MANUAL FOR TRAINING WORKSHOP ON GMO/LMO: HANDLING APPLICATIONS AND INSPECTION

BHUTAN AGRICULTURE AND FOOD REGULATORY AUTHORITY (BAFRA)
MINISTRY OF AGRICULTURE AND FORESTS

FOREWORD

The "Training manual on genetically modified organisms (GMOs): handling applications and inspection", has been prepared with an objective to apprise the biosafety regulators, policy-makers and members of national biosafety committees, with diverse educational backgrounds. It offers background knowledge critical in the process of reviewing biosafety dossiers/applications and decision-making. It also provides information about the global status of GMOs and acquaint the regulator and other stakeholders with the international conventions and agreements relevant to biotechnology and biosafety.

The document has been developed under the National Biosafety Framework Project, Bhutan Agriculture and Food Regulatory Authority (BAFRA) with the financial support from the United Nations Environment Program (UNEP) and the Global Environment Facility (GEF).

Karma Dorji
Director General

Contents

CHAPTER 1
INTRODUCTION TO GENETICALLY MODIFIED ORGANISMS

1.1 What are Genetically Modified Organisms (GMOs)/Living modified Organisms (LMOs)?

In modern biotechnology, a Genetically Modified Organism (GMO) is that in which the basic genetic material (DNA) has been artificially altered or modified to improve the attributes or make it perform new functions.

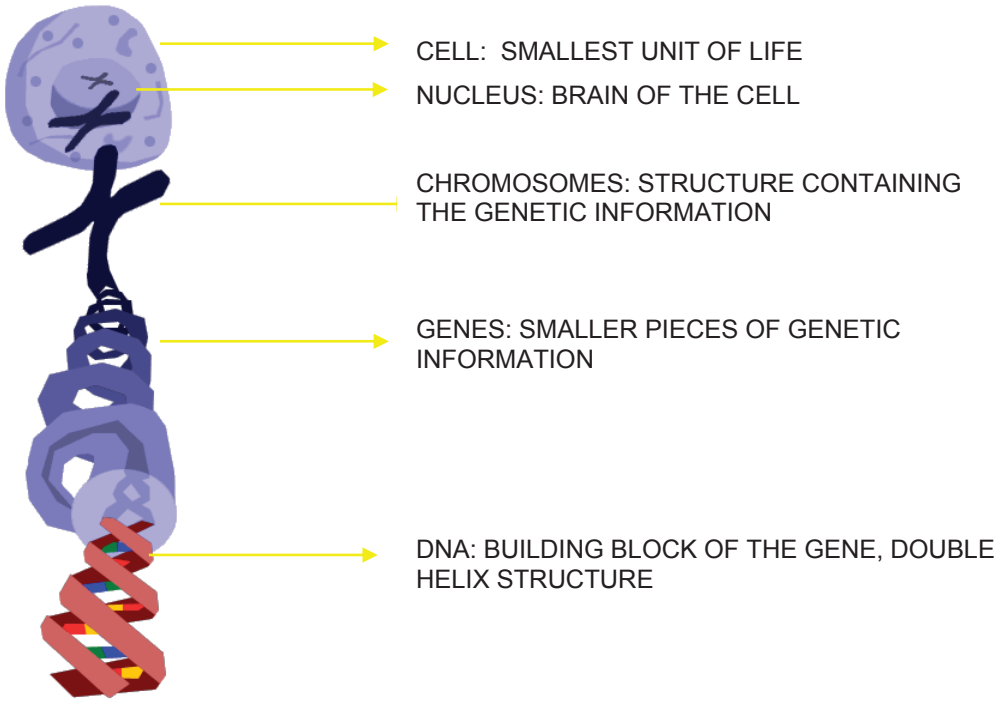
The term Living Modified Organism (LMO) is defined as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. In everyday usage, LMOs are usually considered to be the same as GMOs, but definitions and interpretations of the term vary widely.

GMOs/LMOs form the basis of a range of products and commodities. Products derived from GMOs/LMOs include processed products containing dead modified organisms or non-living GMO components i.e. certain vaccines; drugs; enzymes; food additives; and many processed, canned, and preserved foods.

1.2 Basic elements of genetic modification

Genetic modification involving the copying and transfer of genes from one organism to another is possible because 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. Therefore, it is possible to transfer a copy of DNA sequence (or gene) that codes for a particular characteristics into the cell of a different organism. Once the gene is incorporated into the genome of recipient, the resulting organism is considered to be genetically modified and the new characteristics coded by that gene is inherited by subsequent generations. The basic elements of genetic modification are explained below:

a. ***DNA:*** All the living organisms can be modified because of presence of a molecule called Deoxyribonucleic acid (DNA) in every cell of all the organisms. DNA is the molecule that carries the genetic blueprint for life as it stores the genetic information and provides the key chemical information responsible for the inheritance of traits such as size, shape, color, build and other physical attributes of microorganisms, plants, animals and humans. DNA exists in the nucleus of each cell.

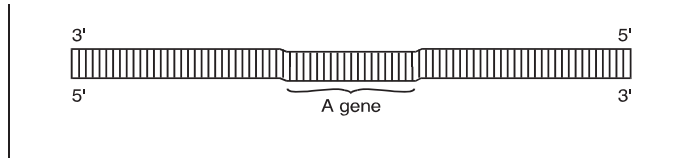


Organization of DNA in the cell

The building blocks of DNA are called bases and they come in four types that can link together in different sequences. The four bases also called nucleotides are adenine, cytosine, guanine and thymine. DNA is double stranded with base pairing between adenine and thymine and cytosine and guanine forming the rungs between the phosphate backbones of the two DNA strands. These two long strands wound around each other in a spiral shape called the double helix. The order of bases on each strand makes up the DNA sequence. The number of possible sequences is almost endless because an individual strand of DNA may contain millions of bases.

b. ***Gene:*** A gene is basically a discrete segment of DNA encoding for set of instructions in the cell and contains all information concerning the form and functions of all living cells that give characteristics to an organism.

Gene as segment of DNA



c. ***Genome:*** An organism's complete set of genes is called the genome. All the cells in an organism carry and identical and complete genome, which means every cell contain at least one copy of every gene, although it may not be active. By switching different combinations of genes on or off cells develop into different types e.g. leaf, root and flower cells in plants or heart, lung and skin cells in animals.

All organisms have genomes of varying sizes; for instance, the human genome has an estimated 60 – 100,000 genes, most plants have about 20,000, a nematode (a microscopic creature) has about 18,000; and the single celled *Escherichia coli* bacterium just over 4,000. The genetic differences among different species as well as organisms within a species lie in the difference in number and sequence of these genes in the DNA/genome.

d. ***Protein:*** Proteins are made up of long chains of amino acids and have a variety of roles in the cell such as structural proteins (e.g. muscles) or enzymes that carry out many of the life processes in plants and animals.

Genes contain the information necessary for assembly of a specific protein. The proteins then function as enzymes to catalyze biochemical reactions, or as structural or storage units of a cell, to contribute to expression of a particular trait. The sequence of events by which the information encoded in DNA is expressed in the form of proteins is via messenger Ribonucleic acid (mRNA) intermediate as shown in the diagram below.

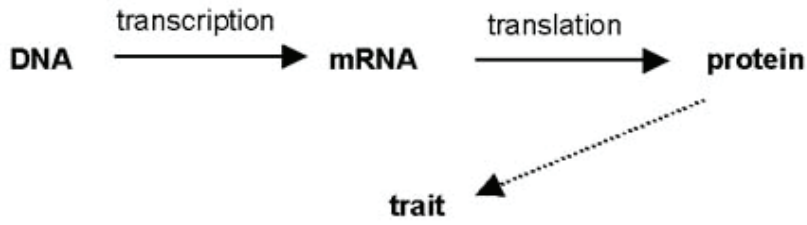


Figure 1: Sequence of events from DNA to protein expression.

Source: http://cls.casa.colostate.edu/TransgenicCrops/how.html

The transcription and translation processes are controlled by a complex set of regulatory mechanisms, so that a particular protein is produced only when and where it is needed.

1.3 Genetic Engineering:

Genetic engineering involves artificial transfer of genes or gene fragments from one organism to another to produce novel traits in the recipient living organism and is also referred as recombinant DNA technology (rDNA). The important tools used in genetic engineering include:

a. ***Enzymes for DNA manipulation:*** The first step in the construction of a recombinant DNA molecule, involves cleaving DNA molecules at specific points and recombining the together again in a controlled manner. The two main types of enzymes commonly used for this purpose are restriction endonucleases and DNA ligases. These enzymes form the backbone of

recombinant DNA technology. Restriction endonucleases cut DNA into defined fragments by targeting junction of specific sequences of the genetic coding and DNA ligases recombine them by consolidating loose bonds for creating large fragments. These enzymes are very specific in their action

b. ***Vectors:*** The function of the vector is to enable the foreign genes to get introduced into and become established within the host cell. Naturally occurring DNA molecules that satisfy the basic requirements for a vector are plasmids and the genomes of bacteriophages and eukaryotic viruses. They are further classified as cloning and expression vectors depending on the stage of genetic engineering at which these vectors are used.

c. ***Expression hosts:*** The functional cell into which the composite DNA molecule carrying the required gene needs to be introduced is called the expression host. The choice of the best host-vector system for the expression and large-scale production of a particular protein is based on considerations of the complexity of the protein to be expressed and the yield and quantities needed.

d. ***Marker genes:*** Marker genes and reporter genes are utilized for selection and identification of the clones. These use phenotypic markers, identification from a gene library and DNA sequencing. DNA sequencing helps in determining the precise order of nucleotides in a piece of DNA.

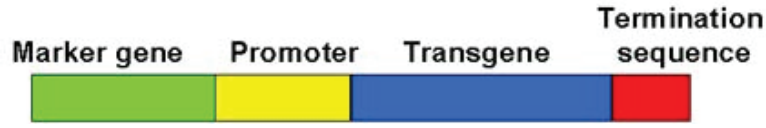
1.4 Development of GMOs

There are four steps in developing a GMO.

a. ***Identification of a gene:*** The first step is to identify a particular characteristic from any organism (plant, animal or microorganism) and find out which gene or genes in the organism are responsible for producing that characteristic. This is followed by the use of molecular biology techniques to isolate and copy the gene of interest, Identifying and locating

genes for the required traits is currently the most limiting step in the development of GMOs particularly in plants and animals. Relatively little is known about the specific genes required to enhance yield potential, improve stress tolerance, modify chemical properties etc. Further, identifying a single gene involved with a trait is not sufficient and it is important to understand how the gene expression is regulated, what other effects it might have on the plant, and how it interacts with other genes active in the same biochemical pathway.

b. ***Designing Genes for Insertion:*** Once a gene has been isolated and cloned (amplified in a bacterial vector), it must undergo several modifications before it can be effectively inserted into a host. A simplified representation of a constructed transgene, containing necessary components for successful integration and expression is given below along with the description of components:



Components of a constructed transgene for integration and expression

- A **promoter sequence** must be added for the gene to be correctly expressed (i.e., translated into a protein product). The promoter is the on/off switch that controls when and where in the plant the gene will be expressed.
- The **termination sequence** signals to the cellular machinery that the end of the gene sequence has been reached.
- A **selectable marker gene** is added to the gene "construct" in order to identify plant cells or tissues that have successfully integrated the transgene. This is necessary because achieving incorporation and expression of transgenes in cells is a rare event, occurring in just a small portion of the targeted tissues or cells. Selectable marker genes encode proteins that provide resistance to agents that are normally toxic to plants, such as antibiotics or herbicides. Only those plant cells

that have integrated the selectable marker gene will survive when grown on a medium containing the appropriate antibiotic or herbicide. As for other inserted genes, marker genes also require promoter and termination sequences for proper function.

c. **Transformation:** Transformation is the heritable change in a cell or organism brought about by the uptake and establishment of introduced DNA. The procedure for introduction of DNA into a host depends on the type of expression of host. A brief heat shock, degradative enzymes, electroporation, microinjection of DNA etc. are some of the methods used for incorporation of DNA. The transfer of gene constructs is relatively straight forward in prokaryotic or single eukaryotic cells, whereas the development of entire multicellular organisms such as plants or animals is much more complex as these organisms need to carry foreign genes in all their cells and pass these genes onto their offspring.

d. **Selection:** Following the gene insertion process, selection and identification of the transformed cell is done using marker/reporter genes. For example only the cell expressing the selectable marker gene will survive in a selective media containing an antibiotic or any other compound, depending on the type of marker used. It is assumed that these surviving organisms possess the transgene of interest. These organisms are replicated resulting in a pure culture of recombinant/genetically modified organisms (GMOs). The methods of replication and regeneration vary depending on whether it is a microorganism, plant or animal.

1.5 Genetically Modified Microorganisms (GMMs)

Genetically modified microorganisms were the first organisms to be modified using recombinant technology. Bacteria were used for genetic modification experiments as they are unicellular organisms and their generation or doubling time is short e.g. about 20 minutes for E. coli. The presence of a short genome of less than 5000 genes helps in easier insertion of a new gene

or any other manipulation. As the proteins in bacteria are produced extracellularly (outside the cell), the high expression levels of introduced genes can be obtained. However, the requirements of complimentarily with the protein from original source such as proper folding and post translation modification led to the use of eukaryotic organisms such as yeast and fungi. Further, instead of transfer of a single gene, it was found necessary to introduce a whole set of genes, coding for a series of enzymes in a metabolic pathway, control or 'master' genes and genes to control cell membrane transport mechanisms to facilitate the release of protein into the medium protein.

Genetically modified microorganisms have provided a versatile tool for gene manipulation in not only expression of several commercially important proteins but also for making other GMOs.

1.6 Genetically modified (GM) plants

GM plants are produced through genetic engineering in which genes that code for desirable traits are transferred from one organism to another. Transgenic plant are a sub set of GM plants that contains a gene or genes of a different species which have been inserted using genetic engineering instead of the plant acquiring them through pollination.

Transgenic technology is similar to conventional breeding in terms of the objective of generating more useful and productive crop varieties containing new combination of genes, but it expands the possibilities by enabling introduction of useful genes not just from within the crop species or from closely related plants, but from a wide range of other organisms. It allows the transfer of one or more genes, in a controlled and predictable way than is achievable in conventional breeding. Transgenic crop plants can therefore incorporate the desired traits more quickly and more reliably than through conventional methods.

There are two main methods of transforming plant cells and tissues. The first one is **The Gene Gun method** (also known as microprojectile bombardment or biolositics). The DNA to be introduced into the plant cells is coated onto tiny particles such as that of tungsten. These particles are then physically shot onto plant cells. Some of the DNA comes off and is incorporated into the DNA of the recipient plant. The second one is **The *Agrobacterium* method** This method uses a bacterium i.e. *Agrobacterium tumefaciens* to introduce the gene(s) of interest into the plant DNA (Figure 2). *Agrobacterium* is a plant pathogen capable of causing tumors in plants through large plasmids called Ti plasmids. When infection occurs, a portion of the Ti plasmid is transferred to the plant cells and is incorporated into the plant genome.

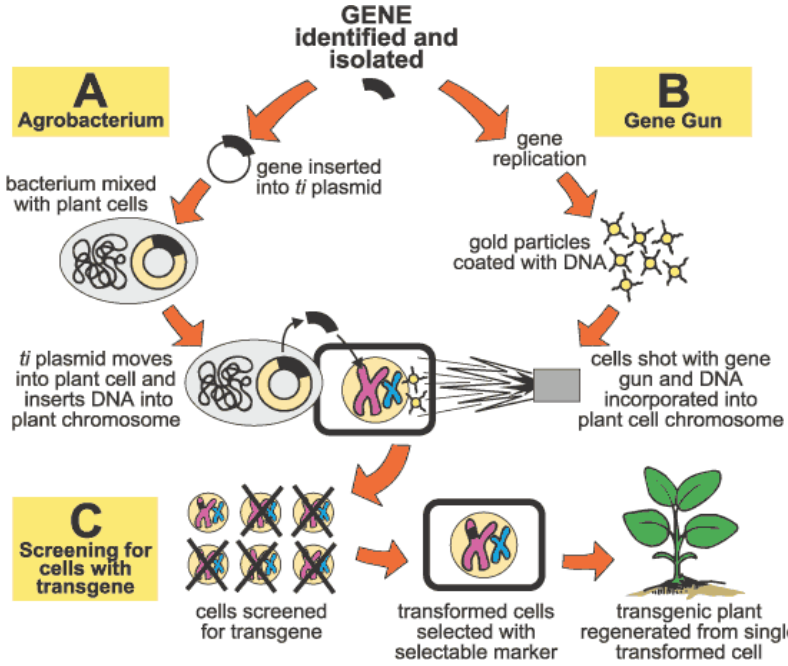


Figure 2: Methods of producing transgenic plant

Source: McKanzie, D (2004). Presentation by AGBIOS

Following the gene insertion process, plant tissues are transferred to a selective medium containing an antibiotic or herbicide, depending on which selectable marker was used. Only plants expressing the selectable marker gene will survive and it is assumed that these plants will also possess the

transgene of interest. Thus, subsequent steps in the process use these surviving plants.

To obtain whole plants from transgenic tissues such as immature embryos, they are grown under controlled environmental conditions in a series of media containing nutrients and hormones by tissue culture. Once whole plants are generated and they produce seeds, evaluation of the progeny begins.

To verify whether the inserted gene has been stably incorporated without detrimental effects to other plant functions, product quality, or the intended agroecosystem, initial evaluation includes attention to activity of the introduced gene; stable inheritance of the gene and unintended effects on plant growth, yield, and quality.

The plant is then crossed with improved varieties of the crop because only a few varieties of a given crop can be efficiently transformed, and these generally do not possess all the producer and consumer qualities required of modern cultivars. The initial cross to the improved variety must be followed by several cycles of repeated crosses to the improved parent, a process known as backcrossing. The goal is to recover as much of the improved parent's genome as possible, with the addition of the transgene from the transformed parent. The next step in the process is multi-location and multi-year evaluation trials in greenhouse and field environments to test the effects of the transgene and overall performance. This phase also includes evaluation of environmental effects and food safety.

Severally commercially important transgenic plants have been developed and commercially cultivated incorporating traits such as insect resistance and herbicide tolerance. Some of the examples of GM plants are as follows:

- **Insect resistant cotton:** Genetically modified insect resistant cotton contains a built in insecticidal protein from natural occurring soil microorganisms *Bacillus thuringiensis* (Bt) that gives protection to cotton from lepidopteron pests. The need for additional insecticide applications for these tests is

reduced or eliminated in Bt cotton as Bt is toxic only to the target pests but do not harm humans, animals, fish, birds or beneficial insects.

- **Herbicide tolerant soybean:** Herbicide tolerant soybean contains a gene that provides resistance to selective herbicides. This GM soybean provides better weed control and improves farm efficiency by optimizing yield, using arable land more efficiently, saving time for the farmers and increasing flexibility of crop protection.

- **GM corn:** Corn being one of the three important grains of the world has been genetically modified incorporating insect resistant as well herbicide tolerant genes. These corn varieties work in similar manner as explained above.

- **Golden rice:** Using genetic engineering beta-carotene gene has been incorporated in rice that lead to the development of rice with enhanced level of beta-carotene. Such rice provides better vitamin A level, thereby reduce its deficiency.

- **GM potato:** GM potato contains gene that provides a built-in protection from the Colorado potato beetle. This potato needs no additional protection for this pest. Likewise virus resistant potato contains genes that provide protection from specific viruses. Virus resistance results in reduced insecticide use that is needed to control insect vectors that transmit viruses.

- **GM papaya:** GM papaya contains a viral gene that encodes for the coat protein of papaya ringspot virus (PRSV). This protein provides the papaya plant built- in protection against PRSV. GM papaya works in a manner similar to virus resistant potato.

1.7 Transgenic Animals

The term transgenic animal refers to an animal in which there has been a deliberate incorporation of external DNA into the genome by genetic engineering, in contrast to the spontaneous mutation. Foreign DNA being

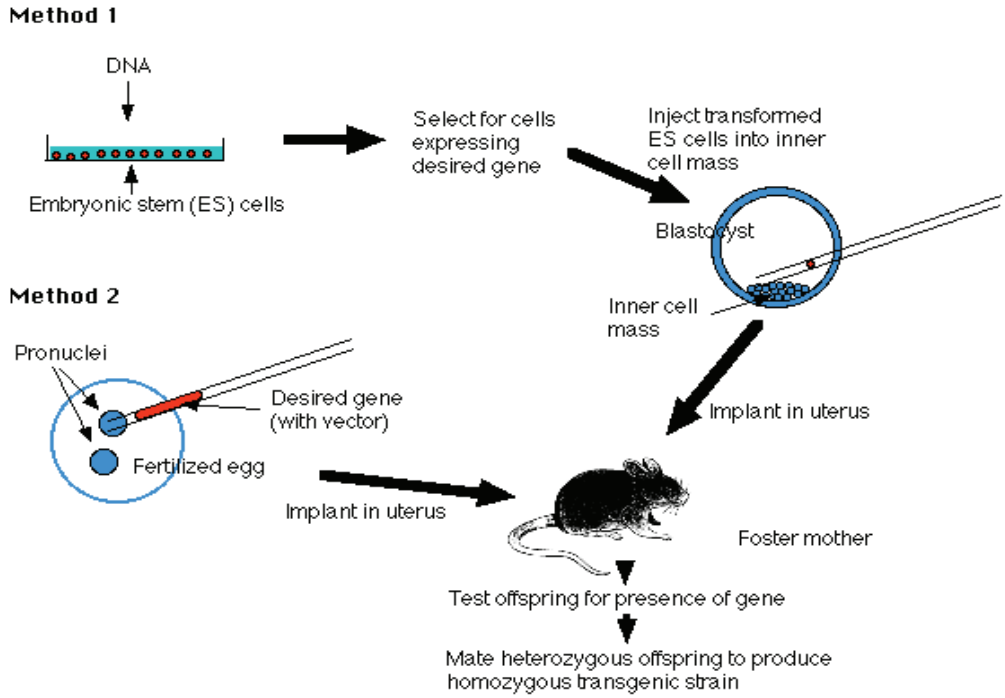
introduced into the animal using recombinant DNA technology should be transmitted through the germ line so that every cell, including the germ cells of animals contain the same modified genetic material. This added DNA may be from another animal of the same species or from a different source altogether. The term “transgenic animal” has now been extended to include knockout and chimeric animals as well, which are produced by deletion of a gene or other modifications in their genome.

In addition to a structural gene, the inserted DNA usually includes other sequences (similar to explained in transgenic plants) to enable it to be incorporated into the DNA of the host and to be expressed correctly by the cells of the host. Developing transgenic animals is more complex than generating transgenic bacteria or plants. Transgenic bacteria are fairly easy to produce, as they are unicellular whereas in case of plants, though multicellular, any transgenic plant cell can be grown into a transgenic plant. However transgenic animals must be generated by altering germ cells (egg and sperm cells) as all other cells i.e. somatic cells are not capable of giving rise to whole new animals although, recently there have been reports of even cloning of animals from single somatic cell.

The two methods commonly used are pronuclear microinjection and embryonic stem cell manipulation.

- **Embryonic stem cell-mediated gene transfer:** This method involves prior insertion of the desired DNA sequence by homologous recombination into an in vitro culture of embryonic stem (ES) cells. Stem cells are undifferentiated cells that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal.
- **Pronuclear microinjection:** This method involves the direct microinjection of a chosen gene construct (a single gene or a combination of genes) from another member of the same species or from a different

species, into the pronucleus of a fertilized ovum. It is one of the first methods that proved to be effective in mammals.



Methods for making Transgenic Animals

Source: http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/TransgenicAnimals.html

Although mice have remained the main species used in the field of transgenic animals for practical reason such as small size, low cost of maintenance, short generation time and genetically fairly well defined, the technology has been extended to various other species such as rats, rabbits, sheep, cattle, pigs, birds, fish etc.

CHAPTER 2
APPLICATIONS OF GMOS

The practical reach of GMOs has grown considerably due to the possibilities to express virtually any kind of coding sequence from any possible source. Sequences from mammals or any other animals, plants, fungi, bacteria or even sequences synthesized *in vitro* can be introduced into and expressed in almost any other organism. The genetic manipulation using genetic engineering is more precise and outcomes more certain over other methods resulting in faster production of organisms with desired traits.

An overview of various applications of genetic engineering is presented below:

2.1 Healthcare:

Healthcare applications of GMOs 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. Some of the prominent areas in healthcare in which GMOs are being used are:

Therapeutics: Recombinant therapeutics produced using GMOs include proteins that help the body to fight infection or to carry out specific functions. Therapeutic proteins are preferred to conventional drugs because of their higher specificity, absence of side effects, less toxigenic and neither carcinogenic nor teratogenic properties.

The first recombinant therapeutic product to be marketed was human insulin introduced by Eli Lilly in 1982. Other therapeutics that have been developed include blood factors, hormones, growth factors, interferons and interleukins. Notable target indications for which recombinant therapeutics have been produced include diabetes, hemophilia, hepatitis, myocardial infarction and various cancers.

Vaccines: Conventional vaccines use attenuated forms of a virus or bacteria to activate the immune system to produce the antibodies against pathogens. rDNA technology provides for the production of purified specific antigen. These are safe and free of side effects when administered as compared to whole cells. Also, rDNA technology ensures a continuous and convenient supply. For example, recombinant hepatitis B vaccine was first approved in 1986 by FDA in USA is now being used world over. Others such as recombinant anthrax vaccine and recombinant pertusis vaccine are under development.

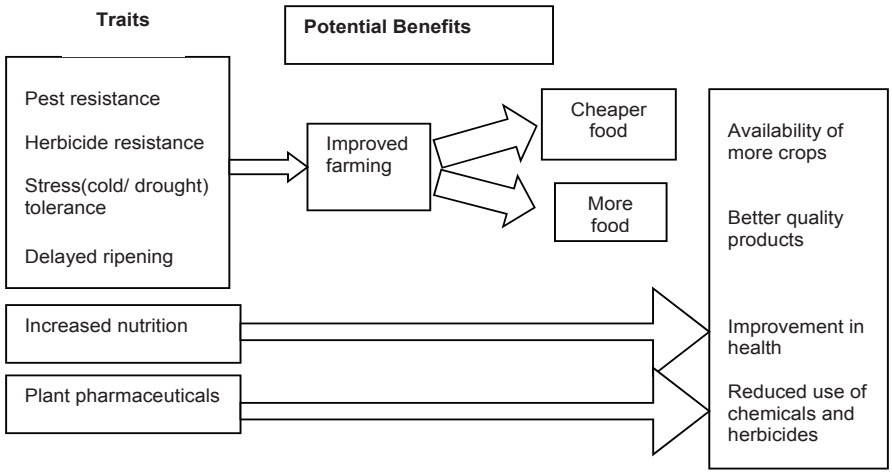
Monoclonal antibodies: Monoclonal antibodies (Mab) are very specific immunoglobins that exhibit a wide range of biological activities. Earlier there were technical problems in Mab production because of host's anti-antibody immune responses. Subsequently humanized monoclonal antibodies have been developed by joining regions of human myeloma protein to the variable region of mouse antibody. For the large-scale production of monoclonal antibodies, expression of monoclonal antibodies genes is accomplished through recombinant DNA technology.

Diagnostics: rDNA technology provides purified proteins such as monoclonal antibodies for highly specific and sensitive immunoassay based diagnostics making it possible to detect many diseases and medical conditions faster and with greater accuracy using biotechnology-based diagnostic tools.

2.2 Agriculture

Application of GMOs in agriculture is primarily for the production of transgenic plants with higher yield and nutritional content, increased resistance to stress and pests.

Potential Benefits of Transgenic Crops



Several commercially important transgenic crops such as maize, soyabean, tomato, cotton, potato, mustard, rice etc. have been genetically modified and the following traits have been imparted:

Herbicide tolerance: Many effective broad-spectrum herbicides do not distinguish between weeds and crops, but crop plants can be modified to make them resistant to herbicides, so as to eliminate weeds more selectively. For example-GM cotton and soyabean resistant to herbicide Roundup^{TM} have been developed. Genes that provide resistance to other herbicides such as sulfonyl ureas, gluphosinates etc. have also been identified and transferred to produce various transgenic plants.

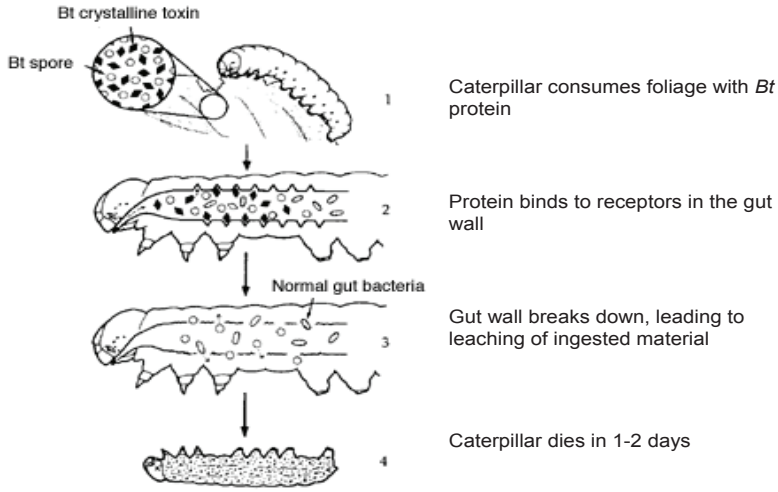
Comparison of a weed-infested soybean plot (left) and Roundup Ready® soybeans after Roundup treatment (right).

Source: http://www.colostate.edu/programs/lifesciences/TransgenicCrops/current.html#Bt/ Monsanto

Insect resistance: Biotechnology has opened up new avenues for natural protection for plants by providing new biopesticides, such as microorganisms,

that are toxic to targeted crop pests but do not harm humans, animals, fish, birds or beneficial insects.

One of the best-known examples is that of commonly found soil bacterium *Bacillus thuringiensis.* The spores of *Bacillus thuringiensis* (Bt) contain a crystalline protein (Cry), which breaks down to release a toxin, known as delta-endotoxin, is highly toxic to lepidopteran larvae. Different Cry genes, also known as Bt genes have been identified, cloned and characterized. Effective gene constructs have made it possible to deliver these genes into plant tissues so that they are expressed at levels high enough to kill the insects. Bt cotton and maize which have increased resistance to boll worms have been developed and cultivated since 1996.



Bt toxin: Mechanism of action

Disease resistance: Plants are susceptible to viral, bacterial and fungal diseases. Much progress has been made in evolving transgenic plants resistant to viruses. For example, expression of a gene that encodes the coat protein of tobacco mosaic virus (TMV) in transgenic tobacco plants has been shown to cause the plants to resist TMV infection. A number of other viral resistant plants species have been developed including squash and potatoes. Genetic engineering of crop plants for resistance to fungal and bacterial infections has been more difficult.

Produce quality improvement: Some of the value added transgenic crops developed via genetic engineering include- tomato varieties exhibiting delayed ripening, transgenic potatoes with increased levels of starch, golden rice containing beta carotene to overcome vitamin A deficiencies, canola

containing high levels of oleic acids and laurate, barley containing feed enzymes, and other vegetables and fruits with delayed ripening as well as modified flavour characteristics.

Transgenic lines of potato with increased levels of starch have been developed by introducing a gene from bacteria for enhancing starch biosynthesis. A promoter from a potato gene that encodes the major protein in potato tubers has been used, so that the expression of the introduced gene is limited to the tuber. Tubers accumulate approximately 3 to 5% more starch than normal potatoes and when they are deep-fried absorb less oil and yield chips having fewer calories.

Resistance to environmental stresses: In addition to the biological challenges to plant growth and development, crops plants need to cope up with abiotic stresses such as drought, cold, heat and soils that are too acidic or saline to support plant growth. While plant breeders have successfully incorporated genetic resistance to biotic stresses such as diseases into many crop plants through crossbreeding, their success at creating crops resistant to abiotic stresses has been more limited, largely because few crops have close relatives with genes for resistance to these stresses.

Therefore crop biotechnology is being increasingly used to develop crops that can tolerate difficult growing conditions. researchers have identified many genes involved in cold, heat and drought tolerance found naturally in some plants and bacteria and are trying to incorporate them in crops.

Plant based pharmaceuticals: Therapeutic drugs to treat cancer, infectious diseases, autoimmune diseases, cardiovascular diseases can potentially be grown in plants. Plant transgenic technology is being used to produce a *plant* that will generate a *seed* that expresses a desired *therapeutic protein*. This seed can *propagate* under the right growing conditions to yield plants and seed stock for producing the desired protein. The desired protein can be extracted from the seed to make a biopharmaceutical. Plant based therapeutics are expected to be much more cost effective.

2.3 Process Industry

The microorganisms and their enzymes are used to make useful products and materials in various industrial processes. rDNA technology has helped in development of improved microorganisms with increased enzyme production thereby improving productivity-to-cost ratio. The yields obtained for recombinant and over expressed enzymes are significantly larger than those products by native strains resulting in increase in production and reduction in cost. Today a growing number of enzymes are produced using GMOs with applications in varied sector such as pulp and paper, food processing, textiles etc.

Plants and microbes have also been genetically modified to produce polyhydroxybutyrate, a feedstock for producing biodegradable plastics. Production of abundant amounts of natural protein polymers such as spider silk and adhesives form barnacles through microbial fermentation using rDNA technology is now possible.

Thus the use of recombinant DNA technology in industrial biotechnology processes can increase the range and quality of products tailored to specific consumer needs and provide ecofriendly alternatives to chemical processes.

2.4 Environment

A vast majority of applications of environmental biotechnology use naturally occurring microorganisms (bacteria, fungi, etc.) to identify and filter manufacturing waste before it is introduced into the environment. Bioremediation programmes involving the use of microorganisms are currently in progress to clean up contaminated air, tracks of land, lakes and waterways.

Recombinant technology helps 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. Recombinant DNA technology also is being used in development of

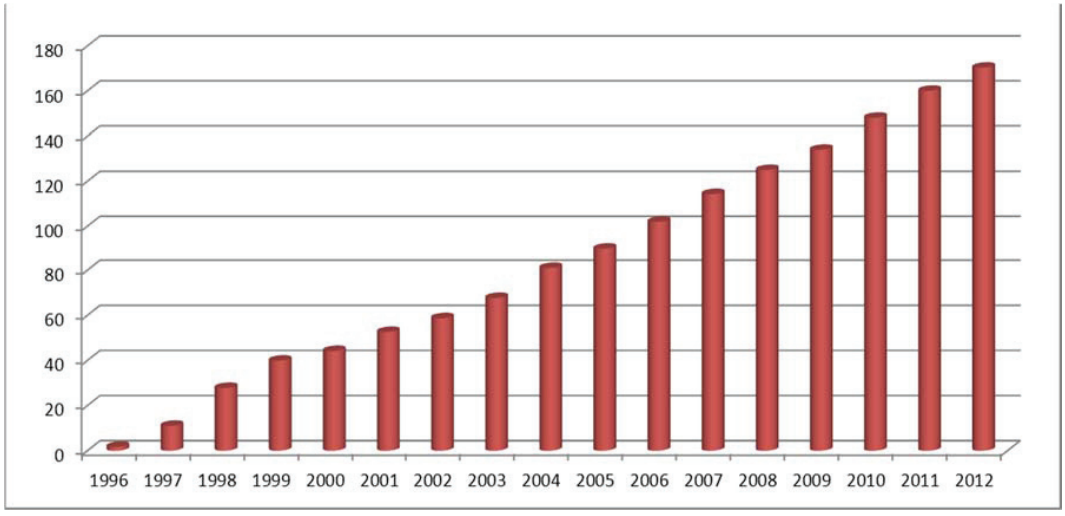
bioindicators where bacteria have been genetically modified as 'bioluminescors' that give off light in response to several chemical pollutants. These are being used to measure the presence of some hazardous chemicals in the environment.

CHAPTER 3
GLOBAL STATUS OF GM CROPS

3.1 What is the present status of development of GM Crops?

GM Crops were first introduced in 1996 and since then the area under cultivation has constantly increased. In the period 1996 to 2012, millions of farmers in ~30 countries worldwide have cultivated GM crops. It has been reported that 170.3 million hectares of GM crops were grown globally in 2012 in 28 countries (Figure 3), of which 20 are developing and 8 are developed countries. In decreasing order of hecterrage under transgenics, USA, Brazil, Argentina, Canada, India, China, Paraguay, South Africa, Pakistan, Uruguay, Bolivia and Philippines grew more than 1 million hectares.

Figure 3. Global area of transgenic crops from 1996 to 2012 (million hectares)



Source: International Service for the Acquisition of Agri-biotech Applications (http://www.isaaa.org)

Table 1: Status of approval of GM crops in various countries in 2012

S. No	Biotech Crop	Country
1	Alfalfa	USA
2	Canola	USA, Canada, Australia, Chile
3	Cotton	USA, Brazil, Argentina, India, China, Paraguay, South Africa, Pakistan, Australia, Burkina Faso, Myanmar, Mexico, Colombia, Sudan, Costa Rica
4	Maize	USA, Brazil, Argentina, Canada, South Africa, Uruguay, Philippines, Spain, Chile, Honduras, Portugal, Czech Republic, Cuba, Egypt, Romania, Slovakia
5	Papaya	USA, China
6	Polar	China
7	Soybean	USA, Brazil, Argentina, Canada, Paraguay, South Africa, Uruguay, Bolivia, Mexico, Chile, Costa Rica
8	Sweet pepper	China
9	Squash	USA
10	Sugarbeet	USA , Canada
11	Tomato	China

Source: International Service for the Acquisition of Agri-biotech Applications (http://www.isaaa.org)

While 28 countries planted commercialized biotech crops in 2012, an additional 31 countries have granted regulatory approvals for GM crops for import and food and feed use. A total of 2,497 regulatory approvals involving 25 GM crops and 319 GM events have been issued by competent authorities in 59 countries, of which 1,129 are for food use (direct use or processing), 813 are for feed use (direct use or processing) and 555 are for planting or release into the environment.

India is fifth in terms of area under cultivation of GM crops with a total area of 10.8 million hectares in 2012, next to USA, Brazil, Argentina and Canada. As of now Bt cotton is the only approved GM crop in India. Bt brinjal, though approved by the regulatory authorities has not been released by the Ministry of Environment & Forests.

3.2 What are the future GM crops in pipeline?

There is intensive research going on to develop GM crops incorporating newer traits, to help the farmers as well as bring more direct benefits to consumers. More than 100 countries across the world are engaged in GM crop research and development programs ranging from laboratory/greenhouse experiments, to field trials, to regulatory approval and commercial production. These include field crops, vegetables, fruits and other plants. In USA alone, a total of 15,845 notifications for trials have been approved between 1992 and 2011. These include more than 150 plants. Some of the examples of the GM crops in the advanced stages of testing include golden rice, drought tolerant corn, mustard hybrids, high yielding crops etc.

CHAPTER 4
SAFETY ASSESSMENT OF GM CROPS

Although the development of GM crops using recombinant DNA techniques is relatively recent, their applications and use is increasing because of advantages over the conventional crops. However as more and more GM crops are being released for field-testing and commercialization, concerns have been expressed regarding the risks arising from their use due to potential risks to both human health and environment.

These apprehensions arise because GM technology crosses the species barrier as compared to classical selection techniques, thereby permitting the gene transfer among microorganisms, plants and animals. There is no evidence that any unique hazards exist in the development of GM crops, because of novel combinations of genes. It is not true that all GM crops are toxic or are likely to proliferate in the environment. However, specific crops may be harmful by virtue of novel combinations of traits they possess. This means that the concerns associated with use of GMOs can differ greatly depending on the particular gene-organism combination and therefore a case-by-case approach is required for assessment of safety concerns.

4.1 Safety concerns

Potential risks from the use of GM crops broadly fall under two categories i.e. risks to human and animal health and risks to environment as described below:

Risks to human and animal health:

Risks to human health are related mainly to toxicity, allergenicity and antibiotic resistance of the new products. The risk of toxicity may be directly related to the nature of the product whose synthesis is controlled by the transgene or the changes in the metabolism and the composition of the organisms resulting from gene transfer. Most of the toxicity risks can be assessed using scientific methods both qualitatively and quantitatively. The introduction of newer

proteins in GM crops from the organisms, which have not been consumed as foods, sometimes has the risk of these proteins becoming allergens. However, it may be noted that there is no evidence that GM crops pose more risks than conventional products regarding the development of allergies. Further, the new GM crops can be tested for allergens prior to the commercial release. For example, when it was found that the consumption of GM soybean with a methionine producing gene from the Brazil nut could trigger an allergic response in those sensitive subjects who were allergic to Brazil nut, the product was not released for sale.

The use of genes for antibiotic resistance as selectable markers have also raised concerns regarding the transfer of such genes to microorganisms and thereby aggravate the health problems due to antibiotic resistance in the disease causing organisms. Although, the probability of such transfer is extremely rare, steps are being taken to reduce this risk by phasing out their use.

There have been apprehensions about danger from eating the foreign DNA in foods derived from GM crops i.e. the pieces of DNA that did not originally occur in that food plant. DNA being present in all living things such as plants, animals, microorganisms is eaten by human beings with every meal. Most of it is broken down into more basic molecules during the digestion process whereas a small amount that is not broken down is either absorbed into the blood stream or excreted. So far there is no evidence that DNA from GM crops has any additional risk to human health than DNA from conventional crops, animals or associated microorganisms that are normally eaten. In cases where the GM crops is to be used for animal feed, the similar concerns as explained above are addressed.

Risks to environment:

Risks to environment due to release of GM crops include impact of introduced traits on the other related species, the potential build up of resistance in insect populations, effect on biodiversity and unintended effects on non-targeted

organisms. Accidental cross breeding between GM crops and traditional varieties through pollen transfer can contaminate the traditional local varieties with transgenes. The consequences associated with such gene transfer may impact intellectual property, increase weediness if transferred to compatible weedy relatives or lead to extinction endangered varieties of the same genera. However, these risks can be anticipated easily and then evaluated by experiments prior to any commercial release. The gene transfer into a crop or the resultant products can actually remain in environment leading to environmental problems e.g. in case of Bt crops, it was suspected that insecticidal proteins can persist in the environments but experiments have proved that these are degraded in the soil. Further there are concerns about possible interaction that may occur between other organisms in the environment following the release of a GM crop. Environmental concerns have also been raised about the development of increased insect resistance, virus resistance and weediness following the introduction of GM crops.

4.2 Concept of risk analysis

Risk assessment evaluates and compares the scientific evidence regarding the risks associated with alternative activities. Risk management develops strategies to prevent and control risks within acceptable limits and relies on risk assessment. In addition to the scientific assessment, it also takes into consideration various factors such as social values and economics. Risk communication involves an ongoing dialogue between regulators and the public about risk and options to manage risk so that appropriate decision can be made.

Risk assessment is a scientific process that makes use of the best up-to-date knowledge and experience. Broad methodologies for risk assessment for modern biotechnology products have been outlined in several international and national guidelines.

It has been generally accepted that details of risk assessment procedures may vary from case to case but there are few logical steps that need to be followed. These are:

(i) Identification of potential adverse effects on human health and/or environment
(ii) An estimation of likelihood of these adverse effects being realized
(iii) An evaluation of the identified risks
(iv) Considerations of appropriate risk management strategies
(v) Assessment of the overall potential environmental impact, including a consideration of the potential impacts that may be beneficial to human health or the environment.

The methodology of risk assessment of GMOs generally covers the characterization of the organisms, effects on pathogenicity, toxigenicity, allergenicity etc., substantial equivalence, effects related to gene transfer and marker genes and ecological effects.

Risk management is the use or application of procedures and means to reduce the negative consequences of a risk to an acceptable level. The risks can be limited by proper handling and use of various preventive measures. Risk management is employed during the development and evaluation of an organism in a systematic fashion in the laboratory, through stages of field-testing to commercialization.

Whereas risk assessment and management procedures are intended to identify and minimize potential negative effects on human health and the environment, risk communication is an integral part of biosafety procedures to ensure public acceptance of GMOs. It is important to interact with public at large about the specific risks and actions taken to alleviate them as insufficient or inaccurate information needs to misperceptions of risk resulting in adverse public opinion.

4.3 Safety Assessment Procedures

Safety assessment of a GM crop is the most important step in the development process of a GM crop. Extensive testing and a long approval process accompany every GM crop introduction. The approval process includes comprehensive risk analysis to ensure food, feed and environmental safety before entering the market place.

Generally safety assessment of a transgenic crop is initiated by determining if the product is substantially equivalent (except for defined differences) to conventional counterpart varieties. Further analysis than focuses on the evaluation of the defined differences by assessing potential safety risks of host plan, gene donor(s) and introduced protein.

Experiments are designed to systematically identify the hazards, to assess risks and to take steps to manage the risk by applying logically valid strategies. Although, information on some of these questions may be available but many questions need to be investigated using appropriate experimental designs in the lab, greenhouse and field trials in a systematic fashion. Toxicity and allergenicity data are generated using the standard protocols devised by national and international agencies.All the data generated by the developing organizations is then submitted in the detailed formats to the government for seeking permission for commercial release of target transgenic crop.

It may be noted that information requirements and analysis by regulatory committees depends on stage and application area of a particular product. Indicative information requirements for review of applications by regulatory authorities for a GM crop are elaborated below in the context of safety assessment.

Table 2: Information required for risk assessment of a GM crop

Particulars	Information required
Molecular Biology Details	• Description of the plant materials to be transformed. • Source of the gene and the cloning strategy followed. • Characteristics of the plant expression vector • Characteristics of the inserted genes with sequence details • Characteristics of the vectors and the transformation system employed with description of sequences used. • Genetic analysis including insert No., Copy No., Insert Integrity, Segregation, Stability of the gene transfer, Description of the expressed gene, Biochemistry of the expressed gene products, authentication of the gene products by physical, chemical, immunological and biological methods etc.
Human Health Considerations	Toxicity • Comparison of the amino acid sequence homology of the newly expressed protein with the known protein toxins and antinutrients • Animal toxicity studies including acute and subchronic **Allergenicity** • Comparison of the amino acid sequence homology of the newly expressed protein to known protein allergans • Heat stability and susceptibility of the expressed protein to pepsin digestion • Studies such as skin sensitization in animals Compositional analysis • Changes in the level of key nutrients, natural toxicants or anti nutrients, secondary metabolites, physiologically active (bioactive) substances, etc. Livestock feeding studies Effect of processing
Environmental Considerations	• Field trial locations and experimental designs followed • 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 organisms • Impact of gene flow by undertaking pollen related studies and crossability studies with sexually compatible relatives

i. ***Molecular Biology Details:*** Development of a GM crop uses three components for genetic manipulation i.e. the selected gene from the donor source which could be the same plant species or other plants,

animals, microbes etc., the vector that is used for transfer of the gene to the target plant and the crop host plant. Therefore, the first step in risk assessment is to examine these three entities, followed by the resultant gene products and the GM crop.

- ***Characteristics of the donor organisms:*** If the donor organism is merely used as a source of well-characterized DNA for a selectable phenotype or a promoter or other control sequence, the characteristics of the donor are not very important for the risk assessment. If, however, the insert contain genes which are biologically active, producing toxins or virulence factors, then information on the gene characteristics in terms of expressed product in the donor organism is extremely important for risk consequence and its assessment.

- ***Characteristics of the host/recipient organisms:*** A thorough knowledge of the host or recipient organism is extremely important in assessment of the risks of the GM crops particularly keeping in view the concept of substantial equivalence as a starting point. The identity of the host must be established and the taxonomy well understood. There should be adequate and documented experience of the safe use of the host organism. The characterization of the host provides the starting point for the risk assessment and also familiarity allows in reviewing comparative data.

- ***Characteristics of the gene insert/ gene construct:*** The properties of the gene insert for the desired trait are extremely important in risk assessment of GM crops. Individual components used in the development of the gene construct i.e. promoters, enhancers, marker and associated (for e.g., terminator)genes are carefully reviewed by the subject-expert members of the designated regulatory committees.

- ***Characteristics of the gene vector and method of transformation:*** The gene vector has to be characterized both for its own potential for infection and for its ability to transfer the insert to organisms other than the intended transfer. The function of the genetic constitution of the vector should be known as this would ensure that the vector is free from sequences that could be harmful to humans / animals or the other living beings of the environment. .

- ***Method of transformation:*** The method (s) of transformation used for introducing the required gene is also considered for the safety assessment on living organisms due to the modified organism

- ***Characteristics of the modified crop variety / hybrid:*** Molecular characterization of the GM crop is used to provide information about the composition and integrity of inserted DNA, the number of copies of inserted DNA, the number of sites of insertion and the expression level of novel proteins over time and in tissues in case of plants. Molecular characterization provides useful information, but cannot by itself answer all questions on safety of GM crops. Hence, this has to be supplemented by studying the possible impact on human health and environment due to the LMO.

- ***Inheritance and stability of the introduced trait:*** The inheritance and stability of each introduced trait that is functional in the modified organism is also determined. For each novel trait, the pattern and stability of inheritance has to be demonstrated as well as the level of expression of the trait by estimation and analysis of the expressed biochemical product such as protein (s) or such desirable substances for attaining the target benevolence. If the new trait is one that does not result in the expression of new or modified biochemical, then its inheritance will have to be determined by examining the DNA insert directly or by measuring RNA transcript production as well as through evaluation of such expressed substance(s) in the progenies of several generations.

ii. ***Human Health Safety assessment:*** 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.

- **Toxicity and allergenicity studies:** Toxicity and allergenicity of gene products are the primary concerns and focus of risk assessment, particularly for GM crops that are to be used as food or feed. The toxicity and allergenicity assessment takes into account the chemical nature and functions of the newly expressed substance and identify the concentration of the substance in the edible parts of the GM crops. The dietary exposure could also take into account while planning these studies. The assessment of potential toxicity focuses on amino acid sequence similarity with known protein toxins and anti nutrients, stability to heat or processing and degradation in appropriate gastric intestinal model system. Appropriate oral toxicity studies are carried out in cases where the expressed biochemical substance(s), present in the food is not similar in chemistry and property to those that have been previously consumed safely in food. For allergenicity, an integrated, step wise, case by case approach is followed to assess the safety of the LMO product, using the generated data on amino acid homology for expressed proteins with known allergens from bioinformatics data base, heat stability, pepsin digestibility etc.

- **Compositional analysis:** Analysis of concentration of the key substances / components of the GM crops is compared with an equivalent analysis of a conventional counterpart crop that is grown and harvested under the same agro-climate and growing conditions. The results are analyzed in the context of range of natural variations for that particular trait parameter. The purpose of this comparison is to establish that the content and concentration of the nutritionally favorable and important substances have not been altered in a manner that would have an adverse impact on human health.

- **Livestock feeding studies:** Appropriate livestock feeding studies are undertaken on a case by case basis to reconfirm the nutritional equivalence of a GM crop with its conventional counterpart in livestock animals.
- **Effect of processing:** The potential effect of food processing is also considered describing the processing conditions used in the production of the end product of manufacture, the possible accumulation of expressed substances in the end-product and their safety to consuming target and non-target organisms.

iii. ***Environmental Safety Assessment:*** Environmental risk assessment of GM crops 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 GM crop. 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 of the agro-ecology as well as to others.

CHAPTER 5
INTERNATIONAL APPROACHES TO BIOSAFETY REGULATIONS

The concerns about potential adverse effects of GM crops on human health and environment have led to development of regulatory regimes that specifically addresses the safety of these products. It may be noted that these regulatory systems have generally been developed piecemeal usually beginning with the voluntary guidelines and standards developed cooperatively by academia, industry and government. Over time, these guidelines and standards were incorporated in Indian statutory instruments, either under existing legislation or under new legislation dealing specifically with gene technology. The regulatory agencies in various countries that look into the regulation and commercialization of GM products are as listed below in Table 3.

Table 3: International regulatory agencies

S. No	Country	Name of the regulatory agency	Function of regulatory agency
1	USA	Animal and Plant Health Inspection Service (APHIS)	Ensures agriculture and environmental safety
		Food and Drug Administration (FDA)	Evaluates food and feed safety
		Environmental Protection Agency (EPA)	Evaluates food safety and environmental issues associated with new pesticides and uses
2	Canada	Canadian Food Inspection Agency (CFIA)	Food, feed and seed regulation
		Plant Biosafety Office	Monitors all confined field trials of novel crop varieties
		Environment Canada	Establishes and monitors biotech products other than agriculture
3	Australia	Office of the Gene Technology Regulator (OTGR)	Regulation of all GM organisms
4.	New Zealand	Environmental risk management authority (ERMA)	Regulates the development, field testing and release of GM organisms
5	Australia/ New Zealand	Food Standards Australia New Zealand (FSANZ)	Develops food standards and standards relating to labeling, composition and contaminants, for food available in Australia and New Zealand
6	European Union (EU)	European Food Safety Authority (EFSA)	Releases for research and development are made under Part B of the Directive, which is used for conduct of experimental filed trials on GM crops; Releases for placing a GM product on the market require consent under Part C of the directive
			EU risk assessment body for food and feed safety

S. No	Country	Name of the regulatory agency	Function of regulatory agency
7	Africa	South African Committee for Genetic Experimentation (SAGENE)	Approves, monitors and advises on the development of genetically modified organisms (GMOs)
8	Philippines	National Committee on Biosafety	Mandates guidelines and approvals
9	Argentina	National Advisory Commission on Agricultural Biosafety (CONABIA)	Is a multidisciplinary advisory group that is responsible for the regulation of products of agricultural biotechnology and it also evaluates the scientific and technical issues of environmental release of GM crops.

Further, in many countries the risk assessment expertise lay in academic and other public sector research institutions. Accordingly most of the countries couple in-house expertise in the regulatory agency with expert advisory committees for development of polices and guidelines as well as for case by case decision making.

Regarding the safety assessment principles and requirements, it has been seen that there is a worldwide consensus by countries on the type of information upon which a safety assessment of GM crops is built, though there are some national and regional variation in emphasis placed on extent of information required by the competent authorities.

In addition to the national regulatory regimes, initiatives have also been taken under the aegis of the Organization for Economic Co-operation and Development (OECD), the Codex *Alimentarius Commission*, World Health Organization (WHO), the Food and Agriculture Organization (FAO) etc for developing principles of safety assessment and Ad Hoc Working Group under the Cartagena Protocol on Biosafety.

The OECD is an intergovernmental organisation that has been working on the issues on biotechnology for more than 25 years. The guidelines for "*Recombinant DNA Safety Considerations"* (referred to as the Blue Book) were prepared way back in 1986, providing one of the first international scientific frameworks for the safe use of organisms derived from rDNA techniques in industry, agriculture and the environment. This was followed by preparation of good developmental principles for small scale field trials of GM

plants and micro-organisms, published under the title "*Safety Considerations for Biotechnology, 1992"* and for large scale trials published under the title "*Safety Considerations for Biotechnology: Scale up of Crop Plants, 1993".*

The work at OECD by a Working Group on Harmonization of Regulatory oversight in biotechnology continues with the objective to ensure that the type of information used in biosafety assessment, as well as the methods to collect such information, are as similar as possible amongst countries. This improves mutual understanding and harmonized practice, which in turn increases the efficiency of the risk/safety assessment process and avoids duplication of effort while reduces barriers to trade. The publication of consensus and guidance documents continues to be a major output of the Working group. These documents constitute a set of practical tools for regulators and biosafety assessors dealing with new transgenic plant varieties and organisms. So far OECD has produced 43 consensus documents[1] for addressing a range of issues particularly the biology of crops, trees and micro-organisms, as well as selected traits that have been introduced in plants. It also maintains a biosafety database which contains approvals/permits issued for experimental field releases of GM organisms in all its 30 member countries.

WHO and FAO provide advice to their member states on scientific and technical issues related to GM foods. In 1990 the scientific expert consultation resulted in formulation of the report titled "*Strategies for Assessing the Safety of Foods Produced using Biotechnology".* Since then several joint expert consultations to build on this body of work have been held covering the principles of safety assessment of foods from GM plants, animals and microorganisms. The scientific advice of the joint FAO/WHO expert consultations serve as the scientific basis for the Codex *Alimentarius* Commission in their work on risk analysis and safety assessment guidelines for GM foods.

[1] Details are available on http://www.oecd.org/document/55/0,3746,en_2649_34385_2500215_1_1_1_1,00.html

The WHO and FAO are jointly responsible for the global food standards body, Codex *Alimentarius* Commission (Codex). It works on the entry of GM foods into the global food market. The Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology[2],which was convened in 2000 has developed standards and guidelines as appropriate, for GM foods. The task force took into account existing scientific work and risk assessment carried out by other agencies including national authorities and international organizations as indicated in the Box 1 below. The work of the task force related to GM foods is summarized in the following two documents that serve as international references on the safety assessment process:

- Principles for the risk analysis of foods derived from modern biotechnology
- Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants

Box 1: A Decade of Consultation on safety assessment of GM foods

UN Food and Agricultural Organization (FAO)
- 1991, 1996, 2000, 2001

UN World Health Organization (WHO)
- 1991, 1993, 1995

International Life Science Institute (ILSI)
- 1996, 1997

Organization for Economic Cooperation and Development (OECD)
- 1993, 1996, 1997, 2000

International Food Biotechnology Council (IFBC)
- 1990

CODEX Alimentarius
- 2003

As per the abovementioned consultations and guidance agreed at the international level, the safety assessment of GM crops is undertaken by comparing the GM product with the non-GM counterpart is referred to as **"substantial equivalence".** The underline assumption of this comparative assessment approach is that the traditionally cultivated crops serve as a baseline for the food/feed safety and environmental safety as they have gained a history of safe use. These concepts of familiarity and substantial

[2] http://www.fsis.usda.gov/codex/Codex_Biotech_Task_Force/index.asp

equivalence were developed by OECD and further elaborated by FAO/WHO (Box 2). Though, it has been acknowledged that the concept of substantial equivalence contributes to a robust safety assessment but it has also been clarified that the concept should represent the starting point used to structure the safety assessment of a GM food relative to its conventional counterpart.

Box 2: Concept of substantial equivalence

Substantial equivalence embodies the idea that if the rDNA product is substantially equivalent to an existing product, safety assessment of the rDNA product should be made in the context of the existing product. That is, a novel food is essentially the same as that found in nature except for the novel trait. The concept is not new as the same principle was used in assessing the safety of new hybrid varieties produced by non-biotechnological means.

Because the degree of equivalence to a previously consumed food is a continuum of "substantial" to unique and not existing in nature, three tiers of degree of equivalence have been proposed.

(a) When substantial equivalence has been established for an organism or food product, it is considered to be as safe as its conventional counterpart and no further safety evaluation is needed.
(b) When substantial equivalence has been established apart from certain defined differences, further safety assessment should focus on these differences: a sequential approach should focus on the new gene product(s) and their structure, function, specificity and history of use. If a potential safety concern is indicated for the new gene product(s), further *in vivo* and/or *in vitro* studies may be appropriate.
(c) When substantial equivalence cannot be established, this does not necessarily mean that the food product is unsafe. Not all such products will require extensive safety testing. The design of any testing program should be established on a case-by-case basis. Further studies, including animal feed trials, may be required.

This concept of substantial equivalence serves as a guiding tool and is incorporated in all international crop/agriculture biotechnology guidelines and policies. Further to this concept, the toxicity protocols to study the toxicological properties and evaluation strategies for potential allergenicity of newly expressed proteins transferred proteins have been developed by OECD and FAO/WHO.

Regarding the environmental safety assessment, the OECD Working Group on Harmonisation of Regulatory Oversight in Biotechnology) has worked on achieving a consensus on the information used in environmental risk assessment as well as the methods of analysis. The Working Group is in the process of developing a new guidance document "Environmental

Considerations for the Risk/Safety Assessment for the Release of Transgenic Plants".

Another major initiative to harmonise the environmental safety assessment requirements for GM crops is under the aegis of the Cartagena Protocol on Biosafety. The CPB addresses the safe transfer, handling, and use of living modified organisms (LMOs). It is the only international environmental agreement that is concerned exclusively with products of modern biotechnology and its interpretation and implementation have had a significant impact on biosafety regulation in developed and developing countries. The Cartagena Protocol entered into force on 11 September 2003 and has been ratified by 162 countries (as of January 26, 2012).[3]

Article 15 of the Protocol requires that scientifically sound risk assessment be performed for decisions on import of LMOs to be released into the environment, and stresses that "recognized risk assessment techniques" shall be taken into account, including guidelines developed by relevant international organizations. The Protocol describes a method in Annex III to the text of the CPB that specifies the safety considerations that must be evaluated, which are quite consistent with similar guidance published by OECD[4] (Table 4).

[3] The text of the Cartagena Protocol can be viewed at http://bch.cbd.int/protocol/text/.
[4] The text of the OECD guidance document on safety considerations is available at www.**oecd**.org/data**oecd**/8/3/2375496.pdf

Table 4: Comparison of information requirements under the Cartagena Protocol risk assessment procedure and under OECD guidelines

Cartagena Protocol on Biosafety	OECD Guidelines
Recipient (host) organism – biology, taxonomy, center(s) of origin, center(s) of diversity, common name, habitat **Intended use** – including changes in use or practice compared with parental organism	**Host organism** – reproductive biology, taxonomy, center(s) of origin, consumption/uses, interactions with other organisms, occurrence and viability of interspecific hybrids and anticipated changes in agronomic practices.
Donor organism(s) – biological characteristics, taxonomic status, common name and source	**Donor organism(s)** – known toxicological or pathogenicity concerns
Vector – identity, source, host range **Insert and characteristics of modification** – genetic characteristics of inserted DNA and function **LMO** – identity of the LMO noting any differences between the biological characteristics of the LMO and the host organism	**Molecular genetic characterization** – identity and source of genes and/or vectors; modification method; composition and integrity of introduced DNA; multigenerational stability (expression) and inheritance of the introduced trait; levels and tissue, or temporal, specificity of expression
Receiving environment – geographical, climatic and ecological considerations, including biological diversity **Detection and identification** – suggested detection methods and their specificity, sensitivity and reliability	**Establishment/persistence (weediness)** – seed dissemination, dormancy, germination, competitiveness, disease resistance and stress tolerance **Gene transfer** – both to other sexually compatible organisms and to unrelated species

The *Ad Hoc* Technical Expert Group (AHTEG) on Risk Assessment and Risk Management under the Cartagena Protocol met for the first time in April 2009 to discuss the development of guidance documents to further support countries in conducting risk assessments of LMOs. The AHTEG has met three times to draft "Guidance on the Risk Assessment of LMOs"[5]. It has additionally established sub-working groups to develop guidance documents on specific aspects of risk assessment and risk management, namely:

[5] The "Guidance on the Risk Assessment of LMOs" is essentially a compilation of the other draft guidance documents being developed by sub-working groups of the AHTEG. The latest version of the document, dated 13 January 2012, can be accessed from http://bch.cbd.int/onlineconferences/discussiongroups_ra.shtml

- Roadmap for Risk Assessment
- Risk Assessment and Risk Management of Living Modified Crops with Resistance or Tolerance to Abiotic Stress
- Risk Assessment and Risk Management of Living Modified Mosquitoes
- Risk Assessment and Risk Management of LMOs with Stacked Genes or Traits genes
- Post-release Monitoring and Long-term Effects of LMOs Released into the Environment
- Risk Assessment of LM Trees

None of the guidance documents developed by the AHTEG and its sub-working groups have so far been finalized.

CHAPTER 6
CARTAGENA PROTOCOL ON BIOSAFETY

6.1 Introduction

Although many countries have enacted national biosafety legislations to ensure the safe use of GMOs and products thereof, biotechnology being a global industry and GMOs traded across borders, international rules are needed as well. Cartagena Protocol on Biosafety is an attempt to produce a globally harmonized regime for biosafety under the Convention on Biological Diversity (CBD). Named after the Colombian city where the final round of talks was launched, the Cartagena Protocol on Biosafety sets out a comprehensive regulatory system for ensuring the safe transfer, handling and use of GMOs subject to transboundary movement. The full text of the protocol may be seen at http://bch.cbd.int/protocol/.

The Protocol deals primarily with GMOs that are to be intentionally introduced into the environment (such as seeds, trees or fish) and with genetically modified farm commodities (such as corn and grain used for food, animal feed or processing). It does not cover pharmaceuticals for humans addressed by other international agreements and organizations or products derived from GMOs, such as cooking oil from genetically modified corn.

The protocol entered into force from September 11, 2003. As of July, 2014, 167 countries have ratified the protocol. India ratified the protocol in January 2003.

6.2 Objective

The objective of the Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of LMOs resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into

account risks to human health, and specifically focusing on transboundary movements.

6.3 Salient Features

The Protocol promotes biosafety by establishing rules and procedures for the safe transfer, handling, and use of LMOs, with specific focus on transboundary movements of LMOs. It features a set of procedures including one for LMOs that are to be intentionally introduced into the environment (advance informed agreement procedure, and one for LMOs that are intended to be used directly as food or feed or for processing. The salient features of the Protocol are as follows:

- **Advance Informed Agreement (AIA) Procedure:** The AIA procedure applies only to the first intentional transboundary movement of any particular GMO intended for introduction into the environment. These include seeds, live animals and other organisms that are destined to grow and that have the potential to pass their modified genes on to succeeding generations. The exporter must provide a notification to the importing country containing detailed information about the LMO, previous risk assessments of the LMO and its regulatory status in the exporting country. The importing country must acknowledge receiving the information within 90 days and whether the notifier should proceed under a domestic regulatory system or under the Protocol procedure. In either case, the importing country must decide whether to allow the import, with or without conditions or deny it within 270 days. Consecutive shipments are not subject to the AIA requirement.

  Further the Protocol`s AIA procedure does not apply to LMOs in transit; destined for contained use and LMOs intended for direct use as food or feed or for processing.

- **Procedures for LMOs Intended for Direct Use as Food or Feed for Processing (LMOs– FFP):** Instead of requiring the use of the AIA procedure, the Protocol establishes a simpler system for the transboundary movement of LMOs intended for direct use as food or feed or processing (LMOs – FFPs) and not as seeds for growing new crops. Under this procedure, governments that approve these commodities for domestic use must communicate this decision to the world community via the Biosafety Clearing- House within 15 days of its decision. They must also provide detailed information about their decision.

  Decisions by importing countries on whether or not to import these LMO-FFPs are taken under its domestic regulatory framework.

- **Risk Assessment:** The Protocol empowers governments to make its decisions in accordance with scientifically sound risk assessments. These assessments aim to identify and evaluate the potential adverse effects that a LMO may have on the conservation and sustainable use of biodiversity in the receiving environments. They are to be undertaken in a scientific manner using recognized risk assessment techniques. While the country considering permitting the import of a LMO is responsible for ensuring that a risk assessment is carried out, it has the right to require the exporter to do the work or to bear the cost. This is particularly important for many developing countries. Annex-III to the Protocol provides details of steps to be followed for risk assessment.

- **Risk Management and Emergency Procedures:** The Protocol requires each country to manage and control any risks that may be identified by a risk assessment. Key elements of effective risk management include monitoring systems, research programmes, technical training and improved domestic coordination amongst government agencies and services. The Protocol also requires each government to notify and consult other affected or potentially affected governments when it becomes aware that LMOs under its jurisdiction may cross international borders due to

illegal trade or release into the environment. This will enable them to pursue emergency measures or other appropriate action. Governments must establish official contact points for emergencies as a way of improving international coordination.

- **Handling, Transport, Packaging and Identification Of LMOs:** The Protocol provides for practical requirements that are deemed to contribute to the safe movement of LMOs. Parties are required to take measures for the safe handling, packaging and transportation of LMOs that are subject to transboundary movement. The Protocol specifies requirements on identification by setting out what information must be provided in documentation that should accompany transboundary shipments of LMOs. It also leaves room for possible future development of standards for handling, packaging, transport and identification of LMOs by the meeting of the Parties to the Protocol.

  Each Party is required to take measures ensuring that LMOs subject to intentional transboundary movement are accompanied by documentation identifying the LMOs and providing contact details of persons responsible for such movement. The details of these requirements vary according to the intended use of the LMOs,

  For LMOs intended for direct introduction into the environment, the accompanying documentation must clearly state that the shipment contains LMOs. It must specify the identity and relevant traits and characteristics of the LMO; any requirements for its safe handling, storage, transport and use; a contact point for further information; and the names and addresses of the importer and exporter. In cases where a government agrees to import LMO-FFPs the shipment must clearly indicate that it “may contain” living modified organisms and that these organisms are not intended for introduction into the environment.

- **Biosafety Clearing House (BCH):** The Protocol established a Biosafety Clearing-House (BCH) as part of the clearing-house mechanism of the Convention, in order to facilitate the exchange of scientific, technical, environmental and legal information on, and experience with, living modified organisms; and to assist Parties to implement the Protocol. In addition to enabling governments to inform others about their final decisions regarding the import of GMOs, the Biosafety Clearing- House contains information on national laws, regulations, and guidelines for implementing the Protocol. The Biosafety Clearing-House also includes information required under the AIA procedure, summaries of risk assessments and environmental reviews, bilateral and multilateral agreements, reports on efforts to implement the Protocol, plus other scientific, legal, environmental and technical information. Common formats are used to ensure that the information collected from different countries is comparable. The Biosafety Clearing-House has been developed as an Internet-based system and can be found at https://bch.cbd.int/.

- **Unintentional Transboundary Movements of LMOs:** When a country knows of an unintentional transboundary movement of LMOs that is likely to have significant adverse effects on biodiversity and human health, it must notify affected or potentially affected States, the Biosafety Clearing-House and relevant international organizations regarding information on the unintentional release. Countries must initiate immediate consultation with the affected or potentially affected States to enable them to determine response and emergency measures.

- **Issue of Non-Parties:** The Protocol addresses the obligations of Parties in relation to the transboundary movements of LMOs to and from non-Parties to the Protocol. The transboundary movements between Parties and non-Parties must be carried out in a manner that is consistent with the objective of the Protocol. Parties are required to encourage non-Parties to adhere to the Protocol and to contribute information to the Biosafety Clearing-House.

- **Capacity Building:** Countries that trade in GMOs need to have the capacity to implement the Protocol. They need skills, equipment, regulatory frameworks and procedures to enable them to assess the risks, make informed decisions, and manage or avoid any potential adverse effects of GMOs on their natural relatives. The Protocol promotes international cooperation to help developing countries and countries with economies in transition to build human resources and institutional capacity in biosafety. Parties are encouraged to assist with scientific and technical training and to promote the transfer of technology, know-how, and financial resources. Parties are also expected to facilitate private sector involvement in capacity building. Biosafety activities under the Cartagena Protocol are eligible for support from the Global Environment Facility – an international fund that was established to help developing countries protect the global environment.

- **Public Awareness and Participation:** It is clearly important that individual citizens understand and are involved in national decisions on GMOs. The Protocol therefore calls for cooperation on promoting public awareness of the safe transfer, handling and use of GMOs. It specifically highlights the need for education, which will increasingly have to address GMOs as biotechnology becomes more and more a part of our lives. The Protocol also calls for the public to be actively consulted on GMOs and biosafety. Individuals, communities and non-governmental organizations should remain fully engaged in this complex issue. This will enable people to contribute to the final decisions taken by governments, thus promoting transparency and informed decision-making.

- **Liability and Redress:** The Protocol contains an enabling provision by which the Conference of the Parties serving as the meeting of the Parties shall, at its first meeting, adopt a process with respect to the appropriate elaboration of international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of living modified organisms. Article 27 of the Cartagena Protocol gives the

Conference of Parties a mandate to begin the development of international regulations regarding liability for damages resulted by the transboundary movement of LMOs and the legal redress available with regard to such damages at its first meeting and complete the development of such regulations within four years. Negotiations began at the first meeting of the Parties in Kuala Lumpur. This long task was completed after six years at the 5th meeting of the Parties. On 16 October 2010, Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol was adopted

The new supplementary protocol includes legally binding regulations with regard to administrative procedures. The new regulations enable the competent authorities of countries that have joined the Protocol to hold liable and demand the restitution of damages from those who distribute products which result in damage to wildlife from living, modified organisms. The full text of the Supplementary Protocol may be seen at http://bch.cbd.int/protocol/supplementary/

- **Institutional Arrangements at the National Level:** Parties are required to designate national institutions to perform functions relating to the Protocol. Each Party needs to designate one national focal point to be responsible on its behalf for liaison with the Secretariat. Each Party also needs to designate one or more competent national authorities, which are responsible for performing the administrative functions required by the Protocol and which shall be authorized to act on its behalf with respect to those functions. A Party may designate a single entity to fulfill the functions of both focal point and competent national authority.

- **Governing Body of the Protocol:** The governing body of the Protocol is the Conference of the Parties to the Convention serving as the meeting of the Parties to the Protocol (COP-MOP). The main function of this body is to review the implementation of the Protocol and make decisions necessary to promote its effective operation.

6.4 BENEFITS OF BECOMING A PARTY TO THE PROTOCOL:

Becoming a Party to the Protocol presents a number of benefits, such as the following:

- Influence on the implementation of the Protocol and shaping of its further development through participation in the decision-making processes of the Conference of the Parties serving as the meeting of the Parties to the Protocol;
- For developing country Parties and Parties with economies in transition, eligibility for financial support from the Global Environment Facility (the financial mechanism for the Protocol) for capacity-building, as well as other support for implementation of the Protocol and participation in its processes;
- Enhanced visibility and credibility of national systems for regulating biosafety within the global community;
- Contribution to harmonized rules, procedures and practices in managing the transboundary movement of LMOs;
- Facilitation of mechanisms and opportunities for governments to collaborate with other governments, the private sector and civil society on strengthening biosafety;
- Improved access to relevant technologies and data, and benefiting from a regular exchange of information and expertise; and
- Demonstration of commitment to conservation and sustainable use of biological diversity through the implementation of biosafety measures.

CHAPTER 7
STATUS OF BIOSAFETY REGULATIONS IN BHUTAN

The first legal instrument dealing with biosafety of GMOs in Bhutan has been a ministerial decree issued by the Ministry of Agriculture and Forests in 2000 that bans all imports of GMOs into the country. The same was issued to ensure that Bhutan is free of GMOs[6]. Subsequently, the provisions related to GM food have been added in the Food Act of Bhutan. Chapter VII, Sections 59 and 60 of the Food Act of Bhutan, 2005 directly address the issue of food safety resulting from genetically modified food. It has provisions to adopt the rules and regulations for addressing the issue of food safety, including that resulting from GMOs/GM food[7]. The Ministry of Agriculture and Forests implements the Food Act and the BAFRA has been designated as the lead agency.

Bhutan acceded to the Cartagena Protocol on Biosafety on August 26, 2002. Subsequently, with the financial support from UNEP/GEF, a draft National Biosafety Framework was prepared in 2006[8]. As part of the process of preparation of the biosafety framework, both the existing and proposed biosafety related legislation were reviewed and are presented in the following Table 5:

Table 5: Biosafety related legislations of Bhutan

Title of law/ regulation	Scope of law/regulation	Responsible agency	Status
Food Act	Addresses the issue of food safety resulting from genetically modified food	Bhutan Agriculture and Food Regulatory Authority (BAFRA)[9]	Adopted in 2005

[6] Wangchuk Tashi (2010)., Harmonizing the Biosafety Framework of Bhutan with National Laws and Policies., Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, Royal Government of Bhutan.

[7] Compendium of RNR sector policy and legislation framework (2010), Policy and Planning Division, Ministry of Agriculture and Forests. http://www.moaf.gov.bt/moaf/?wpfb_dl=144

[8] Draft National Biosafety Framework of the Kingdom of Bhutan (2006), National Environment Commission, Royal Government of Bhutan.

[9] http://www.bafra.gov.bt/

Title of law/ regulation	Scope of law/regulation	Responsible agency	Status
Livestock Act	To ensure that only quality and appropriate breeds of livestock, poultry and fish are introduced and to ensure the units used for semen and embryo production and storage are free from diseases	Ministry of Agriculture and Forests (MoAF)[10]	Adopted in 2001
Plant Quarantine Act	To safeguards agricultural and wild flora from introduced pests, defined as "any form of plant or animal life, or any pathogenic agent, injurious or potentially injurious to plants or plant product." In particular it ensures that all imported plants are quarantined and screened prior to entry into the country.	BAFRA	Adopted in 1993`
Draft Regulation on Biosafety	To provide for the assessment, management and control of potential risks associated with the genetically modified organisms (GMOs) and products thereof, and activities associated with them, in order to enable the country to benefit from modern biotechnology and at the same time to protect the biodiversity and people of Bhutan from their potential negative or adverse effects.	BAFRA	Draft form
Seeds Act	To regulate import and export of Agriculture seeds, to prevent introduction of plants and diseases and to promote seed industry in the country aimed at enhancing rural income and livelihood	Ministry of Agriculture and Forests	Adopted in 2000

[10] http://www.moaf.gov.bt/moaf/

Title of law/ regulation	Scope of law/regulation	Responsible agency	Status
Biodiversity Act	to ensure national sovereignty of the RGOB over genetic resources in accordance with relevant National and International Law	National Biodiversity Centre [11]	Adopted in 2003
National Environment Protection Act	To provide for the establishment of an effective system to conserve and protect environment through the National Environment commission or its successors, designation of competent authorities and independently regulate and promote sustainable development in an equitable manner.	National Environment Commission [12]	Adopted in 2007

The NBF was prepared with an objective to safeguard the biodiversity of the country and the health of its citizens from the potential adverse effects of modern biotechnology and yet at the same time benefit from the safe and proven benefits of this technology. Two major outcomes that emerged from the NBF were firstly that the Bhutan Agriculture and Food Regulatory Authority (BAFRA) was designated as the national competent authority (NCA) for biosafety in the country and secondly a legal system for regulating the safe transfer, handling and use of GMOs resulting from modern biotechnology was initiated. In fact 'Draft Biosafety Rules and Regulations of Bhutan, 2006' were prepared and annexed to the report[13].

Although, the NBF was approved by the government, thereafter a number of changes in governance in Bhutan took place. These included Bhutan's landmark change in governance from a monarchy to a democracy with the adoption of the Constitution of Bhutan in 2008 and installation of the first elected government. The

[11] http://www.nbc.gov.bt/

[12] http://www.nec.gov.bt/

[13] Moore Patricia and Wangchuk Tashi (2011), Review and Revisions of Biosafety Rules and Regulations of Bhutan.

NBF particularly the draft biosafety rules and regulations have subsequently been reviewed to harmonize the NBF with the Bhutan's Economic development Policy, 2010 which brands Bhutan as 'Organic Bhutan' for the world.

In 2011, the cabinet approved the following changes to be considered with regard to the implementation of the biosafety framework:

- GMO plants, animals, seeds, semen and embryos - any GM biological material capable of reproducing - may be banned in Bhutan to protect the country's rich biological diversity and the well-being of the people.
- GMO foods, feeds, and processing (FFPs) can be permitted since Bhutan imports more than 35% of FFPs from neighboring countries and also receives food aid from WFP which contain GMOs.
- The National Biosafety Framework can be revised to reflect these decisions.

Accordingly, a Biosafety Bill of Bhutan has been drafted after a number of consultations. The draft Biosafety bill, 2014 is under discussion in the Parliament. As per the proposed Bill, it shall apply to all GMOs, products derived from GMOs and all stages of import, export and direct use of products having GM content within the country.

CHAPTER 8
DETECTION OF GMOs/LMOs

Generally, GMOs are indistinguishable from non-GMOs to the naked eye. Testing of GMOs is based on detecting the rDNA introduced during the transformation process or on the recombinant proteins expressed in the GMO. Recombinant proteins or rDNA are targets that differentiate GMOs from their non-GMO counterparts. Moreover it is more difficult to detect the novel genes in the processed GMOs or their products. Therefore highly sensitive and specific testing methods are required that can look for the genes (DNA) engineered into the particular organism or the proteins produced in the organism by the introduced DNA.

Analytical methods to detect (qualitative or yes/no answer) and quantify (percentage content) GMOs fall into two main categories: protein-based analysis to detect the specific protein expressed by the transgene in the GMO through the use of ELISA (enzyme-linked immunosorbent analysis) and lateral flow strip tests and DNA-based analysis to detect the specific transgene in the GMO or specific elements associated with the transgene.

For both protein and DNA-based GMO testing 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.

The majority of the methods hitherto developed for detection of LMO and LMO-derivatives focus on detecting DNA, while only a few methods have been developed for detecting proteins or RNA. This has various reasons. DNA can be purified and multiplied in billions of copies in just a few hours with a technique called PCR (polymerase chain reaction). Multiplication of RNA and proteins is a more complicated and slow process. DNA is a very stable molecule, while RNA is unstable. The stability of a protein varies and depends on the type of protein. There is normally a linear correlation between the quantity of LMO and DNA if the genetically modified DNA is nuclear, but not if it is extranuclear (in eukaryotes, chromosomal / extra-chromosomal in prokaryotes). However, there is usually no

such correlation between the quantity of LMO and protein/RNA. Finally, the genetic modification itself is done at the DNA level. At present, the genetically modified DNA is nuclear in all commercialized LMOs.

Protein based methods rely on a specific binding between the protein and an antibody, a molecule related to those protecting our own organism against infections from bacteria and virus. The antibody recognizes the foreign molecule, binds to it, and in LMO detection assays the bound complex is successively detected in a chromogenic (colour) reaction. This technique is called ELISA (enzyme linked immunosorbent assays). The antibody needed to detect the protein cannot be developed without access to the purified protein. This protein can be purified from the LMO itself, or it can be synthesized in a laboratory if the composition of the protein is known in detail.

RNA based methods rely on specific binding between the RNA molecule and a synthetic RNA or DNA molecule (called a primer). The primer must be complementary to the nucleotide sequence at the start of the RNA molecule. The result is a double stranded molecule similar to DNA. Usually binding between the RNA molecule and the primer is followed by conversion of the RNA to a DNA molecule through a process called reverse transcription. Finally the DNA can be multiplied with PCR or it can be translated into as many as 100 copies of the original RNA molecule and the procedure can be repeated by using each copy as a template in a technique called NASBA (nucleic acid sequence-based amplification). The specific primers needed for the procedure cannot be developed without prior knowledge of the composition of the RNA molecule to be detected.

DNA based methods

DNA based methods are primarily based on multiplying a specific DNA with the PCR technique. Two short pieces of synthetic DNA (primers) are needed, each complementary to one end of the DNA fragment to be multiplied. The first primer matches the start and the coding strand of the DNA to be multiplied, while the second primer matches the end and the non-coding (complementary) strand of the DNA to be multiplied. In a PCR the first step in a cycle involves separation of the two

strands of the original DNA molecule called ‘denaturation’. The second step involves binding of the two primers to their complementary strands, respectively termed ‘annealing’. The third step involves making two perfect copies of the original double stranded DNA molecule by adding the right nucleotides to the end of each primer, using the complementary strands as templates called ’extension’. Once this cycle is completed, it can be repeated, and for each cycle the number of copies is doubled, resulting in an exponential amplification. After 20 cycles the copy number is approximately 1 million times higher than at the beginning of the first cycle. However, after a number of cycles the amount of amplification product will begin to inhibit further amplification, as will the reduction in available nucleotides and primers for incorporation into new amplification products. This effect is often referred to as the plateau effect.

One of the most commonly applied techniques for demonstration of the presence of a DNA or RNA is gel electrophoresis, a technique that allows the amount and size of the DNA/RNA to be estimated. This may eventually be coupled to digestion of the DNA with restriction enzymes that e.g. are known to cut a PCR fragment into segments of specified sizes. A more sophisticated technique involves determination of melting point profiles, by means of e.g. SYBR Green I, a dye that when intercalating double stranded DNA emits fluorescent light. When the temperature is increased, the DNA strands begin to separate. This leads to a corresponding reduction in fluorescence that can be measured directly e.g. on a real-time thermal cycler (PCR machine). The melting point is more characteristic of a specific DNA sequence than its size, but complete sequencing (determination of the order of nucleotides) of the DNA/RNA allows for more specific determination of the origin of the molecule. A fourth alternative is to use short synthetic molecules (similar to primers but called probes) and allow these to bind (hybridize) to the DNA/RNA. If appropriately designed, a probe will be able to discriminate between the correct molecule (sequence) and almost any other DNA/RNA molecule. Labeling of molecules with fluorescence, radioactivity, antibodies or dyes facilitate detection of the present molecules. For LMO analyses, gel electrophoresis and hybridisation techniques are currently the most commonly applied techniques.

The specificity of currently available DNA based methods for GMO detection can be divided into four categories (1) screening methods that can detect a wide range of LMOs without identifying the LMO, (2) screening methods that can detect a certain type of genetic modifications ie the transgene, 3) construct specific methods that can be used to identify the LMO and 4) event specific methods. In addition PCR-based LMO analyses usually include testing for presence of DNA from the particular species of interest, e.g. soybean DNA. Sometimes (in the absence of amplifiable DNA from the particular plant species) LMO analyses also include testing for presence of amplifiable (multicopy) DNA from plants or Eukaryotes, e.g. chloroplast DNA or nuclear ribosomal small subunit genes (18S like), beta-actin. Different parts of the rDNA sequence are targeted in individual analytical steps of GMO testing.

Category1-screening methods: Screening methods target a part of the rDNA sequence that is present in many GMOs, such as the regulatory sequences of promoters, terminators. The goal is to determine their presence or absence and at the same time limit the cost of analysis. The targets of screening methods are selected based on information regarding the genetic elements composing different GMOs. The promoter and terminator elements used to transform most of the currently approved GMOs are the Cauliflower Mosaic Virus promoter (P-35S) and the *Agrobacterium tumefaciens* nopaline synthase terminator (T-Nos). Although, other promoters and terminators have also been used, almost all GM plants contain at least one copy of the P-35S, T-35S and/or the T-Nos as a part of the gene construct integrated in its genome. Consequently, methods detecting one of these elements are popular for screening purposes .

Category 2 methods like those that can detect the CryIA(b) or EPSPS gene can tell us more than the category 1 screening methods, but will not be suitable for identification of the specific LMO. The synthetic CryIA(b) gene has been integrated with different specific regulatory elements (promoters and terminators) in the various LMOs containing the gene. Currently it is therefore possible to identify the LMO with category 3 detection methods targeting the junctions where the gene and regulatory elements are fused and called ‘Construct specific’. . However, in the future, even

these junctions may be found in more than one LMO. With currently available technology it has not yet been possible to control where in the genome of a plant the insert is integrated. If the same insert is integrated into the genome of the same type of organism several times, the likelihood of integration of the insert in the same place in the genome two times is usually negligible. Consequently, the junction between the integration site and the insert will be unique for each transformation event. Category 4 methods detect these regions and will remain specific for the transformation even when the same construct has been integrated into the same plant species many times. However, category 4 methods are under development, and none has yet been published. When two LMO are crossed, e.g. two different approved genetically modified maize cultivars, the resulting hybrid offspring may possess the genetic modifications from both parent cultivars. This phenomenon is called "gene stacking. None of the above four categories of analysis methods will be able to identify cases of gene stacking. Instead, cases of gene stacking will give results indistinguishable from the separate detection and identification of each of the parental cultivars in the sample.

Scheme of GMO Testing

Testing of GMOs is usually performed in a stepwise system. In the first step the screening tests using screening methods are performed. If a screening result is negative, the analysis is concluded and no action on the sample tested is required. If the result of the screening test is positive, it is not known which GMO is present, therefore a second step is needed to identify the specific GMOs in the sample. After their identification using event-specific tests, the legal status of the GMOs present in the sample is determined. If all GMOs present are authorized, a third step is performed: quantification of each individual GMO detected in the sample to define whether its content is above or below the labeling threshold set in legislation. f the content is above the threshold, the product has to be labeled.

With multiplex PCR-based methods several target DNA sequences can be screened for and detected in a single reaction. Although in principle, standard PCR methods may be combined in the same reaction, in practice this will often create an unacceptably high risk of producing incorrect results from analyses of real samples.

Firstly, each method may require different reaction conditions, e.g. different temperature regimes or different reagent concentrations. Secondly, the combination of primers from different methods may increase the risk of amplifying DNA fragments other than the target fragments. Thirdly, when more than one target fragment is being amplified in a PCR reaction, the two fragments (amplicons) will compete for reagents etc. Normally, a fragment present in a large number of starting copies will out compete another fragment that may be present only in very few numbers. However, if two amplicons are amplified with significantly different efficiency this may also have a severe impact on the final ratio of the two amplicons, e.g. if the starting copy numbers were more or less the same for both. Consequently, development of multiplex assays requires careful testing and validation. After the PCR the resulting pool of amplified DNA fragments needs to be further analysed to distinguish between the various amplicons. This may be done by the use of specific hybridisation probes (possibly also during PCR in real-time assays), by gel electrophoresis and comparison of fragment sizes or by the use of specifically labeled primers. The advantage of multiplex methods is evidently that fewer reactions are needed to test a sample for potential presence of LMO-derived DNA

Quantitation methods using PCR

PCR based quantitation can be performed either after completion of the PCR (end-point analysis), or during the PCR (real-time analysis). End-point analyses are usually based on comparison of the final amount of amplified DNA of two DNA targets, the one to be quantified and a competitor (an artificially constructed DNA that is added in a small and known quantity prior to the PCR amplification and that is co-amplified with the target that is to be quantified). This is called competitive quantitative PCR and it requires that the two DNA targets are amplified with equal efficiency since otherwise the final amount of product is not linearly correlated with the starting amount. A dilution series of the DNA to be analysed is prepared, and a constant amount of the competitor is added. After completion of the PCR the resulting amplification products are visualized through gel electrophoresis and when both DNA targets yield the same amount of product it is assumed that the starting amount was also the same. By setting up two competitive PCRs, one for the LMO (e.g. Roundup Ready soybean) and one for the species of interest (e.g. soybean),

and including competitors in both, the quantity of LMO relative to the species can be estimated by extrapolation from the degree of dilution and concentration of the competitors. Such assays are referred to as quantitation by double competitive PCR.

For the quantification of GMOs, taxon-specific methods targeting sequences confined to the particular taxon of interest are needed. Quantification is done by relating the content of a taxon-specific sequence to the content of rDNA, determined by an event-specific method. In this way, the percentage of GMOs
in relation to individual plant species or taxon is evaluated. Real-time PCR (qPCR) is by far the most widely used technique for detection, identification, and quantification of GMOs. In real-time analyses the amount of product synthesised during PCR is estimated directly by measurement of fluorescence in the PCR reaction. All qPCR systems rely upon the detection and quantification of a fluorescent reporter, the signal of which increases in direct proportion to the amount of PCR product in a reaction. Various chemistries for the generation of fluorescent signal have been developed: intercalating or sequence-unspecific DNA labeling dyes (SYBR ®Green), primer-based technologies (AmpliFluor, Plexor, Lux primers), and technologies involving double-labeled probes detecting hybridization of the probe to the target (molecular beacon) and hydrolysis of the probe (TaqMan, CPT, LNA, and MGB) (Buh Gasparic et al. 2010; Weighardt 2006)

Although TaqMan and SYBR Green are the most commonly used in routine laboratories, other chemistries are rapidly developing. The idea is that with the use of fluorescence it becomes possible to measure exactly the number cycles that are needed to produce a certain amount of PCR product. This amount corresponds to the amount producing a fluorescence signal clearly distinguishable from the background signal and measured well before the plateau effect becomes a problem. The number is called the Ct-value. Then by comparison of Ct-values for the LMO target sequence, e.g. Roundup-Ready soybean 3' integration junction, and the reference gene, e.g. soybean lectin, it becomes possible to estimate the ratio of the LMO target sequence to the reference sequence in terms of difference in number of cycles needed to produce the same quantity of product. Since one cycle corresponds to a doubling of the amount of product, a simple formula can be presented to estimate the ratio in percent. While real-time PCR requires more

sophisticated and expensive equipment than competitive PCR, it is faster and (at least sometimes) more specific.

Microarray DNA chip-technology

Microarray technology (DNA chip technology) has been developed in recent years for automated rapid screening of gene expression and sequence variation of large number of samples. Microarray technology is based on the classical DNA hybridisation principle, with the main difference that many (up to thousands of) specific probes are attached to a solid surface. Different formats are known, *e.g.* macroarrays, microarrays, high-density oligonucleotide arrays (gene chips or DNA chips) and micro-electronic arrays. In DNA chips, short oligonucleotides are synthesised onto a solid support, whereas in DNA arrays, PCR products, corresponding to either genomic DNA or cDNA sequences, are deposited onto solid glass slides (microarray) or nylon membranes (macroarray). Micro-electronic arrays consist of sets of electrodes (capable of generating a current) covered by a thin layer of agarose coupled with an affinity moiety. These techniques are developing rapidly and have many advantages but also some limitations, at least at present. Since the techniques are very sensitive and still under development, they are limited to expert laboratories.

Protein-based methods

The common protein based test methods use antibodies specific for the protein of interest. Immunoassay technologies with antibodies are ideal for qualitative and quantitative detection of many types of proteins in complex matrices when the target analyte is known. Both monoclonal (highly specific) and polyclonal (often more sensitive) antibodies can be used. Detection methods based on the immunological detection of proteins or on the comparison of protein patterns (e.g. one- or two-dimensional gel electrophoresis) require that the sample or the protein of interest is not significantly degraded. Thus, the application of protein-based detection methods is limited for the raw material.

Immunoassay is the current method for detection and quantification of new (foreign) proteins introduced through genetic modification of plants. The crucial component of an immunoassay is an antibody with high specificity for the target molecule (antigen). Immunoassays can be highly specific and samples often need only a simple preparation before being analysed. Moreover, immunoassays can be used qualitatively or quantitatively over a wide range of concentrations. Similar to herbicide bioassays, immunoassays require separate tests for each trait in question. Making a valid identification of the foreign protein in LMOs using immunoassays depends on the availability of the particular proteins for development of the antibodies, which is the essence of the assay. The antibodies can be polyclonal, raised in animals, or monoclonal, produced by cell cultures. Commercially available polyclonal antiserum is often produced in rabbits, goats or sheep. Monoclonal antibodies offer some advantages over polyclonal antibodies because they express uniform affinity and specificity against a single epitope or antigenic determinant and can be produced in vast quantities. Both polyclonal and monoclonal antibodies may require further purification steps to enhance the sensitivity and reduce backgrounds in assays. The specificity of the antibodies must be checked carefully to elucidate any cross-reactivity with similar substances, which might cause false positive results. Immunoassays are utilising the specific binding of the antibody to the antigen. Thus, the availability of antibodies with the desired affinity and specificity is the most important factor for setting up test systems. The reaction between the antigen and antibody is detected through a second antibody preferably reacting with another epitope on the antigen. The second antibody carries a label that can be detected or can generate a detectable signal

ELISA (Enzyme Linked Immunosorbent Assay).

In ELISA the antigen-antibody reaction takes place on a solid phase (microtiter plates). The antibodies raised against the genetically modified protein component of LMO are bound to each well of a ELISA plate. Antigen and antibody react and produce a stable complex, which can be visualised by addition of a second antibody linked to an enzyme. Addition of a substrate for that enzyme results in a colour formation, which can be measured photometrically or recognised by eye

It is important to remember that ELISA and lateral flow sticks are trait-specific and thus cannot identify a LMO where several varieties may have the same trait incorporated. Therefore, the immunoassays in general can be considered as screening methods. Since the same target protein can be found in different LMOs, antibody-based assays may not be discriminating (for example, the maize varieties Bt-176, BtII and Mon810 contain the same Cry protein). Thus, at present, PCR based methods are the methods, which allow the most precise LMO identification and have the highest sensitivity, in terms of detection limits.

A list of useful websites for detect of GMOs is listed below

Table 6: List of websites useful for detection of GMOs

S.No	Source	Website
1	The Biosafety Clearing-House(BCH)	http://bch.cbd.int/
2	Centre for Environmental Risk Assessment (CERA) database of safety information	http://www.cera-gmc.org/?action=gm_crop_database
3	BioTrack Product Database.	http://www2.oecd.org/biotech/
4	International Service for the Acquisition of Agri-biotech Applications (ISAAA)	http://www.isaaa.org/
5	United States Regulatory Agencies Unified Biotechnology Website.	http://usbiotechreg.nbii.gov/
6	European Union (EU) GM food and feed	http://ec.europa.eu/food/food/biotechnology/gmfood/index_en.htm
7	European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF)	http://gmo-crl.jrc.ec.europa.eu/
8	GMO register	http://gmoinfo.jrc.ec.europa.eu/
9	GMO Compass	http://www.gmo-compass.org/eng/gmo/db/
10	Codex AlimentariusCodex Committee on Food Labeling (CCFL) Codex Committee on Methods of Analysis and Sampling (CCMAS).	http://www.codexalimentarius.net/web/index_en.jsp
Detection Method Databases		
11	Compendium of reference methods for GMO analysis	http://ihcp.jrc.ec.europa.eu/our_activities/gmo/gmo_analysis/compendiumreference-methods-gmo-analysis
12	GMOMETHODS.	http://gmo-crl.jrc.ec.europa.eu/
13	Crop Life International Detection Methods Database.	http://www.detection-methods.com/
14	Chinese GMO Detection Method Database (GMDD)	http://gmdd.shgmo.org/

The limitations of current detection methods

The limits of detection and quantification are method specific but also depend on the sample that is being analysed. We can distinguish between three types of detection and quantification limits: 1) the absolute limits, i.e. the lowest number of copies that must be present at the beginning of the first cycle to obtain a probability of at least 95% of detecting/quantifying correctly, 2) the relative limits, i.e. the lowest relative percentage of GM materials that can be detected/quantified under optimal conditions, and 3) the practical limits, i.e. the limits applicable to the sample that is being analysed (taking into consideration the actual contents of the DNA sample and the absolute limits of the method) methods that sometimes can be used to identify the LMO, 4) transformation event.

Validation of current detection methods

The need for powerful analytical methods for routine detection of LMOs by accredited laboratories has called attention to international validation and preparation of official and non-commercial guidelines. Among these guidelines are preparation of certified reference material (CRM,) sampling, treatment of samples, production of stringent analytical protocols, and extensive ring-trials for determination of the efficacy of selected LMO detection procedures. Validation of methods is the process of showing that the combined procedures of sample extraction, preparation, and analysis will yield acceptably accurate and reproducible results for a given analysis in a specified matrix. For validation of an analytical method, the testing objective must be defined and performance characteristics must be demonstrated. Performance characteristics include accuracy, extraction efficiency, precision, reproducibility, sensitivity, specificity, and robustness. The use of validated methods is important to assure acceptance of results produced by analytical laboratories. The validation must meet International standards such as ISO 5725

Uncertainties involved in GMO analysis has been well described in the ‘Guidance document on Measurement of Uncertainty (MU) for GMO analysis. This document gives guidance to GMO testing laboratories how to estimate the variability in the quantitative analytical results obtained by Real time PCR. In order to be able to

judge whether the analytical result exceeds the threshold the MU must be estimated and reported along with the result. The value obtained after subtracting the MU is used to see whether it falls within the legal specification. This guidance document is available at https://ec.europa.eu/jrc/sites/default/files/eur22756en.pdf

A compendium of reference methods for GMO analysis, 2010' has been produced jointly by the European Union Reference Laboratory for Genetically Modified Food and (EURL-GMFF) and the European Network of GMO Laboratories (ENGL). This compendium provides a state-of-the-art of the detection methods applied n GMO analysis. All these methods have been validated according to International standards ISO 5725 and /or IUPAC protocol. This compendium is available at http://ucbiotech.org/resources/methods/jrc_reference_report_2010_11_gmo_analysis_compendium.pdf

Further reading

1. Buh Gasparic M, Tengs T, La Paz J, et al (2010) Comparison of nine different real-time PCR chemistries for qualitative and quantitative applications in GMO detection. Anal Bioanal Chem 396:2023–2029.
2. Food Control (1999), Volume 10 (6) Special Issue' Detection Methods For Novel Food Derived From Genetically Modified Organism
3. GMO Detection Methods and Validation
4. **GMO M**ethods: **The European Union Database of Reference Methods for GMO Analysis"**, Bonfini, Laura; van den Bulcke, Marc H.; Mazzara, Marco; Ben, Enrico; Patak, Alexandre Journal of AOAC International, Volume 95, Number 6, November-December 2012 , pp. 1713-1719(7).
5. Holst-Jensen A (2007) Sampling, detection, identification and quantification of genetically modified organisms (GMOs). In: Pico Y (ed) Food Toxicants Analysis. Techniques, Strategies and Developments. Elsevier, Amsterdam, Netherlands.
6. Holst-Jensen A (2009) Testing for genetically modified organisms (GMOs): Past, present and future perspectives. Biotechnol Adv 27:1071–1082.
7. How to reliably test for GMOs www.springer.com/cda/content/.../cda.../9781461413899-c1.pdf?
8. http://www.vetinst.no/Qpcrgmofood/otherPubs.htm

9. International Organization for Standardization (1994) ISO 5725:1994. Accuracy (trueness and precision) of measurement methods and results. International Organization for Standardization, Geneva, Switzerland.
10. International Organization for Standardization (2005a) ISO 21569:2005 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Qualitative nucleic acid based methods . International Organization for Standardization, Geneva, Switzerland.
11. International Organization for Standardization (2005b) ISO 21570:2005 Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Quantitative nucleic acid based methods. International Organization for Standardization, Geneva,Switzerland.
12. International Organization for Standardization (2005c) ISO 21571:2005. Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction. International Organization for Standardization, Geneva, Switzerland.
13. International Organization for Standardization (2005d) ISO/IEC 17025 - General requirements for the competence of testing and calibration laboratories. International Organization for Standardization, Geneva, Switzerland
14. International Organization for Standardization (2006) ISO 24276:2006. Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions. International Organization for Standardization, Geneva, Switzerland.
15. Thompson M, Ellison SLR, Wood R (2002) Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis (IUPAC Technical Report). Pure Appl Chem 74:835–855.
16. Trapmann S, Burns M, Broll H, et al (2009) Guidance Document on Measurement Uncertainty for GMO Testing Laboratories. v2. Luxembourg: European Communities, 2009; Report No.: Report EUR 22756 EN/2.
17. Waiblinger HU, Grohmann L, Mankertz J, et al (2010) A practical approach to screen for authorised and unauthorised genetically modified plants. Anal Bioanal Chem 396:2065–2072.
18. Weighardt F (2006) Quantitative PCR for the detection of GMOs. In: Querci M, Jermini.M, Van den Eede G (eds) Training course on the Analysis of Food Samples for the Presence of Genetically Modified Organisms. 2006 th edn.

Luxembourg: Office for Official Publications of the European Communities, Luxembourg, Luxembourg.

CHAPTER 9
INFORMATION SOURCES

I. International Websites

Cartagena Protocol on Biosafety (CPB)

This is the main site for information about the CPB, including the background and full text of the agreement, articles, updated list of signatures and ratifications, meetings, documents, the Biosafety Clearing House and various databases such as capacity-building activities etc. The governing body of the Protocol is the Conference of the Parties (COP) to the Convention serving as the Meeting of the Parties (MOP) to the Protocol. The main function of this body is to review the implementation of the Protocol and make decisions or provide necessary guidance to promote its effective operation.

Biosafety Clearing-House

As per the article 20 of the Cartagena Protocol on Biosafety, the Biosafety Clearing House (BCH) has been established which serves as an information exchange mechanism to assist Parties to implement its provisions and to facilitate sharing of information and experiences with Living Modified Organisms (LMOs). It provides a “one – stop shop” where users can readily access or contribute relevant biosafety related information with an objective to assist governments to make informed decisions regarding the importation or release of LMOs. The BCH also facilitates scientific and technical cooperation between parties and stakeholders by allowing interested stakeholders to access or contribute information on existing biosafety capacity building activities, thus facilitating coordination and synergy between various initiatives. For industry and other stakeholders the BCH allows easy access to information vital to their activities including details of the national contacts, relevant laws and regulations governing LMO activities and the decisions and declarations made by Parties, especially regard to transboundary movements.

The central portal of BCH is at http://bch.biodiv.org. All the Parties to the Protocol are

expected to participate in BCH either by providing information through the central portal or setting up their own websites linked to the central portal.

OECD - BioTrack

BioTrack Online, the web site of OECD's Programme on the Harmonization of Regulatory Oversight in Biotechnology, was created in 1995. This site focuses on information related to the regulatory oversight of products of biotechnology. BioTrack Online aims, not only to help member country governments and industries with biotechnology product notifications/assessments, but also to make the information, developed by the Working Group on Harmonization of Regulatory Oversight in Biotechnology, accessible to all who need it, including non-OECD countries that might find it useful.

The central portal of BCH is at http://bch.biodiv.org. All the Parties to the Protocol are expected to participate in BCH either by providing information through the central portal or setting up their own websites linked to the central portal.

Food and Agriculture Organization (FAO)

The Food and Agriculture Organization (FAO) of the United Nations has the mandate to raise levels of nutrition, improve agricultural productivity, better the lives of rural populations and contribute to the growth of the world economy. As a knowledge organization, FAO creates and shares critical information about food, agriculture and natural resources in the form of global public goods. But this is not a one-way flow. FAO plays a connector role, through identifying and working with different partners with established expertise, and facilitating a dialogue between those who have the knowledge and those who need it.

During the period 2002-10, FAO had undertaken an intense activity of biosafety capacity development, centered largely on enhancing the capacities of regulators and others involved in the implementation of biosafety frameworks, along with other components. A Biosafety Resource Book was prepared to serve as reference material for biosafety regulators, policy-makers and members of national biosafety

committees that can be readily consulted beyond the training events, when the need arises.

World Health Organization (WHO) – Biotech Foods

WHO has been addressing a wide range of issues in the field of biotechnology and human health, including safety evaluation of vaccines produced using biotechnology, human cloning, and gene therapy. This site briefly describes the activities of WHO in regard to biotechnology and food safety.

Codex Alimentarius Commission (CAC)

The Codex Alimentarius Commission (CAC), established jointly by FAO and WHO in 1963 develops harmonized international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair trade practices in the food trade. The Codex standards are based on the best available science assisted by independent international risk assessment bodies or ad-hoc consultations organized by FAO/WHO. The Ad Hoc Intergovernmental Task Force on Foods derived from Biotechnology that was convened in 2000 with the objective to develop standards and guidelines for GM foods summarized their work in two documents namely Principles for the risk analysis of foods derived from modern biotechnology and Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.

II. Regulatory Websites in Other Countries

ARGENTINA

National Advisory Commission on Agricultural Biosafety (CONABia)

CONABia under the Ministry of Agriculture is responsible for harmonizing policies relating to biosafety. It is a multidisciplinary advisory group that overlooks the regulation of products of agricultural biotechnology and also evaluates the scientific and technical issues of environmental release of GM crops.

Association of Southeast Asian Nations (ASEAN)

The Association of Southeast Asian Nations (ASEAN) countries forms of the world's largest mega biodiversity centre. The ASEAN overlooks the biosafety policy formulation and implementation across member countries i.e. Cambodia, Myanmar, Thailand, Singapore, Philippines, Vietnam, Bruni Darussalam etc.

AUSTRALIA

The regulatory framework of Australia involves the Office of the Gene Technology Regulator, Food Standards Australia New Zealand and Australian Government Department of Agriculture, Fisheries and Forestry.

Office of the Gene Technology Regulator (OGTR)

The OGTR is the competent authority for implementing the legislation of the Gene Technology Act, 2000 that regulates all dealings with live and viable GMOs in Australia, including research, manufacture, import, production, propagation, transport and disposal of GMOs. The OGTR maintains a record of GMO and Gm product dealings on its website. The complete list of all GMO dealings approved by the Regulator and of all GM product approvals notified to the Regulator by other product regulators are available at OGTR.

Australian Government Department of Agriculture, Fisheries and Forestry (DAFF)

The Australian Quarantine and Inspection Service (AQIS), that forms part of DAFF functions in regulation of imports for quarantine purposes, inspection and certification of exported primary products consistent with the importing country's quarantine requirements and Australian export legislation and imported food inspection to meet Australian food standards.

Food Standard Australian and New Zealand (FSANZ)

FSANZ is a bi-national Government agency that carries out safety assessments on a case-by-case basis, which means each new genetic modification is assessed individually for its potential impact on the safety of food developed and food standards that cover the food industry in Australia and New Zealand. It also administers the Australia New Zealand Food Standards Code, which regulates the use of ingredients, processing aids, colorings etc and covers composition of some foods e.g. dairy, meat and beverages as well as standards developed by new technologies such as genetically modified foods. FSANZ is responsible for labeling both packaged and unpackaged food, including specific mandatory warnings or advisory label.

BRAZIL

National Technical Commission on Biosafety (CTNBio)

CTNBio under the Ministry of the Science and Technology has regulatory duties related to biotechnology and biosafety. CTNBio is responsible for the safe implementation of the research and development in biotechnology and for submission of applications to the National Committee. It shall monitor and notify the National Committee and competent authorities in case of any harm posed to people or to the environment.

CANADA

In Canada, the Canadian Food Inspection Agency and Health Canada share responsibility for regulating novel agricultural products. The federal government regulations ensure the practical benefits of biotech products in a way that protects health, safety and the environment.

Canadian Food Inspection Agency (CFIA)

CFIA is responsible for assessing the safety of plants with novel traits, animal feeds and animal feed ingredients, fertilizers and veterinary biologics. CFIA along with Health Canada shares responsibility for regulating products derived from biotechnology.

Health Canada

Health Canada is responsible for assessing the human health safety of products derived through biotechnology including foods, drugs, cosmetics, medical devices and pest control products.

Environment Canada

Environment Canada is a science-based department. It establishes and monitors biotech products other than agriculture that are regulated in Canada. It provides the science and technology information needed for making informed decisions about the environment.

EUROPEAN UNION (EU)

European Food Safety Authority (EFSA)

The European Food Safety Authority (EFSA) of European Union (EU) overlooks the risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks.

The Directive 2001/18/EC of the EU contains part B relevant to release for research and development which is used for conduct of experimental filed trials on GM crops and part C relevant to release for placing a GM product on the market require consent.

USA

Three federal agencies of USA the United States Department of Agriculture, Environmental Protection Agency and Food and Drug Administration share primary responsibility for regulating biotechnology in the United States.

United States Department of Agriculture (USDA)

The USDA reviews biotechnology derived applications, which contain or are produced using potential plant pests. It also regulates veterinary biologics, which are products derived from living sources, such as blood products and vaccines, and is largely responsible for assuring the safety of meat and poultry products.

Environmental Protection Agency (EPA)

The Environmental Protection Agency regulates biotechnology-derived plant or microbial pesticides or new chemical substances.

U.S. Food and Drug Administration (FDA)

The FDA regulates foods and feed derived from new plant varieties (GMOs) as well as conventional products. The biotechnology site carries extensive documentation on regulations, labeling, consumer information, and products approved for commercial sale.

UGANADA

Uganda National Council for Science and Technology (UNCST)

Uganda National Council for Science and Technology (UNCST), under the Ministry of Finance Planning and Economic Development coordinates the biosafety regulatory system in Uganda. The Council is mandated to facilitate and coordinate the development and implementation of policies and strategies for integrating

Science and Technology (S&T) into the national development process. The council established the National Biotechnology Committee (NBC) with the mandate to provide technical advice on biosafety issues, including the assessment of individual applications for activities with GE organisms.

GENERAL INFORMATION

AgBioWorld

This site provides information about technological advances in agriculture to the developing world; particularly to teachers, scientists, journalists, and the general public on the relevance of agricultural biotechnology to sustainable development; maintains the declaration of "Scientists In Support Of Agricultural Biotechnology," and offers a discussion list serve.

African Biosafety Network of Expertise (ABNE)

The African Biosafety Network of Expertise (ABNE) is an Africa-based, Africa-led initiative established by the AU/NEPAD's Office of Science and Technology. ABNE is a continent-wide service network that was officially approved in 2008 by the African Ministerial Council on Science and Technology (AMCOST) to promote advancement of science and technology for agricultural development in Africa. ABNE biosafety services aim to empower African regulators with science-based information, targeting the members of National Biosafety Committees (NBCs), Institutional Biosafety Committees (IBCs), and Plant Quarantine Officers (PQs) so that they can make informed decisions on biotechnology products.

Centre for Environmental Risk Assessment (CERA)

The database by Centre for Environmental Risk Assessment (CERA) on safety information (formerly hosted by AGBIOS) is an excellent source of information on safety assessment studies reviewed by regulatory authorities for approval of genetically engineered plants. The database includes not only plants produced using rDNA technologies, but also plants with novel traits that may have been produced

using more traditional methods, such as accelerated mutagenesis or plant breeding. These latter plants are only regulated in Canada.

GMO Compass

This database contains information about every genetically modified plant that has been approved or is awaiting approval in the EU. Information on the food and feed produced from the respective GM plant is also available.

International Life Sciences Institute (ILSI)

ILSI is a nonprofit, worldwide scientific research foundation seeking to improve the well being of the general public through the pursuit of sound and balanced science. It works towards understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. This site lists ILSI publications pertaining to biotechnology.

International Centre for Genetic Engineering and Biotechnology (ICGEB)

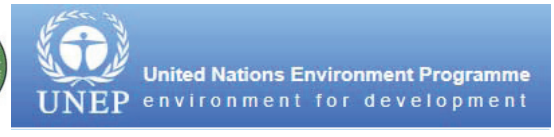
The role of the International Centre for Genetic Engineering and Biotechnology (ICGEB) is to promote the safe use of biotechnology world-wide, with special regards to the need of the developing world. It has played, since its inception, an important role on issues related to biosafety and the sustainable use of biotechnology.

The Biosafety Unit of the ICGEB is dedicated to biosafety and risk assessment for the environmental release of genetically modified organisms. It offers information on biosafety concerns, upcoming meetings and training courses, and a regularly updated index of selected scientific articles published on biosafety and risk assessment from 1990 onward. This site also carries an outstanding collection of links to databases on GMO releases, scientific bibliographies, decision support systems, patents, and numerous other topics.

International Service for the Acquisition of Agri-biotech Applications (ISAAA)

The International Service for the Acquisition of Agri-biotech Applications. ISAAA's objectives are the transfer and delivery of appropriate biotechnology applications to developing countries and the building of partnerships between institutions in the South and the private sector in the North, and by strengthening South-South collaboration.

The primary site describes ISAAA's activities and initiatives in biosafety, food safety, intellectual property, and technology transfer. The Global Knowledge Center on Crop Biotechnology section (http://www.isaaa.org/activities/ knowledge_center.htm) is organized into several main areas. Global Network provides a status of biotechnology in the developing countries of Asia, South America, and Africa. Crop Biotech Update is a weekly summary of world developments in agricultural biotechnology for developing countries. Separate pages cover GM products and biotechnology issues.



NATIONAL BIOSAFETY FRAMEWORK PROJECT
BHUTAN AGRICULTURE AND FOOD REGULATORY AUTHORITY
MINISTRY OF AGRICULTURE AND FORESTS
Thimphu, Bhutan
Post Box 1071

PABX – 975-2-327031/325790/325993;
Fax No. 975 – 2 - 327032/335540;
bafraheadoffice@gmail.com
Edition 1, 2014

Website: www.bafra.gov.bt