DEPARTMENT OF AGRICULTURE
SOUTH AFRICA

GUIDELINE DOCUMENT FOR WORK WITH GENETICALLY MODIFIED ORGANISMS

Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997)

May 2004

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0001
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Foreword by Ms Thoko Didiza, MP and Minister for Agriculture and Land Affairs

According to the National Biotechnology Strategy, South Africa can be summarised as follows: "South Africa has a solid history of engagement with traditional biotechnology. It has produced one of the largest brewing companies in the world; it makes wines that compare with the best; it has developed many new animal breeds and plant varieties, some of which are used commercially all over the world and it has competitive industries in the manufacture of dairy products such as cheese, yoghurt, baker's yeast and other fermentation products".

However, in spite of the achievements from traditional biotechnology, South Africa has failed to extract value from the more recent advances of the technology, such as genomics, bioinformatics and proteomics. The majority of South Africans have not benefited from recent advances in biotechnology, largely due to the political history of the country where large sectors of the population could not access services and technologies in order to respond to agricultural challenges.

The National Biotechnology Strategy is designed to stimulate growth of biotechnology industries within South Africa to enable us to take full advantage of this technology and in turn maintain sustainable development. In order to achieve this successfully, a governmental agency will champion biotechnology, built human resources proactively and develop scientific and technological capabilities in this field. In addition, successful commercialisation of public sector-supported research and development (R&D) will require strong linkages between institutions within the National System of Innovation and a vibrant culture of innovation and entrepreneurship, assisted by incubators, supply-side measures and other supporting programmes and institutions.

Government has identified agriculture as one of the sectors of the economy that require special attention because of its potential to contribute to the objectives of higher growth rates and job creation, but also for its potential in addressing other national imperatives such as improved access to and affordable health care, sufficient nutrition at low cost and the protection of our rich environment. With the vision of a united and prosperous agricultural sector, the Department of Agriculture acknowledges the diversity of the agricultural sector and aims to ensure a place and role for all farmers in a united sector. This includes sectors taking advantage of genetic engineering, provided that the technology is applied in a regulated manner.

All activities with genetically modified organisms in South Africa are regulated under the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997). This Act provides for measures to promote the responsible development, production, use and application of genetically modified organisms to ensure that all activities involving the use of genetically modified organisms are carried out in such a way as to limit possible harmful consequences to the environment. The Act also makes provision for the determination of requirements and criteria for risk assessments that will ensure that genetically modified organisms are appropriate and do not present a hazard to the environment or human and animal health.

The GMO Act is administered by the Directorate Genetic Resources within the national Department of Agriculture and makes provision for a Registrar, two regulatory bodies, i.e. the Advisory Committee and Executive Council, and inspectors. The Registrar is responsible for administration of the Act, the Advisory Committee for evaluation of risk assessment data within every application and the Executive Council for taking a decision on whether a specific activity should be authorised or not. Inspectors appointed in terms of the Act monitors authorised activities with GMO's across the country.

Sections 4 and 5 of the Act stipulate the objectives, powers and duties of the Executive Council. One provision made in Section 5 is the development and publication of guidelines for all uses of GMO's. It is in accordance with this provision, as well as the aim to establish appropriate procedures for the notification of specific activities involving the use of genetically modified organisms, that the Department of Agriculture has, through the assistance and recommendations

of the Advisory Committee and Executive Council, produced the guidelines provided for in this document.

These guidelines aim to provide general information on the provisions of the Act, functioning of the bodies appointed in terms of the Act, how applications are processed and provide assistance to the applicant on how to apply for a permit. The guidelines will aid in public understanding of the administration of the Act and increase transparency towards the regulation of GMO's in SA. I therefore want to express my sincere gratitude and appreciation to the Advisory Committee and Executive Council, and the Registrar for GMO's, for their commitment in developing these guidelines.

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1. Introduction

1.1 The national Department of Agriculture has prepared the following guideline document in consultation with the Advisory Committee and the Executive Council appointed under the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997). All activities involving genetically modified organisms (GMO's) are subject to the Regulations under Section 20 of the GMO Act.

1.2 These guidelines complement the Regulations of the GMO Act, are in accordance with the regulations and the Act and should be utilised in conjunction with the regulations and the Act. These guidelines also provide information on risk assessment and risk management procedures.

1.3 The Annexures to the guidelines contain information on specific topics discussed in the document. Please take note that these guidelines are not exhaustive and will be updated when necessary and to include new scientific information, particularly when the Genetically Modified Organisms Act, 1997 (Act No. 15 of 1997) has been reviewed. Application forms for any activity with GMO's in South Africa can be obtained from the Department of Agriculture's website at www.nda.agric.za.

1.4 All risk assessments will be approached in a precautionary way and that every decision on a proposed activity will be taken on a case-by-case basis.

1.5 To obtain further information on any of the sections contained in the guidelines or on completion of the application forms, please contact the office of the Registrar.

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Directorate Genetic Resources
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2. Responsibilities

2.1 Responsibilities of applicant/permit holder

a) To comply with all applicable regulations established in SA.
b) To prepare a folder for submission to the Registrar with each application for experimental release or commercial production including all pertinent and required information on the GMO to be released.
c) Ensure that persons involved in distribution of a GMO product are adequately trained, such that they are capable of providing a user with advice on efficient and safe use. This also applies in the event that the distributor is at arms length to the owner of the product.
d) Notify the Registrar of any problem related to the release and use of the GMO, voluntarily corrective action and, when requested by Registrar, help to find solutions to any problem.

2.2 Responsibilities of companies/farmers growing GMO's

2.2.1 Farmers should:

a) Maintain appropriate records of GMO varieties, area planted and pesticide use.
b) Respect and obey indications and requirements related to risk management, including refugia and other agronomic practices, intended to prevent or delay the evolution of resistance in pests.

c) Comply with any signed agreement regulating the production, saving and distribution of GMO.
d) When growing GMO's, which involve the use of a pesticide, follow the regulatory rules for the particular pesticide and specific use.
e) Adhere to all other regulations regarding handling and cultivation of plants.

3. Potential effects resulting from activities with GMO's

3.1 The potential impact that activities with GMO's can have on the environment and human health can be categorised in mainly three sections. These include –

i) Intended and unintended phenotypic changes
ii) Intended and unintended ecological effects
iii) Intended and unintended effects on human health and welfare.

3.2 The different potential effects are outlined in Table 1 (Source: Manual for assessing ecological and human health effects of genetically engineered organisms – Reference 9.9). This table lists six general classes of potential and intended phenotypic modifications, representative kinds of intended or unintended ecological effects resulting from the modifications, and a few examples of affected human enterprises and matters of environmental protection.

3.3 The aim of this table is to remind the user of the great range of alterations and effects that need to be considered when planning an activity with GMO's. Please note that the effects resulting from a single modification may not necessarily be confined to only one category and the applicant should consider all possible effects that a modification can have.

This table is not exhaustive and new data will be incorporated when necessary.

Table 1: The relationship between the potential and intended phenotypic modifications, unintended or intended ecological effects and effects on human health and welfare.

TYPE OF EFFECT	EXAMPLES OF POTENTIAL/INTENDED PHENOTYPIC CHANGES	EXAMPLES OF INTENDED/UNINTENDED ECOLOGICAL EFFECTS	EXAMPLES OF EFFECTS ON HUMAN HEALTH AND WELFARE
Metabolism	▪ Individual growth rates ▪ Energy metabolism, pathways and rates ▪ Photosynthetic and chemosynthetic pathway structures and rates ▪ Rates of nutrient uptake and cycling ▪ Amounts and types of nutrients used ▪ Use of pollutants as nutrients, and pollution degradation ▪ Nitrogen fixing pathways and rates ▪ Carbon dioxide consumption ▪ Tolerance of elevated CO_2 ▪ Expression of novel proteins or metabolites, and increased metabolic wastes ▪ Production of antibiotics, or biological toxins such as that from *Bacillus thuringiensis* (Bt toxin) ▪ Antibiotic or pesticide resistance	▪ Altered feeding rates and efficiencies ▪ Altered rates of nutrient cycling and biological energy transfers ▪ Altered rates of photosynthesis and carbon fixation and plant productivity ▪ Modified rates and patterns of nitrogen fixing ▪ Shifts in competitive abilities among species ▪ Changes in the degree of pesticide and antibiotic resistance among target and naturally occurring species, and spread of antibiotic resistance genes by lateral transfer ▪ Release of antibiotics, toxins, or increased concentration of novel metabolites ▪ Decrease or increase of biological diversity	▪ Changes in agricultural productivity ▪ Changes in forest production and timing of tree harvesting cycles ▪ Changes in stock composition and productivity of fisheries ▪ Increased dependence on aquaculture ▪ Increased intensity and variety of food allergies due to novel proteins, hormones or other metabolites, or altered levels of normal proteins and hormones and other metabolites

TYPE OF EFFECT	EXAMPLES OF POTENTIAL/INTENDED PHENOTYPIC CHANGES	EXAMPLES OF INTENDED/UNINTENDED ECOLOGICAL EFFECTS	EXAMPLES OF EFFECTS ON HUMAN HEALTH AND WELFARE
Tolerance of physical factors	▪ Temperature ▪ Humidity or moisture ▪ Soil chemical and physical properties, including nutrients and water potential ▪ Light intensity ▪ Salinity ▪ pH (acid/base) ▪ Water chemistry ▪ Pressure ▪ Oxygen, carbon dioxide, and other gases such as those of anaerobic environments ▪ Toxic chemicals/pesticides/antibiotics ▪ Heavy metals (e.g. mercury	▪ Geographical relocation, expansion or concentration of preferred habitats for species and ecological communities ▪ Changed species/population phenology (seasonal timing of life cycles), including patters of growth, development, and breeding ▪ Altered geographical ranges of species ▪ Altered patterns of dispersal and migration ▪ Increased and change in routes and extent of biomagnification (concentration) of toxic substances, including heavy metals ▪ Changed composition and diversity of ecological communities	▪ Change in geographical or local constraints on crop production ▪ Changes in geographical or local constraints on disease vectors, pathogens, pests or pollinators ▪ New threats or persistence and abundance of terrestrial and aquatic wildlife ▪ Increased invasiveness of noxious or weedy species ▪ Loss of genetic diversity in natural populations
Morphology or architecture of organisms	▪ Animal shape, size, colour ▪ Internal and surface geometry of unicellular algae and protozoa ▪ Antigenicity of disease organisms and parasites ▪ Skeletons and appendages ▪ Leaf shape, pattern of plant nodal extension and branching, flower structure, branching and frond geometry of macrophytic algae ▪ Spines, hairs, trichomes and other protective devices ▪ Bacteria cell-wall characteristics ▪ Mosaic segments of virus ▪ Cell structure, organs, organ systems ▪ Unicellularity, multicellularity	▪ Altered species interaction: predator/prey, herbivory, competition ▪ Mate recognition ▪ Changes in bacterial cell walls and some antibiotic resistances ▪ Altered virus/host interaction ▪ Changed crop plant architecture ▪ Increase or decrease in plant protection against pathogens and herbivores	▪ Increase or decrease in virulence of pathogens ▪ Gains or losses in plant yields through changes of achritechture (e.g. dwarf varieties of rice and wheat) ▪ New problems in conservation ▪ New opportunities for horticultural innovation

TYPE OF EFFECT	EXAMPLES OF POTENTIAL/INTENDED PHENOTYPIC CHANGES	EXAMPLES OF INTENDED/UNINTENDED ECOLOGICAL EFFECTS	EXAMPLES OF EFFECTS ON HUMAN HEALTH AND WELFARE
Behaviour	▪ Reproduction ▪ Territoriality ▪ Migration, navigation or orientation ▪ Chemosensory abilities, including pheromones and allochemicals ▪ Motility/locomotion ▪ Animal communication ▪ New kinds and levels of plant secondary compounds ▪ Colonisation ▪ Phatogenicity of bacteria, virus and fungi ▪ Mutualisms/coevolution ▪ Pollination ▪ Photoperiodism ▪ Foraging patterns and feeding specialisation and rates ▪ Social behaviour, communicable and co-operative living, “altruism”	▪ Altered breeding patterns and cycles, and mate-recognition systems ▪ Change in population abundance and species assemblages ▪ Altered population dynamics and phenology ▪ Changes in self-compatibility and incompatibility of plants ▪ Changes in rates, plant species spectrum, and effectiveness of pollination ▪ Increases and decreases in pathogenicities and patterns of disease transmission	▪ Changes in local and geographical patterns of abundance of wildlife, game and commercially harvested species ▪ Alterations in agricultural productivity ▪ Increase or decrease in human, animal and plant health as behaviours of pathogens, disease vectors and pollinators change
Factors controlling or regulating natural populations	▪ Novel disease resistance ▪ Reduced predation or parasitism ▪ Habitat preferences, extensiveness of preferred and secondary habitats ▪ Antibiotic or biocide sensitivity and resistance ▪ Extinction, local or global ▪ Increases or decreases in fitness	▪ Altered population and community dynamics ▪ Release from pre-existing ecological limits or establishment of new limits ▪ Changed disease transmission ▪ Lateral transfer of antibiotic and toxin resistances among bacteria ▪ Changed tropic interactions ▪ Increase or decrease in pest and pathogen populations and the attendant problems	▪ Decline or loss of therapeutic effectiveness of antibiotics ▪ Origin of new pests, weeds and pathogens (especially plant virus modification)
Demography, life history, population	▪ Population fitness ▪ Average life cycle patterns (simple or complex)	▪ Altered population and community dynamics ▪ Shifts in the composition of ecological	▪ New problems in pest and pathogen control ▪ Epidemiological problems

TYPE OF EFFECT	EXAMPLES OF POTENTIAL/INTENDED PHENOTYPIC CHANGES	EXAMPLES OF INTENDED/UNINTENDED ECOLOGICAL EFFECTS	EXAMPLES OF EFFECTS ON HUMAN HEALTH AND WELFARE
genetics and evolution	▪ Mode of reproduction: sexual, asexual, or alternating between these two ▪ Frequency of reproduction ▪ Average rates and patterns of embryonic and larval development ▪ Patterns of metaphorphesis ▪ Age of reproductive maturity and age of last reproduction ▪ Fertility and fecundity ▪ Survival rates with age (survivorship), average longevity ▪ Net and intrinsic rates of change in population size and density ▪ Age-structure of population ▪ Social organisation, kin selection and inclusive fitness ▪ Substratum affinities ▪ Patterns of dormancy, diapause, aestivation, hibernation, and spore and seed banks ▪ Sex, sex ratios, mating types ▪ Population genetic structure, genetic recombination within populations ▪ Genotype-environment interactions and correlation ▪ Pathogens host ranges ▪ Vector host ranges and competence ▪ Geographical arrays of conspecific populations (metapopulations) ▪ Specialised genetic exchange (sexual) mechanisms of bacteria (transduction, transformation, conjugation, retrotransposons, conjugative transposons, other mobile elements) ▪ Gene flow among conspecific populations ▪ Hybrid zones and geographical clines ▪ Genetic exchange between species and phylogenetic lineage	communities and local biological diversity ▪ Increased or decreased fitness of populations ▪ Increased or decreased pop8ulation sizes and dentsities ▪ Increased or decreased populations fluctuations, population stability ▪ Altered age-structure in populations ▪ Micro-evolutionary changes set in motion in the GMO population or surrounding natural populations ▪ Changes in spatial and temporal distribution of population and species ▪ Altered genetic structure of the GMO population and their parental populations, if the two are sympatric (conspecific introgression) ▪ Increase interspecies hybridisation ▪ GMO evolution due to mutation, genetic exchange and natural selection	▪ Commercially harvested and/or game species yield change ▪ Conservation and wildlife management practices require adjustment ▪ Design of wildlife refuges and nature preserves require reconsideration and possibly change ▪ Mitigation procedures become necessary to protect biological diversity and the genetic diversity of natural populations

4. Possible routes of dispersal into the environment

When compiling an application to conduct a certain activity with GMO's, the applicant must consider all possible routes by which the GMO may be dispersed into the environment, through the duration of the activity, regardless of whether the dispersal was intended or unintended, in order to determine the appropriate level of containment required.

4.1 Natural routes of dispersal

- Flowing water
- Subsurface flowing water
- All birds, flying animals (bats), earthworms (arthropods), spiders (arachnids), etc.
- Wind
- Terrestrial vertebrates, especially mammals (fur)
- Terrestrial and flying insects
- Rafting on logs and larger floating islands broken away from shorelines, on lakes, rivers and seas
- Ocean and lake currents
- Atmospheric circulation with subsequent deposition as rain, etc.
- Autonomous locomotion
- Tornadoes, cyclones, hurricanes, floods, etc.
- Influent/makeup water
- Effluent/drawdown water
- Waste slurries
- Aerosols

4.2 Routes of dispersal due to human activity

- Shipping at sea and on large lakes and rivers
- Via ballast water and sediments
- On all the surfaces and crevices of boats below the water line
- On surfaces above the water line
- Floating oil and gas drilling platforms
- Aircraft
- Ground transport (including agricultural equipment such as tractors)
- Recreational boats
- Containers used to transport live organisms
- Containers used to transport food
- Transport of crop seeds, cuttings, and nursery stock
- On and in human bodies or clothing
- Trash, refuse or garbage
- Agricultural livestock
- Sewage systems
- Navigation canals allowing active dispersal of mobile organisms
- Transfer of water between municipalities and regions, for domestic and industrial use and irrigation
- On cleaning materials and in dust

5. Risk assessment

i) Before any activity with GMO's may be undertaken, an identification of risks to human health and the environment and the potential impacts of the GMO should be undertaken. This process is called risk assessment. Risk assessments should be conducted on a case-by-case

and step-by-step basis. For e.g. one cannot assume that because it is regarded as low risk to release GM-sorghum in the United States, that it is also safe in Africa, its centre of origin. The step-by-step principle refers to the fact that during initial work with an organism, where little is known and there is a high level of uncertainty, a conservative approach to risk management would normally be adopted. However, every opportunity should be taken to gather data on the performance of the GMO under the more restrictive conditions in order to be able to form judgements about the future safety if the control measures are relaxed.

ii) A properly conducted risk assessment should reveal all hazards posed by the GMO, a comprehensive description of such hazards, how the identified hazards could be realised, the likelihood and frequency that harm will result should the hazards be realised and, an overall evaluation of the risk and the type, significance and magnitude of impacts should the hazards be realised.

iii) The level of detail to be considered during a risk assessment will depend on circumstances. All risk assessments have to be "suitable and sufficient". For a simple operation involving a well-known and well-understood organism, the hazards will be relatively low and may it be possible to declare the result of the assessment almost at first glance. For a complex operation involving dangerous organisms about which there is a lot of uncertainty, the assessment will have to be extensive and may involve the acquisition of new data.

iv) Note that it is always permissible to assume the worst and act accordingly if there are any doubts present when performing the assessment. In other words, if there is a range of risk for a given organism and activity within which you are uncertain of the level of risk, you may simply choose to apply control measures appropriate to the upper bound of the range applicable.

v) A risk assessment should always be reviewed if there is any reason to suspect that the initial assessment is no longer applicable due to significant changes in the activity, or the acquisition of new scientific knowledge.

5.1 Risk assessment with regard human health aspects

This section deals in general with the issues that need to be considered when determining the effects of a GMO on human health. The following issues should be taken into account –

a) Possible modes of exposure (i.e. skin contact, ingestion, inhalation of aerosols, etc.)
b) Concerns with regard to
   - Toxic, allergenic and pathogenic effects
   - Product hazards.
   - Transfer of antibiotic resistance, etc.
c) Concerns related to human activities, such as –
   - diseases caused and mechanism of pathogenicity, including invasiveness and virulence.
   - communicability.
   - infective dosage.
   - host range, possibility of alteration.
   - possibility of survival outside of human host.
   - presence of vectors or means of dissemination.
   - biological stability
   - antibiotic resistance patterns.
   - allergenicity
   - availability of appropriate therapies, etc.

(d) Susceptibility of humans exposed to the GMO (e.g. immunosuppressed status)

Although there are various methods available to undertake risk assessment for human health, one can make use of the Brenner Scheme, which deals primarily with human health issues. More information on this Scheme can be found in Section 5.4.1.

5.2 General requirements for food safety assessments.

5.2.1. General**:** An assessment of the safety of genetically modified organisms when intended for food or/or feed shall be conducted on a case-by-case basis. Guidance is given in this document. Feed although not intended for human consumption may enter the human food chain unintentionally and would therefore be considered as human food.

Applications for licensing of all genetically modified organisms must be directed to:
The Registrar: Act 15 of 1997
Directorate: Genetic Resource,
Department of Agriculture
Private Bag X 973
PRETORIA 0001

Tel 27 012 319 6536
Fax 27 012 319 6329

5.2.2. Codex Alimentarius Commission (www.codexalimentarius.net.)

The Codex Alimentarius Commission approved the following principles and guidelines:
A Principles for the risk analysis of foods derived form modern biotechnology.

B Guidelines for the conduct of food safety assessment of foods derived form recombinant-DNA plants including the assessment of possible allergenicity.

C Guidelines for the conduct of food safety assessment of foods produced using recombinant – DNA micro-organisms.

The Codex Alimentarius principles and guidelines are accepted as policy for food safety requirements by the Department of Health, South Africa (www.doh.gov.za)

5.2.3. The applicant must submit data and information according to the following requirements.

5.2.3.1. Presentation of food safety data
In order to facilitate the evaluation of data, the following are requested of the applicant:

a. Summary
The applicant must submit a comprehensive summary of all results preferably in table format.

b. Data
The applicant should submit a complete package of the data required for food safety assessments. The data shall be concisely presented.

- Each volume should contain a table of contents, mentioning the title and page numbers;
- An index of the each package of data must be provided in a separate volume should more than one volume be submitted;
- The contents must be entered under the appropriate headings. Each entry should include the date of submission, originating laboratory, company name, author, title of the paper and location in the submission such as page and volume number;

c. Reporting of data
The format and content of the report shall be as follows:

- Each report should include a copy of the Good Laboratory Practice (GLP) certificate and must provide the name of the study director, date of completion, statements on Good Laboratory Practices (GLP) and Quality Assurance (QA), signature of the senior scientific responsible person and certification by the applicant, or an authorized agent of the applicant, as a complete and unaltered copy of the report provided by the test laboratory;
- Summary of the test results;
- Full description of the test procedures and methods, including deviations and reasons for the deviations;
- Reporting of the results and evaluation of specific tests including all data, information and analyses done;
- Discussion of positive and negative results. Explanations must be given, based on sound scientific principles. Conclusions arrived at by the study author must be included;
- Appropriate statistical methods shall be used to summarize experimental data, to express trends, and to evaluate the significance of differences in data obtained from different test groups. All data averages or means shall be accompanied by standard deviations;
- References must be supplied for the statistical and other methods employed for analysing data, and for any published literature used in developing the test protocol, performing the testing, making and interpreting the observations, and compiling and evaluating the results;
- A description of the testing methods must be supplied. Toxicological methods describe by the OECD. Methods other then those of the OECD would also be considered. Where applicable, analytical methods described by Codex Alimentarius are recommended.

d. Literature studies
The following information is required when literature is submitted to substantiate the submission:

- A copy of the journal article or the publication;
- Motivation for inclusion of the published information;
- Assessment of the published information; and
- Reference at appropriate places in the text.

e. Registration status
Full details of the registration status in other countries specifically with respect to food safety issues.

5.2.3.2. Requirements for the conduct of food safety assessment of foods derived from recombinant-DNA-plants

The applicant is advised to study the Codex Alimentarius documents. The framework for submitting data is the following:

A) Description of the recombinant-DNA plant;
B) Description of the host plant and its use as foodstuff;
C) Description of the donor organisms;
D) Description of the genetic modification(s);
E) Characterisation of the genetic modification(s);
F) Safety assessment:
   - Expressed substances (non-nucleic acid substances)

- Compositional analysis of key components
- Evaluation of metabolites
- Food processing
- Nutritional modifications and

G) Other considerations

5.2.3.3. Additional data and information

a. Exposure assessment
An exposure assessment based on South African food intake data must be submitted. This must be accompanied by a calculation of the Safety Factor

b. Use of antibiotic resistance marker genes
An assessment of the presence of antibiotic maker genes will be conducted. The availability of any clinically used antibiotics in South Africa is a specific consideration.

c. Animal feed
A food safety assessment is applicable to all applications for licensing of a genetically modified organism whether applied to food and/or feed.

5.3 Environmental risk assessments

i) The main objective of an environmental risk assessment of GMO's is to identify possible effects on the environment from growing these plants. Risk identification is only the first step in a conventional risk assessment, the other steps being risk characterisation (magnitude of the risk), exposure assessment (in this context an estimate of likelihood or frequency of identified risks) and finally risk communication.

ii) Risk assessment takes into account the results of the three steps to provide an estimation of the likelihood of any adverse effect occurring, as well as an estimate of the magnitude of harm that might result. This risk assessment may be quantitative or qualitative. The latter has prevailed in previous cases with approval of GMO's, because the complexity of biological systems makes it difficult to pursue a quantitative approach. Much of the needed information for a risk assessment can be obtained from practical experience with traditional crops growing in the same environment, but in some cases further experimentation is needed particularly regarding gene flow and fitness.

iii) In accordance with the Act, the applicant is required to deliver the relevant information and the Executive Council will then base their evaluation upon this information, combined with expert opinions (Advisory Committee) and public input. The objective of the following section is, however, only to identify potential adverse ecological risks to the environment.

5.3.1 Delimitation

This section of the guidelines is confined to dealing with the environmental risk assessment of GM-plants and is based on a strictly scientific and technical approach. The risk assessment must be performed on a case-by-case basis and adapted to the local conditions and agricultural production system. Relevant aspects related to GMO's such as food and feed safety, pleiotropic effects associated with transgenes, ethical concerns and socio-economic consequences are not considered in this section.

5.3.2 Potential risks of GM-plants, in particular herbicide resistant crops (HRC) and insect resistant crops (IRC).

Kindly note that undertaking a risk assessment may also include potential benefits from the introduction of the GMO, and not only negative effects, in order to provide for a balanced view.

i) The GM-plant may establish itself beyond its agricultural boundaries and growing season and become a weed in the succeeding crops.
ii) The GM-plant may inter-cross with non-transgenic relatives growing in the same or adjacent areas, depending on cross pollination characteristics and agents such as wind or by insects. In some instances where the population size of native relatives is low, genes from the transgenic crop may come to dominate the native population and lead to their extinction. The compatibility between the HRC/IRC and non-target species is of utmost importance in this regard. In the instance of the GM-plant crossing with wild relatives, this should be considered in context of (a) inter-crossing that might already have occurred between the non-transgenic crop and the wild relative (are the risks any different in the case of a GM crop?) and (b) any likelihood of real adverse effects on the environment as a result of such inter-crossing.
iii) The GM-plant may have botanical identical or closely related species that can hybridise with the plant, either in the adjacent ecosystems or in the agro-ecosystem. Hybridisation could lead to the spread of introduced genes into non-transgenic crops, gene stacking in volunteer plants and transfer of introduced traits to weedy or wild species.
iv) The continued use of HRCs with their associated herbicide over large areas for several years may unintentionally change the composition of the weed flora by selecting for naturally tolerant weeds. This is particularly important in monocultures or in cropping systems with limited crop rotation or minimum tillage. However, this is only valid if it takes into account the potential impact of changes in herbicide usage compared with the current situation. Herbicides have been used routinely in agriculture for many years so the issue of herbicide resistance in weeds is not new.
v) The potential beneficial and negative effects of HRC and IRC should be mentioned (e.g. less loss of topsoil as a result of minimal tillage in the case of HRC, and less impact on non-target insects through the use of IRC as an alternative to indiscriminate chemical spraying)
vi) The engineered traits may increase fitness of volunteers or weedy hybrids, thus making a crop turn into a weed that can interfere with future crop production or aggravating the negative impact of existing weed species. The incorporation of resistance into a non-target species may also alter its competitive ability and displace other native species.
vii) Intensive use of GM-plants to control pests or diseases may result in the selection of insect or microbial strains resistant to this method of control.

5.3.3 General information desirable for risk assessment of GM-plants

i) Information related to the GMO
- Taxonomic description and scientific name
- Cultivar's name
- Diagnostic phenotypic and genetic markers
- Description of geographic distribution and of the natural habitat of the plant
- Potential for gene flow and exchange with other plants
- Ecological and physiological traits
  - Generation time in natural ecosystems, sexual and asexual reproductive cycle
  - Information on survival, including the incidence of volunteers and the ability to form perenniating structures (propagules)
- Pollination mechanism (s)
- Longevity of pollen or seed
- Methods of vegetative reproduction
- Any mechanism to limit propagation (e.g. male sterility)
- History of previous releases or uses of the GMO

ii) Information inserted genetic material in the GMO and related to the genetic modification process
- Methods used for the modification
- Description of the inserted genetic material and vector construction

- Sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question
- Description of genetic trait(s) or phenotypic characteristics, particularly new traits and characteristics which may be expressed or no longer expressed
- Stability of the genetic trait(s)
- Rate and level of expression of the new genetic material
- Cumulative effects of more than one insert in the event (e.g. stacked events, such as MON810 x NK603)
- Description of identification and detection techniques

iii) Information on the receiving environment
- Geographical location of the site
- Proximity to protected habitats or areas
- Proximity to compatible, related species
- Climatic characteristics and flora and fauna of the region
- Description of target and non-target ecosystems likely to be affected
- Any known planned developments or changes in land use in the region which could influence the environmental impact of the released crop
- Description of ecosystems to which the GMO could be disseminated
- Distance from human settlements
- Prevailing winds
- Proximity of surface water

iv) Information related to the interactions between the GMO and the environment:
- Characteristics affecting survival, multiplication and dissemination
- Studies of the behaviour and characteristics of the GMO and their ecological impact
- Post release genetic transfer capability from the GMO into organisms in the affected ecosystems
- Likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the GMO
- Description of genetic traits, which may prevent or minimise dispersal of genetic material.
- Routes of biological dispersal and known or potential modes of interaction with the dissemination agent.

v) Potential environmental impact
- Potential for excessive population increase in the environment
- Competitive advantage of the GMO in relation to the unmodified recipient
- Anticipated mechanism and result of interaction between the released plant and wild and weedy relatives
- Known or predicted effects of non-target organisms on the environment, impact on population levels of all potential competitors.

5.3.4 Information required with regard to the conditions of experimental release

i) Description of the proposed release including the purposes and foreseen products
ii) Foreseen dates of the release and time planning of experiment including frequency and duration of release
iii) Size of the site
iv) Method to be used for the release
v) Quantities of GMO to be released
vi) Method of cultivation and description of general agricultural practices
vii) Post-release treatment of the site
viii) Techniques which will be applied for the elimination or inactivation of the GMO upon experiment completion
ix) Information on and results of previous releases of the GMO, especially at different scales in different ecosystems.
x) Problems associated with volunteer plants

- Is the crop known to leave volunteers in succeeding crops?
- Does the crop have weedy traits?
- What is the characteristic of the volunteer plants?
- Can the volunteer be easily controlled? Explain how.
- Is a herbicide used for the control of non-transgenic volunteers in succeeding crops?
- Is the volunteer able to establish in the wild?
- Does the trait present in the volunteer increase the fitness of the volunteer compared to non-transgenic volunteers?

xi) Hybridisation with weedy or wild relatives

- Is the crop strictly self-pollinating?
- Is the trait maternally inherited?
- Do hybrids occur between the crop and weedy/wild relatives?
- Do these relatives occur in the proximity of the crop?
- Do the relatives and the GM-crop have similar flowering periods?
- Do the GM-crop or relative set seed?
- Is there a severe fitness penalty in the first generation backcrosses?
- Does the trait give hybrids, backcrosses or wild relatives an advantage on arable land or wild habitats?
- Does the hybrid or backcross have the same trait as the GM-crop?
- Can the hybrid or backcross be controlled by means of herbicides or through any other means?
- Is the hybrid or backcross able to survive and establish in the wild?

xii) Hybridisation with non-GM-crops

- Is the crop strictly self-pollinating?
- Is the trait maternally inherited?
- Can pollen be dispersed to and fertilised by other crop varieties?

xiii) Built-up resistance in insects

- Does the GM-crop comprise a minor proportion of the local area planted with non-transgenic varieties of the crop?
- Does the GM-crop contain different ingredients active against the harmful insect?
- Is expression of the trait confined to a selected growth stage of the crop and how long does this growth stage last?
- If resistance occurs, is expression of the trait associated with a significant fitness penalty for any resistant insects?
- Can resistant insects be controlled by other control measures?

xiv) Dispersal in time and space

The transfer of genes involves hybridisation, and is followed by the subsequent establishment and persistence of the hybrid. The transfer of genes to different entities, i.e. non-transgenic crops, wild non-weedy relatives, weedy relatives and volunteers may be influenced by the following elements-

- Transfer of genes to non-transgenic crops:
  - Consider the release of pollen and the possibility of male sterility
  - The proximity between crops relative to pollen dispersal distance
  - Synchrony of flowering
  - Seed set of the crop
- Release of pollen to wild non-weedy relatives:
  - Identification of the centre of origin or diversity of the crop
  - Presence of wild relatives in the natural habitat of the non-GM crop
  - The release of pollen from the GM-crop (male sterility)
  - Presence of cross compatible wild species
  - Proximity in distance between crop and natural habitat
  - Synchrony of flowering
  - Will the transferred trait control insects, which are natural enemies of the wild species?
  - Whether the insect is a significant constraint to the abundance of the wild species.

- Transfer of genes to wild relatives
  - Release of pollen from the crop (effect of male sterility)
  - Presence of cross compatible weedy species
  - Synchrony of flowering
  - Seed set
  - Seeds that will survive and be incorporated into seed banks. Deposits into seed banks will be influenced by (a)seed dormancy after seed shed and (b)cultural practices (post harvest harrowing)
  - GM-hybrids being backcrossed into wild species. This will depend on (a) the first generation of backcrosses and (b) the presence of the wild species and GM-hybrids in succeeding crops.
  - GM-wild species which are not suffering from a fitness penalty. This occurrence will depend on the potential of these species to become a problematic weed problem, and it will require an increased fitness compared to non-transgenic weeds.
- Transfer of genes to volunteers
  - The management of GM-volunteers is in most cases no different than those practices used for non-transgenic volunteers.
  - Considerations should be given to whether the GM-volunteer may become a weed problem under conventional circumstances, posing difficulties to control.
  - It should be determined whether the GM-volunteer's seed have the potential to accumulate in the soil and build up a persistent seed bank.

xv) Possibility of the GMO becoming a weed outside agriculture

- The possibility of GMO's becoming weeds outside agriculture depends on whether volunteer plants can establish as feral populations in neighbouring non-agricultural areas.
- It should be determined is the volunteer plant have biological and ecological attributes that will make it invasive of natural habitats
- Determine whether the trait does indeed confer any advantage to the crop outside agricultural boundaries.

xvi) The presence of seed banks

The presence of a seed bank can influence the environmental risks imposed by the GM-crop in the following manner:

- A seed bank may enable a weed or volunteer to spread in time.
- A seed bank may facilitate
  - Cross breeding of volunteer and succeeding crops of the same species by allowing the crop to grow as a volunteer
  - Cross breeding of volunteer and compatible weedy species by allowing the crop to come into contact with the weed in a succeeding crop
  - Emergence of volunteers which may not be controlled by the herbicide to which the GM crop was resistant

xvii) Development of resistance in other potentially harmful organisms

- Planting of GM-crops and using a selective set of herbicides may result in new selection pressures on weeds for herbicide tolerance.
- Similarly a selective pressure for the development of resistance may develop in certain insects.

xviii) Effects on non-target organisms

- Non-target organisms can range anything from the following –
  - Insects harmed from direct feeding on the crop
  - Insects harmed from feeding on pollen dispersed from the crop to the leaf surface of host plants
  - Predators to harmful organisms, which are feeding on insects that are negatively affected by the GM-crop
  - Predators feeding on insects which are not negatively affected by eating the GM-crop, but the toxins from the GM-crop affect the predators negatively

- Predators feeding on weed species which are controlled during planting of GM-crops.

- Micro-organisms or other soil dwelling organisms harmed by metabolising debris from the GM-crop.
- The applicant should determine the extent to which non-target organisms will be affected, positively and negatively, when introducing the GMO into the receiving environment. If the introduction will lead to a noticeable fluctuation in the population size of the organisms and have a major positive or negative impact for the natural or agro-ecosystems, this should be addressed in the application.
- Due to a lack in knowledge of many of the effects on non-target organisms, the applicant should consult scientific experts to conduct an evaluation of the impact that the organism has on non-target organisms. This should be done on a case by case basis.

5.3.5 Information required in the case of application for placing in the market

i) Name of product and names of GMO contained therein
ii) Name and address of manufacturer in country of origin
iii) Specificity of the product including the appropriate environment and geographical area of the country for which the product is suited
iv) Estimated production or import to the country
v) Proposed packaging (to prevent unintended release during storage or at a later stage)
vi) Proposed labelling in the official language(s) of the country including information on handling and agricultural use.

5.3.6 Information on monitoring and control of release

Extensive international discussions are ongoing regarding traceability with respect to food. The Codex Ad Hoc Task Force on Foods derived from Biotechnology reached consensus on the following: Article 21 of the Principles for the risk analysis of foods derived from modern biotechnology – risk management reads: “Specific tools may be needed to facilitate the implementation and enforcement of risk management measures. These may include appropriate analytical methods: reference materials; and, tracing of products for the purpose of facilitating withdrawal from the market when a risk to human health has been identified or to support post-market monitoring in circumstances as indicated in paragraph 20’

Paragraph 20 reads “ post marketing monitoring may be an appropriate risk management measure in specific circumstances. Its need and utility should be considered on a case-by-case basis, during risk assessment and its practicability should be considered during risk management. Post market monitoring may be undertaken for the purpose of:

- verifying conclusions about the absence or the possible occurrence, impact and significance of the potential consumer health effects; and
- monitoring changes in nutrient intake levels, associated with the introduction of foods likely to significantly alter nutritional status, to determine their human health impact”

Monitoring is therefore subjected to a need for monitoring identified in the risk assessment.

Possible monitoring aspects to take into consideration are -
i) Methods for tracing the GMO and monitoring its effects
ii) Specificity, sensitivity and reliability of monitoring techniques
iii) Techniques for detecting transgenes introgressed into non-target plants
iv) Methods and procedures to avoid and minimise the spread of the GMO beyond the site of release or the designated area for use
v) Methods and procedures for controlling the GMO in case of unexpected spread.

5.4 Environmental risk assessment of genetically modified micro-organisms

i) This part is intended to provide guidance on the risk assessment, for environmental safety, of work with modified bacteria, fungi, cell cultures, etc. The risk assessment must include the identification of any potential harmful effects, characteristics of the proposed activity, the severity of the potential harmful effects and the likelihood of them occurring. After the assessment is complete, appropriate containment and control measures must be indicated.

ii) Risk assessments of genetically modified micro-organisms will vary in the amount of detail necessary to draw conclusions about the hazards related to the activity, the likelihood that they will give rise to harm and the control measures required. Simple activities involving low hazard, well-known and well-understood organisms may normally need less detailed considerations than complex activities involving hazardous and less familiar organisms. For example, the risk assessment of an activity that involved the cloning of viral polymerase genes to replace the polymerase gene of a pathogenic virus would be considered as complex and would therefore at least require (for each construct) consideration of the following factors:

- the properties of the parental virus, including the extent to which the infectivity or tissue tropism of the virus may be limited at the level of expression of the virus polymerase;
- the known properties of each of the polymerase genes being cloned;
- the likelihood that each gene would substitute for the function of the wild-type polymerase;
- details of the precise manner in which each gene would be cloned and the consequences that this might have for the expression of the gene as compared to the wild-type polymerase;
- the likelihood that the modified virus may have an altered tropism or infectivity.

iii) For detailed information and guidance in conducting risk assessments of activities with micro-organisms, the Regulations on Hazardous Biological Agents of the Health and Occupational Safety Act, 1993 should be consulted.

iv) In addition to selecting appropriate control measures, the risk assessment procedure includes the classification of all activities involving genetically modified micro-organisms into one of four risk groups (classes), i.e. Risk Group 1, Risk Group 2, Risk Group 3 and Risk Group 4. The assignment to a risk group must be made on the basis of the outcome of the risk assessment process. More details on the classification of activities are contained in paragraph 5.4.2.

v) Recognising that containment measures form a continuum rather than four discrete levels, many activities will require control measures, which fall somewhere between two levels. For instance, the risk assessment may show that an activity requires laboratory level 2 containment with the addition of negative air pressures and HEPA filtration of extract air. This implies that the activity lies between levels 2 and 3. In such a case the classification should be to the higher level (in this case containment level 3)

5.4.1 Risk assessment of genetically modified micro-organisms using the Brenner Scheme

i) As mentioned before, the risk assessment of genetically modified micro-organisms involves the assignment of appropriate containment and control measures on the basis of both human health and environmental factors. In the majority of cases the containment and control measures appropriate for the protection of human health and safety will also be sufficient to protect the environment.

ii) One method of assessment that may be used by the applicant to provide for the protection of human health and safety, is the Brenner Scheme. This scheme provides a method to determine the risks associated with a particular combination of inserted DNA, vector and host organism.

iii) Although the Brenner system can be extremely useful, it should be pointed out that there are instances where it does not give a reliable indication of the appropriate containment level. Examples include:

- Cloning of genes that alter or exacerbate existing pathogenic traits, e.g. pathogenic determinants, or clinical use of the antibiotic resistance genes whose dissemination might prejudice clinic use of the antibiotic
- Work with host strains where there is uncertainty over the level of attenuation
- Work that does not involve a construct formed in a classical way, from a plasmid vector and an inserted coding sequence, e.g. deletion mutants, certain cell fusion.

iv) Please note that this scheme does not constitute a complete risk assessment, but only gives an indication of the level of containment appropriate for human health. Furthermore, this is not the only manner in which risk assessments may be conducted and other methods may be used.

v) The Brenner scheme considers three characteristics of the GMO before a decision can be made on an efficient containment level, viz. –

A. Access

- The access factor is an indication of the likelihood that the GMO could enter and survive in a human. Depending on the GMO, various routes of entry should be considered, as well as the properties of the vector.
- The value assigned under this section should also take into account the structure and stability of the vector in the final GMO, the frequency of mobilisation and the capacity of the final GMO to colonise a human.
- If the attenuated or disabled strain of an acknowledged pathogen is used, data supporting an alteration of the hazard group of the pathogen must be made available with the application.
- Please refer to Table 2 for indications of the most appropriate access factor for a particular GMO/vector combination.

Table 2: Access factors for host/vector combinations

Vector	Host Especially disabled[1]	Disabled or non-colonising[2]	Pathogenic, colonising or wild type [3]
Non-mobilisable[4]	10^{-12}	10^{-9}	10^{-3} /1
Mobilisation defective[5]	10^{-9}	10^{-6}	10^{-3} /1
Self mobilisable[6]	10^{-3}	10^{-3}	1

[1] *Especially disabled host* means one whose growth requires the addition of specific nutrients not available in humans or outside of the culture media and is sensitive to physical conditions or chemical agents present in man or the environment. This definition applies to certain specific organisms with an extended history of safe use, as well as some strains of *E. coli* K12 and cell or tissue culture systems where the vector does not have the ability to infect or transfer DNA to other cells.

[2] *Disabled or non-colonising hosts* means a multiple auxotroph or their host which is unlikely to persist in the gut, lung or survive outside of the culture media, e.g. most strains of *E. coli* aK12 and other species.

[3] *Pathogenic or colonising hosts* includes all other hosts. A value of 1 applies if it is pathogenic or non-pathogenic but able to colonise humans. A value of 10^{-3} is appropriate if it is wild type and capable of survival outside of culture.

[4] *Non-mobilisable vectors* are Bom^- , (Nic^-), Mob^- , and Tra^- . They include E *coli* plasmid vectors such as pUC, pAT153, pACYC184, pBR327 and pBR328 and their derivates.

[5] *Mobilisation defective vectors* are usually Bom^+ but Mob^- and Tra^- . They include *E. coli* plasmid vectors such as pBR322, pBR325, RP4DI, pACYC177 and p15A and their derivates.

B. Expression

- Expression is a measure of the anticipated or known level of expression of the inserted sequence.
- A probability of 1 is appropriate when the expression system is designed to produce at a maximum rate (include all systems which produce either >10% soluble protein or >200mg l^{-1} protein) in the host.
- Please refer to Table 3 for examples of expression factors that might be applied to an initial cloning experiment on the basis of the known properties of the promoters contained in the vector or insert and their likely activity in the GMO.
- Some vector systems utilise a promoter which is not recognised by normal host RNA polymerases, e.g. T3, T7 or SP6 promoters. When cloning into these vectors, the expression factor should be that appropriate for the level of expression, which is anticipated in the absence of the correct polymerase, i.e. 10^{-6} or 10^{-9} .

Table 3: Relative values for the expression factor for an initial cloning experiment.

	Expression factor
Deliberate in-frame insertion of expressible DNA downstream of a strong promoter (e.g. P_L , P_R , *tac, trp, lac, Cm)* with the intention of maximising expression (e.g. vectors pDS-5, pUC8-I, pUC9-I).	1
Insertion of expressible DNA downstream of a strong promoter (see above) with no attempt to maximise expression.	10^{-3}
Insertion of expressible DNA into a site of limited promoter activity (e.g. *Bla* promoter in pBR322)	10^{-6}
Insertion of expressible DNA at a site specifically engineered to prevent expression (e.g. pDOC55, pNH series)	10^{-9}
Non-expressible DNA, e.g. DNA with no foreseeable biological effect or gene containing introns which the host is unable to process.	10^{-12}

C. Damage

- This factor is a measure of the likelihood of harm being caused to a person by exposure to the GMO.
- This factor should be considered independently from the Access and Expression factors. However, this factor becomes mostly important when these factors allow for a significant dose of the active product to be generated within the body of the exposed person.
- The assessment of potential damage should be linked to the known or suspected biological activity and to the levels and nature of the product required to elicit this activity.
- The Damage factor should in particular reflect health considerations such as the activity of the expressed protein and any toxic, allergenic or pathogenic effects caused by the GMO. Attention should also be given to bacterial or human fusion proteins, which might induce autoimmune disease in persons, sensitised to the protein.
- The biological activity of a product may be dependant on the host cell system in which the product is expressed, and the full biological activity of other molecules will be dependant on post-translational modifications, glycosylation or renaturation, which will only be achieved in certain host organisms (usually animal cells)
- Consideration should also be given to whether the protein is synthesised as an inactive fusion product or not.
- Table 4 contains examples that might be of use when assigning the appropriate damage factor.

Table 4: Recommended values for Damage factors

	Damage factor
A toxic substance or pathogenic determinant that is likely to have a significant biological effect	1
A biological active substance which might have a deleterious effect if delivered to a target tissue, OR a biological inactive form of a toxic substance which, if active, might have a significant biological effect	10^{-3}
A biologically active substance which is very unlikely to have a deleterious effect or, for e.g. where it could not approach the normal body level (e.g. less than 10% of the normal body level).	10^{-6}
A gene sequence where any biological effect is considered highly unlikely either because of the known properties of the protein or because of the levels encountered in nature.	10^{-9}
No foreseeable biological effects (e.g. non-coding DNA sequence)	10^{-12}

D. Assignment of containment

- An indication of the GMO's potential to cause harm to human health is determined by multiplying the individual values allocated under Access, Expression and Damage.
- This value can be used to assign a particular containment level, making use of Table 5.
- The containment levels that are indicated in this table might be used when considering the likelihood of harm from occurring, as it largely determines the level of exposure to the GMO.

Table 5: Provisional containment levels for human health

Overall value	Containment level
10^{-15} or lower	1
10^{-12} or lower	2
10^{-9} or lower	3
10^{-6} or lower	3 or 4*
Greater than 10^{-6}	4
* case by case	

5.4.2 Classification of biological agents

i) Biological agents can be classified, based on their inherent risks, into different risk groups, which are analogous to the levels of containment. These classifications presume ordinary circumstances in the research laboratory or growth in small volumes for diagnostic and experimental purposes.

ii) The classification into risk groups and the derivation of containment and control measures, though related, are separate procedures. Nevertheless, most group 1 genetically modified micro-organisms will probably only require containment level 1. If the organism is classified into group 2 and the assessed level of containment is above level 1, then both classification and containment level should be checked to make sure that they are correct. It is quite

possible that they are; assignments to a particular containment level do not determine the classification of the GMO.

iii) In classifying GMO's (as opposed to any other class of biological agent) the classification must consider the following –

- The recipient or parental organism (host)
- The vector
- The insert (cloned) DNA
- The final GMO

iv) The inherent risks of biological agents (conventional or genetically modified) are further determined on the basis of several factors. These factors include:

- the severity of disease it cause
- the routes of infection
- its virulence and infectivity
- the existence of effective therapies
- possible immunisation
- presence/absence of vectors
- quality of the agent and whether the agent is indigenous
- possible effects on other species.

v) In the following paragraphs a brief outlay is given of the precautions applicable to each of the containment level. However, please take note that these containment levels are designed for micro-organisms and cannot be extended to other GMO's. For further guidance and more information on the classification of hazardous biological agents, please consult the Regulations for Hazardous Biological Agents of the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993), available at the following address:

The Registrar
Department of Labour
Private Bag X117
Pretoria
0001
Tel: +27 +12 309 4374
Fax: +27 +12 320 5112

a) Criteria for classification into Risk Group 1
   - Agents posing low individual and community risk.
   - A biological agent that is unlikely to cause disease in healthy workers or animals.

b) Criteria for classification into Risk Group 2
   - Agents posing moderate individual risk and limited community risk.
   - An agent (pathogen) that can cause human or animal diseases but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.
   - Laboratory exposures to these agents rarely cause infection leading to serious disease; and effective treatment and preventative measures are available.
   - The risk of spread of these agents is limited.

c) Criteria for classification into Risk Group 3
   - Agents posing a high individual risk but low community risk.
   - A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences.

- A pathogen that does not ordinarily spread by casual contact from one individual to another.
- Pathogens that can be treated by anti-microbial or anti-parasitic agents.

d) Criteria for classification into Risk Group 4
- Agents posing a high individual risk and high community risk.
- A pathogen that usually produces very serious human or animal disease, often untreatable.
- A pathogen that may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

Table 6 illustrates the group classification and the corresponding range of possible containment levels:

Table 6: Containment levels applicable to each risk group

Risk Group	**Containment level necessary to control risk**
Risk Group 1	Level 1 (or less)
Risk Group 2	Level 1 + additional measures from containment level 2 Level 2 (without additional measures)
Risk Group 3	Level 2 + additional measures from containment level 3 Level 3 (without additional measures)
Risk Group 4	Level 3 + additional measures from level 4 Level 4 (with or without additional measures)

For more information on the requirements at each containment level please refer to next section – Containment of biohazards.

5.4.3 Containment of biohazards

5.4.3.1 Physical containment levels – small scale use

Four levels of containment appropriate to the four risks groups for infectious agents used in small scale, are defined below. Each containment level indicates the physical requirements as well as the operational requirements necessary. It remains the responsibility of the principal investigator or laboratory director and institution to require a higher level of containment for specific manipulations if these manipulations will appreciably increase the hazard of infection. For quick reference on the most important containment measures required at different levels, please refer to Table 7 at the end of this section.

The requirements listed below are based on the containment levels as stipulated in the Regulations on Hazardous Biological Agents of the Health and Occupational Safety Act. However, additional requirements may have been incorporated into each level.

i) Containment level 1

- Applies to basic laboratories handling Risk Group 1 agents.
- Requires no special design features beyond those suitable for a well-designed and functional laboratory.
- Biological safety cabinets are not required.
- Work may be done on an open bench top and containment achieved through the use of normal practices employed in a basic microbiology laboratory.

a) Physical requirements
- The facility may be a room separated from public areas by a door, which remains closed at all times.
- Coating on walls, ceilings, furniture and floors should be cleanable.
- There may be no windows that can open near working areas or containment equipment, and which are equipped with fly screens.
- There are no special air handling requirements beyond those concerned with proper functioning of the biological safety cabinets and those required by building codes.
- Hand-washing facilities must be provided preferably near point of exit.
- Separate hanging areas for street clothing and laboratory coats should be provided.
- Eye wash stations may be required by local statute.

b) Operational requirements
- Basic laboratory safety practises should be followed.
- Where chemical disinfection procedures are practised, effective concentrations and contact times must be employed.
- Chemical disinfectants must be replaced regularly.

ii) Containment level 2

- Suitable for work with agents from Risk Group 2.
- In addition to requirements of containment level 1 the following are required:

a) Physical requirements
- The laboratory does not have to be separated from other activities in the same building.
- Post a biohazard sign with appropriate information on entrance to lab, if required by risk assessment.
- Laboratory furnishings and work surfaces must be impervious and readily cleanable.
- Coat hooks should be provided near the exit.
- An autoclave should be available in or near the laboratory.
- Laboratory doors must be self-closing.
- Access should be restricted to authorised personnel only

b) Operation requirements
- Class I or II biological safety cabinets are appropriate for all manipulations of agents, which may create aerosols.
- Biological safety cabinets (follow SABS specifications – Annexure 10.3) must be tested and certified according to separate standards.
- Air from cabinets may be recirculated to the room only after passage through a HEPA filter.
- Centrifugation must be carried out using closed containers or aerosol proof safety heads or cups that only open in biological safety cabinets.
- Animals/insects, which have been experimentally infected, must remain in the laboratory or appropriate containment facility.
- An emergency plan for handling spills of infectious materials must be developed and ready for use whenever needed.
- Workers must be educated and drilled in the emergency plan.
- Vacuum lines used for work involving the agent must be protected from contamination by HEPA filters.
- Laboratory coats are to be worn only on the laboratory area and not outside. Coats that fasten in front are permissible.
- Special care should be taken to avoid contact of infectious materials with skin, e.g. wearing of gloves.

- Contaminated glassware may not leave the containment; decontamination must be carried out using procedures that are effective.
- If there is no autoclave or incinerator in the laboratory: contaminated glassware should be wiped off with disinfectant, chemically or double bagged and transport to the autoclave in durable, leak-proof containers which are closed and wiped of with disinfectant before leaving the laboratory.
- Service personnel and cleaning staff who enter the laboratory must be informed of the hazards they might encounter. Cleaning staff must only clean the floor. Laboratory staff have the responsibility for rendering the facility safe for routine cleaning. Periodic disinfection should be done at regular intervals.
- Cleaning and maintenance staff should receive immunisation and medical surveillance if appropriate.

iii) Containment level 3

- Suitable for work with agents from Risk Group III.
- Laboratory staff must receive special training in the safe handling and manipulation of the agents used in the laboratory.
- The laboratory must undergo annual performance evaluation, testing and verification.
- The laboratory requires special design and construction.

a) Physical requirements

The following conditions are required in addition to containment levels 1 and 2

- The laboratory should be located away from general work areas and have controlled access from other areas.
- Entrance must be through a lockable changing room with self-closing doors.
- A body shower should be within the containment perimeter.
- Air in the laboratory should be held at negative pressure relative to the surrounding areas at all times in such a way that a directional airflow is created by air entering through all entry and exit areas.
- The laboratory should be provided with a dedicated supply and exhaust system that is sealed.
- Air discharged may not be circulated back into either the air supply system of the laboratory, or the building or adjacent buildings.
- Provided there is a dedicated sealed exhaust system, air may be exhausted from the laboratory to the exterior of the building without HEPA filtration. At the discharge point the exhausted air must be dispersed away from the air intake and populated areas.
- When air is exhausted by means of a dedicated exhaust system, it must be passed through HEPA filters before discharging into the main building exhaust air ventilation-system. This exhaust housing must be designed to allow in situ decontamination, and pass annual testing and certification by aerosol challenge and scan techniques.
- A control system must be in place that ensures that the laboratory does not become positively pressurised relative to the surrounding area.
- When the supply of air is not provided by a dedicated system, airtight back-draft dampers or HEPA filters must be installed in the supply system. The supply must be interlocked with the exhaust system.
- Biological safety cabinets must be installed in a manner that does not interfere with the air balance of the cabinet or room. Thimble unit connections are recommended.
- A dedicated hand-washing sink with foot, knee or automatic controls should be located near the exit.
- A pass-through or stand-alone autoclave should be located in the work zone.
- Laboratory furnishings must be kept to a minimum.
- Work surfaces must be impervious, readily cleanable and resistant to chemical disinfectants.
- Seal all penetrations for services in the floors, walls and ceiling.

- Air supply or exhaust systems should be provided with manual dampers at room perimeter that may be closed as required to permit gas decontamination.
- Water supply to the laboratory should be provided with reduced pressure back flow preventers.
- HEPA filters should be fitted on all ventlines.
- Dunk tanks may be provided at the containment perimeter.
- Sink and floor drains must be piped separately to the main building drain and appropriately labelled. Floor drains are not recommended.
- Infectious materials must never be placed in sinks or floor drains.
- Autoclave condensate drains should have closed connections and go directly to a sanitary sewer.
- Animal care facilities for small animals: disposal of wastes will not differ from other contaminated laboratory materials.
- Laboratory windows should be sealed and unbreakable.
- Backup power must be provided to critical items.

b) Operational requirements

The following conditions are required in addition to the requirements for containment levels 1 and 2

- Staff must be fully trained in handling pathogenic and other hazardous material, as well as in the use of safety equipment, disposal techniques, the handling of contaminated waste and emergency procedures.
- Staff must change to dedicated solid front clothing on entry to facility, removed on completion of work and autoclaved prior to laundering.
- Personal protective clothing (head covers or foot covers) must be used.
- Appropriate respiratory protection should be considered depending on the infectious agents involved.
- Showers may be required depending on the infectious material used.
- Personal belongings may not be taken into the laboratory.
- Gloves must be worn.
- All activities involving infectious materials must be conducted in biological safety cabinets or appropriate combinations of personal protective and physical containment devices.
- Centrifugation should be conducted in closed containers using aerosol proof safety heads or cups that are loaded and unloaded in the cabinets.
- Effective disinfectants should be available at all times.
- Store all Risk Group 3 agents in the containment level 3 facility.
- Effective pest control programme must be in effect.
- Written protocols must be provided and posted within the laboratory outlining operational protocols, waste disposal, disinfection procedures and emergency responses.
- An existing medical surveillance programme appropriate to the agents used, which includes serum storage for all personnel, must be working in the laboratory. A reporting structure should be in place for accidents and exposures to infective agents or other incidents or unusual occurrences in the operation of the laboratory.
- Authorised maintenance and service personnel must abide by the same operational protocols as laboratory staff and be accompanied by staff when entering the laboratory.
- Containment level 3 facilities and systems must be tested for contamination capability upon completion of construction and at least annually thereafter.

iv) Containment level 4

- Physical and operational requirements are highly specified.
- This is the highest level of containment and represents an isolated unit functionally independent of other areas.
- Requires an airlock for entry and exit, Class III biological safety cabinets or positive pressure ventilated suits, a laboratory support area and a pressure ventilation system in addition to the physical and operational requirements of containment levels 1-3.

a) Physical requirements
   - A laboratory physically separated from other laboratories or consists of an isolated zone, which is monolithic in construction with all penetration to floors, walls and ceilings sealed with non-shrinking sealant.
   - A laboratory designed to accommodate a minimum of 2 persons at all times, all laboratory equipment, long term storage of cultures and maintenance of infected animals.
   - Entry must be through an airlock system, with manual alarm overrides available.
   - Change rooms must be contiguous with the containment perimeter of the structure and have a personnel shower and/or a chemical shower.
   - All drain traps must be kept filled with an effective disinfectant and be connected to a liquid waste effluent system.
   - All air access to any sewer and ventilation lines must be fitted with HEPA filters or equivalent equipment.
   - All gas services must be fitted with HEPA filters, equivalent equipment or back-flow preventers to prevent egress of contaminated material.
   - Water supply systems must be provided with back-flow preventers.
   - All windows must be sealed and be of break resistant glass.
   - A double door autoclave preferable serviceable from the outside of the facility. The autoclave should have read-out charts inside and outside the laboratory and have operating controls inside the laboratory.
   - A dunk tank which is chemically resistant and of a suitable size for the passage of anticipated laboratory materials may be required for Class III cabinet lines laboratories, if indicated by the risk assessment.
   - Dedicated hand-washing sink with foot, knee and automatic controls should be located near the exit.
   - The facility must be equipped with a two-way intercom system.
   - A closed circuit television system should also be considered.
   - All liquid effluent from the facility must enter a waste effluent treatment system for sterilisation, which is mechanically and biologically monitored.
   - Ventilation must occur by an independent, dedicated, sealed supply and exhaust air system, which is not recirculated.
   - Exhaust air must pass through at least 2 HEPA filters mounted in series in sealed housings designed to allow in situ decontamination and testing by aerosol challenge techniques.
   - Supply and exhaust systems must maintain directional (inward) airflow and pressure differentials and interlocked to prevent pressurisation in the event of exhaust fan failure.
   - A supply air system must be equipped with HEPA filter.
   - The facility should be fully equipped with manometers and other monitoring devices and audible and visual alarms capable of being monitored by both laboratory and maintenance staff.
   - The facility should be provided with Class I, II or III tested and certified biological safety cabinets, which must be installed in an manner that does not interfere with the air balance of the cabinet or room.
   - If a positive-pressure-ventilated-suit type of operation is used, a life support system with full alarming, back-up breathing air, emergency power and a chemical shower

facility are required. Positive pressure suits must be used whenever agents are worked with outside a Class III cabinet.

- Alarms, ventilation and other critical systems must be on separate electrical circuits with an emergency backup.
- A support area adjacent to Level 4 containment facility is required for all non-hazardous laboratory manipulations.

b) Operational requirements

- Only fully authorised personnel may enter the laboratory; all must sign a logbook and maintain and record all entries electronically.
- A competent person should be outside the laboratory at all times when work is undertaken to assist during an emergency.
- Wearing of protective clothing, gloves and impermeable footwear are required, no street clothing may be worn under the protective clothing. On exit personnel must shower and re-dress in street clothing.
- Keep small laboratory animals and insects under experimentation in ventilated cabinets having all output air HEPA filtered.
- Special care should be taken with regard to large animals.
- Store all Level 4 agents within the containment zone.
- Remove all materials through an autoclave or placed it in a double, unbreakable sealed container, the outside of which will be disinfected.
- Where equipment and apparatus are not compatible with heat sterilisation, materials may be removed via dunk tanks or air locks with suitable decontamination procedures.
- All manipulations must be performed in Class III biological safety cabinets or in Class I or II biological safety cabinets used in conjunction with one-piece, positive-pressure-ventilated suits.
- Prepare contingency plans for emergencies, which may include responses to biological, toxic or hazardous spills, and fire and life-threatening situations must be prepared and reviewed by all personnel.
- A written reporting system for laboratory accidents and exposures must be in effect.
- Implement a serum storage programme for all laboratory and support personnel along with a full medical surveillance and treatment programme.
- Level 4 facilities and its systems must be tested for containment capability upon completion of construction and annually thereafter.

Table 7: Containment and control measures for small-scale activities with GM-micro-organisms.

Containment and Control Measures	**Containment Level 1**	**Containment Level 2**	**Containment Level 3**	**Containment Level 4**
Building / Physical Measures:				
The workplace separated from other activities in the same building	No	No	Yes	Yes
The workplace maintained at an air pressure negative to atmosphere	No	No, unless mechanically ventilated	Yes	Yes
Input air and extract air to the workplace are to be filtered using HEPA or equivalent	No	No	Yes, on extract air	Yes, on input and double (2 stage in series) on extract air
Surfaces impervious to water easy to clean and	Yes, for bench	Yes, for bench	Yes, for bench and floor (and	Yes, for bench, floor, walls and

Containment and Control Measures	Containment Level 1	Containment Level 2	Containment Level 3	Containment Level 4
resistant to acids, alkalis, solvents and disinfectants			walls for animal containment)	ceiling
An observation window, or alternative present so that occupants can be seen	No	No	Yes	Yes
Efficient vector control e.g. rodents and insects	No	Yes, for animal containment	Yes, for animal containment	Yes
The workplace sealable to permit fumigation	No	No	Yes	Yes
Effluent from sinks and showers collected and inactivated before release	No	No	Optional	Yes
Work Practice Measures:				
Biohazard signs and level of work posted	No	Optional	Yes	Yes
Access restricted to authorised persons only	No	Yes	Yes	Yes
Personnel trained in both routine and emergency procedures	Yes	Yes	Yes	Yes
Laboratory door closed when work is in progress	Optional	Optional	Yes, should be locked when room is unoccupied	Yes, door to be kept locked
Personal protective equipment protective clothing Gloves RPE	 Yes Optional No	 Yes Optional No	 Yes Optional No	 Yes Yes Yes
Protective clothing decontaminated before laundering	No	Optional	Yes	Yes
Smoking, eating, drinking and the application of cosmetics prohibited in workplace	Yes	Yes	Yes	Yes
Laboratory to contain its own equipment	No	No	Yes, so far as is reasonably practicable	Yes
Equipment and control measures tested and maintained	Yes	Yes	Yes	Yes
Viable material, including any infected animal, to be handled in biological safety cabinet or isolator or other suitable container	No	Yes, where aerosol produced	Yes, where aerosol produced	Yes (Class III cabinet)

Containment and Control Measures	Containment Level 1	Containment Level 2	Containment Level 3	Containment Level 4
Monitoring for the relevant organisms outside	Optional	Optional	Yes	Yes
Safe storage of GMO's	Yes	Yes	Yes	Yes, secure storage
Contaminated waste to be inactivated prior to disposal	Optional	Yes, by validated means	Yes, by validated chemical or physical means	Yes, by validated physical means
Autoclave available in the laboratory	Optional	Optional	Optional	Yes, double ended
Incinerator for disposal of animal carcasses	Optional (for animal containment)	Accessible (for animal containment)	Accessible (for animal containment)	Yes, on site (for animal containment)
Decontaminated and washing facilities provided	Yes	Yes	Yes	Yes
Personnel shower before leaving laboratory	No	No	Optional	Yes

*Optional indicates that the requirement is to be determined based on the risk assessment

5.4.3.2 Physical containment levels – large-scale/industrial use

This guidance does not preclude the use of other approaches. Where there is no specific requirement, alternative methods may be applied so long as the risks are adequately controlled. Please note that large-scale processes should be considered in terms of their unit operations and that a number of engineering control measures may be required.

As in the case with containment at small scale, please consult the Regulations on Hazardous Biological Agents of the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993), available from the Department of Labour at the address indicated below.

Department of Labour
Private Bag X117
Pretoria
0001
Tel: +27 +12 309 4374
Fax: +27 +12 320 5112

For quick reference on the appropriate containment for activities at large scale, please refer to Table 8 at the end of this section.

i) Containment Level 1

- Follow the measures as described by Basic Laboratory Safety Practices (Annexure 10.1).
- Good hygiene must be applied at all times.
- Containment will be determined by the degree to which contact of the GMO with humans and the environment needs to be limited, and should be based on the risk assessment.

(a) Physical requirements
- Production or factory floor areas must be separated from offices, laboratories and other facilities.
- Good hygiene is advisable and buildings must be easily cleanable.
- Mechanical ventilation is not normally needed, although it may be used. For some processes a positive air pressure is needed to maintain product integrity. Consider localised airflow units which give product and operator protection.

(b) Operational requirements

- Viable GMO's should be contained in a system, which includes physical barriers to separate them from the general environment. The need for a closed system will depend on the risk assessment, but is normally not needed.
- Release of the GMO's into the work place and wider environment should be minimised during processes such as addition of materials, mixing or transfer. The acceptable degree of minimisation is to be determined by the risk assessment.
- Seals used on equipment should be designed to minimise release so that contamination of the workplace and wider environment is limited appropriately and harm will not result.
- If the risk assessment indicates that harm may result if viable GMO's are released, they should be inactivated before removed from containment.
- Equipment and control measures should be tested and maintained at appropriate intervals.
- Workers should be trained in both routine and emergency procedures.
- Washing facilities should be provided for personnel.
- Work clothing should be provided if necessary.
- Release of GMO's into the work place should be minimised during sample collection.
- Waste should be disposed of in a safe manner.
- There is no need to treat exhaust gases.
- There is no need for emergency plans, although it is good practice to have procedures drawn up.
- Accidents and incidents should be recorded.
- Monitoring is unlikely; however, where there is risk to human health and environmental safety from process organisms outside the closed system, monitoring for viable process organisms should be carried out.

ii) Containment level 2

(a) Physical requirements

- Activities should be undertaken in controlled areas, which are separated from offices, laboratories and other facilities, and where cross traffic is limited.
- Good standards of hygiene are required.
- The controlled area should be ventilated to minimise air contamination. Mechanical ventilation may also be used.
- HEPA filtration of any input and extract air is usually not needed, but filtration of extract air may be necessary where there is a risk to the wider environment from the release of the GMO.
- Where there is risk from catastrophic loss of containment the facility should be designed to contain spillage of the entire contents of the fermenter.

(b) Operational requirements

- Viable GMO's should be contained in a closed system, which includes physical barriers to separate them from the work and wider environment.
- Pipework and valves should be designed with the emphasis on leak-tightness as well as on cleanability.
- Where the needle/septum method is used procedures should be carefully though out to avoid needle puncture injury.
- Static seals on equipment should be designed so as to minimise release.
- Agitator seals would normally be single or double faced mechanical seals.
- Fixed or retractable instrument sensors may be used.
- Any relief system design needs to be considered carefully, e.g. chains of venting systems. Pressures Vessels Regulations requirements must be met.

- Bulk culture fluids should not be removed from the closed system unless the viable GMO's have been inactivated by validated means.
- Equipment and control measures should be tested and monitored.
- Workers should be appropriately trained in both routine and emergency procedures.
- Access should be restricted to nominated personnel when this is indicated by the risk assessment.
- Hand washing facilities, ideally with foot or elbow operated taps, should be provided. Emergency showers and eye wash stations are useful.
- When indicated by risk assessment, a biohazard sign must be posted at entrances.
- Work clothing should be provided and ideally kept in a separate locker.
- Release of GMO's into the work place should be minimised during sample collection. The receiving container should also be designed to minimise aerosols.
- Infected waste and effluent containing viable GMO's should be inactivated by validated means prior to final discharge.
- Exhaust gases should be treated so as to minimise release.
- Filters should be able to be removed safely for protection of maintenance engineers.
- If indicated by the risk assessment, as a result of any foreseeable accident, the health and safety of persons outside the premises may be affected or if there is any risk to the environment, an emergency plan must be drawn up.
- Accidents and incidents should be recorded and immediately reported to a competent person.
- Where there is risk to human health or environmental safety for process organisms outside the closed system, monitoring for viable process organisms should be carried out.

iii) Containment Level 3

(a) Physical requirements

- Most activities should be carried out in controlled areas that are separated from offices; laboratories and other facilities and which are away from general circulation routes.
- High standards of hygiene should be maintained.
- Where indicated by risk assessment, a continuous airflow into the facility should be maintained when work is in progress.
- Extract air is normally filtered through HEPA filters and must be filtered when there is a risk of harm from not doing so.
- Inlet and extract systems can be alarmed, interlocked and indicated.
- When indicated by risk assessment, the controlled area should be sealable to permit fumigation.
- The facility should be designed to contain spillage of the entire contents of a fermenter.

(b) Operational requirements

- Viable GMO's should be contained in a closed system, which includes physical barriers to separate them from the general environment.
- Inoculation of seed vessels should be performed so as to prevent release, and closed systems such as stainless steel transfer vessels should be used.
- Bulk culture fluids should not be removed from the closed system unless validated chemical or physical methods have inactivated viable GMO's.
- Leak testing should be performed using halogens.
- Workers need to be trained to a high standard in both routine and emergency procedures.
- Access should be restricted to nominated personnel. Access via changing rooms and a system of control, which prevents unauthorised access, should be in place.
- Hand washing facilities should be provided, preferable with foot and elbow operated taps. Emergency showers and eyewash stations are worth considering.

- Biohazard signs must be posted at entrances.
- Protective clothing should be worn and changed provided at each entry into the controlled area.
- Data transferring must preferable occur by electronic means.
- Sampling should be performed using a closed aseptic technique and release of GMO's should be prevented.
- Exhaust gases should be treated so as to prevent release. This will involve HEPA filtration with 0.2μm filter cartridges and often 2 filters in series.
- Spray towers, cyclone separators, off-gassing through disinfectants and impingement filters are not recommended.
- An emergency plan must be drawn up and should include procedures to deal with spillage.
- Accidents, spills and exposures to infective material need to be immediately reported to and recorded by a competent person. All accidents must also be reported to the Health and Safety Executive.
- Monitoring of viable process organisms should be carried out.

iv) Containment level 4

(a) Physical requirements

- Activities to be carried out within purpose built controlled areas, which are physically separated from any other activity.
- Scrupulous levels of hygiene are to be maintained.
- The controlled area must be ventilated to minimise air contamination and an air pressure negative to the atmosphere must be maintained.
- Input and extract air should be filtered through HEPA filters, a single filter for input and two filters mounted in series for extracted air.
- The controlled area must be sealable to permit fumigation.
- The controlled area must be designed to contain the entire content of a fermenter and allow for physical inactivation. Drainage channels are not appropriate.

(b) Operational requirements

- Viable GMO's must be contained in a fully closed system that prevents release.
- Any addition of materials to the closed system or transfer of viable GMO's to other closed systems must be performed so as to prevent release. The use of sterile needle / septum techniques are not to be used.
- Before bulk culture fluids are removed from the closed system, viable GMO's must have been inactivated by validated means.
- Equipment and control measures must be tested and maintained at appropriate intervals.
- Workers must have specific training in working in the facility as well as the use of safety equipment and handling of the GMO's concerned.
- Access must be restricted to authorised personnel only. Entry must be via a changing room/lobby area (airlock), which is itself ventilated and maintained at an air pressure negative to the outside of the facility, but positive with respect to the work area. All entrances need to be locked.
- Decontamination and washing facilities must be provided. Personnel must shower before leaving the controlled area.
- Biohazard signs must be posted at each entrance.
- A complete change of protective clothing must be worn, a change being provided for each entry.
- Data should preferable be transmitted by electronic means.
- Only closed aseptic techniques are acceptable when taking samples with release of GMOS prevented at all times.
- All effluent must be inactivated by validated physical means prior to final discharge.

- All exhaust gases must be HEPA filtered to prevent release.
- Emergency plans must be drawn up and include procedures to deal with spills.
- Accidents, spills and exposures to infective materials are to be immediately reported to and recorded by the competent person or the person responsible who needs to take appropriate measures specified in the local rules. Accidents must also be reported to the Health and Safety Executive.
- A monitoring programme should be instigated in the work area and immediate surroundings.

Table 8: Containment and control measures for large scale activities with GM-micro-organisms

Containment and Control Measures	**Containment Level B1**	**Containment Level B2**	**Containment Level B3**	**Containment Level B4**
Building Design:				
Closed system located within a controlled area	(Not applicable)	Optional	Optional	Yes, and purpose built
The controlled area adequately ventilated to minimise air contamination	No	Optional	Optional	Yes
The controlled area maintained at an air pressure negative to atmosphere	No	No	Optional	Yes
Input and extract air to the controlled area HEPA filtered	No	No	Optional	Yes
The controlled area sealable to permit fumigation	No	No	Optional	Yes
The controlled area designed to contain spillage of the entire contents of the closed system	No	Optional	Yes	Yes
Fermentation Methods, Equipment and Utilities:				
Viable micro-organisms contained in a system which physically separates the process from the environment (closed system)	No	Yes	Yes	Yes
Addition of materials to a closed system and transfer of viable micro-organisms to another closed system performed so as to:	(Not applicable)	Minimise release	Prevent release	Prevent release
Equipment seals designed so as to:	Minimise release (if seals used)	Minimise release	Prevent release	Prevent release
Bulk culture fluids not removed from the closed system unless the viable micro-organisms have been	(Not applicable)	Inactivated by validated means	Inactivated by validated chemical or physical	Inactivated by validated chemical or physical

Containment and Control Measures	Containment Level B1	Containment Level B2	Containment Level B3	Containment Level B4
			means	means
Maintenance:				
Equipment and control measures tested and maintained	Yes	Yes	Yes	Yes
Management System/ Work Practices:				
Personnel to be trained in both routine and emergency procedures	Yes	Yes	Yes	Yes
Access restricted to nominated personnel only	No	Optional	Yes	Yes, via airlock
Decontamination and washing facilities provided for personnel	Optional	Yes	Yes	Yes
Personnel shower before leaving the controlled area	No	No	Optional	Yes
Biohazard signs posted	No	Optional	Yes	Yes
Personnel wear protective clothing	Yes, work clothing	Yes, work clothing	Yes	Yes, a complete change
Protective clothing decontaminated before laundering	No	Optional	Yes	Yes
Smoking, eating, drinking and the application of cosmetics prohibited in controlled areas	Yes,	Yes	Yes	Yes
Sampling Procedures:				
Sample collection performed so as to:	Minimise release	Minimise release	Prevent release	Prevent release
Sampling by closed aseptic technique	No	Optional	Yes	Yes
Waste Handling and Gas Emission:				
Effluent from sinks and showers collected and inactivated before release	No	No	Optional	Yes
Effluent treatment before final discharge	Optional	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactivated by validated physical means
Accidents / Emergency Plans:				
Emergency plans prepared	No	Optional	Yes	Yes

Containment and Control Measures	Containment Level B1	Containment Level B2	Containment Level B3	Containment Level B4
Documented spillage procedures drawn up	Optional	Yes	Yes	Yes
Monitoring:				
Monitoring for processed organisms outside primary containment	Optional	Optional	Yes	Yes

*Optional indicates that the requirement is to be determined based on the risk assessment

5.4.4 Risk assessment of genetically modified viruses and viral vectors

(The term animal includes both vertebrates and invertebrates.)

i) This section is an abbreviated version of the guidelines used by the United Kingdom. For more detailed information please refer to Part 1 and 2 of the *Compendium of Guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification*

ii) The following are basic procedures, which may be followed to conduct an assessment of genetically modified viruses and viral vectors. These procedures include -

a) Determination of the predicted properties of the GM virus in order to assess if there are any potential mechanisms by which it could represent a hazard to human health.

The following aspects should be taken into account -

- Hazards associated with the vector
- Particular care must be given to the assessment of vectors with an actual or potential ability to infect humans or human cells
- Viral vectors with reduced pathogenicity
  - To prevent/minimise exposure to a biological agent that can cause harm, the agent should be substituted by a less hazardous agent wherever practicable.
  - To determine whether a viral vector is adequately disabled, the possibility of reversion or complementation should be considered, and it must be confirmed that the virus is still disabled after the modification.
  - The likelihood of reversion will depend on the mechanism of attenuation – deletant mutants are less likely to revert to wild type than point mutations or conditional lethal mutants.
  - Insertion of a gene into a site of any disabling mutant is expected to reduce the likelihood of recombination events resulting in the generation of a replication competent virus expressing the gene, and therefore increasing the effective biological containment.
  - Experiments using viral vectors that do not usually infect human cells in culture, and for which there is no evidence of human infection, are considered to represent a minimal risk to the operator and a containment level 1 is sufficient to protect human health.
- Hazards arising directly from the inserted gene product
  - The insertion of an additional nucleic acid sequences into viral vector can give rise to potential adverse effects, resulting from the direct effects of an expressed gene product, or as a consequence of an alteration in the overall properties of the GMO.
  - Particular attention should be given to the level of expression and the site of insertion of the gene, and whether it is a known or suspected pharmacological or physiological effect. This should include the possibility of effects other than those being sought in the construction.

- Particular attention should also be given to inserted sequences which could result in an alteration in growth, replication or differentiation of cells

- Hazards arising from the alteration of existing pathogenic traits
  - Adverse effects may result through the alteration of existing pathogenic traits as a result from the product of an inserted gene acting alongside existing pathogenic determinants, or as a result of the modification of the normal viral genes which affect pathogenicity.
  - The following points should be considered when assessing the hazards arising through the alteration of existing pathogenic traits –
  - Alteration of tissue tropism or the host range (A change may occur in the structure of the receptor binding site and tissue tropism may be affected by alterations in the transcriptional control of viral genes)
  - Increase in the effectivity or pathogenicity (The modified virus may show an altered susceptibility to host defence mechanisms.)
  - Recombination or complementation (The possibility that a recombination or complementation may influence any disabling feature or attenuation of the viral vector after infection.)
  - Availability of prophylaxis or therapy (The possibility that viral susceptibility to anti-viral drugs may be affected by genetic modification.)

b) Consideration of the likelihood that the GM virus could actually cause harm to human health.

Factors that come into play when considering the likelihood, include an analysis of the probability that rare events may occur and a judgement as to the fitness of the modified virus. In some cases it will be possible to assign a frequency to an event – precise, approximate, semi-quantitave or descriptive.

The ability of a GM virus to establish through an in vivo infection and the efficiency of substances in vivo propagation - consideration should be given to the ability of the virus to spread as this could influence the level of containment.

c) The assignment of general control measures necessary to safeguard human health, i.e. the allocation of a provisional level of containment

The first step in assigning control measures is to determine whether the virus is suitable for work in one of the main containment levels. The assignment of a containment level in most cases will correspond to the level of containment appropriate for the parental organism. Where it is predicted that the modified virus will be considerably more hazardous than the parental virus, a higher containment level should be applied.

Once a containment level is indicated, the next step is to decide whether the minimum requirements of the chosen containment level is adequate or whether some additional measures should not be applied. In all cases the principles of good microbiological practice and good occupational safety and hygiene should be applied.

Additionally to the above, the following is recommended for work conducted with viruses capable of infecting human cells –

- Apply measures to prevent cross contamination during laboratory work to minimise the possibility of adverse consequences resulting from recombination or complementation. E.g. do not use the same bottle of medium for culturing different virus infected cell lines, and discourage laboratory workers from sharing bottles of medium.
- Conduct tests to detect the presence of adventitious agents and replication competent virus (RCV) to determine the absence of RCV in virus stocks.

- In order to minimise the risk of accidental colonisation with infected cell lines, users should not infect cultures of their own cells, nor as a general rule, those of their immediate family or other members of the laboratory.
- The responsible person for the laboratory should ensure that there are adequate laboratory rules, which give effective guidance on the maintenance of laboratory discipline and on avoiding accidental inoculation.
- All workers should be trained in good laboratory techniques before commencing work with GMO's and be fully aware of the potential hazards associated with the work.

d) Consideration of the nature of work to be undertaken, and the assignment of additional control measures if required

At this stage of the risk assessment the nature of the work to be conducted must be taken into consideration. Will the work involve any non-standard operations that may involve risks that are not accounted for in the general requirements of a containment level?

If the work will involve non-standard operations that will generate risks that are not accounted for in the provisional containment level, additional control measures should be applied.

e) The identification of hazards to the environment and assignment of additional containment measures to protect the environment.

The primary consideration in this section should be whether the virus is capable of infecting animals. If the virus cannot infect any species other than humans, the risk assessment should include a statement to this effect together with some justification. Risks to the environment can in this case be assumed as negligible.

If the virus may infect any animal, then the risk assessment should consider the risks posed to the environment. Attention should be given to viruses that are known to be pathogenic to wildlife.

Any additional risks to the environment caused by the modification or the inserted sequence should be assessed by consideration of the following points –

- Survivability – will the GM-virus result in an altered survivability in the environment?
- Alteration of tissue tropism or host range – Is the modification likely to alter the tissue tropism or host range of the recombinant virus?
- Increase in infectivity or pathogenicity – Will the GM-virus increase pathogenicity or infectivity? Will there be an altered susceptibility to host defence mechanisms?
- Effects on other organisms – Does the inserted sequence code for a protein with known or suspected inhibitory, detrimental or other physiologically active effects on other organisms?
- Environmental release – Do you know all possible routes of escape into the environment?
- Availability of control agents – Will the virus susceptibility to control agents be affected by the modification? E.g. vaccination or norm immune status.

The possibility of accidental releases and survival of the GMO in the environment must always be considered. If the virus is to be used at high levels of containment because of the potential risks imposed to human health and safety, it is likely that the control measures assigned will also be sufficient to protect the environment.

In the case where the virus is known to have a limited survivability in the environment or is known not to infect South African hosts, the likelihood of the hazard being realised and causing harm, can be considered to be low or effectively zero.

6. Containment measures for GMO's conducted in greenhouses

i) The containment measures that are provided in this section is to serve as a simple and convenient reference on the appropriate biosafety and containment levels applicable for activities with GMO's in a greenhouse. The information in this section was taken from the book by Traynor, Adair and Irwin named *A Practical Guide to Containment* (herein after referred to as the *Guide*. The complete text of this Guide is available in the ISB (Information Systems for Biotechnology) Web site – http://www.isb.vt.edu. Print copies are also available at no charge – order forms may be obtained from the ISB Website, or you can send your request by e-mail to isb@vt.edu. Orders may also be faxed to 540 231 4434.

ii) When activities with genetically modified plants (GM-plant) are to be conducted in greenhouses, the predominant goal of containment is to prevent the GM-plant from escaping into the environment. To assign appropriate containment for activities with GM-plants in greenhouses, the activity must be categorised into a certain biosafety level. In Section 6.1 a brief description is given of each biosafety level, as described in the National Institutes of Health's *Guidelines for Research Involving Recombinant DNA Molecules.* For examples of activities in each level, please refer to Section III of the Guide.

6.1 Biosafety levels

i) Biosafety Level 1 for plants (BL1-P)

This level provides for a low level of containment and activities that normally involve GM-plants that are not able to survive and spread into the environment. Additionally, in the event that the GM-plant is accidentally released into the environment, it would pose no environmental risk.

This level is appropriate for plant-associated GM micro-organisms that cannot spread rapidly and are not known to have any negative effects on either natural or managed ecosystems.

ii) Biosafety level 2 for plants (BL2-P)

Experiments with GM-plants and associated organisms which, if released outside the greenhouse, could be viable in the surrounding environment but would have a negligible impact or could be readily managed, are categorised in BL2-P. This level is therefore required for GM-plants that may exhibit a new weedy characteristic or that may be capable of interbreeding with weeds or related species growing in the vicinity.

Transgenic experiments involving the entire genome of an indigenous infectious agent or pathogen will also be assigned to this level. This level is also appropriate for activities with transgenic plant-associated micro-organisms that are either indigenous to the area and potentially harmful to the environment but manageable, and to transgenic plant-associated micro-organisms that are exotic but have the potential for causing serious harm to managed or natural ecosystems. BL2-P also applies to experiments using plant-associated transgenic insects or small animals as long as they pose no threat to managed or natural ecosystems.

iii) Biosafety Level 3 for plants (BL3-P)

Facilities designed to accommodate activities at BL3-P are designed to prevent the accidental release of transgenic plants, plant pathogens, or other organisms that have a recognised potential for significant detrimental impact on the environment. This level applies to experiments that involve transgenic plants or organisms that contain genes coding for vertebrate toxins.

Activities that use transgenic microbial pathogens of insects or small animals that associate with plants and has the potential to cause harm to the local environment, will also be categorised within this level.

iv) Biosafety Level 4 for plants (BL4-P)

This level is recommended for activities with certain exotic, readily transmissible infectious agents that are potentially serious pathogens of major South African crops.

v) In Table 9 a comparison of the standard practices for containment of plants in greenhouses, is given for each of the biosafety levels.

Table 9: Containment of plants in greenhouses

BL1-P	BL2-P	BL3-P	BL4-P
Discretionary access; Hinged or sliding entry doors	Access limited to individuals directly involved with experiments; Hinged or sliding entry doors; locks at entry doors	Access restricted to required personnel only; Double set of self-closing, locking doors	Access restricted; secure locked doors (double set of self-closing, locking doors with air-lock); record kept of all entries/exits; clothing change; the only means of entry/exit is via a shower room through airlock
Personnel must read and follow instructions	Personnel must read and follow instructions	Personnel must read and follow instructions	All who entered should be advised of the hazards and safeguards
Procedures followed must be appropriate for the organism	A greenhouse manual must be in place to advise of consequences and outline contingency plans	A greenhouse manual must be in place to advise of consequences and outline contingency plans	A greenhouse manual must be prepared and adopted; Personnel are required to follow contingency plans
Records must be kept of the experiments in the facility	Records must be kept of experiments as well as movement in/out of the greenhouse	Records must be kept of experiments as well as movement in/out of the greenhouse	Records must be kept of experiments as well as movement in/out of the greenhouse
	Containment is required for movement in/out of containment	Containment is required for movement in/out of containment, as well as external decontamination	Special packaging containment required for movement in/out; Airlock or decontamination is required for removal
			Supplies and materials must enter through a special chamber
Must biologically inactivate experimental organisms at the end of the experiment	Must biologically inactivate experimental organisms at the end of the experiment, and de-contaminate gravel periodically	Must biologically inactivate experimental organisms at the end of the experiment (including water runoff), de-contaminate equipment and	Decontaminate experimental materials prior to removal from area by autoclave/other means; All runoff water must

BL1-P	BL2-P	BL3-P	BL4-P
		supplies	be collected and decontaminated
A pest control program must be in place	A pest control program must be in place	A pest control program must be in place	Chemical control program for pests and pathogens must be in place
Appropriate caging and precautions must be in place to prevent escape of motile organisms	Appropriate caging and precautions must be in place to prevent escape of motile organisms	Appropriate caging and precautions must be in place to prevent escape of motile organisms	Appropriate caging and precautions must be in place to prevent escape of motile organisms
	Erect a sign stating restricted experiment in progress, mention plant names, persons responsible and special requirements	Erect a sign stating restricted experiment in progress, mention responsible person, special requirements, and a biohazard symbol if there is a risk to humans	Erect a sign stating restricted experiment in progress, mention responsible person, special requirements, and a biohazard symbol if there is a risk to humans
		Minimise aerosol creation to reduce contamination	Standard microbial procedures to decontaminate equipment and containers must be in place
		Wear protective clothing to minimise dissemination, and wash hands before leaving facility	Street clothing must be removed; complete change into lab clothing which is autoclaved before laundering
			Report and record all accidents
Framing may be aluminium, steel, wood or pipe	Framing may be aluminium, steel, wood or pipe	The structure of the greenhouse should be rigid; a wind resistant frame; internal walls, ceilings and floors should be resistant to liquids and chemicals	The structure should be reinforced; the frame rigid; the walls, floors and ceilings from sealed internal shell that is resistant to liquids and chemicals
Glazing – standard greenhouse glass or plastic material	Glazing – standard greenhouse glass or plastic material	Glazing – Laminated, strengthened, sealed	Glazing – double-paned, laminated, strengthened, sealed
Screening – if used, use standard 30 mesh fly screen	Screening – use standard 30 mesh or higher fly screen	Screening – not permitted	Screening – not permitted
Ventilation – use roof/side vents, fans, cooling pads, fog system	Ventilation – use roof/side vents, fans, cooling pads, fog system	Ventilation – separate negative pressure system; air supply fans with back-flow damper; exhaust air HEPA filtered	Ventilation – Air-conditioned and HEPA filtered; closely monitored negative pressure, no roof or side vent allowed
Benching – any material; solid or porous bottoms	Benching – any material; solid or porous bottoms	Benching – seamless water and chemical resistant bench tops	Benching – seamless water and chemical resistant bench tops

BL1-P	BL2-P	BL3-P	BL4-P
Floors – gravel; soil; concrete; impervious walkways	Floors – impervious material; collection of runoff water may be required, depending on the organism used	Floors – Impervious material; for microbes the runoff water must be collected and decontaminated	Floors – sealed floors as part of the internal shell; runoff collection and decontamination
Drains – discharge into groundwater or sanitary/storm sewer	Drains – discharge into groundwater or sanitary/storm sewer	Drains – make provision for collection and decontamination of runoff	Drains – Runoff collection and sewer vents filtered
	Autoclave must be available	Autoclave must be within the facility; hand washing with hands free on/off; filtered vacuum lines; disinfectant traps for liquid lines	Double-door autoclave; self-contained vacuum system; in-line filters and back-flow protection for all liquid/gas services

More information on the terms glazing, sealing, screening, negative air-pressure, etc. can be obtained in Section IV of the *Guide*.

vi) Greenhouses that offer high-level BL3-P and BL4-P containment are expensive to built and operate. The cost of greenhouse containment at these levels may therefore be prohibitive for many institutions. Other means of high level containment may however be obtained through the use of growth chambers or growth rooms.

6.2 Biological containment

i) Additionally to the containment measures explained above, other precautionary measures can be taken to prevent the unintended dissemination of GMO's from the greenhouse to the environment.

ii) Possible biological containment for GM-plants in greenhouses

One or more of the following procedures can prevent dissemination of genetic material by means of pollen dispersal or seed dispersal:

- Cover/remove flower and seed heads
- Harvest plant material prior to sexual maturity or use male sterile lines
- Control the time of flowering so that pollen shed does not coincide with the receptive period of sexually compatible plants nearby
- Ensure that cross-fertile plants are not within the pollen dispersal range of the experimental plant
- Use genetic modification techniques that localise transgenes in non-propagative parts

iii) Possible biological containment for micro-organisms in greenhouses

Effective physical containment of bacteria, viruses and other microbes can be extremely difficult because they cannot be seen, and once dispersed, cannot be recovered. Biological measures therefore often provide better containment.

The following methods may help to prevent dissemination of genetically modified micro-organisms from greenhouses –

- Avoid creating aerosols when inoculating plants with transgenic microbes
- Provide adequate distance between an infected plant and another susceptible host , especially if the micro-organism can be disseminated through air of by leaf contact

- Grow experimental plants and microbes at a time of the year when nearby susceptible plants are not growing
- Eliminate vectors for insect-borne micro-organisms
- Choose micro-organisms having an obligate association with the host plant
- Genetically disable the micro-organism to minimise survival and reproduction
- Treat or evaporate runoff water

iv) Possible biological containment for insects in greenhouses

Insect and mite containment is very difficult in a greenhouse, however, the following procedures can be used to prevent dissemination of arthropods and other small animals –

- Choose or create non-flying, flight-impaired or sterile strains
- Conduct experiments at a time of year when survival of escaping organisms is impossible
- Choose organisms that have an obligate association with a plant not found in the vicinity
- Treat or evaporate runoff water to eliminate viable eggs and larvae
- Avoid use of small-sized insects in experimental greenhouse cages
- Destroy pollinating insects in experimental cages after pollen transfer to eliminate potential for dissemination of transgenic pollen into the environment

7. RISK MANAGEMENT

i) Risk management with regard to activities involving GMO's will be determined by the results obtained during a risk assessment. Appropriate risk management measures will vary from case to case and should be established in combination with the risk assessment. The type of organisms involved and the manner in which they will be released will also play a role in assigning appropriate risk management measures.

Please note that the measures below are only an indication of possible containment measures that can be used. The applicant may propose the type of measures to be used, however the applicability of the proposed measures and any additional measures will be determined by the Executive Council.

ii) The type of barriers used to obtain containment will in large depend on the type of activity. For e.g. if the aim of the activity is to use the GMO within a laboratory and there is no intention for release, the risk management measures will be different than for activities which involve the deliberate release of the GMO into the environment (trial release). In the case of deliberate release, the aim would not be to obtain containment per se, but to control the risk of potential harm from occurring by implementing appropriate risk management measures.

iii) There are four types of barriers that can be used. Please note that different barriers or a combination of different barriers will be applicable in different situations.

a) Physical or chemical barriers
These barriers are manipulations of the physical or chemical factors, which will induce a 100% mortality in one or more stages during the life cycle of the GMO.

b) Mechanical barriers
Mechanical barriers are made up of mechanical structures, which can be either stationary or moving barriers that will physically keep the GMO from escaping from the activity site. Additionally mechanical barriers can be placed in series at critical locations in the trial site to increase the strength of the barrier.

c) Biological barriers

These are barriers created through measures that would prevent the GMO from reproducing at the site of activity, and would reduce the possibility of survival of the GMO in the environment if released. Biological barriers are usually not sufficient on their own and one needs to add physical, chemical or mechanical barriers to obtain efficient containment.

d) The scale of the project as barrier
This can only be applicable for the research phase of testing a GMO. E.g. if the number of GMO's at the research site were kept so small that an escape into the environment would not impose the risk associated with the identified hazard. If the GMO is a self-fertilising hermaphrodite or a true pathogen, this barrier is not applicable as the escape of only one individual could result in the establishment of a whole population.

7.1 General risk management measures with regard to the deliberate release of GMO's into the environment

This section provides possible risk management measures in order to control the risk of potential harm occurring from a deliberate field release. Please note that the measures listed here are not exhaustive.

a) General risk management measures which may be used for plants
- Reproductive isolation through –
  - spatial separation
  - temporal separation
  - biological prevention of flowering
  - removal of reproductive organs
  - bagging flowers
  - making use of sterile plants
- Controlling the dispersal and persistence of reproductive structures such as seeds.
- Destroying all volunteer plants after harvest for a certain period after completion of the release, with the period depending on the type of plant involved.
- Installation of bird netting if necessary
- Prevention of access to burrowing animals by installing buried liners or suitable barriers

b) General risk management measures which may be used for animals
- Confinement by means of fences, filters, islands or ponds
- Reproductive isolation by using sterile animals
- Isolation from feral animals of the same species
- Controlling the persistence and dissemination of the reproductive structure such as larvae or eggs
- Installation of bird netting if necessary
- Prevention of access to burrowing animals by installing buried liners or suitable barriers

c) General risk management measures which may be used for micro-organisms
- Confinement by using organisms that do not have the ability to grow or survive in the environment
- Minimising gene transfer by using organisms that do not contain self-transmissible mobilisable or transposable genetic elements, or by ensuring that the inserted trait is incorporated into the chromosome.

d) Additional risk management measures that may be used

- Appropriate information and training to personnel involved in the release
- Apply monitoring procedures in such a manner to ensure that efficient steps can be taken in the event of unexpected release
- Control the dissemination and gene flow of the organism from the release site
- Maintain sufficient access control to the release site/facility through -
  - Supplying an ID-badge with a photo of each worker. No person may enter the site/facility without showing the badge.
  - Supply security training to all personnel
  - Installing security alarms
  - Erect signs and warnings at the entry sites
  - Maintain written security plans
  - Maintain adequate records of all activities with in the site or facility
  - Make all visitors sign in and out
  - Accompany all visitors to and from the site/facility.

7.2 Additional risk management options that may be used with herbicide resistant crops (HRC)

The options noted in this section only highlights the management options that may be used to control the development of herbicide tolerance, and does not exclude other risk management measures necessary when conducting a deliberate release with GMO's. Please take note that the options listed here are not the only options available, and the applicant may make use of alternatives.

The following risk management measures may be used –

- Identification of weed problems in the field (i.e. the species present and density of weeds, whether the weed species can easily cross with the GM-crop, what the seed longevity is for the potentially resistant weed)
- The use of cultural or non-chemical weed control methods to decrease selection pressure from the herbicide tolerant crop herbicide (i.e. ploughing prior to sowing, delaying planting an pre-emergence spray with a non-selective herbicide, use crop seed free from weed seed, and prevent seed production in weeds by cutting prior to seed set)
- Rotate with herbicides or use mixtures (i.e. avoid continued use of herbicides having the same mode of action unless it is integrated with other weed control practices, use non-selective herbicides prior to crop emergence to control early flushes of weeds and in case of metabolic resistance, decisions must be made on a case by case basis)
- Tailor the weed control program to weed densities and or economic thresholds
- Follow label instructions provided by the seed supplier, including recommended use rates and application timing.
- Monitor results of herbicide applications while being aware of any trends or changes in the weed population present.
- Maintain detailed field records so that cropping of GM-crops and herbicide history is known.
- If resistance does occur, seek ways to limit further seed production of the tolerant plants by eradicating the remaining weed population if they are growing in patches. This can be done in order to limit build-up and the spread of seed in the soil, and to limit the field to field movement of resistant populations.

7.3 Additional risk management options that may be used with insect resistant crops (IRC)

This section contains recommendations for managing resistance when growing crops modified to convey resistance against insects, however managing insect resistance is not the only aspect to be considered in the risk management strategy proposed for insect resistant crops. This section merely highlights the management options that may be used to control the development of insect resistance per se.

The following risk management measures may be used –

- The use of a refugia (area) of non-GM-crop
- By practising an Integrated Pest Management System to preserve the natural enemies of the harmful insects. The basic principles of integrated control combines biological and chemical control options, i.e. assessment of infestation of insects, control based on thresholds and the protection and usage of the naturally occurring enemies of the harmful insect.
- Monitoring the GM-crop and to contact the seed provider if resistance problems are suspected or experienced.

8. DEFINITIONS AND ABBREVIATIONS

Accident	Any accident involving the unintended general release of a GMO, which could have an immediate or delayed adverse impact on the environment.
Aerosol	Liquid droplets or solid particulars dispersed in a gaseous medium. A gaseous suspension of ultra microscopic particles.
Applicant/notifier	The party (e.g. seed producer or importer, agro-chemical company, farmers' organisation or research institute) that requests permission to deliberately release or introduce a GMO in a country.
Authority	A governmental institution, organisation or entity officially designated in terms of the GMO Act to deal with matters arising from the responsibilities set forth in the Guidelines.
Bacillus thuringiensis (B.t.)	Naturally occurring bacteria present in soil and used successfully by home gardeners and organic farmers to control certain insects. When ingested by a target insect, the protein produced by B.t. controls the insect by disturbing the digestive system.
Biohazard	A potentially dangerous infectious agent.
Biological containment	Genetic or physical impediments to the infectivity and or survival of a micro-organism or eukariotic cell.
Bioproducts	A product derived from or produced by cells or organisms
Competitiveness	A plant's ability to exploit essential elements such as light, water and plant nutrients at the expense of other plants.
Congeners	Refers to species belonging to the same genus.

Conspecific	Refers to individuals or populations of the same species.
Contained use	Any activity in which organisms are genetically modified or in which such GMO's are cultured, stored, used, transported, destroyed or disposed of and for which physical barriers or a combination of physical barriers together with chemical or biological barriers or both are used to limit contact thereof with the environment.
Crop production system	A particular agricultural method, including monocultures, rotations and polycultures, and their associated practices such as tillage plant protection and harvesting.
Decontamination	A process whereby viable micro-organisms are removed from solutions, surfaces or materials by filtration, heating, radiation or chemicals.
Eukaryotic cell	A cell with definite nucleus
Ecosystem	A complex of organisms and their environment, interacting as a coherent unit (natural or modified by human activity, e.g. agro-ecosystem) to maintain a flow of energy and to acquire, store and recycle nutrients.
Fitness	Reproductive success or the proportion of genes an individual leaves in the gene pool of a population.
Gene flow	The transfer of genes (specifically, alleles) from one population to another by way of interbreeding of individuals in the two populations.
Gene pool	All of the alleles available among the reproductive members of a population from, which gametes can be drawn.
General release	The introduction of GMO's into the environment by whatever means, where the organisms are no longer contained by any system of barriers and are no longer under any person's control, so that the organisms is likely to survive and be disseminated.
Genetic engineering	The technique of removing, modifying or adding genes to a living organism; also called recombinant DNA (rDNA) technology or genetic modification.
Genetically modified organism (GMO)	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.
Hazard	Intrinsic biological, chemical or physical characteristic of a GMO, which could lead to an adverse impact on the environment.
HEPA filter	High Efficiency Particulate Air filters (99.97% efficient removal of 0.3μm particles).

Herbicide	A chemical substance or mixture of substances designed to control weeds.
Herbicide resistant crop (HRC)	A crop plant that by genetic modification(s) or breeding has acquired resistance towards a herbicide it would otherwise be sensitive to.
Impervious	Not affording passage
Inactivation	Any process that destroy the ability of a micro-organism or eukaryotic cell to replicate.
Infection	Invasion and multiplication of micro-organism in body tissue which may or may not be clinically apparent.
Insect resistant crop (IRC)	A crop that by genetic engineering has become protected from damage by one or more harmful insects.
Insecticide	A chemical substance or mixture of substances that controls insects that harm crop production or prevents their damage.
Introgression	The transfer of genes from one population to another by backcrossing.
Invasiveness	The ability of a plant to spread and become established over large areas, displacing existing vegetation.
Micro-organism	Microscopic living entity, which can be viruses, prokaryotes or eukaryotes.
Marketing	The theory or practise of commercial selling, i.e. supplying or making available to third parties.
Monitoring	The maintaining of regular surveillance over, the checking of, the warning about or the recording of a situation or process.
Pathogen	A biological agent cell capable of producing disease.
Pest	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plant or plant products.
Pesticide	Refers to any substance or mixture of substances intended to prevent, destroy or control any pest, including substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest or protect the commodity from deterioration during storage and transport.
Physical containment	The confinement of a micro-organism or eukaryotic cell to prevent or minimise its contact with people and /or the environment.

Procaryotic cell	A cell that is lacking a true nucleus or nuclear membrane.
Trial release	The deliberate release of a GMO into the environment in the open under conditions where the degree of dissemination of the GMO is limited by chemical or physical barriers or by built-in barriers which prevent the survival of such organisms in the environment.
Resistance	In the case of plant populations, their inherited ability to grow and reproduce normally when exposed to high doses or levels of a specific agent (e.g. herbicide or insect attacks), which normally would harm plants.
Risk	The probability of causing or incurring a loss or damage or an adverse impact or a misfortune.
Risk assessment	The qualitative or quantitative evaluation of risks resulting from the release of genetically modified plants or products containing GMO's.
Seed Banks	accumulations of ungerminated seeds in the soil representing the balance between the seed rain (seeds that fall or are dispersed from fruits) and seed losses through germination, predation and death. (Archibold 1989)
Spread	Expansion of the geographical distribution of plants containing a genetically modified gene.
Sterilisation	An act or process of destroying all forms of microbial life on and in an object.
Tolerance	When referred to organisms, it is an increased ability of a biotype to endure damage, survive and reproduce after a limited exposure to a specific stress factor (in this context, herbicide applications or insect attack) compared to other biotypes of the species. Tolerance is often a polygenetic inherited trait.
Transgene	A gene or DNA fragment from one organism that has been stably incorporated into the genome of another organisms of interest.
Transgenic	See Genetically Modified Organism (GMO)
Vector	A plasmid, bacteriophage, etc. that can be used to transfer DNA sequences from one organism to another.
Volunteer	A crop plant regenerated from seed or propagules left after a previous harvest and which can act as a weed in the present crop.
Weed	A plant that is growing where humans do not want it.

9. REFERENCES

9.1 Archibold, O.W. 1989. Seed banks and vegetation processes in coniferous forests. In *Ecology of soil seed banks.* Eds. M.A. Leck, V.T. Thomas, and R.L. Simpson. London: Academic Press.

9.2 Canadian Food Inspection Agency Website Information – Plant Biosafety Office.

9.3 Cartagena Protocol on Biosafety to the Convention of Biological Diversity.

9.4 Compendium of Guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification, Directorate of Science and Technology, UK (www.open.gov.uk)

9.5 Guidelines and Procedures for work with genetically modified organisms - February 1998. Compiled by the South African Committee for Genetic Experimentation (SAGENE).

9.6 Guidelines for the analysis, assessment and management of associated risks – working draft of May 2000. Compiled as part of the International Standards for Ecological Risk Assessment of Herbicide or Insect Resistant Crops

9.7 Guidelines for the Regulation of Veterinary Biologics produced by Biotechnology. Food Production and Inspection Branch, Agriculture Canada. Samagh, B.S. ,1989.

9.8 Laboratory Biosafety Guidelines, 2nd Edition 1996. Health Canada – Health Protection Branch/Laboratory Centre for Disease Control.

9.9 Manual on assessing ecological and human health effects of genetically engineered organisms, Part one: Introductory Materials and Supporting Text for Flowcharts. Compiled by the Scientist' Working Group on Biosafety, 1998. The Edmonds Institute, Washington, USA.

9.10 Manual on assessing ecological and human health effects of genetically engineered organisms, Part two: Flowcharts and Worksheets. Compiled by the Scientist' Working Group on Biosafety, 1998. The Edmonds Institute, Washington, USA.

9.11 Netherlands Guidance Notes. Directive 90/220/EEC (14 November 2000).

9.12 Opinion: European Commission, Opinion of the Scientific Steering Committee: Risk Assessment in a Rapidly Evolving Field - The Case of Genetically Modified Plants. October 2000.

9.13 Paper: Agricultural and Environmental Biotechnology Commissions (UK): Risk Assessment for Releases and Marketing of GMO's in the EU.

9.14 Paper: UK Advisory Committee on Releases to the Environment - Commercial use of GM crops in the UK: The potential wider impact on farmland wildlife.

9.15 TRAYNOR, P.L., ADAIR, D. AND IRWIN, R. 2001. Greenhouse Research with Transgenic Plants and Microbes, A Practical Guide to Containment. Sexton Printing St Paul, Minnesota.

9.16 United Nations Environment Programme (UNEP) International Technical Guidelines for Safety in Biotechnology – December 1995.

9.17 USDA website on Agricultural Biotechnology at www.usda.gov.

10. ANNEXURES

ANNEXURE 10.1

BASIC LABORATORY PROCEDURES

The following procedures are regarded necessary to ensure that all activities within a laboratory are conducted in a safe and precise manner.

1. All laboratory personnel must understand the biological and other hazards with which they will come in contact through their normal work in the laboratory, and be trained in appropriate safety precautions and procedures.
2. A laboratory safety manual must be prepared and adopted. The laboratory director or supervisor is responsible to ensure that the manual identifies known and potential biohazards and specifies practices and procedures to eliminate or minimise such risks.
3. Training in laboratory safety must be provided and competence in safe technique demonstrated before work is allowed with hazardous agents or toxins.
4. Laboratories should have a Biological Safety Officer (BSO) and/or a Biological Safety Committee whose responsibility includes ensuring that all work is carried out in accordance with the safety practices established at the Institution. (Refer to Annexure 10.2)
5. Duties of the BSO should include providing technical advice on safety procedures and equipment, developing emergency plans, conducting safety inspections, providing biosafety training, conducting or supervising testing of containment systems and providing guidance and information related to compliance with pertinent regulations. Refer to Annexure 10.2)
6. Laboratories must be kept neat, orderly, clean and storage of materials not pertinent to work should be minimised.
7. Protective laboratory clothing must be available and worn properly fastened by all personnel, including visitors, trainees and others entering or working in the laboratory. Protective laboratory clothing must not be worn in non-laboratory areas. Suitable footwear with closed toes and heels, preferably with non-slip soles must be worn in all laboratory areas.
8. Gloves must be worn for all procedures that might involve direct skin contact with toxins, blood, infectious materials or infected animals. Hand jewellery, which would interfere with glove functioning, should be removed. Gloves should be removed carefully and decontaminated with other laboratory wastes before disposal. Reusable gloves may be used only where necessary and must be appropriately decontaminated.
9. Eyewear must be worn when necessary to protect the face and eyes from splashes, impacting objects, harmful substances and UV light or other rays.
10. Eating, drinking, smoking, storing food, personal belongings or utensils, applying cosmetics and inserting or removing contact lenses are not permitted in any laboratory work area. Contact lenses should be worn only when other forms of corrective eyewear are not suitable. The wearing of jewellery should be discouraged in the laboratory.
11. Oral pipetting of any substances is prohibited in any laboratory.
12. Long hair must be tied back or restrained.
13. Hands must be washed after gloves are removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.
14. Work surfaces must be cleaned and decontaminated with a suitable disinfectant (e.g. 70% ethanol) at the end of the day and after any spill of potentially dangerous material. Loose or cracked work surfaces must be replaced or repaired.
15. All technical procedures must be performed in a manner that minimises the creation of aerosols.
16. All contaminated or infectious liquid or solid materials must be decontaminated before disposal or reuse. Contaminated materials that are to be autoclaved or incinerated at a site away from the laboratory, must first have the outside of the container disinfected chemically or be double bagged. (E.g. Liquid biohazards should be added to 10% bleach, and incubated for 30 minutes and then autoclaved at 135°C for 20 minutes).
17. Access to the laboratories must be strictly limited, especially containment levels 3 and 4. Decisions on entry into containment level 1 and 2 laboratories should be at the discretion of

the laboratory director or principal investigator. Children under the age of 16 years old should not be permitted in the laboratory or support area. Pregnant women or immuno-compromised people who work in or enter the laboratory should be advised of the associated risks.

18. Hazard warning signs, indicating the risk level of the agents being used, must be posted outside each laboratory, when indicated by the risk assessment. Where infectious agents used in the laboratory require special provisions for entry, the relevant information must be included in the sign. The agent must be identified and the name of the laboratory supervisor and other responsible persons as well as any special conditions for staff entry, must be listed.
19. The use of needles, syringes and other sharp objects should be strictly limited. Needles and syringes should be used only for parenteral injection and aspiration of fluids from the laboratory animals and diaphragm bottles. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Procedures should be performed in a biological safety cabinet; needles should be promptly placed in a puncture-proof container and decontaminated, preferable by incineration or autoclaving, before disposal.
20. All spills, accidents, and overt or potential exposures must be reported in writing to the laboratory supervisor or acting alternate as soon as circumstances permit; this person should file this report with management and the appropriate BSO or committee. Appropriate medical evaluation, surveillance, and treatment should be sought and provided as required. Actions taken to prevent future occurrences should be documented.
21. Baseline serum for laboratory and other at-risk personnel should be collected and stored, if required by the risk assessment. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
22. Laboratory workers should be protected by appropriate immunisation where possible. Levels of anti body considered to be protective should be documented. Particular attention must be given to individuals who are or may become immuno-compromised, as vaccine administration may be different than for immunologically competent adults.

ANNEXURE 10.2

CLASSIFICATION AND OPERATION OF A FACILITY

1. As a preface to this section, it should be noted that the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993) and the Hazardous Substances Act, 1973 (Act No. 15 of 1973) place responsibilities on employers and employees in relation to all hazards at work. This includes responsibilities that may arise from genetic modification work. Guidelines and regulations in this regard may be obtained at the following address –

   Department of Labour
   Private Bag X117
   Koedoe Building Room 320
   Pretorius Street
   Pretoria
   0001
   Tel: +27 +12 309 4400
   Fax: +27 +12 320 5112 or 2808

2. A laboratory facility or large-scale facility must be classified as containment level 1,2,3, or 4. The requirements for each containment level are specified in section 5 of the guidelines. A glass house facility must meet the specifications as laid out in section 6.

3. For each facility a responsible person(s) and/or a biological safety officer responsible for the work or particular aspects of work which fall under the Act, must be identified. The person(s) identified as responsible for the work in question must assess, or cause to be assessed, the risk that might arise during work.

4. The Biological Safety Officer should have experience in working within a containment laboratory or with similar practices, but the absence of such experience should not necessarily preclude the appointment of an individual who is otherwise well suited for the position. He/she must be appropriately trained and provided with technical assistance as necessary. Appropriate deputising arrangements should also be made.

5. The Biological Safety Officer must further act as adviser to the head of the establishment or department in all matters relating to the containment of biological hazards and the safety of staff. In the event that the BSO is involved as principal investigator, appropriate deputising arrangements should be made.

6. The Biological Safety Officer should not be the head of the establishment or department.

7. The Biological Safety Officer must carry out regular safety audits and supervise a regular testing program for all exhaust protective cabinets and HEPA filters when these are part of the laboratory equipment.

8. The Biological Safety Officer is to be answerable to the head of the establishment or department, in so far as genetic modification work is concerned, for -

   i) ensuring that the local rules are followed;
   ii) all aspects of training in appropriate laboratory practice;
   iii) investigating all accidents, spillage etc. in the laboratory and taking what action he/she considers necessary. Each incident and the action taken must be recorded, together with the name of the personnel involved;

iv) the safe storage of GMO's, and pathogenic or potentially pathogenic material, and also for ensuring that an inventory of these is maintained;
v) the appropriate transport of all GMO's (transfer of organisms constructed at containment level 2 or above should be recorded);
vi) liaison with the Supervisory Medical Officer;
vii) ensuring that laboratories are appropriately disinfected prior to the start of a new experiment or the entry of maintenance personnel. Appropriate disinfection could range from swabbing down work surfaces to complete fumigation;
viii) physical security of the laboratory.

9. Before agreeing to a new entrant working in a containment laboratory the Biological Safety Officer must be satisfied that the individual concerned is familiar with the local rules and the correct use of the laboratory equipment. The new entrant must have training in good laboratory techniques, preferably externally, but a prescribed period of in-house training may suffice. A responsible member of the laboratory staff must supervise the work of all new entrants.

10. In laboratories for which he/she is responsible, the Biological Safety Officer must maintain a list of all people who are working there.

11. No-one may enter the containment area (other than in an emergency) for cleaning, servicing of equipment, repairs or other activities outside the normal work of the laboratory unless a responsible member of staff has previously been informed and, in containment level 2 or above, laboratory surfaces have been disinfected.

12. The responsible member of staff in charge of experiments is

i) answerable at all times to the Biological Safety Officer for the safe execution of the work in progress and
ii) responsible for ensuring the day-to-day cleanliness of the laboratory.

13. Appropriate protective clothing must be worn in the containment area. When working in an exhaust protective cabinet, gloves should always be worn.

14. Protective clothing designated for use in a containment laboratory must not be worn outside the facility.

15. Each establishment must draw up local rules. The local rules must include information on such matters as:

i) selection and training of laboratory staff and supervision of work;
ii) policy of disinfection (see Annexure 10.4) and procedures for the disposal of potentially infective material;
iii) guidance for ancillary and maintenance staff, contractors and visitors;
iv) maintenance and test procedures for ventilation systems, high efficiency particulate air (HEPA) filter, microbiological safety cabinets (see Annexure 10.3) and other safety equipment;
v) health surveillance which should, where appropriate, include screening procedures including the immune status of the individual, sickness investigation, issues of medical contact cards, immunisation procedures, maintenance of baseline serum samples from staff;
vi) the duties of the biological safety officer.

ANNEXURE 10.3

BIOLOGICAL SAFETY CABINETS

1. Reference should be made to the "Proposed Compulsory Specification for Biological Safety Cabinets (Classes I, II and III)", Government Gazette 15 June 1990, obtainable from SABS under reference no. VC8041 (2001). This gives a full description of the three types of safety cabinets, class I, II and III. The testing and maintenance of these cabinets is also covered in the SABS Code of Practice for the Installation, Post Installation Tests and Maintenance of Biological Safety Cabinets (SABS 0226 2001).

   Both of these standards can be obtained at the following address –

   SABS Standards Sales
   Pretoria
   Tel: 27 12 428 6841 / 6482
   Fax: 27 12 428 6928
   E-mail: sales@sabs.co.za

2. The class II cabinet is designed to control airborne contamination of the work while at the same time reducing the exposure of the operator to airborne particles, which are dispersed inside the cabinet during the work procedures. In the simplified form of cabinet these two functions are achieved by a recirculating downward flow of filtered air over the work area; part of this airflow is exhausted to the atmosphere through a HEPA filter and make-up air is drawn into the cabinet through the open work front.

3. Before a cabinet is selected, the user should assess the need for protection of the work and relate this to the operator protection factor that can be achieved in the prevailing conditions of use. The cabinet must be correctly sited and should be used only by appropriately trained personnel.

4. Careful working procedures will be essential and access to the laboratory should be restricted whilst the cabinet is in operation. The inward airflow that is drawn through the aperture of open-front cabinets (class I and II) can be disturbed by, for example, sudden movements of the arms of the operator, turbulence around the equipment placed inside the cabinet, persons moving across the front of the cabinet, by air movements in the room and changes in air pressure. Disturbances of this kind may affect the level of protection for the operator particularly when a class II cabinet is used since this type generally has a lower inward air velocity through the upper areas of the work front than a class I cabinet. The protection factor provided by the cabinet should therefore be determined. Class I and II cabinets must not be used at containment level 4.

5. Provided that a class II cabinet gives a protection factor of 1.5×10^5 or better (see Government Gazette, 15 June 1990), and if it can be shown that this level is achieved consistently (by testing at regular intervals), a class II cabinet may be used for some work at level 3 where protection of the work is essential. Consideration should always be given however to the use of a class III cabinet that will provide a high level of protection for both the work and the operator.

ANNEXURE 10.4

DISINFECTION

1. Disinfection generally refers to the use of chemical agents to destroy the potential infectivity of a material, but does not imply the elimination of all viable micro-organisms.

2. Effective disinfection is dependent upon the following factors:

   i) The activity : the effectiveness of a particular disinfectant varies with the micro-organism (Table at the end of this Annexure)
   ii) Concentration : the "use-dilution" is the correct concentration for effective disinfection in particular circumstances e.g. spillage, discard jars. The effective concentration may be dependent upon the age of the solution, as once diluted disinfectants lose effectiveness with time.
   iii) Contact : intimate contact for a sufficient period of time must be maintained between the disinfectant and the contaminated article, e.g. air bubbles should be removed from submerged articles.

3. The disinfectants most commonly used are hypochlorites, clear phenols and alcohol. In the following paragraphs more information is on each disinfectant. Less often disinfectants used are aldehydes and surface-active agents.

   i) Hypochlorites
      - E.g. Jik
      - Hypochlorites have a wide spectrum of antimicrobial activity and are rapid in action but they are corrosive, inactivated by organic matter and decompose once diluted.
      - Recommended dilutions are as follows:

        General use : a solution containing 1000-ppm available chlorine
        Discard jars : a solution containing 2500-ppm available chorine
        Spillage : a solution containing 10000-ppm available chlorine

        Organic chlorine-releasing compounds, e.g. chloramine, have the advantage that chlorine is not lost so readily and so exert a more prolonged antimicrobial effect.

   ii) Clear Phenols
      - E.g. Hycolin, Stericol, etc.
      - Phenols are non-corrosive and have a wide range of activity but may be ineffective against non-lipid viruses. Some phenols are affected by organic matter and their antimicrobial activity may also be reduced by hard water Phenols should be used at the manufacturer's recommended use-dilution but should not be stored diluted.

   iii) Alcohols
      - E.g. 70% ethanol, 60% isopropanol
      - Alcohols give a very rapid kill of bacteria and some viruses, but because they are relatively volatile do not provide a sustained antimicrobial action.
      - Alcohols are flammable and require appropriate precautions in storage and use. They should not be used in microbiological safety cabinets or on large areas.

   iv) Aldehydes
      - Formaldehyde as the vapour or the aqueous solution (formalin) is toxic and is not suitable for general purposes. However it is used for fumigating microbiological safety cabinets and certain rooms (e.g. high containment laboratories).
      - During fumigation containers of other disinfectants should be sealed if the disinfectant is incompatible with the fumigant.

- ❖ Glutaraldehyde is also toxic but has relatively low vapour pressure and is usually used as a solution. It has wide range of activities, including against bacterial spores. It is non-corrosive, but does not readily penetrate organic matter and is not particularly stable once activated.

v) Surface-active agents
- ❖ Only the cationic and amphoteric detergents have any antimicrobial activity, and these are regarded as being more bacteriostatic than bactericidal.
- ❖ They are relatively non-toxic and non-irritant but are inactivated by organic matter and anionic detergents e.g. soap.
- ❖ Quaternary ammonium compounds form the basis of the majority of cationic detergents e.g. Cetrimide, Roccal.
- ❖ Only a limited range of amphoteric detergents has been produced as antimicrobial agents e.g. Tego.

4. When selecting a disinfectant its toxicity to humans and the appropriate health and safety precautions should be considered. Different disinfectants must not be mixed together or used in combination unless the possibility of hazardous reactions or the formation of toxic products has been properly assessed.

5. Arrangements should be made for appropriate procedures and training to ensure that suitable disinfectants, at the correct dilutions are available at the point of use. There are advantages in limiting the number of different disinfectants available in the workplace to the minimum necessary, in order to avoid confusion and to reduce costs. Once a disinfectant has been selected, in-use tests should be carried out to monitor not only the performance of a particular disinfectant but also the way in which it is used, for example to detect dilutions wrongly made up or not made up freshly, the use of dirty containers and incompatible reagents for example, certain type of detergent.

Table: Activities of some common classes of disinfectants (note: the specific activity of a particular disinfectant must be assessed on a case-by-case basis)

	Vegetative Bacteria	**Active against Bacterial spores**	**Fungi**	**Lipid Viruses**	**Non Lipid Viruses**
Hypochlorites	+	+	1	+	+
Phenols	+	-	+	+	2
Alcohols	+	-	-	+	2
Aldehydes	+	+	+	+	+
Surface-active agents	+	-	1	2	2

1. Limited anti-fungal activity.
2. Depends on the virus.

ANNEXURE 10.5

RECOMMENDATION DOCUMENT

The chairperson of each review will compile a recommendation document.
The format of the document will be as outlined below.
(The applicant has to ensure that the necessary information is contained in the application to enable the review committee to comment on each item with regard to the application. Failure to supply all the information will result in the application being withheld until all requested information has been submitted.)

A: Details of the review committee
Provide the full names, institutions and expertise involved in the review committee.

B: Summary of the application
Particulars of the applicant's request

i) Name of the applicant
ii) Title of the application
iii) Reference number given by the Registrar's office
iv) Short description of the applicant's request
- The intended use
- Purpose of the use
- Scale of use

v) Short description of the genetic modification
- What it is?
- How developed?
- Stability of integration?

C: Procedures followed during evaluation of the application
Stipulate all dates and actions involved during the evaluation process from the moment that the review chairperson receives the application.

D: Safety issues assessed:

1. Food and feed:
   - Toxicological studies
   - Allergenicity studies
   - Compositional analysis
   - Nutritional analysis
   - Pathogenicity
   - Feeding trials
   - Any other
2. Environment:
   - Weediness/invaseness
   - Gene flow
   - Altered plant pest potential
   - Non-target organisms
   - Impact on biodiversity
   - Any other

E: Non-safety issues
- Experimental design
- Sociological factors
- Economical factors
- Any other

F: Risk management
Proposed risk management measures that should be incorporated into the permit.

G: Recommendation
Provide a recommendation to the Executive Council

H: Appendix
Attach individual review reports

ANNEXURE 10.6

REVIEWERS' CHECKLISTS

This checklist may be used by any member of the sub-committee with the necessary expertise, and may be attached to the report submitted by the reviewer.
These checklists are intended to assist the reviewers, but take note that (a) a particular application may not contain all types of data listed and (b) there may be additional data provided by the applicant, or required by the reviewer, that is not included in this list.

SECTION I – CHECKLIST FOR NORTHERN BLOT DATA

	YES	NO	COMMENT
Does the Northern blot have a figure number and title?			
Are lanes labelled on the blot?			
Does the figure legend describe each lane of the blot, including a description of the following for the RNA that was loaded: ▪ What type of material was loaded (e.g. total purified RNA, poly-A RNA, crude prep, total plant extract)? ▪ Source of the material loaded (e.g. transformation event, tissue, development stage, any prior treatments to induce gene expression, etc.)? ▪ Quantity of material loaded in each lane? ▪ Quality of material loaded in each lane?			
Does the text or figure legend describe how RNA was extracted prior to electrophoresis?			
Does the blot have appropriate positive and negative control lanes - ▪ Positive control consisting of a dilution series of control RNA complemented with wild type RNA of the same tissue (this control is especially relevant for blots used to substantiate the absence of expression); ▪ Positive control purified RNA; ▪ Negative control - the unmodified parental line or variety; ▪ Check for loading differences using a probe for a "constitutive" mRNA.			
Is the gel system and Northern hybridisation protocol described in the text or in the cited literature reference? Are any modifications of the cited protocols described in the petition (application) text?			
Is the position of molecular size standards on the gel indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on the blot?			
Is there a description of the probe that was used for the hybridisation? If so, is the description adequate (in the text on in the figure) to enable one to interpret the results?			
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are differences between samples or treatments?			
Are any superfluous bands or background signals properly explained?			

SECTION II – CHECKLIST FOR SOUTHERN BLOT DAT

	YES	NO	COMMENT
Does the Southern blot have a figure number and title?			
Are lanes labelled on the blot?			
Does the figure legend describe each lane of the blot, including a description of the following for			

	YES	NO	COMMENT
the DNA that was loaded on the gel:			
▪ Type of DNA loaded (e.g. entire plasmid, restriction fragment)? ▪ Source of DNA loaded (e.g. transformation event, tissue, etc.)? ▪ Restriction digestions of DNA prior to loading gel? ▪ Quantity of material loaded in each gel? ▪ Quality of the material loaded in each gel?			
Does the gel have appropriate positive and negative control lanes – ▪ Positive control consisting of a dilution of a series of control DNA complemented by wild type DNA of the same tissue; ▪ Positive control purified transformation vector; ▪ Negative control – the unmodified parental line or variety.			
Is the gel system and Southern hybridisation protocol described in the text or in the cited literature referenced? Are any modifications of the cited protocols described in the petition (application) text?			
Is the position of the molecular size standards indicated, and do they cover an appropriate size range for the fragments that are expected to the detected on the blot?			
Was an entire plasmid used as the probe for the hybridisation? If so, is the plasmid described adequately in the text or in a figure to enable one to interpret the results?			
Was a restriction fragment used as the probe for the hybridisation? If so, is the restriction fragment described adequately in the text or in a figure to enable one to interpret the results?			
Are any superfluous bands or background signals properly explained?			

SECTION III – CHECKLIST FOR RNA DOT BLOT DATA

	YES	NO	COMMENT
Does the Dot blot have a figure number and title?			
Are lanes labelled on the blot?			
Does the figure legend describe each lane of the blot, including a description of the following for the RNA that was loaded: ▪ What type of material was loaded (e.g. total purified RNA, poly-A RNA, crude prep, total plant extract)? ▪ Source of the material loaded (e.g. transformation event, tissue, development stage, any prior treatments to induce gene expression, etc.)? ▪ Quantity of material loaded in each lane? ▪ Quality of the material loaded in each lane?			
Does the text or figure legend describe how RNA was extracted prior to blotting onto the solid support?			
Does the blot have appropriate positive and negative control lanes – ▪ Positive control consisting of a dilution series of control RNA complemented with wild type RNA of the same tissue (this control is especially relevant for blots used to substantiate the absence of expression); ▪ Positive control of purified RNA ▪ Negative control – the unmodified parental line or variety.			
Is the blot system and hybridisation protocol described in the text or in the cited literature			

	YES	NO	COMMENT
reference? Are any modifications of the cited protocols described in the submitted text?			
Is there a description of the probe that was used for the hybridisation? If so, is the description adequate (in the text on in the figure) to enable one to interpret the results?			
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are differences between samples or treatments?			

SECTION IV – CHECKLIST FOR WESTERN BLOT DATA

	YES	NO	COMMENT
Does the blot have a figure number and title?			
Are lanes clearly labelled?			
Does the figure legend describe each lane of the blot, including a description of the following for the protein that was loaded: ▪ What type of material was loaded (e.g. pure, crude, total plant extract)? ▪ Source of the material loaded (e.g. transformation event, tissue, development stage, any prior treatments to induce gene expression, etc.)? ▪ Quantity of material loaded? ▪ Quality of the material loaded?			
Is the protein extraction method adequately described in either the text or the legend?			
Is the antibody or antiserum preparation protocol adequately described in the text, including an adequate description of the antigen and its purity? Has the specificity of the antibody or antiserum been determined and described in the text or in a cited literature reference?			
Is the gel system and blotting protocol adequately described in the text or in a cited literature reference?			
Is the position of the molecular weight standards indicated, and do they cover the appropriate range for the proteins expected to be detected on the blot?			
Does the blot include appropriate positive and negative controls – ▪ Positive control consisting of a dilution series of control protein complemented with wild type material of the same tissue (this control is especially relevant for blots used to substantiate the absence of expression); ▪ Positive control of purified protein; ▪ Negative control – the unmodified parental line or variety.			
Was a normal serum control conducted?			
Are any superfluous bands or background signal properly explained?			
If quantitative analysis is performed, has the methodology or citation to such been provided, and have a sufficient number of replicates or samples been tested to determine whether there are differences between samples or treatments?			

SECTION V – CHECKLIST FOR PCR DATA

	YES	NO	COMMENT
Does the PCR gel have a figure number and title?			
Are lanes labelled on the gel?			

	YES	NO	COMMENT
Does the figure legend describe each lane of the gel, including a description of the following for the DNA that was loaded: ▪ What type of material was loaded (e.g. plasmid fragment, amplified DNA)? ▪ Source of the material used in each reaction loaded (e.g. transformation event, tissue, development stage, any prior treatments to induce gene expression, etc.)? ▪ Quantity of material loaded? ▪ Quality of material loaded?			
Is the position of the molecular weight standards indicated, and do they cover an appropriate size range for the fragments that are expected to be detected on gel?			
Does the text or figure legend describe how PCR amplification was performed prior to electrophoresis?			
Is there a description of the primers used for amplification in the text or in the figure sufficient to enable one to interpret the results?			
Does the gel have appropriate positive and negative control lanes - ▪ Positive control might demonstrate specificity of the primers and the ability to amplify the appropriate size band; ▪ Negative controls might include amplification with DNA from the unmodified parental line or variety, and amplification in absence of DNA template; ▪ Check for amplification of a control fragment from the plant sample (to show that PCR is working, especially if it is intended to show absence of a specific DNA); ▪ Mix plant DNA with plasmid DNA (I copy control) to demonstrate that the PCR is working properly.			
Was an entire plasmid or a restriction fragment used as the positive control template and is it adequately described in the text or in the figure legend for interpretation of the PCR results?			
Is the gel system and PCR protocol described in the text or in a cited literature reference? Are modifications of a cited protocol described in the text?			

SECTION VI – CHECKLIST FOR ELISA DATA

	YES	NO	COMMENT
Does the table have a number and a title?			
Are all entries clearly identified in the table and described in the text or table legend?			
Is the sample preparation described?			
Is the antibody or antiserum preparation protocol adequately described in the text, including a description of the antigen and its purity? Has the specificity of the antibody or antiserum been demonstrated and described in the text or in a cited literature reference?			
Is the ELISA protocol used described in the text or cited in the scientific literature? Any modifications to a cited protocol must be described.			
Were appropriate positive controls (e.g. purified protein) and negative controls (e.g. normal or preimmune serum, non-transformed plant material) used?			
When ELISA is being used to quantify protein expression in transformed tissues: ▪ Was a method for the determination of protein concentration in tissue samples presented in the text or in a cited literature reference? ▪ Were a standard curve prepared and the limit of detection indicated?			

	YES	NO	COMMENT
▪ Have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments? Was statistical analysis performed?			

SECTION VII – CHECKLIST FOR ENZYME ASSAYS

	YES	NO	COMMENT
Does the figure (or table) have a number and title?			
For graphical representations or tables, are the axis or columns labelled and the units indicated?			
Does the scale of the figure accurately represent and allow interpretation of the data?			
Does the legend or text describe: ▪ The substrate and amount used for the reaction? ▪ The quantity and origin of the enzyme? ▪ The temperature and pH?			
Does the text or legend describe the extraction and purification of the enzyme and the degree of purification achieved?			
If the enzyme used in the assay has not been isolated from the transformed plant but is derived from an expression system, has adequate data been presented to demonstrate its substantial equivalence to the plant expressed enzyme?			
Have the assay method and relevant information concerning the enzyme been provided in the text or in a cited literature reference?			
Are appropriate controls included in the assay?			
Has the stability of the enzyme and possible presence of enzyme inhibitors in different tissue extracts been taken into account in the design of the assay or the interpretation of the data?			
When relevant to the safety assessment, have the kinetics of the enzyme been calculated and where possible compared to published data?			
When quantitative analysis is performed, have a sufficient number of replicates or samples been tested to determine whether there are significant differences between samples or treatments? Was statistical analysis performed?			