ENVIRONMENTAL RISK ASSESSMENT FRAMEWORK

for genetically modified organisms:

a guidance document

environment & tourism

Department:
Environmental Affairs & Tourism
REPUBLIC OF SOUTH AFRICA

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Acronyms

DEAT	Department of Environmental Affairs & Tourism
EIA	Environmental Impact Assessment
ERA	Environmental Risk Assessment
GMO	Genetically Modified Organism
I & AP's	Interested and Affected Parties
ICM	Integrated Crop Management
NBSAP	National Biodiversity Strategy and Action Plan
NEMA	National Environmental Management Act
NEMBA	National Environmental Management: Biodiversity Act
NGO	Non-Governmental Organisation
SOE	State of Environment

Director General's Foreword

The South African government, through the Department of Science and Technology is making large investments in Biotechnology Research and Development to establish South Africa as a world-class player in the global quest for new knowledge that uses biotechnology to solve major problems and that capitalizes on the technology to improve quality of life. Biotechnology is being targeted by both industrialized and developing countries as one of the most important drivers of jobs and economic and social progress in the 21st century. Within our own continent, NEPAD has developed a Science and Technology Strategy which identifies biotechnology as a key tool for sustainable development in Africa. The growing importance of biotechnology applications requires robust regulatory regimes that nurture innovation and build public trust and confidence.

Biotechnology refers to a set of biological techniques that use living organisms or their constituent parts to make a product or run a process. A Genetically Modified Organism(GMO) is a living organism (plant, animal or microorganism) that has been genetically altered using genetic engineering techniques. Applications of biotechnology are far-reaching and will fundamentally change traditional approaches to health care, agriculture and environmental sustainability. The environment is constantly under pressure from a number of developments including those in the field of agriculture. As a result, there is a need to ensure that while generating and harnessing appropriate technologies capable of meeting the global food insecurity challenge, environmental matters are given due consideration. The cultivation of genetically modified (GM) crops is changing agriculture practices in a number of developed and developing countries. Biotechnology has immense potential for improving quality of life, particularly through advancement in agriculture and health care. Notwithstanding these significant contributions to social and economic development, genetic engineering and its resultant Genetically Modified Organisms (GMOs) can put a strain on our biodiversity resources.

DEAT is undertaking a number of initiatives in terms of environmental and regulatory issues in order to achieve the following vision: "Biosafety challenge managed to enhance the quality of life of South Africans in terms of environmental safety, social and economic development by positioning South Africa as a responsible world leader in biotechnology." This guideline document is aimed at contributing to the broader understanding of environmental Biosafety considerations.
The process of developing this guideline document has entailed a number of multi-stakeholder consultations involving research and development partners; the various industry sectors; civil society; scientific advisory bodies as well as an internal coordinating mechanism within government.
This initial version of the ERA framework has focused on plants, however, in the near future, additional guidance will be made available on new areas of biotechnology development, namely Genetically modified trees, fish and viruses.

The main pressures on biodiversity result from land use changes (usually associated with increasing populations); unsustainable use and exploitation of natural resources (especially fisheries, agriculture, and forestry); global climate change; and industrial pollution. At the same time, biotechnology is introducing new organisms and their effect on existing organisms and habitats also needs to be considered.

South Africa is signatory to a number of international instruments, notably the Cartagena Protocol on Biosafety and the Convention on Biological Diversity, and as a result DEAT has endeavored to apply international best practice through the guidance document. The Environmental Risk Assessment Framework provides an explanation of how DEAT apply internationally recognized risk analysis practice in the context of our legislation that is the National Environmental Management Biodiversity Act 10 of 2004. As such, this document is a key reference for those working with genetically modified organisms in South Africa and the general public to help understand how environmental risks are identified and assessed. The ERA framework is aimed at increasing mutual understanding among regulators, applicants and the public at large on environmental Biosafety considerations. This in turn will ensure improved environmental safety, while reducing unnecessary delays in decision making. A feature of biotechnology that distinguishes it from other technological innovations is its basis in genetic manipulation and, consequently, the strong — and, at times, polarized — views in society about some applications of this technology. This is no different in South Africa, and as a result a concerted effort has been made to engage all stakeholders in the development of this guideline document. The DEAT experience suggests that open engagement with different opinions and values helps to reveal a more complex and diverse picture of public attitudes and interests, allowing policy-makers to see ways forward.

I would like to take this opportunity to express my gratitude to all those who have provided advice and feedback during our consultation processes. It is my sincerest hope that this guideline document will make an important contribution to environmental risk assessment. I look forward to further input and debate on the ongoing evolution of this important reference document.

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DEAT

SECTION 1: INTRODUCTION, SCOPE AND AIMS

1.1 General Introduction

In recognition of the potential role of biotechnology in addressing the sustainable development imperatives of South Africa, a National Biotechnology Strategy was adopted in 2001. South Africa had a long history of engagement of traditional biotechnology and as a result the progression into third generation biotechnology entailing genetically modified organisms was a natural progression. In order to support these developments, South Africa has a stringent biosafety regulatory system that ensures that the technology is utilized in a manner that causes minimum disruption to the environment while at the same time addressing the country's sustainable development goals and imperatives. South Africa is also a signatory to the Cartagena Protocol on Biosafety, and therefore has an obligation to implement an effective system to monitor and regulate Genetically Modified Organisms (GMOs). South Africa believes that in the future, applications of biotechnology may contribute to the mitigation of the environmental impacts of agriculture and therefore continues to invest in capacity building initiatives to this end. However, concerns have been raised about the possible negative impacts of widespread planting of GMO crops on South Africa's rich and unique biodiversity, highlighting the need to strengthen legislation, decision-making, monitoring and enforcement (Pretty, 2001).These concerns also underscore the need to take a precautionary approach to the release of GMOs into the environment, especially in biodiversity priority areas. In the case of agricultural crops consideration has to be given as to whether the crop is indigenous or exotic – this distinction is important in terms of environmental and biodiversity impacts. Any restrictions deemed applicable to GM crops in "biodiversity priority areas" should be based on concerns that are generally applicable to agricultural activity at large. This means that the same restrictions that would already apply to conventional agriculture in those areas will apply to GM crops in these same areas. Conventional agriculture is considered to be the baseline. It is important that policy and legislation between sectors is aligned, that adequate and relevant information on GMOs is made available to interested and affected parties and decisions regarding release of GMOs into the environment are transparent.

1.1.1 National Biodiversity Strategy and Action Plan

The National Biodiversity Strategy and Action Plan (NBSAP) sets out a framework and a plan of action for the conservation and sustainable use of South Africa's biological diversity and the equitable sharing of benefits derived from this use. The goal of the NBSAP is to conserve and manage terrestrial and aquatic biodiversity to ensure sustainable and equitable benefits to the people of South Africa, now and in the future.

The NBSAP has five strategic objectives that were identified in order to meet its overall goals. One of these Strategic Objectives calls for integrated terrestrial and aquatic management to minimize the impacts of threatening processes on biodiversity. GMOs together with land degradation, and the spread of invasive alien species have been included in the list of threats or drivers of negative environmental change (Paoletti & Pimentel,1996). Through the consultative processes under the NBSAP a number of outcomes were identified together with the related activities. Outcome 3.5 of the NBSAP addresses the effective management and control measures to minimize the potential risks to biodiversity posed by GMOs.
Integrated and adaptive management needs to be applied to processes that threaten biodiversity to ensure a continued flow of ecosystem goods and services. There are existing strategies and programmes, which can be strengthened through more systematic integration of biodiversity priorities in their operations.

1.2 Scope of this Guidance Document

The scope of this guidance document is Environmental Risk Assessment (ERA) and management of all activities

with GMOs released into the environment. The focus of this guidance is on environmental risk assessment of genetically modified plants as most of the current applications and experience available both nationally and internationally has been on plants.

This guidance document is not intended to be prescriptive or to promote specific technologies but rather provide guidance on the minimum requirements for ERA and management. The approaches listed here are not meant to be definitive or exhaustive. Addressing these questions within any one application is neither a requirement nor a prescription for the successful passage of products through the regulatory system. DEAT will continue to consider applications for environmental release of GMOs on a case-by-case basis.

1.3 Aim of Guidance Document

This Guidance document is aimed at achieving the outcomes identified in the NBSAP:

- Ensuring institutional cooperation and coordination to deal with the potential risks posed by GMOs released into the environment.
- Developing and implementing effective measures for management and control of potential risks associated with GMOs by ensuring a transparent science based process for decision making.
- Sharing information and providing support to all stakeholders to ensure adoption and implementation of highest Biosafety standards.

1.4 DEAT Legislative Framework

1.4.1 National Environmental Management Act 107 of 1998

The legislative framework provided by the National Environmental Management Act, 1998 (NEMA) (Act 107 of 1998) introduced a new era of management of the environment. NEMA defines “environment” as the surroundings within which humans exist, which is made up of:

(i) the land, water and atmosphere of the earth;
(ii) micro-organisms, plant and animal life;
(iii) any part or combination of (i) and (ii) and the interrelationships among and between them; and
(iv) the physical, chemical, aesthetic and cultural properties and conditions of these that influence human health and well-being.

Chapter 1 of NEMA sets out the National Environmental Management principles. Key among these is that environmental management must place people and their needs at the forefront of its concern, and development must be socially, environmentally and economically sustainable. Specific reference to biodiversity considerations are as follows:

- that the disturbance of ecosystems and loss of biological diversity are avoided, or, where they cannot be altogether avoided, are minimized and remedied;
- that the development, use and exploitation of renewable resources and the ecosystems of which they are part of do not exceed the level beyond which their integrity is jeopardized; and
- sensitive, vulnerable, highly dynamic or stressed ecosystems, such as coastal shores, estuaries, wetlands and similar systems require specific attention in management and planning procedures, especially where they are subject to significant human resource usage and development pressure.

NEMA has several provisions that are of relevance to GMOs. NEMA stipulates a ‘risk-averse and cautious approach’ to avoid, minimize or remedy the disturbance of eco-systems and loss of biological diversity. Environmental management decisions should take into account the impact of the decisions taken on all people, as well as promote participation of interested and affected parties, take place openly and transparently, and be appropriate in relation to the assessment of social, economic and environmental costs and benefits. Inter-governmental co-ordination and harmonization of policies, legislation and actions relating to the environment is required.

NEMA contains provisions which set out the requirements for integrated environmental management. Under NEMA, an activity which will significantly affect the environment will only be authorized after considering, investigating and assessing the impact of such activity on the environment, socio-economic conditions and cultural heritage. This applies even to cases where authorization is governed by alternative legislation, such as the GMO Act. However, NEMA further lists certain activities which may not be commenced without prior authorization (such authorization requiring an EIA). Details of these activities will be conveyed to the applicant after a review of the baseline information for the GMO under assessment (see Section 4).

1.4.2 National Environmental Management: Biodiversity Act 10 of 2004 (NEMBA)

The Biodiversity Act provides for:

(i) the management and conservation of biological diversity within the Republic and of the components of such biological diversity;

(ii) the use of indigenous biological resources in a sustainable manner; and

(iii) the fair and equitable sharing among stakeholders of benefits arising from bioprospecting involving indigenous biological resources.

Chapter 5 Of NEMBA: Species and organisms posing potential threats to Biodiversity in Part 3 of Section 78 specifically deal with Genetically Modified Organisms. The purpose of Chapter 5 of NEMBA is "to ensure that environmental assessments for purposes of permits in terms of the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997), are conducted in appropriate cases in accordance with Chapter 5 of the National Environmental Management Act. This therefore means that NEMBA is intended to augment rather than to duplicate the provisions of the GMO Act 15 of 1997. The relevant provisions are intended to ensure that in appropriate cases, EIAs compliant with the requirements stipulated by NEMA are carried out for the purposes of issuing permits under the GMO Act. The provisions will take effect in cases where 'the Minister has reason to believe' that a trial release or general release of a GMO into the environment under a permit applied for under the GMO Act 'may pose a threat to any indigenous species or the environment'. In such cases, the release must be treated as if it were a listed activity under NEMA and the requirements for an EIA in accordance with the NEMA provisions must be followed before a permit may be issued. The onus rests on the Minister to communicate his/her belief about the potential threat of the GMO release concerned to the Registrar of GMOs as soon as possible after the application has been received by DEAT (Peterson et al.,2000).

The other relevant provision of the Act concerns the role of the South African National Biodiversity Institute, an institution established by the Biodiversity Act to assist the government in achieving the objectives of the Biodiversity Act. As one of its functions, the Institute must 'monitor and report regularly to the Minister on the impacts of any genetically modified organism that has been released into the environment, including the impact on non-target organisms and ecological processes, indigenous biological resources and the biological diversity of species used for agriculture' (Nap et al,2003).

1.5 The Importance of Biodiversity

South Africa is blessed with enormous biological wealth, both in terms of numbers of species, and the use value (actual and potential) of these species. The diversity of peoples, topography, climate and geology of the country also ensures a wide variety of landscapes, scenic vistas, lifestyles and knowledge. These natural and cultural resources underpin a large proportion of the economy and many urban and rural people are directly dependent on them for jobs, food, shelter, medicines and spiritual well being.

In South Africa, terrestrial, inland water and marine ecosystems and their associated biodiversity are widely used for commercial, semi-commercial and subsistence purposes through both formal and informal markets. While some of this use is well managed and/or is at levels within the capacity of the resource for renewal, much is unsustainable. "Use" in this case refers to direct use, such as collecting, harvesting, hunting, fishing, etc. for human consumption and production, as well as more indirect use such as ecotourism (bird watching, photography, etc.).

Biodiversity is an intrinsic feature of natural ecosystems, which supply us with an array of ecosystem services on which we depend. These services include the provision of food, water purification, nutrient cycling and the development and protection of soils. The links between biodiversity and ecosystem services are complex, but it is increasingly recognized that losses of biodiversity may lead to reduced ecosystem resilience (Millennium Ecosystem Assessment ,2004). For example, in the provision of an ecosystem service such as pollination, different organisms provide services to different plants. Biodiversity provides ecosystem resilience so that even when different species provide the same services, their efficiency will differ under different prevailing conditions i.e. some will do better during wet seasons and others will do better during dry seasons. Often a suit of pollinators provide a better service than one species on its own. Negative impacts on biodiversity have negative consequences for ecosystem processes and functions leading to insecurity and reduced quality of life for all. Degraded ecosystems increase our vulnerability to disasters and negatively affect the economy. The worst affected sectors of society are often the rural and urban poor, and those who depend directly on the environment for their livelihoods.

1.5.1 Agricultural Biodiversity

There are many definitions of biodiversity but the most appropriate for this document is the definition of biological biodiversity that can be defined as the diversity or variety of plants and animals and other living things in a particular area or region. In practice biodiversity suggests sustaining the diversity of species in each ecosystem as one plans human activities that affect the use of the land and natural resources. The biggest threat to biodiversity is habitat destruction. Increasing crop productivity on the land already under cultivation would prevent or at least reduce habitat destruction. One way to increase farm yields is by improving seed, produced either by traditional crop breeding or by modern biotechnology.

In addition to biodiversity in the wild, there is the biodiversity of organisms used for farming and other human activities. In agriculture, 7 000 species of plants are used by farmers somewhere in the world, but only 30 species provide 90 percent of our calorific intake (intake as observed by Haywood in 2000). Within these dominant crop species, there are many hundred thousands of varieties (landraces, cultivars) adapted to local climates, farming practices, and cultural predilections like taste, color, structure, ability to store the products. Much of this large crop diversity is important for providing the initial material for breeding. However, it must be recalled that the genetic diversity found in crops is much less broad than the genetic diversity observed in plants or animals living in the wild, which points to the importance of wild species for agricultural breeding programs. The top three crops are wheat, rice and maize with around 500 million tons annual production each.

The agricultural sector has had the most profound impact on natural habitat across South Africa. The clearing of natural vegetation for crop cultivation has impacted on all biomes. In some areas, this impact has been dramatic. The renosterveld (lowland fynbos) of the fynbos biome is Critically Endangered, having been cleared for vineyards, orchards and wheat. Sweet grasslands of the Free State, Gauteng and Mpumalanga have been extensively cleared for maize, potatoes and other crops, while tracts of east coast grasslands and thickets have been cleared for crops such as sugar cane, pineapples and chicory. This has led to declines of up to 80% of some bird species. However, some threatened grassland bird species are able to survive in crop-growing areas, for example, where crops are mixed with pastoralism on tracts of natural veld.

SECTION 2: ENVIRONMENTAL RISK ASSESSMENT (ERA) OF GENETICALLY MODIFIED PLANTS

2.1 Unpacking Environmental Risk Assessment

Risk assessment is the overall process of identifying the sources of potential harm (hazard) and assessing both the seriousness (consequences) and the likelihood of any adverse outcome that may arise. It is based on hazard, consequence and likelihood assessments leading to an estimation of risk. The concept of Environmental Risk Assessment includes the identification and evaluation of possible adverse effects of GMOs on the conservation and sustainable use of biodiversity

Environmental risk assessment is a process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors (U.S. EPA, 1992a). The process is used to systematically evaluate and organize data, information, assumptions, and uncertainties in order to help understand and predict the relationships between stressors and ecological effects in a way that is useful for environmental decision making.

Table 2.1. ERA PHASES.

PHASE 1: Estimate risk for each identified GMO
PHASE 2: Evaluate the probability of occurrence of risk
PHASE 3: Evaluate the scale of risk, should it occur
PHASE4: Decision making i.e. whether to release the GMO into a confined 'area" (glasshouse, production plant) or into the environment based on the assessment of risk in the above 3 PHASES, or that no release take place at all
PHASE 5: Appropriate Risk Management measures stipulated
PHASE 6: Risk Communication

Environmental risk assessment requires assessing harm not only to individuals and populations within a species but also to interactions within and between species in the context of biological communities and ecosystems. There may also be the potential for harm to the physical environment. Information can be sourced from research fields including botany, zoology, entomology, mycology, microbiology, biochemistry, population genetics, genetics, molecular biology, agronomy, weed science, ecology, chemistry, hydrology, geology and knowledge of biogeochemical cycles, and therefore requires consideration of complex dynamic webs of trophic interactions (Conner, 2003).
In accordance with the precautionary approach, the following general principles should be followed when carrying out the environmental risk assessment:

- the identified characteristics of the GMO and its use that may have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations;
- the risk assessment should be carried out in a scientifically sound and transparent manner based on available scientific and technical data;
- the risk assessment should be carried out on a case by case basis; this implies that the required

information may vary depending on the type of the GMO, its intended use and whether the environment where it is to be released already contains GMOs;

- The risk management measures employed should be proportional to the chosen level of protection, should be non discriminatory, consistent, based on an examination of potential benefits and costs;
- if new information on the GMO and its effects on the environment becomes available, the environmental risk assessment should be re-examined in order to:
    - o determine whether the risk level has changed;
    - o determine whether there is a need to amend the risk management or where necessary take appropriate measures to withdraw the authorization of the GMO in question.

Although ERA has emerged as a specific area in its own right, it is actually complementary to the Environmental Impact Assessment (EIA) and forms an integral part of Integrated Environmental Management (Table 2.2).

Table 2.2. Similarities in the basic principles of the EIA procedure and the ERA framework.

Generic Environmental Impact Assessment Process	Environmental Risk Assessment
Accountability for information and decisions taken.	Risk manager is accountable.
Open, participatory approach.	Participatory approach from planning to risk communications.
Consultation with interested and effected parties.	Risk communication occurs with interested and affected parties.
Considers alternative options.	Alternative options are considered in remediation approaches.
Ensures that social costs of developing proposals will be outweighed by social benefits.	Includes cost-benefit analysis.
Opportunity for public and specialist input in decision-making.	Risk communication between risk managers and public/Interested and Affected Parties (I&APs)in decision-making.
Includes uncertainty.	Includes uncertainty.

2.2 Tiered approach to ERA

The ERA is conducted on the basis of two sets of information. The first set of information to be submitted will include molecular, agronomic and morphological characteristics of the GMO in question. The second set of information is the comparative data of the GMO and its conventional counterpart as well as information about the species' major interactions with other life forms in its production range in South Africa (the concept of substantial equivalence). This data should be collected through appropriate testing and analysis conducted under South African conditions, although data from other countries or conditions could also be considered.

The ERA for GM plants relies on a tiered process of both testing and subsequent assessment. This process proceeds from well-controlled, focused, laboratory studies conducted under very conservative assumptions regarding exposure potential, to less certain field studies and monitoring that seek the manifestation of hazard under real world conditions. Because controlled laboratory studies are conservative indications of the likelihood for the effect to be manifested under real world conditions (that is, of risk), the majority of GM crop ERAs conducted to date have relied on laboratory studies. In cases where confirmatory field studies and monitoring

were conducted, laboratory study findings have proven adequate to determine that there is reasonable certainty of no harm associated with environmental release.

A summary of the methodology and process of the tiered approach to environmental risk assessment is shown in Table 2.3. By making use of this tiered approach unnecessary delays in applications for contained use, field trials or commercial release can be avoided as there is a natural progression from one stage into any of the other. Duplication of information will also not be necessary. It should however be noted that this data should be collected through appropriate testing and analysis conducted under South African conditions.

Table 2.3. Tiered environmental risk assessment.

Environmental risk assessment tiers	Methodology of risk assessment	Evaluation of risk assessment and monitoring
Tier 1: Hazard Identification: Glasshouse/contained use These studies would normally be conducted under controlled laboratory, growth room or glasshouse conditions in order to measure effects in relation to known exposure levels.	Phase 1 – Consideration of each of the inserted genes and sequences, individually/ and combinations thereof 1.Hazard identification→ type of potential adverse effect(s): possibility of inserted gene products causing toxicity or allergenicity. 2.Likelihood estimation→ influenced by many different factors e.g. characteristics of inserted gene, of the recipient organism and the scale of the activity. 3.Consequence evaluation. Phase 2 – consideration of GM plant 'as a whole'. Phase 3 – consideration of risk management and determination of overall risk. Phase 4 – risk estimation and risk management based on the scientific knowledge at the time of the application but it should be minimal if not nil as it is for contained use	Phase 1 – Consideration of each of the inserted genes and sequences, individually 1.Hazard identification addresses two closely related topics: which new genotypic or phenotypic characteristics of the GM plant may cause adverse effects on the environment; what scientific scenarios could theoretically lead to these adverse effects. 2.Estimation of likelihood of any of the aspects of hazard identification happening. 3.Consequence evaluation. Phase 2 – evaluate indirect effects of the GM plant on organisms not directly exposed to the GM plant but one or two steps removed in the food chain i.e. likely exposure. Phase 3 – Easily controlled as the GM plant has been grown under controlled laboratory or glasshouse conditions and have to follow the regulations specified by the respective permit application. Phase 4 – As specified in the permit application – it is simple and straightforward.

Environmental risk assessment tiers	Methodology of risk assessment	Evaluation of risk assessment and monitoring
Tier 2: Trophic layer effects: **Field trials** Exposure Studies: Trials are established, simulating the cultivation of the GM plant, in order to quantify actual levels of exposure of different biota and to determine likely ecological adverse effects due to the GM plant and its management, in comparison with equivalent non-GM materials and their management.	Phase 1 – Consideration of each the inserted genes and sequences individually/and combinations thereof 1. Hazard identification → type of potential adverse effect(s): possibility of inserted gene causing any of the following: toxicity or allergenicity; weediness; increased susceptibility to pathogens; effects on target and non-target organisms; spread of GM plant → any adverse effect resulting from outcrossing or gene flow; changes in agricultural practices . 2. Likelihood estimation → influenced by many different factors e.g. characteristics of inserted gene, of the recipient organism and the scale of the activity. 3. Consequence evaluation. Phase 2 – Consideration of GM plant 'as a whole' Phase 3 – Consideration of risk management and determination of overall risk. Phase 4 – Risk estimation and risk management → based on the scientific knowledge at the time of the application but it should be minimal if not nil as it is for contained use	Phase 1 – Consideration of each of the inserted genes and sequences, individually/and combinations thereof 1. Hazard identification – In cases where the field trial application follows a contained use application done in South Africa only those aspects that are different from the contained use need to be addressed e.g.; spread of GM plant → any adverse effect resulting from outcrossing or gene flow taking in consideration that outcrossing is a natural process that happens between plants growing in nature; changes in agricultural practices. 2. Estimation of likelihood – in terms of 'highly likely', 'likely', 'unlikely', 'highly unlikely', 'negligible' or 'effectively zero'. In cases where likelihood does not result in a clear conclusion the 'worst case scenario' should be assumed. 3. Consequence evaluation – this is better achieved using the concept of 'baseline'. Phase 2 – Evaluate likely exposure. Phase 3 – Easily controlled as the GM plant has been grown under controlled laboratory or glasshouse conditions and have to follow the regulations specified by the respective permit application. Phase 4 – As specified in the permit application – it should be straightforward and a simple environmental monitoring plan should be included.

Environmental risk assessment tiers	Methodology of risk assessment	Evaluation of risk assessment and monitoring
Tier 3: General release / commercial release	Phase 1 – Consideration of each the inserted genes and sequences individually/and combinations thereof 1. Hazard identification→ type of potential adverse effect(s): possibility of inserted gene causing any of the following: toxicity or allergenicity; weediness; susceptibility to pathogens; effects on target and non-target organisms; spread of GM plant→any adverse effect resulting from outcrossing or gene flow; changes in agricultural practices. 2. Likelihood estimation → influenced by many different factors e.g. characteristics of inserted gene, of the recipient organism and the scale of the activity. 3. Consequence evaluation. Phase 2 – Consideration of GM plant 'as a whole'. Phase 3 – Consideration of risk management and determination of overall risk. Phase 4 – Risk estimation and risk management → based on the scientific knowledge at the time of the application but it should be minimal if not nil as it is for contained use.	Phase 1 – Consideration of each of the inserted genes and sequences, individually/and combinations thereof 1. Hazard identification – In cases where the commercial release application follows a field trail application done in South Africa only those aspects that are different from the field trial release need to be addressed eg; spread of GM plant → any adverse effect resulting from outcrossing or gene flow; changes in agricultural practices. 2. Estimation of likelihood – in terms of 'highly likely', 'likely', 'unlikely', 'highly unlikely', 'negligible' or 'effectively zero'. In cases where likelihood does not result in a clear conclusion the 'worst case scenario' should be assumed. 3. Consequence evaluation – this is better achieved using the concept of 'baseline' Phase 2 – Evaluate likely exposure. Phase 3 – Easily controlled as the GM plant has been grown under controlled laboratory or glasshouse conditions and have to follow the regulations specified by the respective permit application. Phase 4 – As specified in the permit application – it should be straightforward and a simple environmental monitoring plan should be included.

Tiers 1 and 2 identify the potential hazards while Tier 3 identifies the likey exposure levels so that the actual risk can be estimated,

2.3. Quality of data / submissions / conclusion

An environmental risk assessment contained in an application for release of genetically modified organisms should ideally:
(a) identify and evaluate the potential damage to the environment, whether direct or indirect, immediate or delayed, which may arise from the release or marketing of genetically modified organisms;
(b) include bibliographic reference and indications of the methods used where applicable;
(c) where the genetically modified organisms contain antibiotic resistance markers, the environmental risk assessment shall include an examination of the particular risks of damage to the environment which may be posed by the deliberate release or marketing of those genetically modified organisms.

SECTION 3: ENVIRONMENTAL SAFETY ASSESSMENT CRITERIA

This section of the guideline document will review the environmental safety assessment criteria that are generally accepted globally when evaluating applications for release of GMOs into the environment.

In conducting an ERA, the most current conceptual and empirical knowledge and peer-reviewed science on transgenic biology must be utilized. Applications are encouraged to clearly describe the test procedures followed in developing the test data, including test methods, reference products, quality control, quality assurance procedures, appropriate statistical analysis, together with bibliographic references, including numbered patents, where these are appropriate. Field trials should be conducted in a manner consistent with the proposed production practices of the GMO in question. The generation of field trial data should be produced using statistically valid experimental designs and protocols. The applicant may be asked to submit details of field trial protocols, including experimental designs and sampling procedures .

Some of the impacts of GMOs are already known, but given the fact that GMOs are the result of a relatively new technology, which is likely to grow and develop rapidly in future, it is possible that a potential impact could remain obscure. This means that an activity associated with GMOs could carry an unacceptable risk to biodiversity. Such activities need to be managed and the risks assessed, and measures put in place to minimize unacceptable impacts.

While it is accepted that GMOs have a role to play in the sustainable development imperatives of South Africa there are a number of basic environmental safety criteria, some of which have been prioritized in the following table (Table 2.4).

Table 3.1. Safety Criteria for Environmental Risk Assessment of Genetically Modified Organisms.

BIOTIC ENVIRONMENTAL SAFETY CRITERIA	
Gene transfer /flow	Persistence and invasiveness
	Selective advantage or disadvantage
	Potential of the GMO to become a weed to agriculture or be invasive to natural habitats
Potential impact on biodiversity	Interaction between GM and Target Effects
	Interaction between GMO and non target organisms
	Impact on biodiversity, landraces and agro-biodiversity
Effects of bio-geochemical processes	Impact on carbon dioxide evolution
	Adverse impact on biological nitrogen fixation
	Impacts on organic matter turn over
Change in agricultural practices	Changes in application of pesticides, biocontrol agents
	Crop rotation systems
ABIOTIC ENVIRONMENTAL SAFETY ASSESSMENT CRITERIA	
Alterations of climatic conditions	Altered production of green houses gases Altered sensitivity to climatic conditions such as cold/ heat
Altered sensitivity to or tolerance of abiotic fractions of soil	Salinity, mineral nutrients and mineral toxins
Alteration of mineralization	Exudates changing soil pH
Physical environment	Persistence of the products or by-products of GMOs that has a negative effect

3.1 Baselines to prevent environmental harm

The assessment of the risk of a GMO needs to be placed in the context of existing agricultural activities, whether non-GM or organic, and which also have the potential to cause adverse environmental effects. The baseline against which the risk of a GMO can be compared in the South African environmental context still needs to be firmly established. All agricultural activities result in adverse effects on the environment, and to this end, DEAT is engaging with agriculture as a whole in terms of minimizing impact on biodiversity. The present system being used is one that compares GMOs with conventional crops in the agricultural context.

While the South African National Biodiversity Strategy and Action Plan are committed to stop and where possible reverse biodiversity declines, it is important that the risk assessment process identifies GM crops whose use may lead to increasingly intensive agricultural management practices. It is thus critically important to embark on research programmes that monitor the impact of GMOs on the environment so as to put in place appropriate risk management practices, thereby assisting in the development of baselines. This research will provide a greater understanding of arable ecology that can be used to underpin this process (Poppy, 2004).

3.2 Persistence and Invasiveness

Once invasive species have become established, the cost of control and eradication is enormous, diverting funds from important social and development needs. The uncontrolled spread of invasive species across our ecosystems can be likened to the spread of diseases caused by viruses or bacteria, or the spread of agricultural pests. A coordinated approach to prevent the introduction of new invasive species would include preventing the entry of pests and pathogens. Indeed, many species imported into the country carry pests and diseases, which can spread to indigenous species (for example, freshwater fish have been impacted in this way). There is need to pay particular attention to aquatic ecosystems, to implement the recently developed ballast water policy and prevent inter-basin (inter-catchments) transfer of fish and other aquatic organisms. Equally, the commercial forestry, horticulture, agriculture and pet trade sectors need to be regulated to prevent further unwanted introductions, and encourage the implementation of programmes to limit and contain the spread when such introductions do occur. To this end, the ERA information will include special reference to potential for invasion of natural habitats particularly those in protected areas (Ackhurst et al, 2003).

The overall question to be answered is, while the GM crop itself is not very likely to become a weed, could its pollen create new invasive weeds through spreading the foreign gene to other plants? Gene transfer through pollen is more likely between closely related species. In countries where modern crop plants originated, this kind of transfer is indeed possible and can create a problem (e.g. gene flow from GM sorghum to wild relatives of sorghum). A more pertinent debate may be the risk of gene flow from introduced domestic plants to introduced wild plants, such as brassicas and wild mustards. Studies conducted around the world have shown that cross-pollination falls off rapidly within a short distance of transgenic plants. There is a risk of pollen traveling over several hundreds kilometres for most species, or even over a few kilometres for some wind-pollinated plants such as pine trees.

3.3 Potential for Gene transfer

Genes can be transferred from one organism to another by vertical or horizontal transmission. The frequency of horizontal gene flow may be low even if the gene is not present in the pollen, through soil bacteria taking up DNA from a plant root or leaf, but it is virtually impossible.

Four factors are important in the consideration of the risk of gene transfer through pollen and they include consideration of:

- **Pollen movement:** some plants are fertilized by wind-borne pollen. Some plant pollen is light and can travel considerable distances (several kilometres for pine pollen), but in others it is heavy and rarely moves more than one metre from the plant (corn). Insects or birds pollinate other plants and the behavior of the pollinators will determine the risk of pollen being carried to another plant species (e.g. bees tend to stick with the same plant species while flies will move around more).
- **Pollen viability:** some pollen will remain viable for a long time when stored under dry conditions but, in the field, where it is exposed to humidity and the sun's ultra-violet radiation, most pollen loses its viability rapidly, within hours, after release.
- **Receptivity of other plants:** some flowers are receptive to pollen only for a brief period during the day; others may be receptive for several days. To calculate the risk of how likely plants are to pick up pollen from a neighboring field of GM crops, it is important to know the flowering season and duration of the flowering season of both species.
- **Competition:** even if viable pollen reaches a receptive plant, it still faces competition from other viable pollen, so the risk of gene transfer will depend on the number of other pollen-producing plants in the vicinity. Planting transgenic trees for cropping, shelter or forestry near conservation areas would seem to present the greatest ecological risk, but for most GM crop plants, buffer zones are considered adequate measures to prevent unwanted plant drift (Roy et al., 2003).

3.3.1 Horizontal gene flow/gene transfer - crossing the species barrier

While it is a common belief that crossing different species leads to sterile offspring it is true for most animals but is not always the case in the plant kingdom. Many species, even genera, can be crossed with good seed viability. However, this does not mean that a genetically modified plant can cross with non-genetically modified plants easily, as there are three types of barriers -spatial, temporal and biological - that normally prevent plant species from crossing.

- Spatial barriers intervene between plants growing in different areas or where there is no common pollinator. These barriers are broken when plants or pollen are moved intentionally or accidentally, resulting in new hybrids.
- Temporal barriers arise where plants flower at different times of the year. When breeders induce synchronous flowering they can achieve crosses that would never happen normally.
- Biological barriers reduce the chance of fertilization between species by preventing fertilization or seed development.

Many of our common crop plants have arisen as the result of species crossing. Some of these crosses have been chance events, assisted by man as plants were moved around the globe, overcoming the spatial barrier. It is important to realize that crossing species barriers using standard breeding methods can result in multiple variant genes with unpredictable consequences.

3.3.2 Gene transfer in the soil

Some soil bacteria can take up exogenous DNA from other organisms and incorporate it into plasmids. Such plasmids are present in most bacterial cells and the exchange of plasmids from one bacterial species to another is a natural process. Theoretically, bacteria could take up DNA from a genetically modified plant and transport it along a chain of other microbial species. However the likelihood of this process occurring in nature is extremely low because a whole host of conditions have to be satisfied, including strong selection pressure that favours multiplication of bacteria that carry the exogenous DNA.
While such gene transfers are possible between plants and soil bacteria, there is no evidence to suggest that such exchanges happen more in GM than in normal crops.

3.3 Interaction between the GM plant and Target Insects

An assessment is required of the potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GM plant and target organisms. Data on the comparative susceptibility of the GM plant to pests and diseases compared with that of the non-modified plants are useful indicators of effects, together with observations on agronomic performance during greenhouse and experimental field trials.

3.4 Interaction between the GM Plant and Non-target organisms

An assessment is required of the possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GM plant with non-target organisms (also taking into account organisms which interact with target insects), including impact on population levels of competitors, herbivores, symbionts (where applicable), predators, parasites and pathogens.

Impact should be assessed on non-target species (plant, animals and microbes) in the crop ecosystem (which may include pollinators, beneficial, predatory and phytophagous species), and, if appropriate, the aquatic environment. Studies should be designed in order that sufficient statistical power is obtained to detect possible effects on non-target organisms. Adequate statistical power can be achieved from the proper control of variation and replication, since power depends on sample size, the degree of random variation between experimental units and the chosen significance of the tests. An appropriate approach might be to select a desired level of statistical power and the size of effect to be detected, collect preliminary data to estimate within treatment variability and then to calculate the required sample size for the proposed study. The duration of experiments to assess the risks to non-target organisms should be sufficient to reflect the pattern and duration of exposure that these organisms are likely to experience under field conditions (Perry et al., 2003; Marvier, 2002).

However, it is important that food chain effects due to reductions in target prey species (e.g. declines in parasitoids populations) are differentiated from, for example, population declines due to the effects of GM toxin accumulation in food chains.

Many of the current GMO crops in use in South Africa have been produced through biotechnology to provide their own built-in protection against insect pests. Assessing how these crops and the proteins they produce for protection might affect other organisms is an important part of the safety assessment and regulatory review process. This risk assessment involves examining both the inherent toxicity of the product to species it is not intended to control - called “non-target” species - and whether those species would actually be exposed to the insecticidal protein at harmful levels in nature. A risk of adverse impact is present only if there is inherent toxicity and exposure of the insecticidal protein to a particular species at a high enough level during the use of the product under normal agricultural conditions. The assessment of that risk is based upon a thorough review of the scientific literature, a series of laboratory tests and field studies, when needed, to validate the results of the laboratory testing (U.S. EPA, 1998).

Testing needs to be performed on several different organisms that are relevant to the ecosystem into which the GM crop is being introduced. These tests will indicate whether non-target organisms will be at risk from GMOs containing additional proteins such as Bacillus thuringiensis (Bt) insecticidal proteins. Also, subsequent field studies will need to be carried out to verify if there are any adverse effects to non-target organisms.
Bt genes have been incorporated into many plants, which raised concern that its continuous presence will make it more likely that pest insects will become resistant to it (Tabashnik et al. ,2003; Champion et al., 2003). Such concerns have also prompted further studies to determine how any negative impacts on non-target species could be averted, for example, by avoiding the expression of Bt or other toxins in pollen. South Africa had a high percentage of endemic species long before GMOS were developed. The introduction of plants and animals from around the world has had major impacts on the original ecosystem. Competition with and displacement of native plants in the remaining areas of native bush is a continuing problem.
Paradoxically, genetic modification has the potential to contribute both to the loss of more species and to the better protection of those species that are already endangered.

3.5 Effects on Bio-geochemical processes

An assessment is required of the possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GM plant and target and non-target organisms in the vicinity of the GM plant release(s). The applicant should address, where appropriate, the potential impact on biogeochemical processes as these influence ecosystem function, e.g. in relation to soil microbial communities. Examples of such bio-geochemical processes include, CO2-evolution, organic matter turnover, nitrogen fixation that is relevant to the ecosystem into which the GM crop is being introduced.

Soil fertility strongly influences the growth and productivity of plants. As plant-associated (rhizosphere) and soil microbial communities perform the vital biotransformation that underpins soil fertility, any negative impact(s) on microbial participants in this key compartment would have to be carefully evaluated. This should be assessed on a case-by-case basis with particular reference to the nature of the introduced trait and the consequences of the genetic modification/alteration in the GM plant.
The risk assessment should aim to establish if direct or indirect effect(s) of the genetic modification in the GM plant have any long-term or sustainable deleterious effect on the soil microbial communities and the associated functional activities that are responsible for maintaining soil fertility and plant productivity. The assessment should also address the fate of any (newly) expressed gene products and derivatives in those environmental compartments where they are introduced and which result in exposure of non-target organisms (e.g. in soil after the incorporation of plant material).

Exposure should also be estimated to relevant soil biota (e.g. earthworms, microorganisms, organic matter breakdown) in relation to the impact on decomposition processes. Risk assessment should also include an analysis to determine if a shift occurs in populations of deleterious organisms in the presence of the modified plant.

3.6 Changes in Agricultural Practices

The introduction of herbicide resistant cultivars has, in particular, triggered studies on the impact of changed management practices. Questions arise with respect to the conservation of soil, reduction in tillage, monoculture impacts, etc. It has also been argued that higher productivity of land (assuming that this is achieved through a transgenic crop) can lead to a reduction of demand for cultivated land, which in turn, can release land for conservation purposes (Goklany, 2001).

In considering the negative environmental impacts brought about by changed management practices, one once again considers the likely reversibility of a negative impact, and the likely extent of a positive impact. In the event of these risks being generally reversible and localized, it is viewed that such risks are of a lesser concern, relative to the risks of gene transfer and invasiveness of transgenic organisms. This does not however mitigate the need for a case-by-case consideration, and, where necessary, the implementation of monitoring programs and risk assessment studies.

Positive impacts in management practices on the environment should also be considered in developing an overall assessment of the net effect on the environment. The detrimental effects of “traditional” agriculture to the environment hold a real concern, with massive declines in biodiversity in Europe over the past twenty years; applications of transgenic technology may hold potential to reduce the impact of agriculture on the environment (Johnson, 2000).

3.6.1 Herbicide use

Chemical herbicides are now a standard practice of conventional farming. They offer a cost-effective method of killing weeds and, as an alternative to mechanical cultivation, result in less topsoil lost and lower labour and energy costs. However, their negative effects include reduced soil fertility, water pollution, losses in earthworms and beneficial soil microbes, and a range of effects on human health.
Most GM crops with herbicide resistance have been modified to resist Monsanto’s glyphosate-based Roundup (Roundup Ready soy accounted for more than half of the

global plantings of soy in 1999, (ISAAA, 2001)). Roundup is the world's most frequently used herbicide and many would argue that it is one of the most environmentally friendly herbicides - it breaks down quickly in water and soil into harmless metabolites and is practically non-toxic to animals and humans (Beringer, 2000). Traditional agricultural production practices can lead to the intentional and unintentional stacking of traits. Cross reference to section dealing with stacking under areas of additional consideration – Annexure 1.

3.7 Additional Areas for Consideration

3.7.1 Intentional stacking of GM traits

The presence of a transgene in an organism, regardless of the method used to introduce it, will trigger the requirement for an ERA to be conducted before authorization may be granted by DEAT to release it into the environment. This therefore means that organisms that have stacked traits resulting from either intentional intra specific or inter specific crosses between GMOs already authorized for commercial release will still require comprehensive risk assessments (Hilbeck & Andow, 2004). Annexure 1 of this document highlights the ERA considerations when assessing stacked GMOs.

Stacking of traits with possible incompatible management requirements, possible negative synergistic effects, or where the production of the plant may be extended to a new area of the country, may elicit an ERA. If the parental GMO events have been previously assessed and approved by the GMO Act Executive Council, the evaluation of the combined events produced from their crossing should concentrate on the following points:

Assessment of potential interactions between combined events (Source EFSA, 2006 Consultation Document)

Applicants would need to carry out a risk analysis on the potential for any interactions between the combined events, which could impact on human or animal health and/or the environment.

A step-by-step approach in the risk assessment should be followed:

1. The risk assessment of the combined events should initially consider the characteristics and properties of each transgenic trait individually. This will also give information, where appropriate, on the segregants from the plants with combined events.
2. Risk assessment should consider whether or not the occurrence of combined transgenes presents issues that were not addressed when considering the single events e.g. in parental lines. For example, whether the combination of genes results in altered expression of both endogenous and/or novel traits in a plant.
3. Selection of appropriate comparators - The genetic backgrounds of the controls should be as close as possible to those used in producing the genetically modified plants containing the combined events. The applicant should provide information, which validates the choice of controls used for the various parts of the risk assessment.
4. Comparative compositional analysis - Compositional analysis should be carried out alongside appropriate controls grown in the same location and the experiments designed to yield statistically meaningful data. Where the substantial equivalence of parental lines containing genetically modified events has been fully tested in replicated field trials over at least 2 seasons, one year's field trailing of events combined by crossing is acceptable where geographical localities are representative of the climatic conditions to which such crops will be exposed. Based on the outcomes of this assessment additional follow-up analysis of compositional characteristics over further growing seasons may be required if unexpected differences occur beyond the range of natural variation. On a case-by-case basis, this may trigger further assessment. In terms of the nutrients, anti-nutrients and natural toxins, these need to be analyzed following the OECD published consensus documents on the key components that should be considered in the comparative assessment of new crop varieties of particular species. Measurement of these components can be regarded as the minimum requirement for genetically modified events combined by crossing.

5. Assessment of toxicity, allergenicity and nutritional value - This would include, for example, an assessment of any potential for increased toxicity to humans and non-target organisms or to modifications in nutritional value due to the combination of the events. Any of the above may arise from additive or synergistic effects of the gene products and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways. This will clearly require a case-by-case approach. The appropriate principles of risk assessment as described in the EFSA guidance document also apply to the assessment of genetic modification events combined by crossing.
6. Environmental risk assessment of plants with combined events - The environmental risk assessment (ERA) will take into account the evaluation of the single events and additional data from molecular characterization and compositional analysis when determining potential interactions between genes or between gene products. The risk assessment of hybrids will then focus on the possible environmental effects as a result of these interactions. Any new interaction could result in changes to the physiology of the GM plant and related species and potentially in modified ecological behavior of these plants.

Assessment of the intactness of the inserted loci and phenotypic stability

The requirement is to establish that each transgenic locus in the hybrid is the same as in the original independent transformation event. This information will also be important to confirm the identity of samples used in comparative studies, which would include compositional analysis and any trials involving animals. Intactness of loci and comparisons with insert structures in parental lines should be carried out on material which is representative of cultivars produced for commercial production i.e. which will enter the environment and the food/feed chain. To assess intactness of loci, applicants should use appropriate molecular approaches e.g. Southern blots and PCR analyses and ensure that probes and primes used cover the entire insert and flanking regions.

Stability can be assessed by confirming that traits targeted by the genetic modification events (the phenotypes) remain unchanged. Changes in the expression of the trait/phenotype might indicate a potential stability issue with respect to the transgenes. For example, in the case of an herbicide-tolerant crop, the plants should remain tolerant to the herbicide in question. Any significant change in the expression of the trait/phenotype targeted by the individual transgenes due to the presence of more that one event (e.g. events providing tolerance to biotic and abiotic stress) – in comparison to the expression levels of these transgenes in the parental events – should be taken into consideration during the safety assessment, including the environmental risk assessment (ERA). In such cases and where altered expression of the trait/phenotype is viewed as a potential safety issue further assessment of the expression levels of the transgenes in plants segregating for the transgenic traits is needed.

Applicants should also provide data to indicate the potential for biologically significant levels of target proteins when traits are combined by crossing. This may have consequences for the ERA.

Points to consider for interaction effects

1. Altered toxicity to target organisms and any consequential impact on the development of resistance in t arget organisms;
2. Enhanced toxicity to non-target organisms (including changes in effects on the non-target range);
3. Altered fitness of the GM plant or plants acquiring the transgene combination through gene flow;
4. Enhanced capacity for gene flow and introgression;
5. Altered effects on microbial diversity and activity in relation to biogeochemical cycles.

3.7.2 Antibiotic resistance markers

Specific ecological concerns with respect to antibiotic resistance marker genes relate to the possibility that if the

gene is expressed in the plant, antibiotic resistance might result in a plant or one of its wild relatives becoming a weed, or might disturb the ecological relationships of the plant in another unknown way. The antibiotic resistance gene could also potentially be transferred from the GMO to soil micro-organisms. Any increase in antibiotic resistant soil micro-organisms could lead to a potential increase in human exposure to antibiotic resistant micro-organisms from ingesting them as contaminants of food and water.

Annexure 2 of this document highlights some of the international discussion and consensus on the use of antibiotic resistance markers.

3.7.3 Impact on soil ecosystems

The impact of a GMO on soil ecosystems should be assessed if the altered gene products or the management of organisms containing the gene has a greater potential for harm to a soil system than similar conventional organisms (or potential species in the case of higher plants). The risk assessment should include a full description of the appropriate commercial management regime, including changes in pesticide applications, tillage, rotations and other crop protection measures where these are different from relevant non-GM organisms.

Where risks have been identified to soil systems, direct and indirect effects on target and non-target organisms within the soil must be assessed or summarized from previous relevant studies. The extent to which non-target organisms are exposed either directly or indirectly must be identified. The information given in the application must also be indicative of the likelihood and consequences of horizontal gene transfer to soil organisms. In the case of target organisms within soil systems this may include the possibility of development of resistance to the modified trait (Landis, 2003).

Risks that require management should be defined together with a suitable risk management strategy. An evaluation of the overall risk of the GMO should be made taking into account the proposed risk management strategies.

3.7.4 Socio-economic and cultural considerations

Tese aspects of the impact of GMOs are taken into account by the NEMA provisions, as the conservation of the environment occurs in the socio-economic/human context. The net socio-economic or cultural impacts of GMOs may hold persuasive motivations to off-set the environmental benefits or losses against those of socio-economic/cultural importance. A potentially negative consequence of the importing of GMOs is the reinforcing of an ever-widening skills gap between countries in possession of the technology and the local economy. On the other hand, contribution towards local knowledge and skills generation in this area can be viewed as a positive contribution with respect to the socio-economic impact of their introduction.

3.7.5 Assessment criteria for the evaluation of environmental safety of plants with pharmaceutical properties

The existing regulatory framework for genetically modified plants was not intended for the regulation of production of pharmaceuticals in plants. However, future applications of biotechnology will result in the production of plant varieties that are not developed for the purpose of food, feed or textile fibre production, but rather for the production of pharmaceuticals and industrial compounds.
To enable the environmental safety assessment of GMO intended for pharmaceutical production, the application should address the following issues:

- the identity and origin of the GMO intended for pharmaceutical production;
- the properties of the novel gene and gene product(s);

- the relative phenotypic expression of the GMO;
- anticipated or known effects on the environment;
- potential impacts on human and animal livestock health, resulting from the environmental release;
- measures taken to ensure the safe handling and segregation of the GMO under review from other commodities produced in parallel;
- contingency plans, should the GMO be released outside of its authorized area, or if it has unintentionally entered the food or feed supply chains.

Annexure 3 of this document highlights the key considerations for ERA of plant-made pharmaceuticals.

SECTION 4: ENVIRONMENTAL IMPACT ASSESSMENT FOR GENETICALLY MODIFIED ORGANISMS

The purpose of Chapter 5 of NEMBA is "to ensure that environmental assessments for purposes of permits in terms of the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997), are conducted in appropriate cases in accordance with Chapter 5 of the National Environmental Management Act. This therefore means that NEMBA is intended to augment rather than to duplicate the provisions of the GMO Act 15 of 1997. The relevant provisions are intended to ensure that in appropriate cases, EIAs compliant with the requirements stipulated by NEMA are carried out for the purposes of issuing permits under the GMO Act. The provisions will take effect in cases where 'the Minister has reason to believe' that a trial release or general release of a GMO into the environment under a permit applied for under the GMO Act 'may pose a threat to any indigenous species or the environment'. In such cases, the release must be treated as if it were a listed activity under NEMA and the requirements for an EIA in accordance with the NEMA provisions must be followed before a permit may be issued. The onus rests on the Minister to communicate his/her belief about the potential threat of the GMO release concerned to the Registrar of GMOs as soon as possible after the application has been received by DEAT (Peterson et al.,2000).

4.1 Objectives of Environmental Impact Assessment

The objectives of environmental impact assessment (EIA) is to precisely evaluate the effects imposed by GMOs and their products on environmental quality and ecosystem evolution when undertaking research, commercialization, application of and releasing of GMOs and GM products to agricultural ecosystem,

grasslands, forestry, water environment and other natural elements. Based on these assessments, effective measures will be taken to prevent or minimize adverse effects. Below are detailed requirements for environmental impact assessment as stipulated by NEMA:

- Identifying impacts of GMOs and their products on the environment; defining level and frequency of such impacts.
- Evaluating mode of impact.
- Analyzing environmental elements and identifying them for evaluation and protection objectives.
- Description of EIA results according to studies and analyses.
- Comparing and analyzing socio-economic and environmental benefits of various protection measures.
- Proposing impact prevention or mitigation measures.

4.2 Criteria that may be used to trigger a scoping or environmental impact assessment of a GMO

An Environmental Impact Assessment (EIA) may be evoked by the Minister of Environmental Affairs and Tourism in consultation with scientific experts if it is deemed that one of the following criteria is applicable (Marks et al., 2003):

Scope of application includes release with a focus inter alia on one or more of the following:

- GM in question will result in changes in conventional use – e.g. pharmaceuticals in plants, biofuel production;
- GM will result in substantial changes in current agricultural practices and pest (medical, veterinary, agricultural) management practices e.g. expansion into new agricultural areas;
- GMO where there is prior evidence of changes in the agro-ecosystem dynamics that may lead to substantial changes in current agricultural practice such as evidence of secondary pest emergence or evidence of resistance development;
- potential negative impact on threatened or protected organisms listed in terms of NEMBA
- Release of :
- Indigenous GM organisms
- GM Organisms with cultural or geopolitical significance, or potentially negative socio economic impact e.g. grapes and cassava
- GMOs that have wild indigenous relatives
- GMOs that have non indigenous weedy relatives
- GMOs that have the potential to become invasive e.g. bentgrass; fish (aquaculture)
- Release of modified microorganisms that are expected to have a significant negative effect on the environment
- The scope of the application entails any environmental release of GMOs to be used for bio-terrorism.

4.3 Administrative arrangements in the event that an EIA is required

To apply for a permit for the environmental release of genetically modified organisms (GMOs) it is necessary to submit a written application to the Registrar of the GMO Act, in the Department of Agriculture. Part of the application procedures includes the submission of an environmental risk assessment of the GMO in question. This risk assessment is a prerequisite for environmental release.

In the event that the GMO application in question meets with any of the criteria listed in section 4.2 above, a further application should be lodged with DEAT which administers the EIA regulations in terms of NEMA.

SECTION 5: MONITORING GM PLANTS RELEASED INTO THE ENVIRONMENT

Section 5 of the Guidance document is aimed at highlighting the monitoring of GMOs released into the environment.

The Biodiversity Act introduces the legislative context for environmental monitoring of GMOs. Under Chapter 2 of the Biodiversity Act, the newly established South African National Biodiversity Institute (SANBI) "must monitor and report regularly to the Minister on the impacts of any genetically modified organism that has been released into the environment, including the impact on non-target organisms and ecological processes, indigenous biological resources and the biological diversity of species used for agriculture." Thus, under this provision, SANBI will need to ensure that there is some monitoring of GMOs released into the environment and to report to the Minister of Environmental Affairs and Tourism about any impacts of those GMOs on the environment. In order to effect this provision, additional monitoring obligations will be imposed in permits for activities with GMOs and the results of that required monitoring will be reported to SANBI to be used in its analysis and reporting to the Minister of Environmental Affairs and Toursim for use in decision making.

5.1 Monitoring of GMOs released into the environment

The objectives of an environmental post market monitoring plans are:

- to confirm that any assumption regarding the occurrence and impact of potential adverse effects or benefits of the GMO or its use in the environmental risk assessment are correct; and
- to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated or intended in the environmental risk assessment.

5.2 Case specific monitoring

Case-specific monitoring is intended to assess whether GMO-related adverse effects on the environment occur. It is based on specific risks that a particular GMO could present. Case specific monitoring can therefore be regarded as the continuation of the investigations performed during environmental risk assessment where defined hypotheses on possible anticipated effects are tested. The hypotheses can be confirmed or rejected after a defined period of time, after which case specific monitoring can be terminated. As case specific monitoring is performed in close relation to the cultivation of a certain GMO, it should be possible to draw conclusions about the causes of detected changes. The knowledge obtained may lead to new questions, which have to be answered in specific risk assessment studies. Case specific monitoring helps to reduce remaining uncertainties and its results may influence the environmental risk assessment of new GMOs with comparable properties.

5.3 General surveillance monitoring

General surveillance, on the other hand, is intended to detect unanticipated adverse environmental effects that were not identified and considered during pre-market risk assessment. Results obtained from general

surveillance cannot be linked to any specific attributes of GMO cultivation, since the program provides a general assessment of the state of the environment, independent of any preconception. It can provide information on exceptional environmental changes, and possibly provide basic information to forecast the likely development of the environment. General surveillance is not designed to determine the cause of possible environmental changes, as a multitude of factors could be involved. If environmental changes are observed, and it is considered likely that the cultivation of a specific GMO has caused them, the causality will have to be determined through specific risk assessment studies. DEAT would like to propose that the research to be conducted under the legal mandate of SANBI be of a general surveillance nature.

5.4 Monitoring plan

Along with general release application, the applicant must submit a monitoring plan introducing the objectives of the monitoring and explaining the details of the process itself.

5.4.1 Examples of parameters for monitoring

- Changes in the population of target insects as a result of the toxin produced by the GMO
- Changes in the population of non-target insects as a result of the toxin produced by the GMO -effects on organisms that normally feed on these non-target insects.
- Impact on non-target organisms
- Pollen transfer
- Persistence
- Dissemination
- Insect resistance
- Transfer of antibiotic resistance genes
- Changes in bio-diversity
- Cumulative environmental effects.

Table 5.1: Structure of a monitoring plan

Section 1: Monitoring strategy

- Concept
- Consideration of Environmental Risk Assessment
- Considering background information
- Case-specific monitoring
- Consideration of relevant objectives (according to the results of the ERA)
- General surveillance
- Baseline and controls
- Time scale of monitoring
- Responsibilities

Section 2 Monitoring methods

- Identification of parameters and methods that are valid and fit-for-purpose
- Methods for sampling and analysis
- Use of standardized methods - if applicable
- Adaptation to “state of the art”
- Sampling sites and networks
- Frequencies
- Collection and collation of (single) results/recorded data
- Responsibilities

- Deadlines
- Formats

Section 3 Intended analysis and reporting
- Frequency of the review and discussion of an overall analysis
- Intended analysis of the data
- Consideration of extraordinary conditions
- Statistics
- Intended modalities of reporting and publication
- Communication between applicant, authorities and third parties
- Publication of the results

Resistance Evolution and Management

Resistance evolution is one the key considerations in evaluating environmental risk in the South African context. It is commonly agreed that in the absence of adequate management strategies insect pests are likely to evolve resistance to transgenic insecticidal crops - such as the Bt crops - and cause crop losses.
However, resistance evolution in pests can be addressed using appropriate risk management strategies. Different crops and cropping systems offer different options for resistance management, but a practical management strategy can be developed that is specific to the crop, farming system and the environment in question.

Step 1. Identification of species at risk of resistance

All target species and some non-target species may evolve resistance, but some are more likely to do so than others, depending in part on their association with the crop, likely exposure to the transgene product and the genetic structure of their populations. The idea is to determine which species is at the greatest risk of developing resistance and to concentrate efforts around this weak link.

Step 2. Dose and dominance

These are technical options that determine whether resistance is ‘recessive’ or ‘dominant’. Dose refers to the concentration of transgene product to which the pest is exposed in the plant. If the resistance trait is functionally recessive (a high dose) it will take longer for resistance to evolve and allows more management options than if resistance is dominant.

Step 3. Assessing the degree of risk

Several factors influence the degree of risk, including the frequency of resistance, mating behavior and movement of adults, host plant use, occurrence of natural ‘refuges’, fitness cost associated with resistance, and the regional farming system. A refuge in this context is defined as a place where selection for resistance does not occur.

Step 4. Practical resistance management

Focusing on the weak link, practical and practicable management strategies can be designed. Most of these strategies require some designed implementation of refuges.

These resistance management strategies can be developed before field release of the GM crop. At the same time, critical information gaps are identified, which provides time for observation and research to develop an even more effective resistance management plan. It is important to consider here what post release monitoring of resistance frequencies is needed. Groundwork for that monitoring approach should be done before field release.

Step 5. Monitoring

SECTION 6: REFERENCES

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APPENDIX 1: GLOSARY OF TERMS

Introgression: A foreign gene/DNA is included in a recipient of the surrounding population and this recipient is environmentally fit, survives and (sexually) transmits the gene/DNA further to others in the population, thereby maintaining the pollution event.

GMO/transgenic/ transgene: Genetically Modified Organism: an organism, the genes or genetic material of which has been modified in a way that does not occur naturally through mating or natural recombination or both (GMO ACT, 1997).

LMO: Living Modified Organism- any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (Cartegena Protocol).

Ecological Risk Assessment (ERA): The application of risk assessment techniques to assessing risks to plants, animals and ecosystems. The ERA evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors. The assessment may describe the type, magnitude and probability of the effect and relate to the specific spatial and temporal context (DEAT, 2006).

F1: The first generation progeny of a cross between two parent sources, in the context of this document the F1 would be the progeny resulting from a cross between the GMO and non-GMO.

Biodiversity: "biological diversity" or **"biodiversity"** means the variability among living organisms from all sources including, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part and also includes diversity within species, between species, and of ecosystems;

Indigenous species: means a species that occurs, or has historically occurred, naturally in a free state in nature within the borders of the Republic, but excludes a species that has been introduced in the Republic as a result of human activity;

ANNEXURE 1: ENVIRONMENTAL RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS WITH INTENTIONAL STACKING OF TRAITS

REGULATION OF PLANT BIOTECHNOLOGY PRODUCTS CONTAINING TWO OR MORE TRAITS

Executive Council for Genetically Modified Organisms

Within the framework of the Genetically Modified Organisms Act, 1997

(Act No. 15 of 1997)

BACKGROUND

Combined trait plant biotechnology products (events) are those products containing more than one biotechnology-derived trait, for example insect resistance and another for herbicide tolerance.

Combined plant biotechnology products can be obtained through conventional breeding (crossing of plants carrying individual traits) or through modern biotechnology techniques (Agrobacterium transformation).

Combined traits are more commonly referred to as “stacks”.

PURPOSE OF THIS DOCUMENT

This document shall serve as a framework, in accordance with the provisions of the GMO Act, for the processing of applications for activities with plant biotechnology products containing two or more traits.

REGULATORY REQUIREMENTS IN SA

(i) All stacks, including vector stacks, whether obtained through conventional breeding or modern biotechnology techniques, are considered unique and novel.
(ii) Assessment of stacked trait products will be a science-based safety assessment, with additional safety assessments when the traits are expected to interact, or where they affect the same metabolic pathway.
(iii) The safety assessments previously undertaken for the individual single trait products should be taken into consideration for the combined trait product.
(iv) The Council recognises the approach taken by the World Health Organisation in 1995, which the conclusions of the safety assessments conducted for each of the individual traits apply to the combined trait products when the traits do not affect the same metabolic pathway.
(v) The Council recognises the application of the OECD (Organisation for Economic Co-operation and

Development) unique identifier system to combined trait products.

(vi) The Council may, without jeopardizing the safety to humans, animals and the environment, determine procedures to prevent redundant regulatory reviews of multiple applications for combined trait products (e.g. develop requirements with regard to bridging data).

INTERNATIONAL REGULATORY REQUIREMENTS

All countries consider vector stacks as new products.

Country	Regulatory requirement
Argentina	- Considers all stacks as novel and unique, but considers parent trait's status. - Require extensive regulatory data on the stack, but are considering a bridging regulatory approach.
Australia/ New Zealand	- Regulates stacks at single trait level (for food regulations). - For environmental release (cultivation), requires information on lack of antagonistic effects of the traits (interactions). - Requires developed notifies relevant agency on intent to market.
Brazil	- No specific regulations for stacks. - Requires field trial data to generate bridging data.
Canada	- Notification of intent to commercialise stacked trait crop. - Reserves the right to request data demonstrating substantial equivalence of stacked trait products to the parents.
China	- Regulates products at the single trait level. - No specific stacked trait requirements.
Colombia	- Regulates products at the single trait level. - No specific stacked trait requirements.
EU	- Consider stacked trait products novel, but considers parent trait's status. - Require very extensive bridging regulatory data (Approaching new product).
Korea	- Assess stacks on category basis. - Notification system for import – justification for exemption from further safety assessment (e.g. no change in the stacked trait progeny, no crossing between different species and no change in the resulting human consumption levels). - If product not exempted, separate safety assessments required – efficacious presence of stacked traits confirmed (e.g. through bioassay).

Country	Regulatory requirement
India	- Regulates products at the single trait level. - No specific stacked trait requirements.
Japan	- Assuming no change in progeny, no crossing between different species, no change in the resulting human consumption levels, traits are classified either: 1. Do not alter metabolic pathways of host plants, 2. Promote or inhibit a metabolic pathway of host plants – enhanced nutritional components or inhibit cell wall degradation; or 3. Introduce new metabolites that never existed in host plants. - Require confirmatory bioefficacy and/or expression data.
Mexico	- Assesses stacks on categorical basis (previously regulated as unique products). - Bridging approach for import approval of stacks that fit category 1 and 2: 1 – unrelated traits – require that each trait be previously approved and 2 – related traits with different modes of action (e.g. 2 insect control traits). - Category 3 – Traits that function in the same biosynthetic pathway (i.e. have increased potential for interaction).
Philippines	- Stacks not regulated as new events but assessed on possibility of interaction. - Conducts documentary risk assessment on possible or expected interactions between genes (or gene products) – potential for new allergen/toxins, affect protein compartmentalisation, change phenotypic characteristics, impact on mode of action and protein expression levels for single and combined traits.
Russia	- Regulates products at the single trait level. - No specific stacked trait requirements.
Taiwan	- Has no specific guidelines for stacked trait products. - Stacked trait products treated as novel or unique, regardless of parent trait's status. - Require regulatory bridging data as well as in-country agronomic data
US	- No specific regulations for stacked trait products (previously approved, single trait products). - Environmental Protection Agency regulates Bt x Bt stacks.

*Bridging regulatory approach refers to a regulatory process in which only certain data is required to determine if the stack resulted in any different traits than found in the parent lines (single trait events).

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ANNEXURE 2: CONSIDERATION OF THE USE OF ANTIBIOTIC RESISTANCE MARKERS IN DEVELOPMENT OF GENETICALLY MODIFIED ORGANISMS

The frequency of horizontal gene transfer from GM plants to other organisms is very low for all three groups of antibiotic resistance marker genes considered. This in itself is an important consideration with regard to the risk posed by the use of antibiotic resistance marker genes that can be divided into 3 groups.

Group I

Group I contains antibiotic resistance genes which (a) are already widely distributed among soil and enteric bacteria and (b) confer resistance to antibiotics which have none or only minor therapeutic relevance in human medicine and only restricted use in defined areas of veterinary medicine. It is therefore extremely unlikely (if at all) that the presence of these antibiotic resistance genes in the genome of transgenic plants will change the already existing bulk spread of these antibiotic resistance genes in the environment or will impact significantly on human and animal health. This refers to the following two antibiotic resistance genes:

• *npt*II gene: the substrates of the APH(3')II enzymes include the antibiotics, kanamycin, neomycin, paromycin, butirosin, gentamicin B and geneticin (G 418). The antibiotics of this category which are relevant for human therapy, amikacin, gentamicin (predominantly C1, C1a and C2) and other aminoglycosides and aminocyclitoles, are not substrates for the APH(3')-II enzymes. The *npt*II gene is widely spread in micro-organisms in the environment (Smalla et al., 1993; Leff et al., 1993).

• *hph* gene: hygromycin is not used in human therapy, and there is no cross-resistance with other antibiotics used for human therapy. This antibiotic was originally developed for veterinary use and is still added in some parts of the world to animal feed as an anthelmintic.

Group II

Group II contains antibiotic resistance genes which (a) are widely distributed in micro-organisms in the environment (soil, plant, water and the mammal gut) and (b) confer resistance to antibiotics which are used for therapy in defined areas of human and veterinary medicine. The presence of these antibiotic resistance genes in the genome of transgenic plants will have only a minimal effect on the bulk spread of these antibiotic resistance genes in the environment, and therefore will have a minimal impact on human and animal health, if at all. Their presence in genetically modified plants will thus not contribute to their occurrence in bacteria. This refers to the following antibiotic resistance genes:

• C*m*R gene: chloramphenicol-resistant micro-organisms are widely distributed in the environment, and many of these carry the CmR gene. In the EU, chloramphenicol is rarely used for medical purposes because of the risk of causing aplastic anaemia and has not been authorized for use in food-producing animals.

• *amp*R gene: it is reasonable to assume that almost every person on earth harbours or has harboured Escherichia coli cells containing the ampR gene in their intestinal tract, even without exposure to βlactam antibiotics. This is supported by the observation that approximately 35% of all clinical E. coli isolates are resistant

to ampicillin (Kresken et al., 1999) of which 90%, in turn, are caused by TEM-1 βlactamases (Livermore, 1995). Studies have also demonstrated that approximately 74% of all *E. coli* isolates from cattle and swine are ampicillin resistant (BgVV, 1997). Thus, even in the light of the clinical relevance of ampicillin, the presence of *amp*R (bla gene) in transgenes is not seen to significantly alter the existing pool of already resistant bacteria.

- *aad*A gene: streptomycin and spectinomycin are used in human medicine to a limited extent only (WHO, 1993). However, they still are of importance in human medicine for the treatment of tuberculosis (streptomycin) or gonorrhoea (spectinomycin). *aad*A is to a limited extent prevalent in a range of environmental habitats (Van Overbeek *et al*., 2002).

Group III

Group III contains antibiotic resistance genes which confer resistance to antibiotics highly relevant for human therapy and, irrespective of considerations about the realistic value of the threat, should be avoided in the genome of transgenic plants to ensure the highest standard of preventive health care. This refers to the following antibiotic resistance genes:

- *npt*III gene: for use in human therapy, amikacin is an important reserve antibiotic whose therapeutic importance should not, even potentially, be reduced by the use of the *npt*III gene in the establishment of genetically modified plants.
- *tet*A gene: tetracyclines are characterized by their wide spectrum of action and continue to be of therapeutic importance in human medicine; they are used to control *Brucella, Chlamydia, Mycoplasma, Rickettsia, Vibrio,* etc.

With regard to best practice, regarding therapeutically important antibiotics and the desire to limit the use of antibiotic resistance marker genes, it is recommended the use of antibiotic genes placed in group I (e.g. the *npt*II marker) as they have a 13-year history of safe use in food crops. Furthermore, resistance to antibiotics in group I is widespread in naturally occurring prokaryotic gene pools.

The use of antibiotic resistance marker genes in group II should be restricted to field trial purposes and should not be present in GM plants to be placed on the market. Experimental releases of GM plants are generally confined, being limited in time and space. GM plants in experimental releases are not intended for use in foods or feeds. No hazardous effects on human health and the environment are thus to be expected from the presence of the antibiotic resistance marker genes in GM plants used for experimental releases under approved conditions. Given their current importance in clinical usage, the GMO panel recommends that antibiotic resistance marker genes placed in group III should not be present in GM plants to be placed on the market or in plants used for experimental field trials.

There appears to be an emerging international consensus to evaluate each GMO containing antibiotic resistance marker genes on a case-by-case basis. Irrespective of the scientific conclusions, removal of the antibiotic resistance gene from the final GM plant or use of alternative strategies is now being recommended whenever feasible.

Specific ecological concerns with respect to antibiotic resistance marker genes relate to the possibility that if the gene is expressed in the plant, antibiotic resistance might result in a plant or one of its wild relatives becoming a weed, or might disturb the ecological relationships of the plant in another unknown way. The antibiotic resistance gene could also potentially be transferred from the GMO to soil micro-organisms. Any increase in antibiotic resistant soil micro-organisms could lead to a potential increase in human exposure to antibiotic resistant micro-organisms from ingesting them as contaminants of food and water.

ANNEXURE 3: ENVIRONMENTAL RISK ASSESSMENT OF GENETICALLY MODIFIED PLANT MADE PHARMACEUTICALS

Regulatory requirements for the release of genetically modified plants including those producing pharmaceutical (non-food) products in South Africa

Production of the transgenic plant	Host plant	Nature of the wild type organism including description of the host plant biology growth habit, levels of any toxins, antinutrients, and allergens known to be produced by the plant species. Is the plant species used for food or feed in a raw or processed form?
	Transgene construct	Description of the origin and function of all components of the inserted DNA and the extent to which it has been characterised. Physical map of the construct illustrating the position of each functional component.
	Method of transformation	Description of the gene transfer method including selection methods for the final transformation event.
Analysis of transgenic plant lines prior to release	Presence of inserted sequence	Description of the genetic and resultant phenotypic modifications of the GMO and how this can be verified using sequence information of the inserted DNA.
	Expression	Description of the methods used to determine the expression levels of the protein being produced. Quantitative data characterizing the distribution of the product in the major plant tissues (leaves, roots, stalks, seeds). Comparison of the properties of the natural protein with those of the protein as expressed in the transgenic plant.
	Inheritance	Demonstrate pharmaceutical plant lines are stable in both phenotype and genotype. To demonstrate genetic stability, include data from segregation analysis for the trait of interest as well as from a molecular characterization of the genomic insert and analysis of expression of the intended product. For fertile plants the pattern and stability of inheritance and expression over several generations.

		For infertile plants, demonstrate that the trait is stably maintained and expressed during vegetative propagation over a number of cycles that is appropriate to the crop.
Environmental Impact		Environmental issues affecting the cultivation of transgenic plants are likely to include the application of containment measures to production crops and to their genetic material (such as pollen), and arrangements for the proper disposal of transgenic waste material. The Environmental risk assessment must address: (i) The likelihood of increased persistence or invasiveness in agricultural or natural environments. (ii) Potential for gene transfer. (iii) Any direct or indirect impact on non-target organisms (iv) on human health. (v) Any direct or indirect impact on animal health and consequences for the food/feed chain. (vi) any effects of cultivation techniques specific to the PMP.
Transgenic Banking System	Seed stocks	A transgenic seed bank should be prepared to ensure consistent lot-to-lot growth of the plant and expression of the regulated product. Control over the inventory and disposition of viable seeds to preclude the possibility that such seeds will be used to produce material that could be used for food or feed production. Seed stocks should be stored in aliquots of appropriate volume to allow reasonable accurate accounting of use and disposition. A record of the amount and disposition of any withdrawals from the seed bank should be made.
Confinement of PMPs		Documentation relating to standard operating procedures (SOPs); outlines of production, or other records as appropriate. Ensure plant line is only used for its intended purpose as a source material for a regulated product. When a food or feed plant species that is used: consider mitigation measures including use of genetic markers and restricting the site or timing of expression of the product. For plants that outcross, grow in regions where little or none of the food/feed counterparts are grown. Establish control measures to ensure no inadvertent mixing of plant material with that intended for food or feed including the inadvertent mixing of seed. Determine where such inadvertent mixing could occur.

Cultivation of PMPs	Managing the field trial	Permit from DEAT and Department of Agriculture required to grow pharmaceutical plants in the field. Control over the growing process from planting through harvesting and over the disposition or remaining crops or drop residue and, if required over the subsequent use of the field if for growth of food or feed or as a pasture during subsequent seasons. All persons involved in field growth of the product should be adequately trained. Control measures should include accounting for seed, documentation of size and location of all sites, control of pollen spread, subsequent use of the field and the destruction of volunteers in subsequent growing seasons. Fields should be identified and the use of perimeter fencing to help exclude wildlife and escaped livestock is recommended. All fields are subject to inspection. Appropriate confinement measures for the transport of the source material from the field or greenhouse to the production facility. Containers of harvested material should carry a label that clearly indicates that the material is not to be used for food or feed. Reconciliation of the quantities of material leaving the growing facility and arriving at the production facility should be made.

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