm questionnaire forms or online systems). These should be analysed by the applicant, and the reports submitted at the agreed time intervals (usually annually) to the appropriate authorities. The results of the farm questionnaires will allow the applicant to record the implementation of recommended management and stewardship of the GM plant (*e.g.* good agricultural practice, hazard analyses, critical point compliance) and to identify unanticipated adverse effects.

13.3.3 Importance of a baseline

There is a need for general surveillance plans using both existing and novel monitoring systems to be able to compare impacts of GM plants and their cultivation with those of conventional plants. The baseline is the current *status quo*, *e.g.* current conventional cropping or historical agricultural or environmental data. Direct comparison with non-GM plant reference areas should be used if available, but reference can also be made to the historical knowledge and experiences of the "observer" (*e.g.* farmers, inspectors, wildlife rangers) in relation to the situation prior to the introduction of the GM plant. It will be important to inform observers to report any unusual events and *not* to attempt to anticipate impacts.

There is also a need to take into account the fact that the GM event will occur in a changing genetic background of new varieties which may have an impact independent of the GM event, and thus it is the event that needs to be monitored in any variety.

13.3.4 Data quality, management and statistical analyses

The design of the monitoring programme will influence the quality and usefulness of resulting data. Hence, efforts should be made to ensure that data from all the monitoring systems used can be statistically analysed. Meta-analyses of different datasets might be useful. If relationships between datasets can be identified, it will contribute to the credibility of monitoring.

The general surveillance plan should

- take account of the scale of commercialisation as well as the historical baseline knowledge in the different areas to be monitored,

- consider the geographical areas to be studied and which existing environmental monitoring programmes could be useful for inclusion,
- consider national cultivation registers of GM plants (including co-existence measures) as they can provide useful data,
- describe the generic approach used for data collection, management and exploitation within general surveillance (*e.g.* data from existing networks and questionnaires),
- describe how any unusual adverse effects related to GM plants will be identified, including details of the statistical approach,
- include a comprehensive description of the techniques to be used for data analysis and statistical analysis, including the requirements for statistical significance,
- provide a detailed description of the operational handling of data from different sources into a 'general surveillance database',
- describe the approach to categorise the data (*e.g.* influencing factor, monitoring character) and the method for pooling the results and matching them with data on GM cultivation in time and space,
- contain data from case-specific monitoring that might complement the general surveillance data.

13.4 Reporting the Results of Monitoring

Following placement in the market of a GM plant or product, the applicant has a legal obligation to ensure that monitoring and reporting are carried out according to the conditions specified in the approval. The applicant is responsible for submitting the monitoring reports to the National Biosafety Board. The applicant should describe the methods, frequency and timing of reporting in their monitoring plan.

Although no timeframe for reporting is specified, reports – allowing for case-specific adaptations – preferably should be submitted:

- annually, to confirm that monitoring has been carried out according to the given approval, together with a summary of major preliminary results that are important for short-term feedback on ERA ('annual reports'), and
- periodically (e.g. every third year), to cover longer periods in which observations and data collected are reported and analysed in detail, and which therefore provide more comprehensive accounts that are important for a longer term feedback on ERA ('comprehensive report').

The comprehensive report should include in more detail the results of any relevant monitoring by third parties, including the farmers/ growers, seed companies, independent surveyors, local, regional and

national environmental surveyors. In addition, the applicant should evaluate these results and incorporate full analysis and conclusions in the submitted monitoring report. If appropriate, the applicant should provide access to raw data for stimulating scientific exchange and co-operation.

1. For the flow of information on the cultivation of GM plants, the following procedures should be complied with:
    - All GM seeds must be labelled with the variety, and should also contain information on the construct, the supplier's name and address, full instructions on any specific cultivation requirements, and reporting procedures for any incidents, including the address of the Approval Holder for the marketing of the seeds.
    - The farmer/grower is required to declare the variety, sowing date, amount of cultivated plants and exact geographic location to the national cultivation register.
    - The farmer should record all relevant cropping and management data for that GM plant and these data should be available for inspection.
2. Flow of information in instances where the GM plants are thought to have caused unusual or adverse effects (*i.e.* if adverse effects have been detected in areas where the GM plants are grown or where there is a suspicion that the GM plants may be associated with an incident) the following procedures should be complied with:
    - Farmers should follow the procedure for reporting established by the applicant at the time of purchase of the GM seeds, and provide information to the information point specified therein of any unusual observations without delay.
    - The applicant should immediately take the measures necessary to protect human health and the environment, and inform the National Biosafety Board. In addition, the applicant should revise the information and conditions specified in the application.
    - The applicant may inform external organisations (*e.g.* public institutions), asking them to immediately communicate any adverse effects they may detect to a specified information point.
    - The applicant could carry out a preliminary examination in order to verify whether a GM plant-related effect has really occurred, and provide the National Biosafety Board with a report on the result of its preliminary investigations, including an assessment of potential harm.
    - If information becomes available to the National Biosafety Board which could have consequences for the risks of the GM plant/s to human health or the environment, it will immediately

forward the information to the Minister of Natural Resources and Environment (NRE) and the other relevant ministries.

Where adverse effects on the environment are observed, further assessment should be considered to establish whether they are a consequence of the GM plant or its use, as such effects may be the result of environmental factors other than the placement in the market of the GM plant in question. The National Biosafety Board should inform Minister of NRE of the reported observation, and, together with the applicant and scientific institutions or experts, investigate the causes and consequences of the reported incident. Finally, the National Biosafety Board should submit a report to the Minister of NRE on the extent of any environmental damage, remedial measures taken, liability and recommendations for the future use/ management of the GM plant.

13.5 Review and Adaptation

Monitoring plans should not be viewed as static. It is fundamental that the monitoring plan and associated methodology are reviewed at appropriate intervals as they may need to be modified and adapted depending on the results of the monitoring information collected. The monitoring plan might also be adapted based on an assessment of the appropriateness and cost-effectiveness of the monitoring plan. Implementation of the revised monitoring plan remains the responsibility of the applicant unless otherwise determined by the National Biosafety Board.

13.5.1 Concept and Process of Risk Assessment

The Centre for Environmental Risk Assessment (CERA) of the International Life Sciences Institute Research Foundation adopts the process flow for risk assessment proposed by Wolf *et al.* (2010). The concept and process flow of risk assessment may be summarized as in *Figure 8*.

13.5.2 Problem formulation

The first step in an environment risk assessment (ERA) is problem formulation. In problem formulation, societal values (defined by legislation, laws and regulations), policy goals, scope (the state of the environment in which a situation exists), assessment endpoints are taken into consideration. Through problem formulation, it will be possible to determine only those aspects of the environment which are appropriate to consider for a specific risk assessment of an explicitly stated problem, and subsequently determine the potential hazards (problems) which need to be addressed by the risk assessment in the context of the activity under consideration (CERA, 2011). Problem formulation also establishes suitable methods of analysis to obtain information that will guide the risk assessment.

Problem formulation is divisible into two distinct parts: problem context and problem definition.

13.5.2.1 Problem context

Problem context incorporates external considerations in the form of legislation, regulations and environmental management goals along with other elements of public policy, and identifies the relevant aspects that might be considered in a specific risk assessment. These external considerations are typically independent of the risk assessment itself, and are not case-specific as they apply to a variety of activities at a national or regional level. These public policy considerations are generally broad and wide-ranging, and may be vague. They may also establish specific regulatory or procedural requirements (*e.g.* the consideration of threatened or endangered species). In establishing the problem context, the risk assessor explains how these public policy goals and requirements will be incorporated into the risk assessment process.

The problem context also includes information about the proposed activity or decision which necessitates the risk assessment. In the case of GE crop plants, this usually consists of information about the identity and biology of the plant species, its intended use and the likely receiving environment(s). Using this information, together with the identified environmental policies and goals, the risk assessor can identify assessment endpoints that are potentially useful to the risk assessment. Assessment endpoints are measurable expressions of an environmental value that is susceptible to harm, and can provide evidence of harm if it occurs.

Depending on the specific case, some aspects of problem context may be incorporated into specific regulations, guidelines or risk assessment frameworks. This means that they may simply be handed to a risk assessor prior to beginning a risk assessment. Nevertheless, it is useful for a risk assessor to recognize and understand the components of the problem context in order to ensure that the risk assessment meets its intended purpose. By explicitly addressing the problem context before undertaking a risk assessment, the assessor can help provide clarity to the decision-makers and the public as to how the assessment was done.

13.5.2.2 Problem definition

Problem definition is the process of taking the environmental policy goals, potentially useful assessment endpoints and information about the GM plant and the receiving environment from the problem context to identify specific risk hypotheses that merit assessment. To do this, a risk assessor will generally consider two things: the potential for exposure, and the potential for harm resulting from the exposure. (This is equivalent to steps 2 and 3 of EFSA's model, viz. hazard characterization

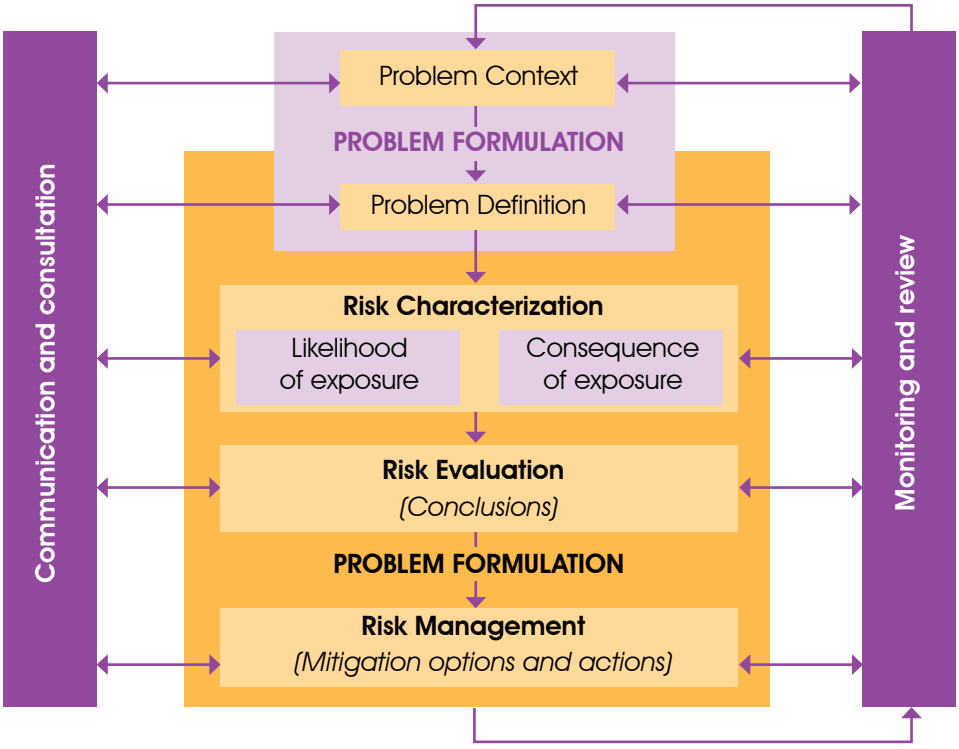


Figure 9. *Process flow for risk assessment (after Wolf et al., 2010)*

and exposure characterization). It is possible that all the environmental values (and their associated assessment endpoints) that were identified in the problem context will merit assessment, but usually one or more of these environmental values can be excluded based on either a lack of exposure or the lack of a reasonable hypotheses explaining how the exposure to the GM plant might lead to a harm (*i.e.* an adverse effect on the assessment endpoint).

Potential exposures can be identified based on the intended use of the GM plant and its biological characteristics. The intended use tells an assessor where the plant will be deliberately released into the environment, and the biology of the plant allows consideration of whether the plant will persist in that environment, or in neighbouring environments through incidental or unintentional introductions. An assessor can also consider the potential for exposure through gene flow to wild relatives based on the biology of the plant, and the presence of any sexually compatible species in the receiving environment.

Exposure is necessary but not sufficient to produce a risk. There must also be a potential mechanism by which the exposure might lead to harm. The amount of evidence supporting the hypothesis prior to the risk assessment will vary depending on the national context, the

potential magnitude of the harm, and the assessor's familiarity with the potential risk. However, a reasonable risk hypothesis should be made explicit. This provides two important logical underpinnings for the subsequent analysis. First, an articulated risk hypothesis allows an assessor to determine what information is necessary or useful to address the potential risk. If the information needed to characterize the risk is not clear, it is an indication that the risk hypothesis may be too vague or inappropriate for the particular risk assessment. Secondly, information that is collected without the benefit of a clear risk hypothesis is often not very useful or informative for risk assessment. Information that highlights potential changes, to the environment or to the GM plant, which cannot be linked to any adverse consequences (*i.e.* inconsequential effects), can generate confusion and distract attention from more relevant information as well as cloud the final results of the risk assessment.

It may be seen that the process flow for risk assessment postulated by Wolt *et al.* (2010) gives opportunities at every stage of ERA for feedback from communication and consultation as well as data from monitoring and review.

CHAPTER

14 ERA OF ACTIVITIES WITH PLANT-ASSOCIATED GENETICALLY MODIFIED MICROORGANISMS

The objective of the risk assessment is to determine the likelihood and the possible consequences of an accidental release of a genetically modified microorganism (GMM) from containment into the environment. In a properly maintained and managed facility with the correct containment measures in place, the likelihood of such a release will be low. However, it is important to identify all possible hazards and consider any routes by which GMM could be released (including waste disposal, equipment failure and spread by humans).

The risk assessment should consider both the environment surrounding the containment facility as well as the wider environment, especially if there is a possibility that GMM could survive and disseminate. The Contained Use Regulations require consideration of whether there may be an adverse effect from interactions of GMM with other organisms at the premises with which it is likely to come into contact. For example, an insect-borne pathogen and its intermediate vector may be present in adjacent laboratories. In such cases, it might be necessary to implement additional controls.

14.1 Hazard Characterization

The following potential hazards to the environment posed by GMM should be considered:

- Hazards associated with the recipient microorganism. These will be particularly relevant where the organism being modified is a plant pathogen or is not indigenous to Malaysia, and therefore could disrupt microbial ecological balance;
- Hazards associated with the inserted gene/element. These will be particularly relevant if the insert encodes a toxic product and could have adverse effects on humans, animals, plants and soil ecology;
- Hazards arising from the alteration of existing traits. These concern the effects of the modification, and will centre upon changes to the survivability and interactions with the host plant of other environmental organisms.

14.1.1 Hazards associated with the recipient organism

The characteristics of the recipient strain that will be of relevance to the final GMM include pathogenicity, virulence, infectivity, toxicity, symbiosis, ability to colonise and ability to compete with indigenous microbes. If the recipient organism is pathogenic or mutualistic, then GMM may also exhibit the same features, albeit potentially altered by the modification.

Particular care must be taken in the assessment of work with pathogens that infect plants that are indigenous to Malaysia. Clearly, there may be major economic risks to consider if work is undertaken on pathogens of plants that are grown commercially. Similarly, work on pathogens that infect indigenous plants or those grown ornamentally may also pose significant hazards to the environment.

In the event of a release, there is potentially a fine balance between the reduced pathogenicity of an attenuated pathogen and the ability to contain an outbreak of a virulent one. If the host organism is present in the receiving environment, then an attenuated strain should be used, if possible or otherwise practicable, as this will reduce the impact of pathological effects in the event of a release. Should a virulent microorganism be used, then careful consideration should be given to the possibility that the pathogen may persist in the environment.

Nonetheless, a pathogen with increased virulence that causes severe disease (or a *hypervirulent* pathogen) might fail to persist, as the disease will be 'self-limiting' due to local 'fade-out' of the host plant population. Conversely, a less virulent strain might be more able to persist and therefore spread further. If a hypervirulent pathogen is to be constructed or used, then this should be fully justified by the risk assessment, and suitable management strategies implemented. These activities carry with them the risk of serious environmental impact and effects upon population structure and density of the host organism, as well as impact upon the wider ecology. Such considerations need to be carefully weighed and all hazards, including the possibility of severe disease and persistence, should be fully accounted for in the risk assessment.

There are a number of modification strategies that can be employed to disable a plant pathogen, or to study the mechanisms of host interactions more safely. These approaches include:

- deletion or mutation of genes that are essential for growth or replication;
- deletion or mutation of genes involved in pathogenesis;
- elimination of intermediate vector transmission by using non-transmissible isolates or altering/removing sequences required;

- studying the molecular mechanisms without using the whole pathogen. For example, studying self-propagating viral RNAs (replicons).

The origin and mechanism of such attenuation should be well understood and form an important part of the risk assessment. In assessing whether a GM plant pathogen is adequately disabled, the possibility of reversion or complementation should be considered. Furthermore, it should be confirmed that GMM is indeed disabled, or remains so, after modification.

The stability of the genetic modification should also be considered, particularly where there is a possibility that an attenuated or disabled GMM might revert to a wild type or pathogenic phenotype and become an environmental hazard. The likelihood of reversion will depend on the mechanism of attenuation, e.g. deletion mutants are less likely to revert than point mutations or conditional lethal mutants. Therefore, the genetic stability of the modification is linked to phenotypic stability, especially where the modification restricts the GMM's ability to survive and to spread.

An organism with a restricted capacity to survive will be under stress in the environment, and there will be a strong selection pressure for the reversion of attenuating and disabling genetic lesions. The possibility that GMM will be genetically unstable outside of the controlled conditions in which it was intended to exist should be taken into account, and consideration be given to any detrimental effects this might cause. In particular, careful consideration should be given to the use of disabled GM plant viruses in conjunction with transgenic plants engineered to complement the genes which are deleted from the viral genome (thus effectively using a 'helper plant'). Such an approach could be used to generate disabled virus vectors, providing an enhanced measure of biological containment. This approach may, however, lead to a selective pressure for recombinant viruses to reacquire the essential genes from the transgenic plant.

Survivability of the organism will be a key attribute. If an organism is not capable of surviving for significant periods in the environment (as may be the case for many of the disabled organisms used in containment), then none of the other hazard areas are likely to come into play. In many cases, a disabled GMM can probably be considered safe from an environmental standpoint as they are biologically, if not physically, contained. Conversely, if an organism can survive and perhaps disseminate in the environment, then other possible hazards should be considered This means that alterations in pathogenicity, and possible adverse effects of any inserted gene products, will also need to be considered.

When assessing whether an organism might survive in the environment, it should be remembered that this includes all types of association with living organisms, as well as the possibility of persisting in soil, water or other sites.

14.1.2 Hazards associated with inserted genes

GMM may be a hazard to the environment by virtue of the properties inherent to the genetic insert, even if the recipient microorganism poses no specific risk. For example, the products of the inserted sequences may have the desired effect in the intended experimental system but nevertheless kill or be detrimental to environmental plant, animal or microbial species. This is particularly relevant for modified microorganisms that can infect plants and express the inserted gene within plant tissues.

Careful assessment will also be required for recipient microorganisms that can remain viable outside of a plant host and secrete potentially hazardous products into the soil or water. It is important to consider any potentially harmful (or beneficial) effects that GMM can have on microorganisms in the soil environment.

> *For example, a soil-borne bacterium expressing and secreting anti-fungal compounds could kill mycorrhizal fungi if it escaped and became established. Similarly, a plant infected with GMM encoding a product that could disrupt mechanisms of mutualism could harm the ecology.*

It is also important to assess the potential for an encoded product to cause adverse effects in animal populations. These considerations primarily apply to those genes encoding products with biological activities, particularly if they are novel and not normally found in plants. Examples of such genes would include those encoding industrial, pharmaceutical, immunogenic, toxic or allergenic products, such as antigens from human or animal pathogens expressed for vaccine development. Such products could have adverse effects on humans and animals in the environment. In particular, if an infectious GMM could lead to the expression of a gene encoding a toxic product in a plant eaten for food by animals, then these animal populations might be reduced.

It is important to consider the properties inherent to the products of a heterologous gene insert in conjunction with the expected characteristics of expression.

> *For example, the gene product might be allergenic or toxic to animals. If the gene is expressed in the leaves or edible parts of an infected plant; then an adverse effect due to contact with or ingestion by animals or humans might be possible. Should the expression of that product be restricted to root tissue, then*

the potential risks posed to grazing animals might be reduced. However, toxic products secreted by root systems or mycorrhizae might have adverse effects on the soil microbial populations, symbiotic organisms and plant health.

The non-coding regulatory regions and signal sequences present in the insert will affect the characteristics of expression. It is important that the effects of these are considered in addition to the biological activity of the expressed product.

Inserted genes may encode products with no specific activity, but will nevertheless have a potentially harmful action within GMM, or due to interactions with the host. For example, an inserted gene could encode a pathogenicity or virulence determinant. This could exacerbate a potentially harmful phenotype of a plant pest or confer pathogenicity on an organism that is otherwise harmless (see Section 14.1.3 below). Furthermore, the insertion of an essential gene from the host plant into a GM virus vector can cause the modified virus to have harmful effects due to post-transcriptional gene silencing. If the virus is carrying an essential gene, this could have adverse effects on the growth of the infected plants, overcome inherent resistance mechanisms, or alter environmental tolerances.

14.1.3 Hazards arising from the alteration of existing traits

The modification may lead to adverse effects arising as the result of alteration of existing traits. This could represent an exacerbation of a pathogenic phenotype, or the disruption of a mechanism that is beneficial to plant, animal or microbial populations. It may arise as the result of the product of the inserted gene acting alone (see Section 14.1.2 above), or in combination with other microbial determinants. Alternatively it is possible that modification of normal microbial genes may also alter pathogenicity.

In identifying any hazards associated with the genetic modification of a microorganism, the following list (non-exhaustive) or mechanisms should be considered:

- ***The modification alters survivability or stability***. A key question will be whether the modification could alter the GMM's ability to survive in the environment as this will affect whether or not other potential risk factors will come into play. Organisms will have varying degrees of survivability. However, modifications may impact upon tolerances to UV, temperature fluctuations and dehydration.
- ***The modification alters infectivity or pathogenicity.*** Consideration should be given to modifications that might affect the pathogenic mechanisms of GMM. For example, the insertion of a known

pathogenicity or virulence determinant into a microorganism might increase the potential for that organism to cause harm in the event of environmental exposure. Special consideration should be given to the insertion of genes encoding products involved in pathogenesis into microorganisms that are not normally harmful.

There are many possible mechanisms by which the inherent pathogenicity of a host organism can be affected, and these may not be directly related to the harmful properties of the encoded products. Unforeseen effects may also be observed while making seemingly innocuous alterations to the genes of the organism. This is particularly relevant in complex systems such as bacteria where genes are often part of a cluster, or encode a component of a regulatory network. Fungal gene regulation systems are also complex, but are poorly understood compared with bacteria. The modification or deletion of one gene may have ramifications beyond the loss or alteration of the known functions of the encoded products. The expression of other genes may be affected, and biosynthetic or signalling pathways may be disrupted, resulting in altered traits.

- ***The modification affects host plant defence mechanisms***. The modification of genes that are involved in subverting host defence mechanisms may affect the susceptibility of plants to infection, constituting an alteration in pathogenesis. For example, products that are secreted by bacteria can be important determinants of pathogenesis in bacteria, and may suppress plant defence mechanisms.
- ***The modification alters tissue tropism or host range.*** Modifications that can alter the types of plant tissue affected, or alter the host range, will require careful consideration. There are many factors that may change the natural tropism[13] or host range of a microorganism. Pathogenic bacteria may also have determinants that affect the host range or the ability to colonise certain sites. During risk assessment, careful consideration should be given to the possible effects on tissues or host plants not normally affected or colonised by the recipient organism, and whether the normal route of transmission of the organism has been altered. It is recognised that the consequences of changes in tropism or host range are difficult to predict. In assessing the risk of manipulations designed to modify tropism, particularly in the case of replication-competent viruses, it should be assumed that they would require a higher level of containment as compared to the recipient strain until the properties of GMM are better understood.

[13] Tropism is taken to mean the intentional alteration of types and location of tissues affected.

- ***The modification alters transmissibility***. A clear distinction should be drawn between the movement of a microorganism within a plant, and transmission between plants. Both may present a hazard, although the risk assessment of the two scenarios may be very different.

  In general, the insertion of gene sequences that are known to facilitate the migration of a plant-associated microorganism within a host will potentially create GMM that is more harmful. Careful consideration should also be given to modifying sequences that will affect the transmission between plants, *e.g.* the DAG motif in potyvirus capsid proteins. Generally speaking, modifications that are expected to bestow additional transmissibility functions should be assumed to result in GMM that is more hazardous.

14.1.4 Transfer of harmful sequences between organisms

There are many mechanisms by which sequences may be transferred between organisms, and the factors that affect the frequency of such events and the likelihood of a harmful consequence are complex. Such issues must be carefully considered in risk assessment. It is important to consider the potentially harmful consequences of sequences inserted into GMM being transferred to other organisms, or that GMM itself may acquire sequences that might result in adverse effects in the environment.

With the notable exception of viruses, the transfer of genetic information present in the genomes of microorganisms is much less likely than if they are present on an episomal form, such as a plasmid or cosmid. The frequencies of successful horizontal gene transfer in the environment are low, even for genes located on plasmids. However, there is a finite possibility that any gene may be transferred, even if the mechanism is just a passive one involving the release of DNA from senescing cells. Therefore, the primary consideration is to concentrate on the possible consequences, rather than on the likelihood of transfer.

The survival of GMM in the environment, either independently or in association with a plant host, may affect the likelihood of nucleic acid sequence transfer to another organism. Consideration should be given to the possibility that there could be selective pressure in the environment that might contribute to the persistence of a sequence or gene, and its acquisition by an organism. There are a number of mechanisms whereby sequences could be transferred or acquired. The possibility that one or more of the following mechanisms might contribute to a potentially harmful sequence being acquired by another organism should be considered:

- ***Sequence mobilisation in bacteria***. This is particularly pertinent to

sequences that are present in a mobilisible or episomal form, such as a bacterial plasmid. Sequences present on bacterial chromosomes are less likely to be transferred.

- ***Introduction of sequences into plant cells***. Transformation of plants with Agrobacterium results in stable integration of genetic material into the plant chromosomes. The genomes of some DNA plant viruses can also become inserted into plant genomic DNA.
- ***Recombination between related viruses***. While the phenotype of the GM virus that is under construction is the primary consideration, some thought should also be given to the possibility that harmful sequences may be transferred as the result of a recombination event. Recombination between plant viruses is common, and could lead to persistence of an inserted sequence in a replication-competent virus. For example, recombination is observed in geminiviruses, and has been correlated with enhanced pathogenicity. Interspecies hybrids will often result in a less virulent virus, but some may be more virulent than their progenitors. If a recombination event could give rise to a harmful derivative of a GM plant virus by restoring previously deleted or mutated genes, then great care should be taken to prevent cross-contamination in the laboratory or in plant growth areas.
- ***Reassortment between segmented plant viruses***. Some viruses have segmented genomes and can achieve genetic variability in nature by 'swapping segments' with related viruses. It is important to consider that cross-contamination in the laboratory, or co-infection of the GMM with a wild-type virus in the environment, could result in the generation of novel strains that could be regarded as harmful.

14.1.5 Phenotypic and genetic stability

The stability of the genetic modification should also be considered, particularly where there is the possibility that GMM attenuated or disabled for growth might revert to a wild-type or pathogenic phenotype and become an environmental hazard. Therefore, the genetic stability of the modification may be linked to phenotypic stability, especially where the modification restricts the GMM's ability to survive and to spread.

The loss of an inserted gene from GMM is unlikely to constitute a hazard. However, inherent genetic instability leading to incorporation of genes elsewhere in the genome of the same GMM could be hazardous. An organism with a restricted capacity to survive will be under stress in the environment, and there will be a strong selection pressure for the reversion of attenuating and disabling genetic lesions. The possibility that GMM will be genetically unstable outside of the controlled conditions in which it was intended to exist should be taken into account and consideration given to any detrimental effects this might cause.

14.2 Exposure Characterization

14.2.1 Likelihood that GMM will be a risk to the environment

The initial stages in the risk assessment process thus far involve identifying those features of GMM that have the potential to cause harm, and the mechanisms by which these hazards could be realised. While it may be possible to draw up theoretical scenarios whereby GMO may be hazardous to the environment, the chances of them being realised should be evaluated and understood.

It is therefore important to consider the likelihood that the identified hazards will be manifested. Factors that come into play are:

(i) judgements as to the overall fitness of GMM;

(ii) the probability that rare events may occur (*e.g.* the likelihood of gene transfer); and

(iii) the severity of the possible consequences.

Estimating the likelihood of a harmful consequence being realised will be difficult where there are no firm data on which to base a judgement. In general, the weight given to information used in these considerations should reflect the quality of the supporting data. Where the likelihood of harm is poorly understood, a *cautious approach* should be adopted until evidence to the contrary has been obtained.

14.2.2 Assessment of likelihood

A key factor in whether or not a hazard will be realised is the environment into which GMM would be released. It is therefore important to consider the nature of GMM in relation to the receiving environment. There may be characteristics of the receiving environment that will contribute to the likelihood of the hazard being manifested, *e.g.* the presence of a suitable host species or soil conditions. For the purposes of using the risk determination matrix, likelihood can be expressed as 'highly likely', 'likely', 'unlikely' or 'highly unlikely'.

Even if GMM could conceivably survive, become established and disseminate in the environment, it may be that the environment itself would not be able to support it. For example, GMM derived from pathogens of plants that are not present in Malaysia would have limited capacity to become disseminated, even if it could survive for extended periods. Similarly, the transmission of some pathogens may require an intermediate vector that might not be present in the country. The possibility of unknown hosts or intermediate vectors should be accounted for, as should the longer-term possibility that such hosts and vectors will become native to Malaysia, *e.g.* as a result of climate change.

However, in general, the risk that such GMM could be a hazard to the environment will be negligible.

14.2.3 Consideration of the ability of GMM to become established

An assessment should be made as to the ability of GMM to become established, how efficient it will be, and its ability to spread within a host, population or ecosystem. This represents an evaluation of the 'fitness' of GMM and should be based upon available scientific knowledge. Any uncertainty should be acknowledged and the precautionary principle followed.

The concept of fitness is difficult to define but will clearly be important in assessing the potential for GMM to cause harm if there were to be a breach of containment. For example, over-expression of a toxin in a bacteria or fungus may make GMM more hazardous than the recipient strain, but the over-expression of that toxin might be deleterious to the metabolism of the organism.

An example relating to fitness has been demonstrated with a number of GMM systems, as there is a tendency for inserted sequences to be deleted. The loss of a gene that confers environmental tolerance would therefore reduce the potential for spread and render the virus less fit. However, extra gene carriage should not automatically be presumed to reduce GMM fitness.

14.2.4 Consideration of the probability that rare events will occur

It is often possible to assign a frequency to a given event, *e.g.* mutation, recombination or sequence mobilisation rates. Often, this can take the form of a precise numerical frequency obtained in-house or through published data.

In many cases, precise evaluation will not be possible or properly supported. An approximate, semi-quantitative or descriptive assessment of the frequency, based upon experience with similar GMM or techniques, could be used in these cases. For example, the likelihood of an attenuated or disabled GMM reverting to wild-type status can be assessed on the basis of the number of discrete events that would need to take place, *i.e*. the more events needed, the less likely it is that reversion will occur.

However, it should not be assumed that failure to observe an event is evidence that it does not occur. As part of such considerations it should be recognised that microorganisms often have extremely short generation times, and adapt to specific environments and selective pressures rapidly.

Mutant genomes are continually being generated, and the effects of selection pressure should be assessed. For example, although variants will often be maintained at low frequencies by negative selection, in a situation where a microorganism can replicate in an environment that differs from that in which it is normally found, the probability of one of the genetic variants becoming dominant will be increased. When undertaking risk assessment of GMM, it is important to have some awareness of this genetic variability. Even if GMM that is initially constructed is not well-adapted to growth in a particular environment or host, there is a possibility that it will adapt as new variants arise. Therefore, it is necessary to proceed with caution and use defective recipient strains wherever possible. This will virtually eliminate problems arising from genetic variability.

When estimating the probability and frequency of events, consideration should also be given to the number of organisms that might be involved in the incident. This will depend on the nature of the experiment. However, the probability that a hazard will be realised will often depend on the number of GMM that are being handled and, consequently, the number that could escape.

14.2.5 Assessment of the possible consequences

After the likelihood of all hazards is assessed, the consequence of each hazard should be estimated. Again, the consequence will depend to a very large extent on the potential receiving environment. In particular, the presence of compatible host plants or species with which GMM may be able to compete will be an important consideration.

Evaluation of the magnitude of potential consequence is difficult because there is inevitably a degree of judgement involved, although a qualitative appraisal of the impact on other species or ecosystems should be possible. For the purposes of using the risk determination matrix (*Table 2*), consequences could be described as being 'major, 'intermediate, 'minor' or 'marginal'. The following descriptions may help:

- **Major consequence**: a major change in the numbers of one or more species leading to negative effects on the functioning of the ecosystem and/or other connected ecosystems (*e.g*. a significant alteration in the turnover of biomass, or supply of nutrients to crops). It is unlikely that the changes would be easily reversible.
- **Marginal consequence**: little or no measurable change in any population, *e.g.* plant, animal or microbial, in the environment or in any ecosystem function. (This does not preclude some fluctuation in indigenous populations as long as this is within the range of that which could be expected naturally).

If the consequences of a hazard are deemed 'major, while the likelihood of the hazard being manifested at all was 'highly unlikely', then there is 'moderate' risk of harm. Thus, a cautious approach to risk determination is adopted, leading to more stringent containment requirements. Likewise if the consequence of a hazard were 'marginal' or 'minor', then even if the likelihood of its manifestation were 'highly likely', the risk of harm would still be 'low' or 'moderate', respectively (see *Table 2*).

14.3 Risk Characterization

14.3.1 Determination of risk

The determination matrix in *Table 2* can be used to estimate the level of risk. This matrix is provided as a tool and is not intended to be a definitive measure of risk.

It may be necessary to evaluate whether any specific control measures (risk management strategies) are required to adequately protect the environment. Containment measures should be applied until the risk of harm is 'negligible'.

14.4 Risk Management Strategies

14.4.1 Containment level needed to sufficiently protect against harm to the environment

It is recommended that the minimum containment level (Containment Level 1, 2, 3 or 4) that is necessary to protect the environment be set. At this stage, it is only an estimate of the containment measures that will be required solely for the purpose of preventing the release of GMM, or to minimise the likelihood that it will become a threat to the environment. Factors that may be relevant to this include:

- containment measures required by any plant quarantine and sanitation conditions needed for work on the **recipient** microorganism where it is an unmodified plant pathogen;
- any identified hazards arising as a consequence of the genetic modification, the severity of any harmful consequences and the likelihood that they might occur (determination of the risk of harm, as given above).

If there are no prescribed containment measures for the recipient organism, then a judgement should be made on whether GMM will be a risk to the environment. If all risks are deemed to be 'low' or 'negligible', then no specific measures will be required. However, if any risk exceeds this level, then control measures should be implemented so that the risk of harm to the environment is reduced to 'low' or 'negligible'.

The applicant should judge which measures listed in the Contained Use

Regulations are appropriate for containment of GMM. The containment level can be set accordingly to safeguard the environment. It is recognised that there is a degree of judgement required in setting 'risk values' and containment measures.

14.4.2 Risk assessment for human health

It is recognised that for many activities with GM plant-associated microorganisms, the risk to humans will automatically be low or negligible. The objective is to identify any plausible hazards to human health and then to assess the likelihood and potential severity of the consequences, should the hazards be realised. Where a hazard is identified, this will most likely be associated with modifications that result in the production of a toxin or allergen. Biomanufacture may involve the transformation or transduction of a plant with GMM, resulting in the production of pharmacologically or immunologically active substances.

14.4.2.1 Mechanisms by which GMO could be a risk to human health

As for ERA, the hazard identification process must include considerations of potentially harmful or adverse effects upon human health that would be mediated by the recipient organism, the products of any inserted genes or the predicted properties of the final GMM. However, assessments should concentrate on hazards arising from modification, rather than those associated with the recipient organism.

The majority of human health hazards will most likely arise where toxic products are secreted by GMM. Alternatively, hazards may arise as a result of modifications that alter the properties of an infected plant. Using GMM as a vector in plants that express biologically active compounds might make them more toxic or allergenic.

Where a potential for harm to humans is identified, consideration should be given to whether direct contact with GMM-contaminated material, or with transduced plant materials (*e.g.* leaves, sap or pollen) might represent a hazard. Consideration may also need to be given to the potential for the products to be expressed in different plant tissues, the consequent routes of exposure and the possibility that these may be altered.

Consideration should also be given to the possibility that microbial or plant post-translational processing may differ from mammalian cells. Therefore, potentially toxic or allergenic human or animal products expressed in microbial or plant systems might be processed differently, and there may be unexpected effects due to presentation of novel confirmations.

14.4.2.2 Likelihood that GMM will be a risk to human health

For each identified hazard, an estimation of the likelihood of it being manifested and the seriousness of the consequence should be made in a way similar to the assessment of environmental risks outlined earlier. GMM may have characteristics that might lead to a potential health hazard, but the chances of them being realised should be evaluated and understood. The risk determination matrix can be used as a tool to evaluate the magnitude of the hazards. This will require an estimation of both the likelihood and consequences of exposure. The matrix is not intended to be a definitive measure of risk, thus the specifics of each case should be carefully considered.

Once again, estimating the likelihood of a harmful consequence being realised will be difficult where there are no firm data on which to base a judgement, and the weight given to information should reflect the quality of the supporting data. Where the likelihood of harm is poorly understood, a precautionary approach should be adopted until evidence to the contrary has been obtained. For the purposes of using the risk determination matrix, likelihood can be expressed as 'highly likely', 'likely', 'unlikely' or 'very unlikely'.

Similarly, evaluation of the magnitude of potential consequence may be difficult as it is inevitable that this will involve a degree of judgement. However, a qualitative appraisal of the impact on humans should be possible. For the purposes of using the risk determination matrix, consequences could be described as being 'major', 'intermediate', 'minor', or 'marginal'.

14.4.2.3 Containment level needed to sufficiently protect human health

It is recommended that the minimum containment level (Containment Level 1, 2, 3 or 4) that is necessary to protect human health be set. At this stage, it is only an estimate of the containment measures that will be required solely for the purpose of safeguarding the well-being of those who may come into contact with the GMM.

The measures implemented for environmental protection may be adequate to protect human health as well. In many cases, the principles of good occupational safety and hygiene and good microbiological practice will also be sufficient for this purpose. However, it may be necessary to evaluate whether any specific control measures are required to protect human health. If necessary, containment measures should be applied until the risk of harm is 'negligible'. It is a requirement of the Contained Use Regulations that all measures deemed by the risk assessment as necessary for the protection of human health be implemented.

The applicant should judge which measures listed in the Contained Use Regulations are required to minimise harm to workers exposed to GMM. The containment level can be set accordingly.

14.5 Review of Procedures and Control Measures

The requirements for the final containment level must be sufficient to control all the potential harmful properties of GMM, and offer sufficient protection for both the environment and human health. All risks must be reduced to 'low' or 'negligible'. The containment and control measures identified so far for environmental and human health protection only broadly define those needed as a function of the properties of GMM itself.

The nature of the activity will also affect the level of risk. Therefore, it is important to take into account the nature of the work, or any non-standard operations, that might increase the likelihood of release or risk of exposure. For example, large-scale growth or harvest of GMM will often mean that large amounts of the organism will be handled, which may result in increased likelihood of release and/or exposure.

If any such operations or activities are likely to generate risks that are not accounted for in the minimum containment measures already applied in reaction to the risk assessments for the environment and human health, then additional control measures should be applied. Equally, it may be that as a result of the nature of the activity, the nature of a risk that is inherent to GMM itself is diminished. For example, if GMM are cultured in a sealed system, then exposure to workers might be much less likely. In these cases, certain control measures might not be required.

The person responsible for the work should be satisfied that the local rules covering the use of laboratories or plant growth facilities are adequate to minimise or prevent viable GMM being released from the containment facility. Moreover, there should be a programme of internal inspections and/or active monitoring to ensure that the local rules are satisfactorily implemented. All workers should be trained in good laboratory or glasshouse techniques before commencing work, and should be fully aware of the potential hazards inherent to the activity. Access to the containment facilities should be limited, where appropriate, to authorised personnel and designated workers.

The maintenance schedule for protective apparatus such as safety cabinets and ventilation systems should be strictly adhered to. It is also important that the fabric of the facility and control measures (*e.g.* mesh guards over drains and vents) are regularly checked for possible breaches in containment. One of the major release routes will be via contaminated waste, and it is therefore important that GMM that pose an environmental hazard are adequately inactivated and appropriately disposed of.

A NOTE ON EMERGENCY RESPONSE PLANS

CHAPTER 15

At the conclusion of ERA, the applicant is required to submit emergency response plans to handle each of the risks which had been identified, characterised and analysed to be significant enough to warrant further attention. For this, the review panel recommends the use of existing biosafety risk assessment forms and related contacts to cover the major risks associated in this section of the guidelines

The emergency response plan for each risk provides instructions on one or more of the following:

1. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect observed during contained use activities.
2. Methods for removing the GM plants, their product/s or plant-associated GMM in the affected areas in the case of an unintentional release.
3. Methods for disposing other plants, animals and any other organisms exposed during the unintentional release.
4. Methods for isolating the area affected by the unintentional release.
5. Details of any other contingency measure that should be in place to rectify any unintended consequences if an adverse effect becomes evident during the contained use activities, or when an unintentional release occurs.

REFERENCES

Center for Environmental Risk Assessment (CERA). 2010. GM Crop Database. *http://cera-gmc.org/index.php?action=gm_crop_database* [August 2011]

CERA. 2011. *Problem formulation case study: Commercial introduction of an herbicide tolerant* Brassica napus in *Canada, 1995 and 2010*. Washington, D.C: CERA, ILSI Research Foundation.

Davis, H. V. 2005. GM organisms and the EU regulatory environment: allergenicity as a risk component. *Proceedings of the Nutrition Society* **64**: 481-486.

EFSA (European Food Safety Authority). 2008. *EFSA Scientific Colloquium Summary Report: Environmental Risk Assessment of Genetically Modified Plants – Challenges and Approaches.*

EFSA (European Food Safety Authority). 2009a. Transparency in Risk Assessment – Scientific Aspects: Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: General principles. *The EFSA Journal* **1051**: 1-22.

EFSA(European Food Safety Authority). 2009b. Scientific opinion on statistical considerations for the safety evaluation of GMOs, on request of EFSA. *The EFSA Journal* **1250**: 1-62.

EFSA(European Food Safety Authority). 2010. Scientific opinion on Guidance on the environmental risk assessment of genetically modified plants. *The EFSA Journal* **1879**: 1-111.

FAO. 2011. *Biosafety Resource Book. http://www.fao.org/docrep/014/i1905e/i1905e00.htm* [August 2011]

Heard, M. S., C. Hawes, G. T. Champion, S. J. Clark, L. G. Firbank, A. J. Haughton, A. M. Parish, J. N. Perry, P. Rothery, R. J. Scott, M. P. Skellern, G. R. Squire, and M. I. Hill. 2003. Weeds in fields with contrasting

conventional and genetically modified herbicide-tolerant crops. I. Effects on abundance and diversity. *Philosophical Transactions of the Royal Society B:Biological Sciences* **358**: 1819-1832.

Joint FAO/WHO Food Standards Programme-Codex Alimentarius Commission. 2003. *Codex Alimentarius: Codex Principles and Guidelines on Foods Derived from Biotechnology*. Rome: Food and Agriculture Organization of the United Nations & World Health Organization.

Kruger, M., J. B. J. Van Rensburg, and J. Van den Berg. 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. *Crop Protection* **28**: 684-689.

Laster, L. L. and M. F. Johnson. 2003. Non-inferiority trials: the 'at least as good as' criterion. *Statistics in Medicine* **22**:187-200.

Losey, J. E., L. S. Raynor, and M. E. Carter. 1999. Transgenic pollen harms Monarch larvae. *Nature* **399**:214.

Marvier, M. 2002. Improving risk assessment for non-target safety of transgenic crops. *Ecological Applications* **12**: 1119-1124.

Marvier, M., C. McCreedy, J. Regetz, and P. Kareiva. 2009. A meta-analysis of effects of Bt cotton and maize on non-target invertebrates. *Science* **316**: 1475-1477.

Moar, W., R. Roush, A. Shelton, J. Ferré, S. MacIntosh, B. R. Leonard, and C. Abel. 2008. Field-evolved resistance to Bt toxins. *Nature Biotechnology* **26(10)**:1072-1074.

OECD. 1993. *Safety Considerations for Biotechnology: Scale up of Crop Plants*. Paris:Organisation for Economic Co-operation and Development.

Perry, J. N. 1986. Multiple-comparison Procedures: A Dissenting View. *Journal of Economic Entomology* **79(5)**: 1149-1155.

Perry, J. N., P. Rothery, S. J. Clark, M. S. Heard, and C. Hawes. 2003. Design, analysis and statistical power of the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology* **40(1)**: 17-31.

Perry, J. N., C. J. F. ter Braak, P. M. Dixon, J. J. Duan, R. S. Hails, A. Huesken, M. Lavielle, M. Marvier, M. Scardi, K. Schmidt, B. Tothmeresz, F. Schaarschmidt, and H. van der Voet. 2009. Statistical aspects of environmental risk assessment of GM plants for effects on non-target organisms. *Environmental Biosafety Research* **8**: 65-78.

Raybould, A. and I. Cooper. 2005. Tiered tests to assess the environmental risk of fitness changes in hybrids between transgenic crops and wild relatives: the example of virus resistant Brassica napus. *Environmental Biosafety Research* **4(3)**: 127-40.

Schuirmann, D. J. 1987. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *Journal of Pharmacokinetics and Biopharmaceutics* **15**: 657-680.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. D. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize:Spodoptera frugiperda (Lepidoptera: Noctuidae) in Puerto Rico. *Journal of Economic Entomology* **103(4)**: 1031-1038.

Tabashnik, B. E., A. J. Gassmann, D. W. Crowder, and Y. Carrière. 2008. Insect resistance to Bt crops: evidence versus theory. *Nature Biotechnology* **26(2)**: 200-202.

Tzotzos, G. T., G. P. Head, and R. Hull. 2009. *Genetically Modified Plants: Assessing Safety and Managing Risk*. New York: Academic Press.

USEPA (United States Environmental Protection Agency). 1998. *Guidelines for Ecological Risk Assessment*. EPA/630/R-95/002F. Risk Assessment Forum, Washington, D.C., USA.

Van Rensburg, J. B. J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *South African Journal of Plant and Soil* **24**: 147-151.

Wellek, S. 2002. *Testing Statistical Hypotheses of Equivalence*. Florida: CRC Press.

Wolt, J. D., P. Keese, A. Raybould, J. W. Fitzpatrick, M. Burachik, A. Gray, S. S. Olin, J. Schiemann, M. Sears, and F. Wu. 2010. Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Research* **19(3)**: 425-436.

APPENDIX 1

NBB REF NO :
(For Office Use)

BIOSAFETY ACT 2007

BIOSAFETY REGULATIONS 2010

NBB/A/ER/10/FORM A

APPROVAL FOR RELEASE ACTIVITIES OF LIVING MODIFIED ORGANISM (LMO) (RESEARCH AND DEVELOPMENT PURPOSES IN ALL FIELD EXPERIMENTS) OR IMPORTATION OF LMO THAT IS HIGHER PLANT

NBB/A/ER/10/FORM A shall be submitted to the Director General as an application for certificate of approval of release of LMO [Research and development purposes in all field experiments - Second Schedule of the Act - 1] or importation of living modified organism (LMO) that is a higher plant (not for contained use activities). Any organization undertaking modern biotechnology research and development shall submit the form through its registered Institutional Biosafety Committee (IBC). The IBC should assess the information in the form prior to submission. Application must be accompanied by the prescribed fees as found in Third Schedule of the Biosafety (Approval and Notification) Regulations 2010. Not all parts in this form will apply to every case. Therefore, applicants will only address the specific questions/parameters that are appropriate to individual applications.

In each case where it is not technically possible or it does not appear necessary to give the information, the reasons shall be stated. The risk assessment, risk management plan, emergency response plan and the fulfillment of any other requirements under the Biosafety Act 2007 will be the basis of the issuance of the certificate of approval by the National Biosafety Board (NBB).

The applicant shall submit 1 original and 6 copies of the application to the Director General. A soft copy of the submitted application (including all supporting documents/attachments, if any) shall also be provided in the form of a CD by the applicant. However, all information that has been declared as Confidential Business Information (CBI) should be omitted from the CD.

Accuracy of information

The application should also be carefully checked before submission to ensure that all the information is accurate. If the information provided is incorrect, incomplete or misleading, the NBB may issue a withdrawal of the acknowledgement of receipt of application without prejudice to the submission of a fresh application. Thus, it is important to provide accurate and timely information that is as comprehensive as existing scientific knowledge would permit, and supported by whatever data available.

Confidentiality

Any information within this application which is to be treated as CBI, as described in the Biosafety Act 2007 in section 59(3) should be clearly marked “CBI” in the relevant parts of the application by providing the justification for the request for CBI. The following information shall not be considered confidential:

a) The name and address of the applicant
b) A general description of the LMO
c) A summary of the risk assessment of the effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health; and
d) Any methods and plans for emergency response

NBB REF NO :
(For Office Use)

Authorization
Please ensure that if this application is being completed on behalf of the proposed user, that the person completing this application holds proper authority to submit this application for the proposed user. Please provide written proof of authorization.

For further information
Please contact the Director General by:
Telephone: 603-8886 1579
E-mail: biosafety@nre.gov.my

The completed forms to be submitted as follows:
The Director General
Department of Biosafety
Ministry of Natural Resources and Environment Malaysia,
Level 1, Podium 2
Wisma Sumber Asli, No. 25, Persiaran Perdana
Precinct 4, Federal Government Administrative Centre
62574 Putrajaya, Malaysia

Please retain a copy of your completed form.

NBB REF NO :
(For Office Use)

APPLICATION CHECK LIST

1. Form NBB/A/ER/10/FORM A is completed with relevant signatures obtained	☐
2. Application assessed and to be sent through the IBC	☐
3. A copy of clearance documents from the Department of Agriculture included (if required)	☐
4. A copy of the clearance document from the state office where the release is to take place	☐
5. Any information to be treated as confidential business information should be clearly marked “CBI” in the application	☐
6. 1 original copy and 6 copies of the completed application submitted. A soft copy of the submitted application (including all supporting documents/ attachments, if any) that do not contain any CBI.	☐
7. Fees as prescribed in the regulation: RM _____ Money order/ Bank draft No: _____ Made payable to the Secretary General of the Ministry of Natural Resources and Environment	☐

Preliminary information

1. Organization:	
2. Name of Applicant:	
3. Position in Organization: Telephone (office): Telephone (mobile): Fax number: Email: Postal Address:	
4. Project Title/ Unique Identification Code:	
5. IBC Project Identification No:	
6. Is this the first time an approval is being applied for this activity?	Yes ☐ No ☐ if no, please provide information in no 7 below
7. I) Please provide the NBB reference no. for your previous notification/application. II) How is this application different from the previous notification/application submitted for this activity? (please provide an attachment if additional space is required)	

NBB REF NO :
(For Office Use)

Details of Agent / Importer

8. Organization name:	
9. Contact Person:	
10. Position in Organization: Telephone (office): Telephone (mobile): Fax number: Email: Postal Address:	

Institutional Biosafety Committee (IBC) Assessment Report for release of LMO (Research and development purposes in all field experiments) or importation of LMO that is a higher plant (not for contained use activities).

This must be completed by the registered IBC of the Applicant's organization

Section A – IBC Details

1.	Name of organization:			
2.	Name of IBC Chairperson:			
	Telephone number:		Fax:	
	Email address:			

Section B – IBC Assessment

3.	Name of principal investigator:	
4.	Project Title:	
5.	Date of the IBC Assessment:	
6.	Does the IBC consider that the principal investigator and every other person(s) authorized to be involved in the field experiment with the LMO have adequate training and experience for the task?	☐ Yes ☐ No
7.	The following information related to this project has been checked and approved	
	a) The objective of the project	☐ Yes ☐ No
	b) The description and genetics of the LMO	☐ Yes ☐ No

NBB REF NO :
(For Office Use)

	c) The risk assessment and risk management, taking into account the risks to the health and safety of people and the environment from the release of the LMO.	☐ Yes ☐ No
	d) The emergency response plan	☐ Yes ☐ No
8.	Has the information been checked by the IBC and found to be complete?	☐ Yes ☐ No
9.	Has the IBC assessed the proposed project? ☐ Yes ☐ No If yes, please append a copy of the IBC's assessment report and indicate the attachment in which details are provided.	

Signatures and Statutory Declaration

The proposed release of LMO (Research and development purposes in all field experiments) or importation of LMO that is a higher plant (not for contained use activities) has been assessed as above and endorsed by the IBC. We declare that all information and documents herein is true and correct. We understand that providing misleading information to the NBB, deliberately or otherwise, is an offence under the Biosafety Act 2007.

Applicant:

Signature: _____ Date: _____

Name as in Identity Card/Passport: _____

Official Stamp:

IBC Chairperson:

Signature: _____ Date: _____

Name as in Identity Card/Passport: _____

Official Stamp:

Head of organization/Authorized representative:

Signature: _____ Date: _____

Name as in Identity Card/Passport: _____

Official Stamp:

NBB REF NO :
(For Office Use)

Part A Risk Assessment

A1 General Information

1. Project Title.

2. Rationale of Project.

3. Project objectives:
   a) Overall Objective

   b) Specific Objective

4. Details of the LMO to be released:
   a) Genus and species
   b) Common name
   c) Modified trait(s)

5. Release site(s) :
   (If more than one location is involved, then the information required in numbers 5, 6, 7, 8 & 9, 10, 11) should be repeated for each location(s) of release)
   a) District(s)
   b) State(s) in which the release(s) will take place

6. Scale of release per release site.
   (Number of LMO involved, size of plot/site etc)

7. Date when the release(s) is expected to commence.

8. Frequency of releases.

9. Date when release(s) is expected to end.

10. For an imported LMO – the date of importation or intended importation, including, if possible, a copy of documentation of clearance or assessment from the relevant authorities like Department of Agriculture (DOA).

11. Description of the proposed activities with the LMO.

12. Name of person(s) authorized to undertake activities with the LMO.

A2 Risk Assessment Information - Parent Organism

(If more than one parent organism of the same species is involved then the information required in this part should be repeated for each parent organism)

13. Details of the parent organism
    If the LMO is the result of a crossing event between more than one species/cultivar/breeding line/variety please include relevant information (for example, LMO crossed with non-LMO or 2 LMOs crossed)
    a) Family name
    b) Genus
    c) Species
    d) Subspecies
    e) Cultivar/Breeding line/Variety
    f) Common name

14. A statement about whether the parent organism has an extended history of safe use in agriculture or in other industries.

15. Information concerning the reproduction of the parent organism:
    a) The mode or modes of reproduction
    b) Any specific factors affecting reproduction
    c) Generation time

16 Information regarding the sexual compatibility of the parent organism with other cultivated or wild plant species.

17. Information concerning the survivability of the parent organism:
    a) Ability to form structures for survival or dormancy including seeds, spores and sclerotia
    b) Any specific factors affecting survivability, for example seasonability

18. Information concerning the dissemination of the parent organism:
    a) The means and extent of dissemination
    b) Any specific factors affecting dissemination

19. Details of the natural habitat of the parent organism and its range.

20. Is the parent organism exotic in Malaysia?

    ☐ Yes ☐ No

21. Is the parent organism naturalized in Malaysia?

    ☐ Yes ☐ No

22. Is the parent organism, or a closely related organism, present at, or near, the site of the proposed release(s)?
    (If more than one location is involved, then the information required in numbers 22 & 23 should be repeated for each location(s) of release)

    ☐ Yes ☐ No

23. If yes, please provide details of the population(s) and the estimated distances between them from the proposed release(s).

24. The potentially significant interactions of the parent organism with organisms other than plants in the ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms.

25. An assessment of whether the parent organism is capable of causing disease or other ill-health in human, plants or animals and, if so, the details of the possible effects.

26. Details of any known predators, parasites, pests or diseases of the parent organism in Malaysia.

27. Details of pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonize other organisms.

28. Is the parent organism resistant to any known antibiotic and if yes, what is the potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy?

29. Is the parent organism involved in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration?

A3 Risk Assessment Information - LMO

30. Details of the modified trait(s) and how the genetic modification will change the phenotype of the LMO to be released.

31. What are the gene(s) responsible for the modified trait(s)?

32. Give details of the organism(s) from which the gene(s) of interest is derived:
(If more than one gene is involved then the information required in numbers 32, 33, 34, 35, 36 and 37 should be repeated for each gene)
    a) Family name
    b) Genus
    c) Species
    d) Subspecies
    e) Cultivar/Breeding line/Variety
    f) Common name

33. Indicate whether it is a:
    a) viroid
    b) RNA virus
    c) DNA virus
    d) bacterium
    e) fungus
    f) animal
    g) plant
    h) other (please specify)

34. Does the gene(s) of interest come from an organism that causes disease or other ill-health in humans, plants or animals? Provide details of the possible effects.

35. Please provide the following information about the gene(s) of interest(s):
    a) Size of sequence of the gene(s) of interest inserted
    b) Sequence of the gene(s)of interest inserted
    c) Intended function of the gene(s) of interest
    d) Number of copies of the gene(s) of interest in the construct
    e) Details of the steps involved in the construction
    f) Provide the map(s) of construct(s) indicating the gene(s) of interests and all other regulatory elements that will finally be inserted in the LMO

36. Please provide the following information about the deleted sequence(s):
    a) Size of the deleted sequence(s)
    b) Function of the deleted sequence(s)
    c) Details of the steps involved in the deletion of sequences from the parental organism
    d) Provide the map(s) of construct(s)

37. The following information is on the expression of the gene(s) of interest:
    a) Level of expression of the gene(s) of interest and methods used for its characterization
    b) The parts of the plant where the gene(s) of interest is expressed, such as roots, stem or pollen
    c) Indicate the part(s) of the vector(s) that remains in the LMO
    d) The genetic stability of the gene(s) of interest

38. A description of the methods used for the genetic modification:
    a) How gene(s) of interest was introduced into the parent organism, or
    b) How a sequence of a gene was deleted from the parent organism

39. If no vector was used for the genetic modification please provide details of how the gene(s) of interest is introduced.

40. If vector(s) was used, please provide the following information:
    (If more than one vector was used, then the information required in 40 should be repeated for each vector).
    a) Type of vector
        i. plasmid
        ii. bacteriophage
        iii. virus
        iv. cosmid
        v. phasmid
        vi. transposable element
        vii. other, please specify
    b) Identity of the vector(s)
    c) Information on the degree of which the vector(s) contains sequences whose product or function is not known
    d) Host range of the vector(s)
    e) Potential pathogenicity of the vector(s)
    f) The sequence of transposons and other non-coding genetic segments used to construct the LMO and to make the introduced vector(s) and insert(s) function in those organisms

41. Details of the markers or sequences that will enable the LMO to be identified in the laboratory and under field conditions. Provide appropriate evidence for the identification and detection techniques including primer sequences of the detection of the inserted gene(s) including marker gene(s).

42. Information (biological features) on how the LMO differs from the parent organism in the following respects:
    a) Mode(s) and/or the rate of reproduction
    b) Dissemination

43. If there is any possibility that the inserted gene(s) in the LMO could be integrated into other species at the release site(s) and the surrounding environment and if so, please provide the following details:
    a) The organism(s) to which the modified trait(s) can be transferred to and the frequency at which it can be transferred
    b) The transfer mechanism involved and the techniques that have been used to demonstrate transfer
    c) Any possible adverse effects of the transfer including
        i. Any advantages the affected organism(s) are likely to have over the number of the species that do not contain the inserted gene(s)
        ii. Environmental risks posed by such an advantage

44. The identification and description of the target organism(s), if any.

45. The anticipated mechanism and result of interaction between the released LMO and the target organism(s).

46. The known or predicted interaction on non-target organisms in the release site(s) and the impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and

pathogens.

47. A statement on whether the modified trait(s) of the LMO will change the capacity of the plant to add substances to, or subtract substances from, soil (for example, nitrogen or toxic compounds) and, if so, details of all such changes.

48. Details of any other possible adverse consequences.

49. Details whether the LMO compared to the parent organism that will confer a selective advantage that can impact on survival in the release site(s), including a statement on how stable those features are.

50. Details of whether the modified trait(s) will confer a selective advantage on the LMO compared to the parent organism and if so, the nature of the advantages including a statement on how stable those features are and under what conditions.

51. Details of whether the gene(s) of interest or any part of the vector(s) has the ability to reproduce or transfer to other hosts and, if so, details of the host range.

52. In relation to human health:
    a) The toxic or allergenic effects of the non-viable organisms and/or their metabolic products
    b) The comparison of the organisms to the donor, or (where appropriate) parent organism regarding pathogenicity
    c) The capacity of the organisms for colonization
    d) If the organisms are pathogenic to immunocompetent persons:
        i. diseases caused and mechanisms of pathogenicity including invasiveness and virulence,
        ii. communicability,
        iii. infective dose,
        iv. host range and possibility of alteration,
        v. possibility of survival outside of human host,
        vi. presence of vectors or means of dissemination,
        vii. biological stability,
        viii. antibiotic-resistance patterns,
        ix. allergenicity, and
        x. availability of appropriate therapies.

53. Details of unintended pleiotropic effects (if any), including undesirable effects on agronomic characteristics of the plant which may result from the expression of the gene of interest(s) in the LMO (for example, reduced fertility, increased prevalence, production losses, grain shedding), including an indication of the likelihood of these events.

54. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.

55. Details of how the genetic modification will change the phenotype of the LMO to be released, including information to demonstrate the effect of the genetic modification.

56. Details of the mechanism of pollen spread (by insect vectors or by other means) in the plant population:
    a) Details of pollen viability for the parent organism and of the LMO
    b) Details of any potential pollinators and their range and distribution in Malaysia
    c) Quantitative data on successful cross-pollination between the parent organism, the LMO and its wild relatives, if available

A4 Information about weeds

57. Details of the members of the family of parent organism that are known to be weeds in any environment.

58. Details of cross-pollination between the species to which the LMO belongs and wild relatives known to be weeds, including a copy of any literature reports that support the information.

A5 Information about the seeds of the LMO

59. A statement on whether the LMO proposed to be released will be allowed to set seed and, if not, whether setting seed is planned for a later release.

60. If the LMO is to be allowed to set seed, will the mature seed normally remain contained within an ear, capsule or pod, so that practically all of the seed can be readily harvested, or is the seed shed soon after it matures?
If the latter, provide an indication of the proportion of seed likely to remain in the release site(s) following harvest.

61. Details of the length of time that the seeds are capable of being dormant and whether it differs from the parent organism.

A6 Characteristics affecting survival of LMO

62. The predicted habitat of the LMO.

63. The biological features which affect survival, multiplication and dispersal.

64. The known or predicted environmental conditions which may affect survival, multiplication and dispersal, including wind, water, soil, temperature, pH.

65. The sensitivity to specific agents (e.g. disinfectant, pesticides, fertilizers, wind, water).

A7 Information about any secondary ecological effects that might result from the release

66. An assessment of possible effects of the proposed release on:
    a) Native species
    b) Resistance of insect populations to an insecticide
    c) Abundance of parasites

A8 Information about resistance of the LMO to a chemical agent (other than selective agents, such as antibiotics, used in strain construction)

67. Details of any environmental risks related specifically to the resistance of the LMO to a chemical agent (for example, a herbicide, but not a selective agent, such as an antibiotic, used in strain construction), where the resistance is a result of the genetic modification.

A9 Information about resistance of the LMO to a biological agent

68. Details of any environmental risks related specifically to the resistance of the LMO to a biological agent (for example, an insect or a fungal disease), where the resistance is a result of the genetic modification.

A10 Information relating to the release site(s)

(If more than one release site is involved, then the information required in this part should be repeated for each release site)

69. The size of the proposed release site(s).

70. The location of the proposed release site(s). Provide site map(s) with national grid reference(s).

71. Details of the reasons for the choice of the release site(s).

72. Details of the arrangements for conducting any other activities in association with the proposed release(s), such as importation of the LMO and transportation of the LMO, to or from the release site(s).

73. The preparation of the release site(s) before the release(s).

74. The methods to be used for the release(s).

75. The quantity of the LMO to be released.

76. The physical or biological proximity of the release site(s) to humans and other significant biota or protected areas.

77. The size of local human population.

78. The local economic activities which are based on the natural resources of the area.

79. The distance to the nearest drinking water supply zone areas and/or areas protected for environmental purposes.

80. The flora and fauna, including crops, livestock and migratory species in the release site(s).

81. The comparison of the natural habitat of the parent organism(s) with the proposed release site(s).

82. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Part B Risk Management

B1 Information on control, monitoring, post-release plans

83. A description of measures (if any) to minimize the effects of any transfer of the modified genetic trait(s) to other organisms.

84. Details of the proposed release site(s) supervision procedures and if necessary any relevant safety procedures designed to protect staff, including a description of procedures for onsite supervision of the release if the release site(s) is located at some distance from the location of the applicant.

85. Details of proposed measures (if any) for monitoring any risks posed by the LMO(s), including monitoring for:
    a) The survival or presence of the LMO, or transferred genetic material, beyond the proposed release site(s), including specificity, sensitivity and reliability of detection methods
    b) Impacts on the characteristics, or abundance, of other species
    c) Transfer of the gene(s) of interest to other species
    d) Any other hazards or deleterious effect

86. Details of proposed procedures for auditing, monitoring and reporting on compliance with any conditions imposed by the NBB.

87. Details of ongoing monitoring to be undertaken after the release(s) are completed.

88. Details of proposed measures to minimize the possible adverse consequences. If no measures have been taken, please give reasons.

89. The methods for elimination or inactivation of the organisms at the end of the experiment and the measures proposed for restricting the persistence of the LMO or its genetic material in the release site(s).

B2 Waste treatment plans

90. Type of waste generated.

91. Expected amount of waste.

92. Possible risks resulting from the waste.

93. Description of waste treatment envisaged and its disposal.

Part C Emergency response plan

94. Methods and procedures for controlling/removing the LMO in case of unintentional release or any adverse effects being realized.

95. Methods for isolation of the area affected.

96. Methods for disposal of other plants, animals and any other thing exposed to the adverse effects

Part D Data or results from any previous release(s) of the LMO

97. Give the following information from the previous applications and releases of the LMO for which the applicant is seeking an approval:
    i. Reference number of each application
    ii. Date of the certificate of approval issued
    iii. Terms and conditions (if any) attached to the approval
    iv. Data and results of post-release monitoring methods and effectiveness of any risk management procedures, terms and conditions and other relevant details
    v. Relevant data if the previous release is on a different scale or into a different ecosystem
    vi. Any other relevant details

98. Details of results of any applications made for approval of the LMO in other countries, including information about conditions (if any) attached to the approval.

99. Details of any previous notifications for contained use activities according to the Biosafety Act 2007 from which the work in this present application has been developed.

100. If the LMO has been previously released overseas, details of any adverse consequences of the release, including identifying references and reports of assessments if any.

APPENDIX 2

NBB REF NO :
(For Office Use)

BIOSAFETY ACT 2007

BIOSAFETY REGULATIONS 2010

NBB/A/ER/10/FORM C

APPROVAL FOR RELEASE ACTIVITIES (SECOND SCHEDULE 2-6) OR IMPORTATION OF LIVING MODIFIED ORGANISM (LMO) THAT IS A HIGHER PLANT AND PRODUCT OF SUCH ORGANISM

NBB/A/ER/10 FORM C shall be submitted as an application for certificate of approval for release activities (SECOND SCHEDULE 2-6) or importation for release of living modified organism (LMO) that is a higher plant and product of such organism(not for contained use activities) . Application must be accompanied by the prescribed fees as found in Third Schedule of the Biosafety (Approval and Notification) Regulations 2010. Not all parts in this form will apply to every case. Therefore, applicants will only address the specific questions/parameters that are appropriate to individual applications.

If the application is for release activities of an LMO or importation for release of an LMO that is a higher plant, please fill up Part A – D.
If the application is for release activities of a product of such organism or importation for release of a product of such organism, please fill up Part E.

In each case where it is not technically possible or it does not appear necessary to give the information, the reasons shall be stated. The risk assessment, risk management plan, emergency response plan and the fulfillment of any other requirements under the Biosafety Act 2007 will be the basis of the issuance of the certificate of approval by the National Biosafety Board (NBB).

The applicant shall submit 1 original and 6 copies of the application to the Director General. A soft copy of the submitted application (including all supporting documents/attachments, if any) shall also be provided in the form of a CD by the applicant. However, all information that has been declared as Confidential Business Information (CBI) should be omitted from the CD.

Accuracy of information
The application should also be carefully checked before submission to ensure that all the information is accurate. If the information provided is incorrect, incomplete or misleading, the NBB may issue a withdrawal of the acknowledgement of receipt of application without prejudice to the submission of a fresh application. Thus, it is important to provide accurate and timely information that is as comprehensive as existing scientific knowledge would permit, and supported by whatever data available.

Confidentiality
Any information within this application which is to be treated as CBI , as described in the Biosafety Act 2007 in section 59(3) should be clearly marked “CBI” in the relevant parts of the application by providing the justification for the request for CBI. The following information shall not be considered confidential:
a) The name and address of the applicant
b) A general description of the LMO

c) A summary of the risk assessment of the effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health; and
d) Any methods and plans for emergency response

Authorization

Please ensure that if this application is being completed on behalf of the proposed user, that the person completing this application holds proper authority to submit this application for the proposed user. Please provide written proof of authorization.

For further information

Please contact the Director General by:
Telephone: 603-8886 1579
E-mail: biosafety@nre.gov.my

The completed forms to be submitted as follows:

The Director General
Department of Biosafety
Ministry of Natural Resources and Environment Malaysia,
Level 1, Podium 2
Wisma Sumber Asli, No. 25, Persiaran Perdana
Precinct 4, Federal Government Administrative Centre
62574 Putrajaya, Malaysia.

Please retain a copy of your completed form.

NBB REF NO :
(For Office Use)

APPLICATION CHECK LIST

1. Form NBB/A/ER/10/FORM B is completed with relevant signatures obtained	☐
2. A copy of the clearance documents from the Department of Agriculture included. (If required)	☐
3. Any information to be treated as confidential business information should be clearly marked "CBI" in the application	☐
4. 1 original and 6 copies of the completed applications submitted. A soft copy of the submitted application (including all supporting documents/ attachments, if any) that do not contain any CBI.	☐
5. Fees as prescribed in the regulation: RM _____ Money order/ Bank draft No: _____ Made payable to the Secretary General of the Ministry of Natural Resources and Environment	☐

Preliminary information

1. Organization:	
2. Name of Applicant:	
3. Position in Organization: Telephone (office): Telephone (mobile): Fax number: Email: Postal Address:	
4. Product Name (commercial and other names) Unique Identification Code:	
5. Type of release activity:	☐ Supply or offer to supply for sale/ placing on the market ☐ Offer as gift, prize or free item ☐ Disposal ☐ Remediation purposes ☐ Commercial planting ☐ Any other activity which does not amount to contained use (please specify)
6. Is this the first time an approval is being applied for this activity?	Yes ☐ No ☐ if no, please provide information in no 7 below

NBB REF NO :
(For Office Use)

7. I) Please provide the NBB reference no. for your previous notification/application II) How is this application different from the previous application submitted for this activity? (please provide an attachment if additional space is required)	

Details of Agent / Importer

8. Organization name:	
9. Contact Person:	
10. Position in Organization: Telephone (office): Telephone (mobile): Fax number: Email: Postal Address:	

Signatures and Statutory Declaration

We declare that all information and documents herein is true and correct. We understand that providing misleading information to the NBB, deliberately or otherwise, is an offence under the Biosafety Act 2007.

Applicant:

Signature: _____ Date: _____

Name as in Identity Card/Passport: _____

Official Stamp:

Head of organization/Authorized representative:

Signature: _____ Date: _____

Name as in Identity Card/Passport: _____

Official Stamp:

NBB REF NO :
(For Office Use)

Part A Living Modified Organism (LMO) that is a Higher Plant

Risk Assessment

A1 General Information

1. Details of the LMO to be released:
   a) Genus and species
   b) Common name
   c) Modified trait (s)

2. Objective(s) of the release.

3. Release site(s):
   (If more than one site is involved, then the information required in numbers 3, 4, 5, 6, 7 & 8 should be repeated for each release site)
   a) District(s),
   b) State(s) in which the release(s) will take place.

4. Scale of release per release site.
   (Number of LMO involved, size of plot/ site etc)

5. Date when the release(s) is expected to commence.
   (Frequency of releases)

6. For an imported LMO – the date of importation or intended importation, including, if possible, a copy of documentation of clearance or assessment from the relevant authorities like Department of Agriculture (DOA), Ministry of Health, Malaysia.

7. Description of the proposed activities with the LMO.

8. Name of person(s) authorized to undertake activities with the LMO.

A2 Risk Assessment Information - Parent Organism

(If more than one parent organism of the same species is involved then the information required in this part should be repeated for each parent organism)

9. Details of the parent organism:
   If the LMO is the result of a crossing event between more than one species/cultivar/ breeding line/variety, please include relevant information (for example, LMO crossed with non-LMO or 2 LMOs crossed)
   a) Family name
   b) Genus
   c) Species
   d) Subspecies
   e) Cultivar/Breeding line/Variety
   f) Common name

10. A statement about whether the parent organism has an extended history of safe use in agriculture or in other industries.

11. Information concerning the reproduction of the parent organism:
    a) The mode or modes of reproduction
    b) Any specific factors affecting reproduction
    c) Generation time

12. Information regarding the sexual compatibility of the parent organism with other cultivated or wild plant species.

13. Information concerning the survivability of the parent organism:
    a) Ability to form structures for survival or dormancy including seeds, spores and scloretia,
    b) Any specific factors affecting survivability (e.g. seasonability).

14. Information concerning the dissemination of the parent organism:
    a) The means and extent of dissemination
    b) Any specific factors affecting dissemination.

15. Details of the natural habitat of the parent organism and its range.

16. Is the parent organism exotic in Malaysia?

    ☐ Yes ☐ No

17. Is the parent organism naturalized in Malaysia?

    ☐ Yes ☐ No

18. Is the parent organism, or a closely related organism, present at, or near, the site of the proposed release?
    (If more than one location is involved, then the information required in numbers 18 & 19 should be repeated for each location(s) of release)

    ☐ Yes ☐ No

19. If yes, please provide details of the population or populations and the estimated distances between them from the proposed release(s).

20. The potentially significant interactions of the parent organism with organism other than plant in ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms.

21. An assessment of whether the parent organism is capable of causing disease or other ill-health in human, plants or animals and, if so, the details of the possible effects.

22. Details of any known predators, parasites, pests or diseases of the parent organism in Malaysia.

23. Details of pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonize other organisms.

24. Is the parent organism resistant to any known antibiotic and if yes, what is the potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy?

25. Is the parent organism involved in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration?

A3 Risk Assessment Information - LMO

26. Details of the modified trait(s) and how the genetic modification will change the phenotype of the LMO to be released.

27. What are the gene(s) responsible for the modified trait(s)?

NBB REF NO :
(For Office Use)

28. Give details of the organism(s) from which the gene(s) of interest is derived :
(If more than one gene is involved then the information required in numbers 28, 29, 30, 31, 32 & 33 should be repeated for each gene)
    a) Family name
    b) Genus
    c) Species
    d) Subspecies
    e) Cultivar/Breeding line/Variety
    f) Common name

29. Indicate whether it is a:
    a) viroid
    b) RNA virus
    c) DNA virus
    d) bacterium
    e) fungus
    f) animal
    g) plant
    h) other (please specify)

30. Does the gene(s) of interest come from an organism that causes disease or other ill-health in humans, plants or animals? Provide details of the possible effects.

31. Please provide the following information about the gene(s) of interest:
    a) Size of sequence of the gene(s) of interest inserted
    b) Sequence of the gene(s) of interest inserted
    c) Intended function of the gene(s) of interest
    d) Number of copies of the gene(s) of interest in the construct
    e) Details of the steps involved in the construction
    f) Provide the map(s) of construct(s) indicating the gene(s) of interests and all other regulatory elements that will finally be inserted in the LMO

32. Please provide the following information about the deleted sequence(s):
    a) Size of the deleted sequence(s)
    b) Function of the deleted sequence(s)
    c) Details of the steps involved in the deletion of sequences from the parental organism
    d) Provide the map(s) of construct

33. The following information is on the expression of the gene(s) of interest:
    a) Level of expression of the gene(s) of interest and methods used for its characterization,
    b) The parts of the LMO where the gene(s) of interest is expressed, such as roots, stem or pollen
    c) Indicate the part(s) of the vector(s) that remains in the LMO
    d) The genetic stability of the gene(s) of interest.

34. A description of the methods used for the genetic modification:
    a) How gene(s) of interest was introduced into the parent organism, or
    b) How a sequence of a gene was deleted from the parent organism

35. If no vector was used for the genetic modification, please provide the detail of how the gene(s) of interest is introduced.

36. If vector(s) was used, please provide the following information:
(If more than one vector was used, then the information required in 36 should be repeated for each vector)

a) Type of vector:
   i. plasmid
   ii. bacteriophage
   iii. virus
   iv. cosmid
   v. phasmid
   vi. transposable element
   vii. other, please specify
b) Identity of the vector (s)
c) Information on the degree of which the vector (s) contains sequences whose product or function is not known
d) Host range of the vector(s)
e) Potential pathogenicity of the vector(s)
f) The sequence of transposons, and other non-coding genetic segments used to construct the LMO and to make the introduced vector(s) and insert(s) function in those organisms

37. Details of the markers or sequences that will enable the LMO to be identified in the laboratory and under field conditions. Provide appropriate evidence for the identification and detection techniques including primer sequences for the detection of the inserted genes including marker genes.

38. Information (biological features) on how the LMO differs from the parent organism in the following respects:
    g) Mode(s) and/or the rate of reproduction
    a) Dissemination

39. If there is any possibility that the inserted genes in the LMO could be integrated into other species at the release site(s) and the surrounding environment, and if so, please provide the following details:
    a) The organism(s) to which the modified trait(s) can be transferred to and the frequency at which it can be transferred
    b) The transfer mechanism involved and the techniques that have been used to demonstrate transfer
    c) Any possible adverse effects of the transfer including
       i. Any advantages the affected organism(s) are likely to have over the number of the species that do not contain the inserted gene(s)
       ii. Environmental risks posed by such an advantage

40. The identification and description of the target organism(s), if any.

41. The anticipated mechanism and result of interaction between the released LMO and the target organism(s).

42. The known or predicted interaction on non-target organisms in the release site(s) and the impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.

43 A statement on whether the modified trait(s) of the LMO will change the capacity of the plant to add substances to, or subtract substances from, soil (for example, nitrogen or toxic compounds) and, if so, details of all such changes.

44. Details of any other possible adverse consequences.

45. Details of whether the modified trait(s) will confer a selective advantage on the LMO compare to the parent organism and if so, the conditions including data on the growth rate with and without the selection pressure and the nature of the advantages including a statement on how stable those features are.

46. Details of the genetic changes, if any, which will be included in the LMO to limit or eliminate any capacity to reproduce or transfer genes to other organism.

47. In relation to human health:
    a) The toxic or allergenic effects of the non-viable organisms and/or their metabolic products
    b) The comparison of the organisms to the donor, or (where appropriate) parent organism regarding pathogenicity
    c) The capacity of the organisms for colonization
    d) If the organisms are pathogenic to immunocompetent persons:
        i. diseases caused and mechanisms of pathogenicity including invasiveness and virulence
        ii. communicability
        iii. infective dose
        iv. host range and possibility of alteration
        v. possibility of survival outside of human host
        vi. presence of vectors or means of dissemination
        vii. biological stability
        viii. antibiotic-resistance patterns
        ix. allergenicity, and
        x. availability of appropriate therapies

48. Details of unintended pleiotropic effects (if any), including undesirable effects on agronomic characteristics of the plant which may result from the expression of the gene of interest(s) in the LMO (for example, reduced fertility, increased prevalence, production losses, grain shedding), including an indication of the likelihood of these events.

49. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.

50. Details of how the genetic modification will change the phenotype of the LMO to be released, including information to demonstrate the effect of the genetic modification.

51. Details of the mechanism of pollen spread (by insect vectors or by other means) in the plant population:
    a) Details of pollen viability for the parent organism and of the LMO
    b) Details of any potential pollinators and their range and distribution in Malaysia

    c) Quantitative data on successful cross-pollination between the parent organism, the LMO and its wild relatives, if available

A4 Information about weeds

52. Details of the members of the family of parent organism that are known to be weeds in any environment.

53. Details of cross-pollination between the species to which the LMO belongs and wild relatives known to be weeds, including a copy of any literature reports that support the information.

A5 Information about the seeds of the LMO

54. A statement on whether the LMO proposed to be released will be allowed to set seed and, if not, whether setting seed is planned for a later release.

55. If the LMO is to be allowed to set seed, will the mature seed normally remain contained within an ear, capsule or pod, so that practically all of the seed can be readily harvested, or is the seed shed soon after it matures?
If the latter, provide an indication of the proportion of seed likely to remain in the environment following harvest.

56. Details of the length of time that the seeds are capable of being dormant and whether it differs from the parent organism.

A6 Characteristics affecting survival of LMO

57. The predicted habitat of the LMO.

58. The biological features which affect survival, multiplication and dispersal.

59. The known or predicted environmental conditions which may affect survival, multiplication and dispersal, including wind, water, soil, temperature, pH.

60. The sensitivity to specific agents (e.g. disinfectant, pesticides, fertilizers, wind, water).

A7 Information about any secondary ecological effects that might result from the release

61. An assessment of possible effects of the proposed release on:
    a) Native species
    b) Resistance of insect populations to an insecticide
    c) Abundance of parasites

A8 Information about resistance of the LMO to a chemical agent (other than selective agents, such as antibiotics, used in strain construction)

62. Details of any environmental risks related specifically to the resistance of the LMO to a chemical agent (for example, a herbicide, but not a selective agent, such as an antibiotic, used in strain construction), where the resistance is a result of the genetic modification.

A9 Information about resistance of the LMO to a biological agent

63. Details of any environmental risks related specifically to the resistance of the LMO to a biological agent (for example, an insect or a fungal disease), where the resistance is a result of the genetic modification.

A10 Information relating to the release site(s)
(If more than one release site is involved, then the information required in this part should be repeated for each release site)

64. The size of the proposed release site(s).

65. The location of the proposed release site(s). Provide site map(s) with national grid reference(s).

66. Details of the reasons for the choice of the release site(s).

67. Details of the arrangements for conducting any other activities in association with the

proposed release(s), such as importation of the LMO and transportation of the LMO, to or from the release site(s).

68. The preparation of the release site(s) before the release(s).

69. The methods to be used for the release(s).

70. The quantity of LMO to be released.

71. The physical or biological proximity of the release site(s) to humans and other significant biota or protected areas.

72. The size of local human population.

73. The local economic activities which are based on the natural resources of the area.

74. The distance to the nearest drinking water supply zone areas and/or areas protected for environmental purposes.

75. The flora and fauna, including crops, livestock and migratory species in the release site(s).

76. The comparison of the natural habitat of the parent organism with the proposed release site(s).

77. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Part B Risk Management

B1 Information on control, monitoring, post-release plans

78. A description of measures (if any) to minimize the effects of any transfer of the modified trait(s) to other organisms.

79. Details of the proposed release site(s) supervision procedures and if necessary any relevant safety procedures designed to protect staff, including a description of procedures for onsite supervision of the release if the release site(s) is located at some distance from the location of the applicant.

80. Details of proposed measures (if any) for monitoring any risks posed by the LMO, including monitoring for:
    a) The survival or presence of the LMO, or transferred genetic material, beyond the proposed release site(s), including specificity, sensitivity and reliability of detection methods
    b) Impacts on the characteristics, or abundance, of other species
    c) Transfer of the gene(s) of interest to other species.
    d) Any other hazards or deleterious effect

81. Details of proposed procedures for auditing, monitoring and reporting on compliance with any conditions imposed by the NBB.

82. Details of ongoing monitoring to be undertaken after the release(s) are completed.

83. Details of proposed measures to minimize the possible adverse consequences. If no measures have been taken, please give reasons.

84. The methods for elimination or inactivation of the organisms at the end of the release and

measures proposed for restricting the persistence of the LMO or its genetic material in the release site(s).

B2 Waste treatment plans

85. Type of waste generated.

86. Expected amount of waste.

87. Possible risks resulting from the waste.

88. Description of waste treatment envisaged and its disposal.

Part C Emergency Response Plan

89. Methods and procedures for controlling the LMO in case of any unintentional release and adverse effects being realized.

90. Methods for isolation of affected area.

91. Methods for disposal of other plants, animals and any other thing exposed to the adverse effects during the unintentional release.

Part D Data or results from any previous release(s) of the LMO

92. Give the following information from the previous applications (successful or unsuccessful) and releases of the LMO for which the applicant is seeking an approval:
    a. Reference number of each application
    b. Date of the certificate of approval issued
    c. Terms and conditions (if any) attached to the approval
    d. Data and results of post-release monitoring methods and effectiveness of any risk management procedures, terms and conditions and other relevant details
    e. Relevant data if the previous release is on a different scale or into a different ecosystem
    f. Any other relevant details

93. Details of results of any applications made for approval of the LMO in other countries, including information about conditions (if any) attached to the approval.

94. Details of any previous notifications for contained use activities according to the Biosafety Act 2007 from which the work in this present application has been developed.

95. Give details of data or results from any previous release of the LMO(s) for which the applicant is seeking an approval, especially the results of monitoring and the effectiveness of any risk management procedures, terms and conditions and any other relevant details.

PART E - Product of Such Organism

E1 General Information

96. The name and address of the manufacturer or distributor of the product.

97. General description of the product:
    a) Type of product
    b) Composition of the product
    c) Physical state of the product

98. For an imported product – the date of importation or intended importation, including, if

possible, a copy of documentation of clearance or assessment from the relevant authorities like Department of Agriculture (DOA), Ministry Of Health, Malaysia.

99. The type of environment and/or the geographical areas within Malaysia for which the product is suited.

100. The type of expected use of the product and the description of the persons who are expected to use the product.

E2 Information regarding proposed labeling of the product (according to Malaysian regulations on the labeling of genetically modified food)

101. Is the product being simultaneously notified to another country?

☐ Yes ☐ No

If yes, please specify.

102. Is the same product marketed in a country outside Malaysia?

☐ Yes ☐ No

If yes, please supply the following information:

a) Name of country
b) Authority which granted consent (if applicable)
c) Conditions under which consent was given (if applicable)

103. Has the product ever been withdrawn from the market of a country?

☐ Yes ☐ No

If yes, please supply the following information:

a) Name of country or countries
b) Reasons for withdrawing the product, if known

104. Has the product been rejected by authorities of a country?

☐ Yes ☐ No

If yes, please supply the following information:

a) Name of country or countries
b) Authority which rejected the product
c) Reasons for rejecting the product, if known

105. Description of identification and detection techniques for the LMO(s) in the product.

E3 Description of the LMO from which the product was derived from

(If the product is derived from more than one LMO, then the information required in numbers 106,107,108,109 & 110 should be repeated for each LMO)

106. Description of the LMO:
a) Genus and species
b) Common name
c) Modified trait(s)
d) Gene(s) responsible for the modified trait(s)

107. Details of the parent organism:
    a) Genus and species
    b) Common name

108. A statement about whether the parent organism has an extended history of safe use in agriculture and other industries.

109. Give the name of the organism from which the gene(s) of interest is derived from:
    a) Genus and species
    b) Common name

110. Indicate whether the organism from which the gene(s) of interest is derived from is a:
    a) virus
    b) bacterium
    c) fungus
    d) animal
    e) plant
    f) other (please specify)

E4 Risk Management of the Product

111. Specific instructions or recommendations for storage and handling of the product.

112. Measures for waste disposal and treatment of the product.

E5 Emergency Response Plan

113. Details of proposed measures to be taken in the event of adverse consequences/ misuse of the product.