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LMOs, BIODIVERSITY AND BIOSAFETY

National and International Regulations

Prepared under

gef UNEP

Phase-II Capacity Building Project on Biosafety

Ministry of Environment
Forests and Climate Change

Ministry of Environment
Forests and Climate Change
Government of India

In association with

BCIL
Biotech Consortium India Limited
New Delhi

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Ministry of Environment, Forest and Climate Change (MoEFCC) and
Biotech Consortium India Limited, New Delhi
under the UNEP/GEF supported Phase II Capacity Building Project on Biosafety

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Introduction

The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity (CBD) is an international treaty governing the movements of living modified organisms (LMOs) resulting from modern biotechnology from one country to another. It was adopted on 29 January, 2000 as a supplementary agreement to the CBD and entered into force on 11 September, 2003. The CPB has been developed in response to advancements in the area of modern biotechnology and associated concerns that LMOs resulting from modern biotechnology may have negative effects on biodiversity and human health. As on January 2019, 171 countries have ratified or acceded to the Protocol. India is a party to CBD and CPB.

Biotechnology, in the form of traditional fermentation techniques, has been used for decades to make bread, cheese or beer. It has also been the basis of traditional animal and plant breeding techniques, such as hybridization and the selection of plants and animals with specific characteristics to create, for example, crops which produce higher yields of grain. Modern biotechnology, meanwhile, employs advanced techniques such as genetic engineering or recombinant deoxyribonucleic acid (rDNA) technology whereby researchers can take a single gene from a plant or animal cell and insert it in another plant or animal cell to give it a desired characteristic, such as a plant that is resistant to a specific pest or disease. Such living organisms that possesses a novel combination of genetic material obtained through the use of modern biotechnology are refered to as LMOs. In everyday usage, LMOs are usually considered to be the same as genetically modified organism (GMO), genetically engineered (GE) organism but definitions and interpretations of the term vary widely.

GMOs/LMOs form the basis of a range of products and commodities used in wide range of applications in healthcare, agriculture, industry etc. Since the release of the first product i.e. human insulin derived from GE bacteria in 1982 followed by commercialization of genetically modified (GM) crops in the mid-90s, products and processes involving modern biotechnology have been extensively used globally. In parallel, there are increasing debates about safety

concerns among various stakeholders related to their impact on health, environment and biodiversity. Many countries with active biotechnology research programmes including India have put in place biosafety regulations to deal with these safety issues.

The Ministry of Environment, Forest and Climate Change (MoEFCC) is the nodal Ministry for implementation of biosafety regulations in India and implementation of CPB. MoEFCC has taken several initiatives for capacity building of multiple stakeholders for creating awareness about CPB and national regulations. Resource documents and outreach material have been prepared as part of UNEP/GEF supported "Phase II Capacity Building Project on Biosafety" being implemented by MoEFCC for regulators, scientists, enforcement agencies, students etc.

In continuing with the same, MoEFCC in association with Biotech Consortium India Ltd. (BCIL), the project coordination unit has prepared booklet for specific categories of stakeholders focusing on their information requirements.

This booklet has been prepared to provide information to stakeholders dealing with biodiversity including central and state Government department, biodiversity boards, experts and other non Government agencies for informing them about key aspects of LMOs and biosafety issues. It has following five sections:

1. LMOs: Applications and status
2. Regulations of LMOs in India
3. Cartagena Protocol on Biosafety
4. Safety assessment methodologies
5. Detection of LMOs

Section 1: LMOs Applications and Status

In general, a LMO is made by taking a gene (a piece of DNA) from one organism and inserting it into the DNA of another organism. Scientists search for genes that correspond to desired characteristics. By inserting these genes into other organisms, scientists can create organisms that display the traits coded for by the gene.

Use of modern biotechnology to make LMOs is based on the fact that chemical composition of DNA is the same in all living organisms (Box 1) . DNA is found in the nucleus of the cells of all living creatures. DNA contains the instructions for making protein which in turn carry out various functions.

Box 1: DNA and Gene

- Deoxyribonucleic acid, more commonly known as DNA, is a complex molecule that contains all of the information necessary to build and maintain an organism. All living organisms have DNA within their cells.
- A gene is a sequence of DNA that contains information that determines a particular characteristic/ trait.
- Genes are units of inheritance that are passed from one generation to the next.
- All organisms have varying number of genes. For instance, the human has an estimated 60-100,000 genes, most plants have about 20,000, a nematode has about 18,000 and a single celled Escherichia coli bacterium just about 4,000.
- The genetic differences among different species as well as organisms within a species lie in difference in number and sequence of these genes in the DNA/genome.

As the genetic code is universal i.e. the DNA of all organism is made up of the same building blocks and is encoded in exactly the same way. The copying and transfer of genes from one organism to another can be done in the laboratory. Hence it is possible to transfer a copy of DNA sequence (or gene) that codes for a particular characteristic into the cell of a different organism. Once the gene is incorporated into the genome of recipient, the resulting organism is considered to be GMO or LMO and the new characteristic coded by that gene is inherited by subsequent generations.

Example of a LMO: Bt cotton

The *cry* gene (also called Bt gene) in the soil bacteria *Bacillus thuringiensis* (Bt) is known to produced insecticidal protiens that are harmful to specific target insects. The insecticidal protiens (crystal protiens) produced by *cry* gene bind to specific receptors on the mid gut of the specific target insects. The *cry* gene from the Bt soil bacteria has been inserted into the DNA of cotton. The Bt Cotton containg the *cry* gene kill the target insects that try to feed on the plant. Due to the highly specific mode of action of the crystal protiens, no harm is caused to humans, animals, fish, birds and beneficial insects. Bt cotton, has been approved for use in a number of countries. India is the fifth largest producer in the world after USA, Brazil, Argentina and Canada.

Safety concerns have been expressed for LMOs produced with the use of modern biotechnology techniques as is with any new emerging technologies. These apprehensions arise because LMOs may contain genes that have crossed the species barriers as compared to classical selection techniques. There is no evidence that any unique hazards exist in the development of LMOs, because of novel combination of genes. However, specific gene organism combination may be harmful by virtue of novel combination of traits they possess and therefore the concerns associated with the use of LMOs differ greatly depending on the particular gene-organism combination. A case by case approach is adopted for assessment of safety concerns. Potential risks from the use of LMOs broadly fall under two categories i.e. risks to human and animal health and risks to environment. Risk to human health may include introducing of any toxins, allergens or other anti-nutrient factors. Risk to environment may include effects on non-target organisms, invasiveness or weediness, resistance development in target organisms or movement of a transgene outside the LMO.

In view of the above, safety of LMOs is evaluated in a comprehensive process that involves several steps. Systematic safety assessment methodologies have been developed at national and international level that give conclusion on whether or not the LMO is as safe as its conventional counterpart. LMOs are permitted to be grown and used only after they have passed safety assessment and are not likely to pose risks for human health and environment.

Genetic engineering is more precise and the outcomes more certain, resulting in faster production of organisms with desired traits. Hence the technology has found applications in several areas such as healthcare, agriculture, process industry and environment for the production of GMOs/LMOs:

a. **Healthcare:** The healthcare applications of LMOs and their products are playing an increasing role in conventional drug discovery as well as opening up new possibilities to prevent, treat and cure many incurable diseases using novel methods of treatment and diagnosis. LMOs have been extensively used for production of therapeutics, vaccines and monoclonal antibiotics. Most common examples of products from use of LMOs include human insulin, hepatitis B vaccine, and interferons.

b. **Agriculture:** The plants are being subject to genetic modification for multiple traits including production of transgenic plants with increased resistance to pest and disease, higher crop yield and nutritional content and increased resistance to abiotic stresses such as draught, salt etc. Most common agricultural crops being cultivated and traded have genes inserted to make them resistant to certain insects or tolerant to different herbicides, as explained below:

   - Herbicide tolerant plants have an introduced gene that allows them to withstand being sprayed with a herbicide that would normally kill the plant. Farmers that grow herbicide tolerant plants can spray their fields with herbicides, killing the weeds but leaving the desired crops.
   - Insect resistant plants like the Bt cotton described before, have an introduced gene that causes the plant itself to produce insecticides that will kill pests that try to eat the plant. Farmers that grow insect resistant plants don’t need to spray as much insecticide to kill the pests that attack their plants.

The application of modern biotechnology in agriculture was started in the 90s. From 1994 to 2016, a total of 67 countries have issued regulatory approvals to GM crops for consumption either as food and/or feed as well as for environmental release. While 24 countries planted GM

crops, an additional 43 countries have granted regulatory approval for GM crops for import as food and feed use. As per the recent reports, 16 GM crops have been cultivated in 24 countries in 2017 on approximately 190 million hectares. The four major crops are maize, soybeans, cotton and canola (rapeseed).

In India, Bt cotton is the only GM crop approved for commercial cultivation in India. The total area under Bt cotton has been increased substantially since it was introduced in 2002. As of now, Bt cotton is cultivated in more than 90% of the area under cotton cultivation in India (Figure 1). Several public and private sector institutions are involved in research and development of GM crops in India. These include brinjal, cabbage, mustard, potato, rice, chickpea, pigeonpea etc.

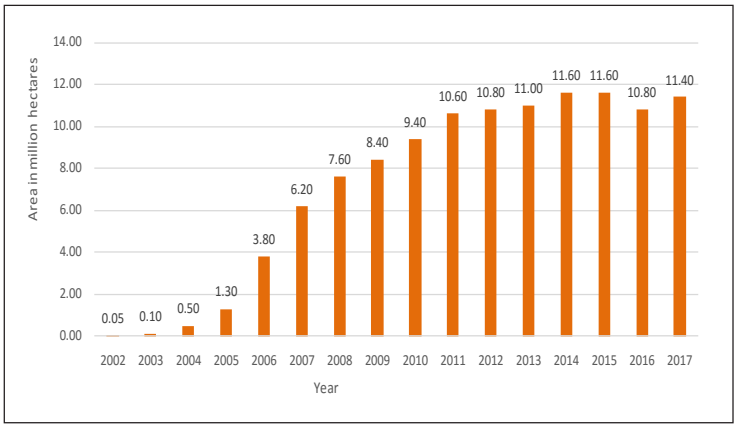


Fig. 1: Area under Bt cotton cultivation in India.

c. Process industry: Useful products and materials through development of improved microorganisms with increased enzymes, bioplastics etc are being developed for specific consumer needs and provide eco-friendly alternatives to chemical processes.

d. Environment: Bioremediation programmes involving the use of microorganisms are currently in progress to clean up contaminated air, tracks of land, lakes and waterways. GMOs help in improving the efficacy of these processes so that their basic biological processes are more efficient and can degrade more complex chemicals and higher volumes of waste materials.

Section 2: Regulations of LMOs in India

The use of LMOs in India is governed by the **"Rules for the Manufacture/Use/Import/Export and Storage of Hazardous Microorganisms, Genetically Engineered Organisms or Cells, 1989"** (Rules 1989) notified under the Environment (Protection) Act, 1986. Rules, 1989 essentially cover entire spectrum of activities involving GMOs and products thereof including the sale, storage, exportation, importation, production, manufacturing, packaging etc.

The Rules, 1989 are implemented by the MoEFCC, the Department of Biotechnology (DBT) of Ministry of Science & Technology and State Governments through six competent authorities viz. (i) Recombinant DNA Advisory Committee (RDAC), (ii) Institutional Biosafety Committee (IBSCs), (iii) Review Committee on Genetic Manipulation (RCGM) and (iv) Genetic Engineering Appraisal Committee (GEAC) (v) State Biotechnology Coordination committee (SBCC) and (vi) District Level Committee (DLC). While the RDAC is advisory in function, the IBSC, RCGM, and GEAC are of regulatory function, SBCC and DLC are for monitoring purposes (Figure 2).

1. Recombinant DNA Advisory Committee (RDAC)	→	Advisory
2. Institutional Biosafety Committee (IBSCs) 3. Review Committee on Genetic Manipulation (RCGM) 4. Genetic Engineering Appraisal Committee (GEAC)	→	Approval
5. State Biotechnology Coordination committee (SBCC) 6. District Level Committee (DLC)	→	Monitoring

Fig 2: Competent authorities notified under Rules, 1989

Guidelines for biosafety evaluation for each step of development process viz. contained use, confined field trials, food safety assessment and environment safety assessment have been prepared by regulatory authorities and are listed in Box 2.

Box 2: Biosafety Guidelines In India

Contained Use

- Recombinant DNA Safety Guidelines, 1990 (Updated, 2017)
- Revised Guidelines for Research in Transgenic Plants, 1998

Confined Field Trials

- Guidelines for Conduct of Confined Field Trials (CFTs) of Regulated GE Plants, 2008
- Standard Operating Procedures (SOPs) for CFTs of Regulated GE Plants, 2008
- Guidelines for Monitoring of CFTs of Regulated GE Plants, 2008

Food Safety Assessment

- Guidelines for the Safety Assessment of Foods Derived from GE Plants, 2008 (Updated in 2012)
- Protocols for Food and Feed Safety Assessment of GE Crops, 2008

Environmental Safety Assessment

- Guidelines for Environmental Risk Assessment (ERA) of GE Plants, 2016
- Risk Analysis Framework, 2016
- ERA of GE Plants: A Guide for Stakeholders, 2016

Others

- Guidelines for generating preclinical and clinical data for rDNA vaccines, diagnostics and other biologicals, 1999
- Guidelines and Handbook for Institutional Biosafety Committees (IBSCs), 2011
- Guidelines on Similar biologics: Regulatory requirements for Marketing Authorization in India, 2012 (updated in 2016)

In India, no person can import, export, transport, manufacture, store, process, use or sell any LMOs, substances or cells except with the approval of GEAC. RCGM is authorised to permit imports only for research purpose. Deliberate or unintentional release of LMOs is not be allowed. Production in which LMOs are generated or used cannot be commenced except with the approval of GEAC.

All approvals are for limited period as per Rules, 1989. GEAC has powers to revoke approvals in case of any new information on harmful effects of LMOs, any damage to the environment that could not be envisaged when approval was given or non-compliance of any conditions stipulated by GEAC. Details of applicable rules, guidelines and decisions of GEAC can be accessed at http://www.geacindia.gov.in/

In addition to the Rules, 1989, provisions in other acts, rules and policies are also applicable to GMOs/LMOs. Relevant details concerning import/export of LMOs are as under:

i. **Plant Quarantine Order, 2003** covers regulation of import of germplasm/GMOs/transgenic plant material for research purpose (Figure 3). The National Bureau of Plant Genetic Resources (NBPGR) has been designated as the Competent Authority to issue the import permits[1] for import of seeds for research purposes after getting permission under Rules, 1989 and to receive import material from customs authorities for quarantine inspection. A phytosanitary certificate issued from exporting country is needed during the imports of transgenic material.

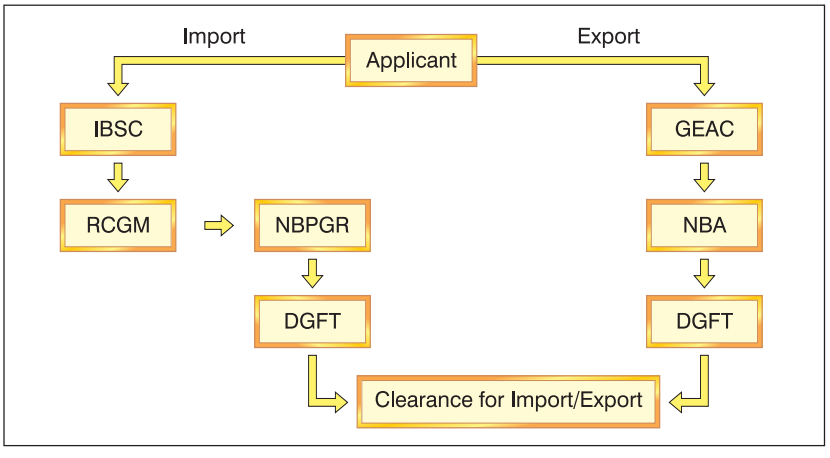


Fig. 3: Procedure for Import/Export of GE Planting Material for Research Purpose.

ii. **Food Safety and Standards Act, 2006,** regulates manufacture, storage, distribution, sale and import of food which includes GM food.

iii. **DGFT Notification Relating to Inclusion of GM Policy in Foreign Trade Policy (2006-09).** At time of imports of a consignment, declaration stating that it contains genetically modified

1 Import Permit: An official document authorizing importation of a consignment in accordance with specified phytosanitary requirements. It is issued by National Bureau of Plant Genetic Resources, New Delhi. In case of GMOs, issue of Import Permit also requires previous authorization by RCGM/GEAC

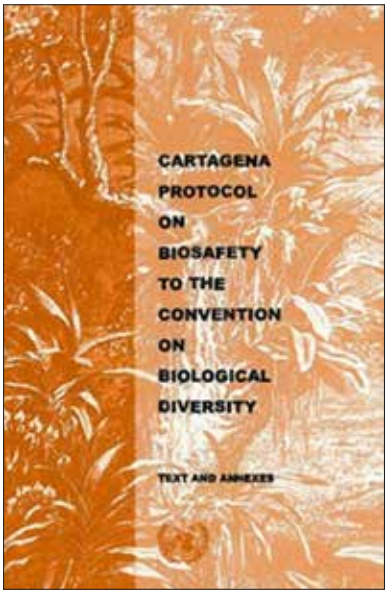
material is needed or importer is liable to penal action under the Foreign Trade (Development and Regulation) Act of 1992.

Pursuant to these acts, rules, policies and guidelines, the mandate of various ministries/ departments is summarised in Table 1 below:

Table 1: Mandate of Ministries/Departments

Ministry of Environment, Forest and Climate Change	• Primarily responsible for conservation and protection of environment, ensuring environmental and human health safety before release of GMOs / LMOs • Nodal agency for implementing Rules, 1989 and the Cartagena Protocol on Biosafety
Department of Biotechnology (Ministry of Science & Technology)	• Nodal department for promoting biotechnology programs • Provides scientific support in implementation of biosafety regulations
Ministry of Agriculture and farmers welfare	• Policies aimed at agriculture growth • Indian Council of Agricultural Research (ICAR) responsible for monitoring agronomic benefits of GM technology • Monitoring post-release performance of GM crops
Ministry of Health and Family Welfare	• Policies aimed at protecting and monitoring human health. • Food Safety and Standards Authority of India responsible for regulating GE foods
Ministry of Commerce and Industries	• Enhance trade with other countries through export/import policies • Nodal agency for implementing DGFT notification on GMOs
Central Board of Excise and Customs, Department of Revenue, Ministry of Finance	• Enforcement of regulation pertaining to transboundary movement of GMOs/LMOs at point of entry

Section 3: Cartagena Protocol on Biosafety



In addition to national regulations, international agreements also impact the activities involving LMOs. A supplementary agreement under the ageis of Convention on Biological Diversity (CBD) was adopted on 29 January, 2000, the Cartagena Protocol on Biosafety (CPB). The CPB entered into force on 11 September, 2003 and has 171 countries Party to it as on March, 2019. India ratified the CPB on January 23, 2003. Ministry of Environment, Forest and Climate Change (MoEF&CC), Govt. of India is the nodal ministry.

The CPB applies to transboundary movement, transit, handling and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

LMOs covered under the CPB are categorized as under:

- LMOs for intentional introduction into the environment (seedlings, trees, animals for breeding, live fish, bacteria or other microorganisms)
- LMOs intended for direct use as food or feed, or for processing (e.g. agricultural commodities-corn, canola, cotton)
- LMOs for contained use (e.g. bacteria for laboratory scientific experiment)

Exemptions under the Protocol include LMOs that are pharmaceutical for humans if they are covered by other international agreements or arrangements and products derived from LMOs such as processed food (e.g. soybean oil, corn flour)

The CPB promotes biosafety by establishing practical rules and procedures for the safe transfer, handling and use of LMOs, with specific focus on regulating the transboundary movement of LMOs. There are 40 Articles in the CPB, which could be categorized into key elements and supporting tools and mechanisms. The four key elements include procedures for transboundary movement of LMOs, risk assessment and risk management, handling, transport, packaging and identification and information sharing (Figure 4).

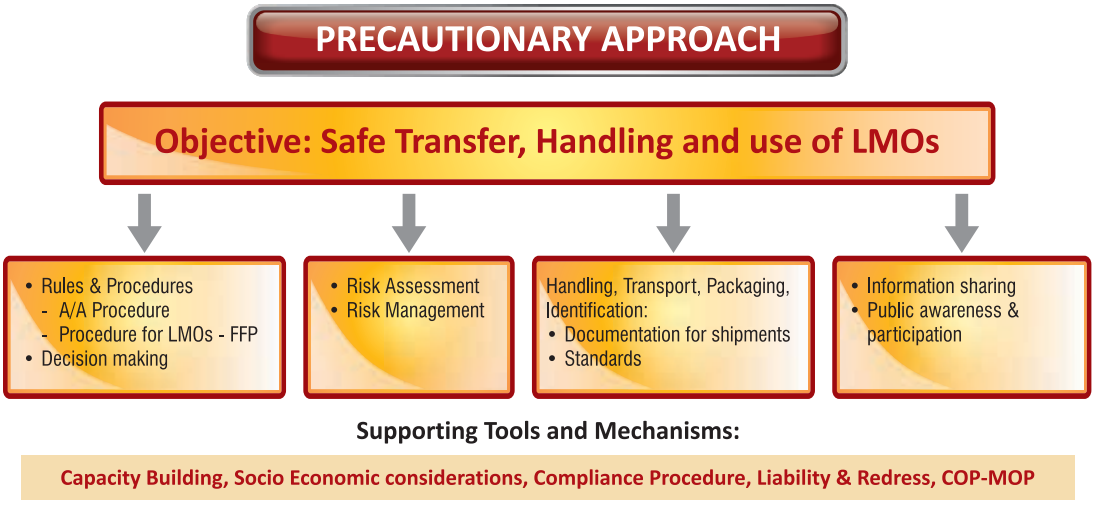


Fig. 4: Key Elements of Cartegena Protocol on Biosafety

Specific procedures have been defined for LMOs for intentional introduction into the environment, that are subjected to advanced informed agreement procedures vs. LMOs for direct use as FFP that may be subjected to a separate procedure. Biosafety protocol requires decisions on import of LMOs for intentional introduction into the environment in accordance with scientifically sound risk assessments. These assessments aim at identifying and evaluating the potential adverse effects of LMOs. The Protocol sets out principles and methodologies on how to conduct a risk assessment. The Protocol also requires Parties to adopt measures and strategies for preventing adverse effects and for managing and controlling risks identified by risk assessments.

The methodology of the risk assessment follows the conventional risk assessment paradigm, beginning with identification of a potential hazard, such as characteristics of an LMO, which may have an adverse effect on biodiversity. Risks are then characterized based on combined evaluation of the likelihood of adverse effects, and the consequences should those effects be realized. Article 15, 16 and Annex III of the CPB outlines requirements that relate to risk assessment and risk management of LMOs as indicated below:

Article 15 on Risk Assessment establishes the basic requirements for risk assessment under the CPB and refers to Annex Ill for further guidance.

Annex Ill sets forth the objectives of the risk assessment, what the risk assessment will be used for, general principles that the risk assessment must follow, the methodology of the risk assessment and particular points to consider when assessing the potential risks of LMO. The general principles include:

- Risk assessment should be carried out in a scientifically sound and transparent manner
- Lack of scientific knowledge of scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk
- Risk associated with LMOs or products thereof, should be considered in the context of risks posed by the non-modified recipient or parental organisms in the likely potential receiving environment.
- Risk assessment should be carried out on a case by case basis

Article 16 on Risk Management deals with the management of risks of those organisms that fall within the scope of the CPB. The CPB requires each Party to manage and control any risks that may be identified by a risk assessment. Parties are required to do the following:

- adopt measures and strategies for preventing adverse effects and for managing and controlling the risks identified by risk assessments
- take measures to prevent unintentional transboundary movements
- ensure that LMOs undergo appropriate periods of observation prior to use
- cooperate in identifying LMOs or traits that may pose risks to biodiversity and take appropriate management measures.

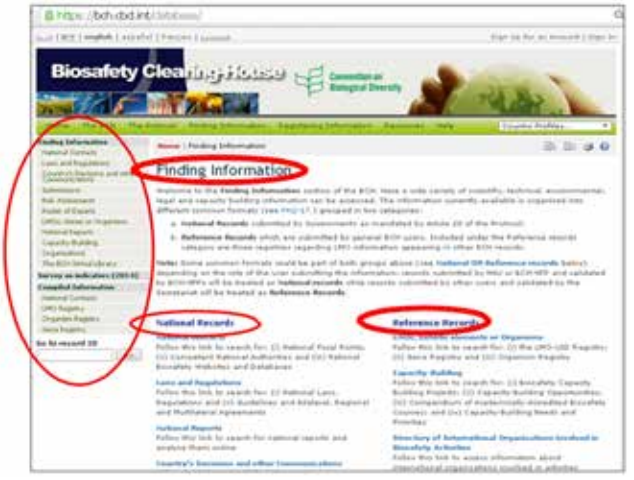
Documentation requirements have been defined for various categories of LMOs viz., LMOs for contained use, LMOs-FFP and LMOs for intentional introduction into the environment. However, all categories require reference to a unique identifier code (Box 3).

Box 3: LMO Unique Identifiers

- Documentation requirements for all categories of LMOs require reference to a unique identifier code. To date, only one unique identification system exists: OECD Unique Identifiers for Transgenic Plants
- OECD Unique Identifier is a simple alpha numeric code that is given to each living modified plant that is approved for commercial use
- Developers of transgenic plants are the ones to assign the unique identifier
- 9- digit code composed of 3 elements separated by dashes
  - 2 or 3 alpha numeric digits to designate the applicant;
  - 5 or 6 alpha numeric digits to design at the transformation event; and
  - 1 numerical digit for verification Example: MON-00810-6M on santo's YieldGardMaize
- Unique identifier codes can be used to search BCH for information about specific LMOs

LMO Quick-links are a tool developed to assist in the identification of LMOs in documentation accompanying their transboundary movement. LMO Quick-links are small image files, which can be easily copied and pasted in documentation accompanying LMOs for the purpose of providing information on a specific living modified organism. LMO Quick-links identify a LMO through the organism's unique identifier (for plants), trade name and a link to the BCH where more information on the LMO is available.

Biosafety Clearing House (BCH) is a website set up under the CPB to facilitate exchange of information on GMOs/LMOs by Parties. It is a repository of up-to-date information on LMOs and biosafety including information about the National laws, regulations, guidelines, competent national authorities and final decisions taken by countries that is Party. It is accessible at http://bch.cbd.int/



Information available on BCH is organized into National Records that are submitted by Parties and Reference Records that are submitted by general BCH users.

To facilitate easier understanding about results of queries involving decisions on LMOs, different icons have been used in the BCH as indicated in Table 2.

Icon	Approval of LMO for
	Intentional introduction into the environment
	Direct use as food
	Direct use as feed
	Processing
	Confined Use
	Pharmaceuticals
	Transit

Table 2: Icons used for conveying information about decisions

Other databases which can be accessed for information on LMOs include OECD BioTrack Online Website (http://www2.oecd.org/biotech/); FAO GM Foods Platform (http://www.fao.org/food/food-safety-quality/gm-foods-platform/en/) and a website containing information about Indian biosafety regulations has been established by MoEFCC.

The website http://geacindia.gov.in provides information about decisions taken by the GEAC, the apex regulatory committee in India.

Section 4: Safety assessment methodologies

As with any new emerging technologies, safety concerns have been expressed with the use of LMO because of the perception that modern biotechnology tools such as genetic engineering lead to creation of new species. LMO and products has been accepted more readily in healthcare, as these are directly beneficial for consumers (e.g. vaccines, medicines with improved treatment potential or increased safety). On the other hand, GE plants have been a subject of intense debate.

Safety concerns associated with the use of GE plants broadly relate to risk to human and animal health, and environment. These differ greatly depending on gene-crop combination, and may include:

- Potential risk of introducing toxins, allergens and other anti-nutrition factors in foods
- Potential likelihood of transgenes escaping from cultivated crops into wild relatives
- Changes in weediness potential
- Interaction with non-target organisms
- Resistance/tolerance of target organisms

Systematic safety assessment methodologies are in place that have been agreed on years of consultations under the aegis of international organizations and agreements viz. FAO, WHO, Codex Alimentarius, OECD and Cartagena Protocol on Biosafety (CPB). Annex Ill to the CPB sets forth the objectives of the risk assessment, what the risk assessment will be used for, general principles that the risk assessment must follow, the methodology of the risk assessment and particular points to consider when assessing the potential risks of LMOs. The key provisions include:

- Risk assessments must be carried out in a scientifically sound manner
- Risk assessments should be comparative
- Risk assessments should be carried out on a case-by-case basis, taking into account the specific circumstances or context for each individual application
- Risk assessments should be made available to the public to ensure transparency of the risk assessment process

Risk assessors use scientific data regarding potential hazards and exposure to assess the

likelihood of adverse impacts on populations of organisms as well as on communities of organisms and their diversity.

In case of a GE plant, a risk may exist if it possesses an introduced trait having the potential to adversely impact individual species, ecosystems or biodiversity and these potential risks must be evaluated before the GE plant may be authorized for widespread planting. The potential changes introduced in GE plants are assessed using comparative risk assessment approach. The underline assumption of this comparative approach is that traditionally cultivated crop has a history of safe use and thus serves as the comparator. As a consequence, safety assessment process gives conclusion on whether or not the GE plant is as safe as its conventional non-GE counterpart.

Impact on human health is studied by analyzing the modified organism for the risks of toxicity, allergenicity, nutritional analysis etc. as relevant to the particular situation of targeted genetic modification. The toxicity and allergenicity assessment takes into account the chemical nature and functions of the newly expressed substance, the concentration of the substance in the edible plant parts and likely dietary exposure. Appropriate oral toxicity studies in laboratory animals are also carried out on a case by case basis. For allergenicity, data is generated on amino acid homology for expressed proteins with known allergens from bioinformatics data base, heat stability, pepsin digestibility etc in an integrated, step-wise manner. Nutritional equivalence is established through detailed compositional analysis by comparing concentration of key components in GE plants with a conventional counterpart that is grown and harvested under the same agro-climate and growing conditions. Livestock feeding studies may also be carried out in specific cases.

Environmental risk assessment of GE plants is undertaken on a case to case basis and there is no single method or model to follow in view of diverse biological properties of crops. Familiarity i.e. knowledge and experience of unmodified plant is basis for comparative risk assessment of a GE plant. Baseline information as documented in biology documents is used as basis for this comparison. Potential changes that are compared include weediness/ invasiveness, gene flow pattern of the introduced trait, impact on non-target beneficial organisms etc. MoEF&CC under the Phase II Capacity Building Project on Biosafety prepared eight crop specific biology (Potato, Tomato, Sorghum, Rubber, Pigeonpea, Chickpea, Mustard and Papaya) to serve as a guiding tool for use in environmental risk assessment. These are in addition to five biology documents on

cotton, rice, brinjal, okra and maize prepared earlier jointly by MoEF&CC and DBT. The safety assessment studies required for commercial release of a GE plant comprise of food and feed safety assessment and the environmental risk assessment coupled with information through the molecular characterization of the GE plant and characterization of the expressed, transgenic proteins (Box 4). Govt. of India is following a case by case safety assessment of GE plants. The information requirement and analysis by regulatory authorities depends on the development stage of a particular product. Data requirement may also vary depending on the crop specific trait and intended use.

Box 4: Broad Information Requirements for Safety Assessment of GM crops

Effect of Genetic Modification and Protein Characterization

- Description of the GM crops
- Description of the biology of the non-modified host plant
- Description of the donor organism
- Description of the genetic modification
- Inheritance and stability of inserted gene(s)
- Molecular characterization
- Function/ specificity/ mode-of-action of expressed protein
- Protein expression levels
- History of safe use and consumption

Food and Feed Safety

- Toxicity assessment by animal toxicity studies such as acute and sub-chronic studies
- Assessment of allergenicity by comparing amino acid sequence homology of the newly expressed protein.
- Heat stability and susceptibility of the expressed protein to pepsin digestion
- Compositional analysis by comparing changes in the level of key nutrients, natural toxicants or anti- nutrients, secondary metabolites, physiologically active (bioactive) substance etc
- Livestock feeding studies
- Effect of processing

Environmental Safety

- Confirmation of expression level of new proteins: Quantify the expression level of the gene product associated with each introduced trait
- Field trial locations and experimental designs
- Description of the phenotype of the transformed plant
- Plant growth and specific observations recorded during the field trials
- Changes in weediness and aggressiveness potential
- Susceptibility to diseases and pests.
- Impact on non-target and beneficial organisms like predators, soil micro flora etc
- Changes in gene flow pattern through pollen flow studies and crossability studies with sexually compatible relatives

Section 5: Detection of LMOs

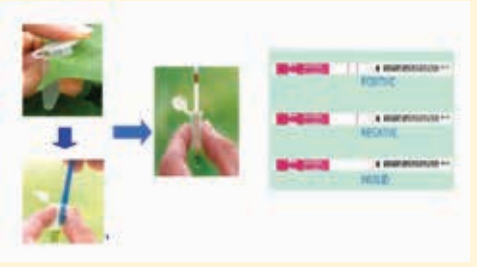
The objective of this section is to introduce the methods used to detect presence of LMO in a sample of seeds or plant material, to identify which LMOs are present and to calculate the quantity. In many instances, this work will not be done by frontline seed inspectors may be performed by seed testing or other laboratories.

Reasons for testing of LMOs in a sample include screening for the presence of LMOs, testing for specific LMOs and quantification of LMOs. For countries that have not approved the import of any LMO, the detection of any GM content in a sample would mean that consignment is not allowed for sale or cultivation. Testing for specific LMOs is done to verify that the LMOs declared in the documentation accompanying the consignment is actually present. Another objective is to test for LMOs that have not been declared as being in the sample. This is important if a country has authorized some LMOs but not others and wants to make sure that unauthorized LMOs are not cultivated in country. Testing for LMOs also used to calculate the quantity of GM content in a sample in the context of labeling.

Methods for detecting LMOs: As indicated in Section 1, a LMO is created by inserting a gene from one organism into the DNA of another organism and this new gene usually leads the organism to produce a protein that gives the organism a desired characteristics. In view of the above, there are two basic approaches to test the LMOs. These include protein-based methods for testing for the proteins produced by the gene that has been inserted into the LMO and DNA based testing for the introduced gene itself.

- **Protein based methods:** These methods can be used for screening (yes/no) and quantification of expressed protein in a LMO using strip test and ELISA based test respectively.
  i) **Strip tests** are simplest of all the detection methods. Strip test kits produced by different companies, include specially coated paper strips that are designed to detect specific proteins produced by different LMOs.

Methodology for Strip Test



Typically, a small sample is first ground into a powder. A liquid extraction buffer, included in the kit is added to a tube along with the powder. The tube is then shaken to allow the maximum amount of protein to be released into the buffer. A small amount of this mixture (referred to as extract) is transferred into vial. The coated paper strip is then placed in the vial. The result monitored as the colour of the strip changes indicating whether or not it is a LMO. Unskilled personnel can easily carry out strip based tests.

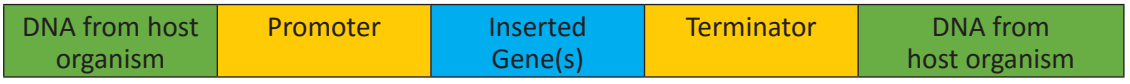
ii) **ELISA based test:** This test uses antibody (polyclonal or monoclonal) raised against a specific protein encoded by transgene. These antibodies are colour coated to enable them to be easily detected and quantified. The kits for ELISA test are also produced by companies that specialize in LMO testing. ELISA kits include plastic plates with number of wells, which are pre-treated so that the protein of interest in the sample will stick to the well.

Methodology for ELISA test

For the ELISA test, an extract is prepared by grinding the sample into a powder and adding an extraction buffer (similar to the process with strip tests). The extract is then added to the wells in a plate. If the extract contains the protein of interest then this protein will stick to the bottom and sides of the well.

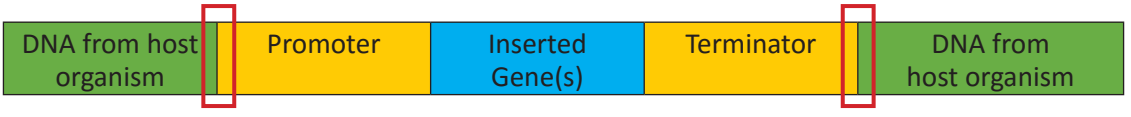
Whether the protein has stuck to the well is not visible to the human eye so additional steps are needed to determine the results of the test. A reagent (a chemical used for analysis and reactions, also provided in the kit) is then added to the wells and it attaches to the protein of interest that is stuck to the well. Finally, the results of the test are visualized with a colour development step. In this step, another chemical is added to the wells, which causes a reaction that changes the colour of the contents in the well. The darker the colour, the higher the concentration of the protein of interest. The Intensity of color is measure using an “ELISA Reader”.

2. **DNA based testing:** The DNA that is introduced into an organisms to create a LMO consists of several components and is known as a gene construct. Components of a gene construct are generally as follows:



DNA-based testing involves testing for any of the components of a gene construct. Some of the components e.g. promoters are widely used in development of different LMO. In such cases detection of a promoter sequence helps in knowing presence of LMO in a sample, but does not allow for specific identification of LMO.

A more specific test for detecting and identifying a particular LMO is to test for the combination of the host organism's DNA and either the promoter or the terminator from the gene construct. This is called 'Event-Specific detection'. Event-specific detection allows identification of the specific LMO in a sample.



DNA based testing involves multiplying/amplifying a specific DNA through polymerase chain reaction (PCR) technique. Specific gene/transgene/elements associated with the transgene can be amplified using a PCR machine. The amplified DNA is then visualized using the gel electrophoresis technique.

DNA methods are highly sensitive and can test for multiple LMOs simultaneously. However, these requires highly skilled personnel, laboratory infrastructure and are more expensive. For both the protein and DNA based detection methods there are several general considerations that include sampling, food matrix effects on protein/DNA extraction, reference materials, method validation, harmonization of standards and access to information database.

In India, several public and private sector organizations have capabilities for detection of LMOs. There are also companies supplying various types of test kits. Four laboratories,

strengthened under Phase II Capacity Building Project on Biosafety have been designated as National Referral Laboratories to detect the presence or absence of LMOs/GMOs under the Seeds Act, 1966.

The laboratories strengthened for detection of LMOs /GMOs include:

1. DNA Fingerprinting and Transgenic Crop Monitoring Lab (DFTCML), Department of Agriculture, Government of Andhra Pradesh, Guntur, Andhra Pradesh
2. ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi
3. Export Inspection Agency (EIA), Kochi Laboratory, Kochi; Kerala
4. Punjab Biotechnology Incubator (PBTI) Mohali, Punjab

More information about detection of LMOs can be seen at http://gmolabs.nbpgr.ernet.in:9090/ maintained by ICAR-NBPGR.

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(कृषि, सहकारिता एवं किसान कल्याण विभाग)

अधिसूचना

नई दिल्ली, 15 नवम्बर, 2017

का.आ. 3604(अ).—केंद्रीय भण्डार बीज नियम, 1966 के नियम 5 के खंड (ग) के साथ पठित बीज अधिनियम, 1966 (1966 का 54) की धारा 4 की उप-धारा (1) द्वारा प्रदत्त शक्तियों का प्रयोग करते हुए, संपूर्ण भारत के लिए इसके प्रकाशन की तारीख से उक्त अधिनियम के अधीन जीवित परिवर्तित जीवों और आनुवंशिक रूप से परिवर्तित जीवों की उपस्थिति का अनुपस्थिति की जांच का पता लगाने हेतु निम्नलिखित प्रयोगशालाओं को राष्ट्रीय सन्दर्भक प्रयोगशाला घोषित करती है, अर्थात्—

(i) डीएनए फिंगर प्रिंटिंग और ट्रांसजेनिक फसल निगरानी प्रयोगशाला (डीएफटीसीएमएल), गुंटूर (आंध्र प्रदेश);

(ii) आईसीएआर-राष्ट्रीय पौध आनुवांशिक संसाधन ब्यूरो (एनबीपीजीआर), पूसा परिसर, नई दिल्ली;

(iii) निर्यात निरीक्षण एजेंसी (ईआईए), कोच्ची प्रयोगशाला (केरल);

(iv) पंजाब जैव प्रौद्योगिकी इनक्यूबेटर (पीबीटीआई), मोहाली (पंजाब)।

[फा. सं. 13-127/2017-बीज-IV]

बी. राजेन्द्र, संयुक्त सचिव

2 THE GAZETTE OF INDIA EXTRAORDINARY [PART II—SEC. 3(ii)]

MINISTRY OF AGRICULTURE AND FARMERS WELFARE

(Department of Agriculture, Cooperation and Farmers Welfare)

NOTIFICATION

New Delhi, the 15th November, 2017

S.O. 3604(E).—In exercise of the powers conferred by sub-section (1) of Section 4 of the Seeds Act, 1966 (54 of 1966), read with clause (c) of Rule 5 of the Seeds Rules, 1968, the Central Government hereby declares the following laboratories as the National Referral Laboratories to detect the presence or absence of Living Modified Organisms and Genetically Modified Organisms under the said Act with effect from the date of publication, for the whole of India, namely:—

(i) DNA Fingerprinting and Transgenic Crop Monitoring Lab (DFTCML), Guntur (Andhra Pradesh);

(ii) ICAR-National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi;

(iii) Export Inspection Agency (EIA), Kochi Laboratory (Kerala);

(iv) Punjab Biotechnology Incubator (PBTI), Mohali (Punjab).

[F. No. 13-127/2017-SD-IV]

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