






Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues

Food and Agriculture Organization of the United Nations
Rome 2009

Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues

Proceedings of the Biotechnology and Biosafety Workshop held
in Gazipur, November 21-30, 2008,
under the FAO TCP/BGD/3102 Project:
"Assistance in the formulation of enabling regulatory measures
for research and sustainable application of biotechnology"
being implemented jointly by FAO and BARC

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Food and Agriculture Organization of the United Nations
Rome 2009

ISBN 978-92-5-106447-4

Correct citation of articles from this publication
Author name, initials. 2009. Title of article. *In:* Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Chowdhury MKA, Hoque MI and Sonnino A (Eds.). pp. .. .. .. © FAO 2009.

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Foreword

Agriculture is the primary base of livelihood and economy in Bangladesh. It relies mostly on rural populations, which represent 74 per cent of more than a 140 million total, and employs about 55 per cent of the economically actives of the country. Over the last forty years, Bangladesh has been part of the successful story of the Green Revolution, particularly with regard to paddy rice (more than 95 percent of all cereals produced) and wheat production, which both increased, respectively from 14.4 to 39.0 million tonnes, and from 0.03 to 1.90 million tonnes.

However, while the total cereals production was increasing by more than 2.5 times fold, the population was rather tripling, from 53 to 140 million people during the same period. Its technology development kept being confined to rice and wheat, while rain-fed agriculture and several important commodities (pulses, jute, vegetables, fruit, oilseeds and livestock) were left almost unattended. Similarly, technologies for integrated management of soil, water and nutrients, and of pests and diseases geared towards sustainable agriculture were not adequately investigated.

To worsen the whole situation, the productivity gains have been decelerating and the production resources, especially land, water, and genetic resources have been shrinking and degrading. In support of this, the total factor productivity for instance, which moved from -3.2 during the period of 1961-1981 to 1.1 during the one of 1981-2000, remains relatively low. Moreover, the biotic and abiotic stresses, as well as natural disasters have intensified, and inequities and technological divides are widening.

If Bangladesh is to face most of these challenges in the years ahead, agriculture will be required to improve its productivity and produce a more diversified food basket, with greater shares of meat, fish, milk, fruits, and vegetables. It can do so only through further systematic intensification of the use of land, water, and labour; and by properly addressing environmental protection issues, consumer concerns of food safety and quality, and the overall enhancement of rural livelihoods.

To achieve this, biotechnologies, which are endowed with high precision and pace of genetic alchemy of crops, livestock, fish and other aquatic species, forest species and agriculturally important microbes, appear to be a viable option to complement all the efforts actively pursued until now. This explains why the National Agriculture Policy (NAP) has emphasized the role of biotechnology in enhancing and sustaining agricultural production and the will of the Government to take advantage of its potentials. As a matter of fact, the NAP has identified it, along with biodiversity, as a priority area and its promotion as one of its 18 immediate objectives.

As a first move into this option, the Government of Bangladesh, UNDP and FAO embarked on the analysis of the status and prospect of the utilization of biotechnologies, especially modern biotechnology, for sustained and enhanced agricultural production and productivity with the project SPPD BGD/02/005/A/08/12 "**Assessment of Utilization and Potential of Biotechnological Advancement for Agriculture Development in Bangladesh**", completed in 2003. Based on the results of this project, a programme ready to be financed entitled "Biotechnological Advancement for Agricultural Development in Bangladesh " (BAADEB) was formulated by the Government.

This programme aimed to judiciously harness biotechnologies for enhanced agricultural productivity and sustainability towards poverty alleviation and food security. Its long-term vision was to promote biotechnologies for ushering in an evergreen revolution through strengthening national capacity in policy, planning, governance, priority setting, undertaking need-based research and technology development and by establishing research-extension-farmer-consumer-market linkages and public-private-NGO-CSO partnerships. It comprised six interdependent and iterative, yet stand alone, sub-programmes: (i) national policy, strategy and governance, (ii) enabling regulatory measures, (iii) institutional support and strengthening, (iv) biotechnological

interventions for food security and poverty alleviation, (v) human resources development, and (vi) awareness raising and information empowerment.

To ensure the effective implementation of this programme, it was necessary that some special attention be paid to the sub-programme dealing with regulatory measures for two reasons at least. Firstly, specific regulatory measures are inherent to biotechnology development. The "Biotechnology Revolution" most commonly known today as the "Gene Revolution" following concerns that have been constantly raised on some of its products, particularly genetically modified organisms (GMO), has brought along the need for interested countries, to develop preventive measures with regard to environment and public health protection. This need is even more acute in developing countries.

Secondly, without setting up a sound regulatory regime, the country will not fully benefit from biotechnology applications; more specifically, it will not have capacities to absorb imported biotechnologies, and will not be capable of conducting any significant research to develop appropriate biotechnologies suitable to its needs. The technical Cooperation Project TCP/BGD/3102 (D) "Assistance in the formulation of enabling regulatory measures for research and sustainable application of biotechnology" was therefore formulated and launched in May 2008.

Under this TCP, a training course of ten working days for 40 research and technical officers was organized during November 21 – 30, 2008. During this training course the following five modules, namely, agricultural biotechnology; ecological aspects of biosafety; biosafety guidelines including risk analysis; post-release monitoring; post-release monitoring; legal aspects, including plant variety protection of two days each were presented and discussed.

Five experts of the Technical Cooperation among Developing Countries (TCDC) programme were recruited to conduct this workshop as the Resource Persons. They prepared training materials consulting the relevant scientific literature and available training tools and by integrating specific themes adapted to the local environment conditions for the training workshop. During the training course participants were engaged in group exercise for various case studies and presented their outcomes.

After the workshop the TCDC experts submitted detailed individual reports on their respective topics which were reviewed by Lead Technical Officer, National Project Director and National Lead Consultant and recommended for its printing. I would like to congratulate both TCDC experts and National Project Management Unit for their efforts to write and compile the whole document to be printed as a book form. Besides the articles written by the TCDC experts, one article has been included on the Status of Relevant Laws and Regulations on Biotechnology in Bangladesh compiled by Liaquat A. Siddiqui, the Legal Consultant of this TCP/BGD/3102 project.

I hope that this valuable document will be useful for the researchers, extension workers, policymakers, students as a reference book. I once again would like to thank my colleagues at Project Management Unit for taking this initiative to develop this valuable document.

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In: Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Chowdhury MKA, Hoque MI and Sonnino A (Eds.). pp. 1-50. © FAO 2009.

Chapter 1: Agricultural Biotechnology

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Introduction

Modern biotechnology, including genetic modification and the production of genetically modified organisms, if properly integrated with other technologies, provides powerful tools for the sustainable development of agriculture, fisheries and forestry, as well as meeting the food needs of an expanding and increasingly urbanized population. These tools cover plant improvement to raise and stabilize yields, to improve resistance to pests, diseases and abiotic stresses such as drought and cold and to enhance the nutritional content of foods. Biotechnology is also being used to develop low-cost disease-free planting materials for crops such as cassava, banana and potato and is creating new tools for the diagnosis and treatment of plant and animal diseases and for the characterization and conservation of genetic resources. Animal feeds and feeding practices are also being changed to improve animal nutrition and to reduce environmental waste.

However, with the portfolio of modern biotechnology applications increasing, there is a crucial need to ensure these tools are used judiciously, and that the progress does not overlook potential risks to human health and the environment. This calls for an objective, science-based evaluation system for determining the benefits and risks of each biotechnology application on a case-by-case basis, and for addressing legitimate concerns for the biosafety of each product or process prior to its release. This includes i) evaluating the possible effects on biodiversity, the environment and food safety, ii) weighing the benefits of the product or process against its assessed risks, iii) monitoring the post-release effects of these products and processes to ensure their continued safety. Such an evaluation must take into consideration the experiences of national regulatory authorities in clearing such products.

This module on agricultural biotechnology, targeting regulators and administrators, reviews the very basic scientific concepts and principles employed in producing GMOs, with specific emphasis on the following key areas:

- Basic concepts of biotechnology
- Genes:- structure and formation
- Promoters, vectors and transformation cassettes
- Plant transformation and selection techniques
- Biotechnology for the improvement of animal breeding
- Genetic engineering of microorganisms of interest to agriculture
- Detection methods for Genetically Modified Organisms

Minimal familiarity with these concepts and principles is critical in ensuring pro-active participation to the process of reviewing dossiers and taking part in decision-making.

Introduction to Biotechnology: Basic Concepts and Definitions

Definition of Biotechnology

The term biotechnology was coined in 1919 by Karl Ereky, a Hungarian engineer. At that time, the term meant all the lines of work by which products are produced from raw materials with the aid of living organisms. Ereky envisioned a biochemical age similar to the stone and iron ages. Now biotechnology is broadly defined as the application of scientific and engineering principles to processing of substances by biological agents to provide goods and services. In this definition, the biological agents are mainly microorganisms, animal and plant cells and enzymes. The substances referred to are renewable materials as well as those produced by the biological agents. The goods and services are products of industries concerned with food, beverages, pharmaceuticals and biomedicals. This definition is applicable to both 'traditional or old' and 'new' or 'modern' biotechnology.

Long before the term "biotechnology" was coined for the process of using living organisms to produce improved commodities, people were utilizing living microorganisms to produce valuable products through the fermentation process. A list of early biotechnology applications follows below in Table 1.

Table 1. Traditional biotechnology application.

Providing bread with leaven	Prehistoric period
Fermentation of juices to alcoholic beverages	Prehistoric period
Knowledge of vinegar formation from fermented juices	Prehistoric period
Cultivation of grapes	Before 2000 BC
Manufacture of beer in Babylonia and Egypt	3rd century BC
Wine manufacturing promoted in Roman Empire	3rd century AD
Production of spirits of wine (ethanol)	1150
Vinegar manufacturing industry	14th century
Discovery of the fermentation properties of yeast properties	1818
Description of the lactic acid fermentation by Pasteur	1857
Detection of fermentation enzymes in yeast by Buchner	1897
Discovery of penicillin by Fleming	1928
Discovery of many other antibiotics	≈1945

Since then biotechnology has rapidly progressed and expanded. In the mid-forties, scale-up and commercial production of antibiotics such as penicillin occurred.

The techniques used were:

(a) isolation of an organism producing the chemical of interest using screening /selection procedures, and

(b) improvement of production yields via mutagenesis of the organism or optimization of media and fermentation conditions. This type of "antique" biotechnology is limited to chemicals produced in nature. It is also limited by its trial-and-error approach, and required a lengthy timeframe (years or even decades) for yield improvement.

About two decades ago, biotechnology became much more of a science (rather than an art). Regions of DNA (called genes) were found to contain information that would lead to synthesis of specific proteins. Each of these proteins have their own identity and function, many catalyze (facilitate) chemical reactions, and others are structural components of entities in cells. If one now is able to express a natural gene in simple bacteria such as *Escherichia coli (E. coli)*, a bacterium living in intestines that has become the model organism for much of biotechnology, one can have this bacterium make a lot of the protein coded for by the gene, regardless of its source. The techniques used for this development include:

(a) isolation of the gene coding for a protein of interest,

(b) cloning of this gene into an appropriate production host, and

(c) improving expression by using better promoters, tighter regulation, etc, together these techniques are known as *recombinant DNA techniques.* These will be discussed at some length in the course.

The commercial implications are that a large number of proteins, existing only in tiny quantities in nature, can now be mass-produced if needed. Also, the yields of biochemicals to be produced can be increased much faster than was possible with classical fermentation. As this approach leads to release of genetically altered organisms into the environment, this part of biotechnology is quite strictly regulated at government levels. The main thrust of this entire course is on the development and implementation of such regulatory frameworks at country level.

About a decade ago, "protein engineering" became possible as an offshoot of the recombinant DNA technology. Protein engineering differs from "classical" biotechnology in that it is concerned with producing new (man-made) proteins which have been modified or improved in some way. The techniques involved in protein engineering are more complicated than before, and involve:

(a) various types of mutagenesis (to cause changes in specific locations or regions of a gene to produce a new gene product)

(b) expression of the new gene to form a stable protein

(c) characterization of the structure and function of the protein produced and

(d) selection of new locations or regions to modify as a result of this characterization

Biotechnology applications are driven by a collection of multidisciplinary fields of activities, commonly referred to as *enabling technologies*. Apart from fermentation and genetic engineering/recombinant DNA technology, other important enabling technologies are plant and animal cell culture technology and enzyme technology. The basis of these enabling technologies are the core disciplines of molecular biology, genetics, microbiology, biochemistry, protein chemistry, chemical and process engineering and computer science.

Table 2. An overview of recombinant DNA based biotechnology.

Double helix structure of DNA is first described by Watson and Crick	1953
Cohen and Boyer develop genetic engineering	1973
The first human protein (somatostatin) is produced in a bacterium (E. coli)	1977
The first recombinant protein (human insulin) appears on the market	1982
Polymerase chain reaction (PCR) technique conceived	1983
Lauch of the Human Genome	1990
The first genome sequence of an organism *(Haemophilus influenzae*) is determined	1995
A first draft of the human genome sequence is completed	2000
Over 40 million gene sequences are in GenBank, and genome sequences of hundreds of prokaryotes and dozens of eukaryotes are finished or in draft stage	2005

Overview of Applications of Biotechnology

Since the discovery of recombinant DNA technology, new techniques and applications have been developed that are benefiting mankind in the areas of agriculture, medicine, environment, industry and forensics. The following sections briefly describe some of these applications and their benefits to society.

Industry

Biotechnology is used to develop alternative fuels. Maize starch is converted by yeast into ethanol, which is used to produce gasohol (a gasoline-ethanol mix). Bacteria are also used to decompose sludge and landfill wastes. Through biotechnology, microbes or their enzymes are used to convert biomass into feed stocks, which are used for manufacturing biodegradable plastics (bioplastics) in their tissues. Organisms (microbes and mammals) are used as pharmaceutical factories for producing chemical compounds that are extracted from their products and processed as drugs and other products. Plant and animal fibers are used in making a variety of fabrics, threads, and cordage. Biotechnology is used to improve the quality and quantity of these products. Biopulping is a technique whereby a fungus is used to convert wood chips into pulp for papermaking.

Health/medicine

In the area of health/medicine, biotechnology is used to develop diagnostic tools for identifying heritable diseases. The results of such diagnoses are used in genetic counseling to aid in making informed choices by parents who are predisposed to the birth of children with genetic abnormalities. Diagnostic tools for pregnancy tests, as well as other tests, have also been developed for early detection. Biotechnology is used to produce more effective and efficient vaccines, antibiotics, and other therapeutics. The famous drug penicillin, is a microbial product. Biotechnology is a $30 billion a year industry that has produced some 160 drugs and vaccines. Furthermore, there are more than 370 biotech drug products and vaccines currently in clinical trials targeting more than 200 diseases, including various cancers, Alzheimer's disease, heart disease, diabetes, multiple sclerosis, AIDS and arthritis.

Through the biotechnology of gene therapy, scientists are making attempts at curing genetic diseases by attempting to replace defective genes with healthy ones. A revolutionary strategy is being developed whereby staple foods such as potatoes, bananas, and others are used as delivery vehicles to facilitate the immunization of people in economically depressed regions of the world.

Environment

Developing and using alternative fuels that burn cleaner improves air quality through reduced pollution of the environment. Microbes are used to decompose and clean up contaminated sites by the technology of bioremediation. The use of disease resistant cultivars makes crop production less environmentally intrusive by reducing the use of agrochemicals.

Forensics

Because of the uniqueness or individuality of any individual, the DNA profile or characteristic pattern of nucleotide distribution is used as a powerful basis of identification of individuals in a population. DNA evidence is used in cases involving paternity disputes and family relationships. The application is used in health care and judiciary systems. In health care it is used to diagnose hereditary diseases to predict the chance of an individual inheriting a disease from an affected parent. It can also be used to detect the predisposition of an individual to a cancer, or chromosomal aberrations. In the judicial system, forensic experts use DNA profiling to identify suspects in criminal cases especially where body fluids and other particles like hair and skin samples can be retrieved. DNA profiling is also used in disputed family relations and immigration cases.

Agriculture

Biotechnology provides a more efficient means of crop and animal improvement. Instead of extensive mixing of genes, as occurs in conventional breeding, biotechnology enables targeted gene transfer to occur. The genome of the recipient individual remains intact, except for the introduced gene, thus accelerating breeding programs. Furthermore, biotechnology

enables gene transfer across natural barriers, breaking down mating barriers and creating a sort of "universal gene pool" or "universal breeding population" accessible to all organisms. Biotechnology is used to improve the yield of crop and animal products and their quality such as flavour and shelf life. In addition to these benefits, biotechnology reduces the need for agrochemicals through disease resistance breeding, thereby reducing environmental pollution from chemical runoff. Increased yields and higher food quality reduce world hunger and malnutrition. Molecular techniques are being used to monitor breeding populations and to diagnose animals and plants infected with diseases. Micropropagation techniques are being used widely to generate clonal materials. Rapid large scale clonal propagation of many plant species including trees is feasible through biotechonology.

Genes, Structure and Function

Genes and Heredity

The study of genes and heredity is called genetics. Heredity phenomena have been interesting to humans since before the dawn of civilization. Ancient people were improving plant crops and domesticated animals by selecting desirable individuals for breeding. Genetics as a set of principles and analytical procedures did not begin until 1860s when an Augustinian monk Gregor Mendel performed a set of experiments that pointed to the existence of biological "factors" responsible for transmitting traits from generation to generation. These factors were latter called genes following the discovery of chromosomes and subsequently linkage in the early twentieth century. Up to this point genetics looked at genes as abstract entities that somehow control hereditary traits. Through pure genetic analysis the inheritance of different genes was studied but the physical nature of the gene remained unknown. Further work revealed that chromosomes consist of DNA (deoxyribonucleic acid) and protein, consequently it was found that DNA was the hereditary material.

DNA was thought to be a simple molecule thus many scientists did not believe that it indeed carried/stored the information about an organism's features. How could such information be passes on from one generation to the next? Clearly, the genetic material must have both the ability to encode specific information and the capacity to duplicate that information precisely. What kind of structure could allow such complex functions in so simple a molecule?

The structure of DNA

Although the DNA structure was not known, its basic building blocks had been known for many years. It had been shown that DNA is composed of four basic molecules called nucleotides, which are identical except that each contains a different nitrogen base. Each nucleotide contains phosphate, sugar (of the deoxyribose type), and one of the four bases. The four bases are adenine, guanine (purines) and cytosine and thymine (pyrimidines).

Watson and Crink in 1953 were the first to succeed in putting the building blocks together and finding a reasonable DNA structure. They used DNA x-ray diffraction patterns produced by Rosalind Franklin and Maurice Wilkins and data from Erwin Chargaff. The X-ray data showed DNA molecule to be very long, thin and helical (spiral like) in shape. Chargaff had established certain empirical rules about the amounts of each component of DNA:

1. The total amount of pyrimidine nucleotides (T + C) always equals the total number of purine nucleotides (A + G).
2. The amount of T always equals the amount of A, and the amount of C always equals the amount of G. But the amount of A + T is not necessarily equal to the amount of G + C.

The structure that Watson and Crink derived from these clues is a double helix. Each helix is a chain of nuclotieds held together by phosphodiester bonds, in which a phosphate group forms a bridge between -OH groups on two adjacent sugar residues. The two chains (helices) are held together by hydrogen bonds. Each base pair consists of one purine and one pyrimidine base paired according to the following rule: G pairs with C, and A pairs T (*Refer to diagrams in the power point presentations*).

Elucidation of the structure of DNA caused a lot of excitement in genetics for two basic reasons. First, the structure suggests an obvious way in which the molecule can be duplicated, or replicated since each base can specify its complementary base by hydrogen bonding. Second, the structure suggests that perhaps the sequence of nucleotide pairs in DNA is dictating the sequence of amino acids in the protein encoded by that gene. In other words, some sort of genetic code may write information in DNA as a sequence of nucleotide pairs and then translate it into a different language of amino acid sequences in protein.

The flow of Genetic Information: The Central Dogma

In the early 1950's, Francis Crink suggested that there was a unidirectional flow of genetic information from DNA through RNA to protein, i.e. 'DNA makes RNA makes protein'. This became known as the central dogma of genetics, since it was proposed without much evidence for individual steps. Now these steps are known, DNA is transcribed to an RNA molecule (messenger RNA), that contains the same sequence information, then that message is translated into a protein sequence according to the genetic code.

The genetic Code

The genetic code is the correspondence between the sequence of the four bases in nucleic acids and the sequence of the 20 amino acids in proteins. In has been shown that the code is a triplet code, where three nucleotides (codon) encode one amino acid. Since there are only 20 amino acids to be specified and potentially 64 different triplets (4^3 =64), most amino acids are specified by more than one triplet and the genetic code is said to be degenerate, or to have redundancy. The genetic code has colinearity, this means that the order of the bases in the DNA corresponds directly to the order of amino acids in the protein. Clearly, if the genetic

code is going to be read like we read a sentence of a book, we need to know where to start and stop. As a start signal all proteins start with the amino acid methionine specified by the codon AUG. However, methionine is found in proteins in other places, not just the beginning. Therefore the translational machinery has to find the correct methionine to start and not just any in the sequence, thus the sequences surrounding the initiation AUG codon is important in the translation process. The end of the translated region is determined by one of three codons which basically encode 'stop'. These are UAA, UAG and UGA. If mutations take place in the DNA which create one of the stop codons instead of an amino acid encoding codon, the results may be catastrophic as the resultant protein will shorter than intended. Such proteins would be referred to as being truncated, and very likely to be non-functional. The region between the start methionine and the first stop codon is referred to as the open reading frame (ORF). Finally, the genetic code is virtually universal. Genes taken from plants can be decoded by animal cells, while genes from prokaryotes can be decoded by eukaryotic systems. Without such a universal nature to the code, genetic manipulation and genetic engineering would be much more difficult than it is.

The Gene defined

Historically, a gene is a heritable unit of phenotypic variation, but from a molecular standpoint, a gene is the linear collection of DNA sequences required to produce a functional RNA molecule, or a single transcriptional unit. Genes can be conveniently assigned to one of two broad functional categories: structural genes and regulatory genes. It is the role of the end product of these genes that distinguishes structural and regulatory genes.

1. *Structural genes* code for polypeptides or RNAs needed for the normal metabolic activities of the cell e.g. enzymes, structural proteins, and receptors.
2. *Regulatory genes* code for polypeptides that form proteins whose function is to control the expression of structural genes. With regard to makeup, these genes are like structural genes.

A gene usually occupies a particular location within the chromosome. This location is defined by specific sequences for the start and termination of its transcription. The gene has a specific effect on the organism's morphology or physiology, can be mutated (i.e. changed), and can recombine with other genes. It is a store of information (in the form of nucleotide base sequence), it does not initiate any action but is acted upon. The complete set of genes of an organism, what is called the genetic constitution of the organism, is its genotype. The physical manifestation or expression of the genotype is the phenotype (i.e. the cell's morphology and physiology). If a particular characteristic, such as brown eye color, is a part of an organism's phenotype that is, if it is expressed, it can be said that the individual has the gene for that characteristic. If, however, a particular characteristic is not expressed, one cannot conclude that the gene is absent because gene expression can be repressed.

Genes may be located on either strand of the double stranded DNA. But, regardless of which strand contains a particular gene, all genes are read in a 5' to 3' direction, and the strand containing the particular gene is referred to as the sense or coding strand.

The arrangement of Genes

In eukaryotic organisms each cell contains more than one DNA molecule packaged into individual chromosomes. Along the length of each DNA molecule/chromosome will be found thousands of genes, and although the spacing of the genes is usually apparently random. In bacteria, it is not unusual to have the need to express several genes that are not the same but are related, in that the proteins which are encoded by the genes are required along a common metabolic pathway. Therefore, as all the gene products are needed more or less simultaneously by the cell, it makes sense for the cell to have all such genes together and have a mechanism to express them together. These clusters of genes are known as operons. The most studied operon is the lactose operon in *E coli.* This operon contains three genes which are contagious on the DNA and are required for the utilization of lactose as a metabolic fuel in the cell. The operon also contains all the control sequences (repressor, promoter and operator) needed to ensure efficient expression of the genes together.

Operons do not occur in higher organisms but some similar genes are found in clusters. Many genes in eukaryotes have a distinctive structural feature. More specifically, the nucleotide sequences contain one or more intervening segments of DNA that do not code for the amino acid sequence of the protein product. These non-translated interrupt the otherwise co-linear relationship between the nucleotide sequence of the gene and the amino acid sequences of the protein it encodes. Such non-translated DNA segments in genes are called introns. The pieces that code for mature mRNA are referred to as exons. During transcriptions, the exons are spliced together from a larger precursor RNA that contains, in addition to exons, interspersed introns. The number of exons coding for a single mRNA molecule depends on the gene and the organism but can be a few as one or as many as 50 or more. The origin of intron/exon structure is thought to be extremely ancient and to predate the divergence of eukaryotes and prokaryotes. However, prokaryotes and small eukaryotes (e.g. yeast) have lost their introns during evolution, perhaps because of the strong selective pressure in the organisms to retain a small genome size.

In addition to the introns and exons, the structural features of the eukaryotic gene include regulatory elements, a promoter region, a transcription start site and a transcription termination site (Fig 2.1). Specific cell proteins in the nucleus can bind to the regulatory element sequences of the gene thus controlling the expression of the gene. The promoter region is the sequence of the gene where the specific transcription machinery (assembly of proteins required for transcription) binds to the DNA in order to start transcription. The start site indicates to the transcription machinery where to start and the termination site indicates were to stop transcription of the gene.

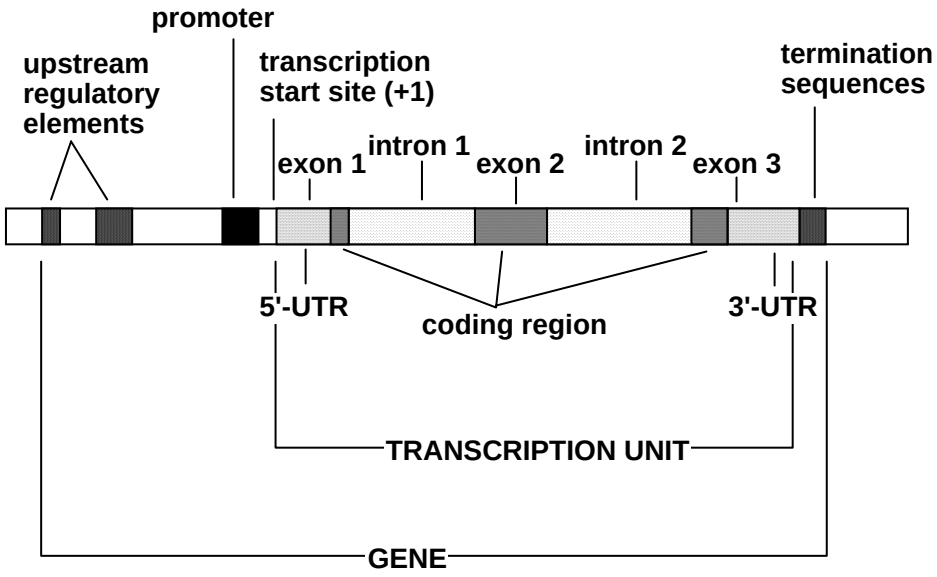

Fig. 2.1. General structural arrangement of the different components making up a eukaryotic gene

Parts of a Gene that are not Transcribed

Promoters

The promoter region of a gene is usually several hundred nucleotides long and immediately upstream from the transcription initiation site and it binds RNA polymerase. There are different types of promoters for different RNA polymerase. Promoters for RNA polymerase II, the polymerase that transcribes genes into mRNA, often contain a consensus sequence 5'-TATA-3', 30 to 50 bp upstream of the site at which transcription begins. Many eukaryotic promoters also have a CAAT box with a GGNCAATCT consensus sequence centered about -75 upstream of the initiation start site. These sequences bind a series of general transcription factors, relatively abundant proteins used to initiate the transcription of nearly all mRNAs. The general transcription factors then facilitate the binding and activation of RNA polymerase II into an activated transcription complex.

Enhancers

Enhancers were described originally as cis-acting sequences that increase transcriptional initiation but, unlike promoters, were not dependent on their orientation or the distance from the transcription start site. It is now apparent that enhancers are generally short (less than 20 to 30bp) sequences that bind specific transcription factors, which then facilitate the assembly of an activated transcriptional complex at the promoter. Most enhancers function whether on the coding or non-coding strand of the DNA (i.e. in either orientation), can act up to several thousand base pairs distant from their promoter target, and are a more general form of cis-

acting regulatory element. Most enhancers are active only in specific cell types and therefore play a central role in regulating tissue specificity of gene expression. Some regulatory elements bind transcription factors that act to reduce transcriptional initiation, and many genes contain a combination of both positive and negative upstream regulatory elements, which then act in concert on a single promoter. This allows gene expression to be controlled very precisely with regard to cell type, developmental stage and environmental conditions. Mutations of gene promoters or enhancers can alter the pattern of expression but not the structure of a particular gene product.

Operators

Operators are nucleotide sequences that lie between the promoter and the structural gene. They are the region of DNA to which repressor proteins bind and thereby prevent transcription. Repressor proteins have a very high affinity for operator sequences. Repression of transcription is accomplished by the repressor protein's attaching to the operator sequence downstream of the promoter sequence (the point of attachment of the RNA polymerase). The enzyme must pass the operator sequence to reach the structural gene's start site. The repressor protein bound to the operator physically prevents this passage, and, as a result, transcription by the polymerase cannot occur.

Attenuators

The attenuator sequences are found in bacterial gene clusters that code for enzymes involved in amino acid biosynthesis. Attenuators are located within so-called leader sequences, a unit of about 162 nucleotide pairs situated between the promoter-operator region and that first structural gene start site of the cluster. Attenuation has a 10 fold effect on transcription. As the level of an amino acid in the cell rises and falls, attenuation adjusts the level of transcription to accommodate the changing levels of the amino acid. High concentrations of the amino acid result in low levels of transcription of the structural genes, and low concentrations of the amino acid result in high levels of transcription. Attenuation proceeds independently of repression, the two phenomena are not dependent on each other. Attenuation results in the premature termination of transcription of the structural genes.

Parts of a Gene that are Transcribed but not Translated

Introns and Splice Junctions

In eukaryotic pre-mRNA processing, intervening sequences (introns) that interrupt the coding regions are removed (spliced out), and the two flanking exons are joined. This splicing reaction occurs in the nucleus and requires the intron to have a 5' –GU, an AG-3' and a branch point sequence. In a two step reaction, the intron is removed as a tailed circular molecule, or lariat, and is degraded. This splicing is directed by RNA-protein complexes known as snRNPs (small nuclear ribunucleoproteins). The snRNPs bind to the conserved sequences to form a spliceosome in which the cleavage and ligation reactions take place.

5' Untranslated Sequences

During the processing of precursor RNA in the nucleus, 3' termini as well as introns are removed. However, precursor RNA always begins with an exon, so that the initial sequences in mRNA are also the first to be synthesized in the precursor RNA. Shortly after initiation of mRNA transcription, a methylguanylate residue is added to the 5' end of the primary transcript. This 5' "cap" is a characteristic of every mRNA molecule, and the transcriptional start or initiation site is also referred to as the capping site. The 5' untranslated region extends from the capping site to the beginning of protein coding sequence and can be up to several hundred base pairs in length. The 5' untranslated regions of most mRNAs contain a consensus sequence of 5' –CGAGCCAUC-3 involved in the intiation of protein synthesis. In addition, some 5' untranslated regions contain "upstream AUGs" that may affect the initiation of protein synthesis and thus could serve to control expression of selected genes at the translational level.

3' Untranslated Sequences and Transcriptional Termination

The 3' end of a mature mRNA molecule is created by cleavage of the primary precursor RNA and the addition of a several hundred nucleotide polyadenylic acid tail. The site for cleavage is marked by the sequence 5' AAUAAA 3' some 15 to 20 nucleotides upstream and by additional uncharacterized sequences 10 to 30 nucleotides downstream. The region from the last protein codon to the polyA addition site may contain up to several hundred nucleotides of a 3' untranslated region, which includes signals that affect mRNA processing and stability. Many mRNAs that are known to have a short half life contain a 50 nucleotide AU rich sequence in the 3' untranslated region. Removal or alteration of this sequence prolongs the half life of mRNA, suggesting that the presence of AU rich sequences in the 3' untranslated region may be a general feature of genes that rapidly alter the level of their expression.

Gene Expression

Genes function through a process called gene expression, a process by which heritable information from a gene is made into a functional gene product, such as protein or RNA. Genes are expressed by being first transcribed into RNA, and may then be translated into protein

RNA

The ribonucleic acid (RNA) is also important in the flow of genetic information. Some viruses use RNA as genetic information. Other organisms that use DNA as the genetic material must first transcribe their genetic information into RNA for the information to be accessible or functional.

RNA is quite similar in structure to DNA. It is a long linear molecule (polymer) that is made up of a limited number of repeating monomers (nucleotides). Each nucleotide is

composed of a sugar, a phosphate, and a base. The sugar is ribose instead of deoxyribose as seen with DNA, hence its name. Unlike DNA, RNA molecules are usually single stranded. RNA molecules have the same bases as DNA except that the pyrimidine base thymine (T) is replaced by uracil (U).

The cell contains three kinds of RNA, namely Messenger RNA (mRNA), Transfer RNA (tRNA) and Ribosomal RNA (rRNA), these correspond to the three basic roles RNA plays in the cell. Firstly, it serves as the intermediate in the flow of information from DNA to protein. The DNA is transcribed (copied) into messenger RNA (mRNA) via an enzyme (RNA polymerase II) and then the mRNA is translated into the protein. Secondly, tRNA molecules serve as adaptors that translate the information in the nucleic acid sequence of mRNA into information designating the sequence of constituents (amino acids) that make up the protein. Finally, the rRNA molecules are important functional components for the molecular machinery (ribosomes) that carries out the translation process.

Transcription and Translation

In all organisms, there are two major steps separating a protein-coding gene from its protein first, the DNA on which the gene resides must be transcribed from DNA to messenger RNA (mRNA), and second, it must be translated from mRNA to protein. RNA-coding genes must still go through the first step, but are not translated into protein.

The first step in gene expression is the ***transcription***, namely the production of a single stranded RNA molecule known as messenger RNA. The nucleotide sequence of the mRNA is complementary to the DNA from which it was transcribed. In other words, the genetic messages encoded in DNA are copied precisely into RNA. The DNA strand whose sequence matches that of the RNA is known as the *coding strand* and the strand from which the RNA was synthesized is the *template strand.*

Transcription is performed by an enzyme called an RNA polymerase, which reads the template strand in the 3’ to 5’ direction and synthesizes the RNA from 5’ to 3’. To initiate transcription, the polymerase first recognizes and binds a promoter region of the gene. Thus a major mechanism of gene regulation is the blocking or sequestering of the promoter region, either by tight binding by repressor molecules that physically block the polymerase, or by organizing the DNA so that the promoter region is not accessible.

In eukaryotes, transcription occurs in the nucleus, where the cell’s DNA is sequestered, the RNA molecule produced by the polymerase is known as the primary transcript and must undergo post transcriptional modification before being exported to the cytoplasm for translation. The splicing of introns present within the transcribed region is a modification unique to eukaryotes. This is a major form of regulation in eukaryotic cells.

Translation is a process by which a mature mRNA molecule is used as a template for synthesizing a new protein. Translation is carried out by ribosomes, large complexes of RNA

and protein responsible for carrying out the chemical reactions to add new aminoacids to a growing polypeptide chain by the formation of peptide bonds.

The genetic code is read three nucleotides at a time, in units called codons, via interactions with specialized RNA molecules called transfer RNA (tRNA). Each tRNA has three unpaired bases known as the anticodon that are complementary to the codon it reads, the tRNA is also covlently attached to the amino acids specified by the complementary codon. When the tRNA binds to its complementary codon in an mRNA strand, the ribosome ligates its amino acid cargo to the new polypeptide chain, which is synthesized from amino terminus to carboxyl terminus. During and after its synthesis, the new protein must fold to its active three-dimensional structure before it can carry out its cellular function.

Regulation of gene expression

Regulation of gene expression refers to the process that cells use to turn the information on genes into gene products. Although a functional gene product may be RNA or a protein, the majority of known mechanisms regulate protein coding genes. Any step of the gene's expression may be modulated, from DNA-RNA transcription modification of a protein.

Vectors, Promoters and Transformation Cassettes

Recombinant DNA Technology : An Overview

Following the elucidation of the structure of DNA and the genetic code, it became clear that many biological secrets were locked up in the sequence of bases in DNA. Technical discoveries in the 1970s led to a new era of DNA analysis and manipulation. Key among these was the discovery of two types of enzymes that made DNA cloning possible. One type called *restriction enzymes,* cut the DNA from any organism at specific sequences of a few nucleotides, generating a reproducible set of fragments. The other type called *DNA ligases,* can covalently join sequences at the termini of restriction fragments. Thus ligases can insert DNA restriction fragments into replicating DNA molecules such as plasmids producing recombinant DNA. The recombinant DNA molecules then can be introduced into appropriate cells, most often bacterial cells, all the descendants from such a single cell, called a clone, carry the same recombinant DNA molecule (Fig 3.1). Once a clone of cells bearing a desired segment of DNA is isolated, unlimited quantities of this DNA can be prepared.

Vectors

A vector is a DNA molecule which can replicate in a suitable host organism, and into which a fragment of DNA may be introduced. Most vectors used in molecular biology are based on bacterial plasmids and bacteriophages. Vectors may need to have the following characteristics:

1. Possess an origin of replication (**ori**), which renders the vector capable of autonomously replicating independent of the main bacterial chromosome.

2. Have a site or sites which can be cleaved by a restriction enzyme, where the foreign DNA fragment can be introduced.
3. There must be convenient markers for identifying the host cell that contains vector with your DNA of interest. A common selection marker is an antibiotic resistance gene. If the host bacteria cells contain the vector then the bacteria will grow in the presence of that antibiotic. Normally the bacteria could not grow in the presence of antibiotic.

In addition to the above features, the vector should be easily introduced into the host organism where it has to replicate and produce copies of itself and the foreign DNA. Furthermore, they should be easy to isolate from the host cell.

Types of Cloning Vectors

Plasmids

Plasmids are circular, double stranded DNA molecules that are separate from a cell's chromosomal DNA. These extrachromosomal DNAs occur naturally in bacteria and in the nuclei of yeast and some higher eukaryotic cells, existing in a parasitic or symbiotic relationship with their host cell. Most naturally occurring plasmids contain genes that provide some benefit to the host cell, fulfilling the plasmid's portion of a symbiotic relationship. For example some bacterial plasmids encode enzymes that inactivate antibiotics. Therefore, a bacterial cell containing such a plasmid is resistant to the antibiotic, whereas the same type of bacterium lacking the drug-resistant plasmid is killed. Plasmids range in size from a few thousand base pairs to more than 100 kilobases (kb).

The plasmids most commonly used in recombinant DNA technology replicate in *E. coli.* Generally these plasmids have been engineered to optimize their use as vectors in DNA cloning. For instance, to simplify working with plasmids, their length is reduced to only ≈3 kb, which is much less than that of naturally occurring *E. coli* plasmids. Most cloning vectors such as pUC18 (Fig 3.1) have a multiple cloning site (MCS), a short region of DNA containing many restriction sites close together (also called polylinker). This allows many different restriction enzymes to be used. In addition to antibiotic resistance gene most modern plasmid vectors use a system for detecting the presence of a recombinant insert, usually the blue/whiteβ-galactosidase system.

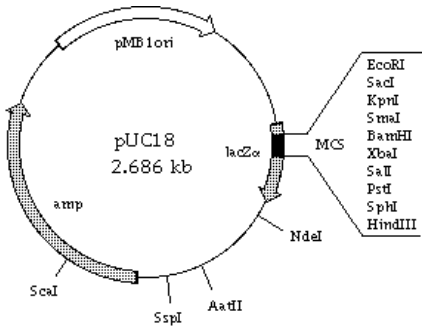

Fig 3.1 pUC18 plasmid cloning vector

Bacteriophage

Bacteriophages,or phages, are viruses which infect bacteria. They can have simple lytic life or more complex lysogenic cycles. One of the best studied phages is bacteriophage λ whose derivatives are commonly used as cloning vectors. The λ phage virion consists of an icosahedral head containing the 48.5kb linear dsDNA geneome, and a long flexible tail. The phage binds to specific receptors on the outer membrane of E. coli and the viral genome is injected through the phage's tail into the cell. The viral genome is linear, its termini are single stranded and complementary. These are called cos ends. The cohesive cos ends rapidly bind to each other once in the cell, produced a nicked circular genome which is repaired by cellular DNA ligase. Much of the central region of the genome is dispensable for lytic infection, and may be replaced by unrelated DNA sequence. There are limits to the size of DNA which can be incorporated into a λ particle, the DNA must be between 75 and 105% of the natural length, i.e. 37 – 52kb. Taking account of the essential regions, DNA fragments of around 20kb (maximum 23kb) can be cloned into λ, which is more than can be conveniently incorporated into a plasmid vector. Another advantage of λ based vectors is that each virion packed with recombinant DNA will infect a single cell. This infection process is about a 1000 times more efficient than transformation with plasmid vectors.

Cosmids

Both λ phage and *E. coli* plasmid vectors are useful for cloning only relatively small DNA fragments. Several other vectors, however have been developed for cloning larger fragments of DNA. One common method for cloning fragments makes use of elements of both plasmid and λ-phage cloning. In this method, called cosmid cloning, recombinant plasmids containing inserted fragments up to 45kb long can be efficiently introduced into*E. Coli* cells. A cosmid vector is produced by inserting the cos sequence from λ-phage DNA into a small *E. coli* plasmid vector about 5kb long. Cosmid vectors contain all the essential components found in plasmids. The cosmid can take foreign DNA inserts that are between 35 and 45kb. Such recombinant molecules can be packaged and used to transform *E. coli.* Since the injected DNA does not encode any λ-phage proteins, no viral particles form in infected cells and no plaques develop on the plate. Rather, the injected DNA circularizes, forming in each host cell a large plasmid containing the cosmid vector and an inserted DNA fragment. Cells containing cosmid molecules can be selected using antibiotic as is done with ordinary plasmid cloning.

A recently developed approach similar to cosmid cloning makes use of larger E. coli viruses such as bacteriophage P1. recombinant plasmids containing DNA fragments up to ≈100 kb can be packaged in vitro with the p1system.

Yeast Artificial Chromosomes (YAC)

YACs are constructed by ligating the components required for replication and segregation of natural yeast chromosomes to very large fragments of target DNA, which may

be more than 1Mb in length. YAC vectors contain two telomeric sequences (TEL), one centromere (CEN), one autonomously replicating sequence (ARS) and genes which act as selectable markers in yeast. YAC selectable markers do not normally confer resistance to toxic substances, as in E. coli plasmids, but instead enable growth of yeast on selective media lacking specific nutrients.

Bacterial Artificial Chromosomes (BAC)

BAC vectors were developed to overcome problems with the use of YACs to clone large genomic DNA fragments. Although YACs can accommodate very large fragments, quite often these fragments turn out to comprise noncontiguous (nonadjacent) segments of the genome and they frequently lose parts of the DNA during propagation (i.e. they are unstable). BACs are generally able to accommodate up to 300 – 350 kb of insert sequence, less than YACs, but they have the advantages not only of stability, but also of the ease of transformation and speed of growth of their E. coli host, and are simpler to purify. The vectors are based on the natural extrachromosomal F factor of E coli, which encoded its own DNA polymerase and is maintained in the cell at a level of one or two copies. A BAC vector incorporates the genes essential for replication and maintenance of the F factor, a selectable marker and a cloning site flanked by a rare-cutting restriction enzyme site and other specific cleavage sites, which serve to enable the clones to be linearized within the vector region, without the possibility of cutting within the very large insert region. BACs are more user friendly than YACs and are now being used extensively in genomic mapping projects.

Promoters

The promoter sequence is the key cis-acting regulatory region that controls the transcription of adjacent coding region(s) into mRNA, which is then directly translated into proteins. Promoters play an important role in the regulation of gene expression at different locations and times during the life cycle of an organism. The study and understanding of the function of their multiple components and the factors associated with their performance have opened up the possibility of modulation of the expression of genes in homologous organisms (i.e. same species) as well as in heterologous organisms (e.g. different species, kingdoms), where foreign promoters together with genes of interest are inserted. As such, promoters have a huge influence in follow-on research and development in biotechnology.

Types of promoters used to regulate gene expression Promoters can be generally divided into:

- ***Constitutive promoters:*** These promoters direct expression in virtually all tissues and are largely if not entirely, independent of environmental and developmental factors. As their expression is normally not conditioned by endogenous factors, constitutive promoters are usually active across species and even across kingdoms.
- ***Tissue-specific promoters:*** These direct the expression of a gene in specific tissue(s) or at certain stages of development. In plants promoter elements that are expressed or

affect the expression of genes in tubers, roots, vascular bundles, other vegetative organs or seeds and other reproductive organs can be found in heterologous systems but it is preferable to work with homologous promoters.

- ***Inducible promoters:*** They are quite popular nowadays because their performance is not conditioned to endogenous factors but external ones that ideally can be artificially controlled. Within this group, there are promoters modulated by abiotic factors such as light, oxygen level, heat, cold and wounding. Since some of these factors are difficult to control outside an experimental setting, promoters that respond to chemical compounds, which are not found naturally in the organism of interest, are of particular interest. Along those lines, promoters that respond to antibiotics, copper, alcohol, steroids and herbicides, among other compounds, have been adapted and refined to allow the induction of gene activity at will and independently of biotic or abiotic factors.

Expression vectors

Cloning a gene encoding a particular protein is only the first of many steps needed to produce a recombinant protein for agricultural, medical or industrial use. The next step is to put gene into a host cell for its expression and the production of protein of interest. For the gene of interest to be expressed in the host cell/organism it must be cloned into a vector that has several distinct sequences/units that provide the different components of a functional gene (see section 2.8 & 2.9). In addition to the characteristics described for cloning vectors, an expression vector must carry a promoter, a polyadenylation and transcription termination sequences within its polylinker. Inserting a coding sequence in proper orientation in between these expression control sequences will result in the expression of the gene in an appropriate host.

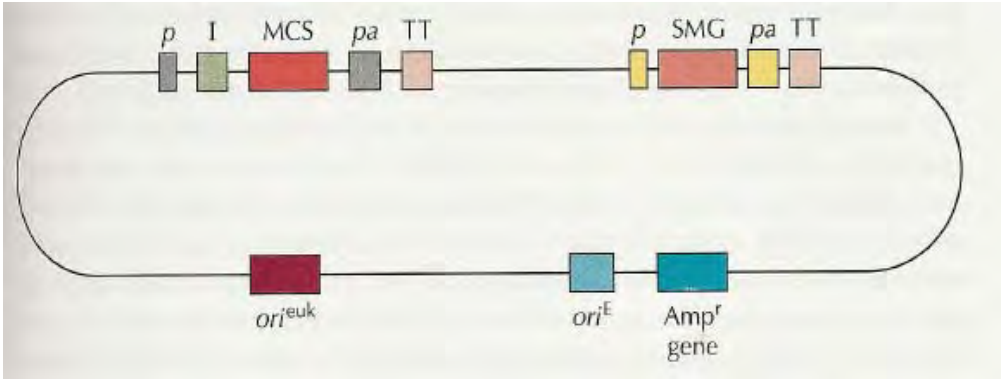

Fig 3.2. Generalised mammalian expression vector. The multiple cloning site (MSC) and selectable gene marker (SMG) are under control of eukaryotic promoter (*p)*, polyadenylation (*pa*), and termination of transcription (TT) sequences. An intron (I) enhances the production of heterologous protein. Propagation of the vector in *E. coli* and mammalian cells depends on the origins of replication ori^{E} and ori^{euk},respectively. The ampicillin gene (Amp^{r}) is used for selecting transformed *E. coli.*

Sometimes it is necessary to fuse some translation control and protein purification elements to the gene of interest (Fig 3.3) or in the expression vector polylinker.

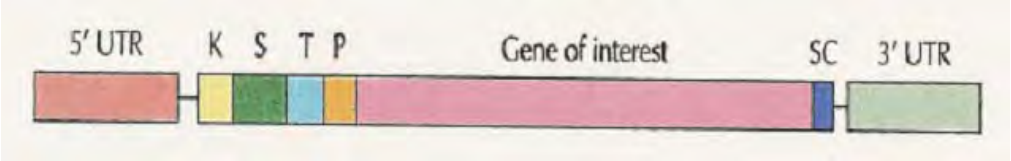

Fig 3.3. A gene of interest fitted with sequences that enhance translation and facilitate both secretion and purification such as a Kozak sequence (K) [5'-ACCAUGG-3', its presence near initiating AUG greatly increases the effectiveness of initiation], signal sequence (S), protein affinity tag (T), proteolytic cleavage site (P), and stop codon (SC). The 5' and 3' UTRs (untranslated regions) increase the efficiency of translation and contribute to mRNA stability.

Plant Transformation and Selection Techniques

Plant transformation

Genetic transformation is the heritable change in a cell or organism brought about by the uptake and establishment of introduced DNA. Transformation encompasses a variety of gene transfer events, characterized by the stability of transformation, the subcellular compartment transformed (nuclear, mitochondrial or plastid) and whether the transferred DNA is integrated into the host genome. Table 4.1 documents the generally accepted definitions of these alternative transformation events.

Table 4.1 Definitions of transformation

Term	Definitions
Stable transformation	Transgene and novel genetic characteristics are maintained during the life of the culture or plant. The transgene is usually, but not necessarily always, integrated into the host genome.
Transient expression	Expression of the transgene is detected in the first few days after its introduction into cells. A subsequent decline in gene activity indicates that expression results from non-integrated DNA.
Integrative transformation	The transgene is covalently integrated into the genome of the host cell. In fertile plants the transgene is inherited by the next generation.
Nuclear transformation	Gene transfere into the nuclear genome of the host cell, as confirmed by cellular fractionation, eukaryotic-type expression or Mendelian inheritance.
Organellar transformation	Gene transfer into the plastid or mitochondrial genome of the host cell, as confirmed by cellular fractionation, prokaryotic-type expression or material inheritance.
Episomal transformation	Viral genomes or 'mini-chromosomes' which replicate independently from the host genome.

Plant Tissue Culture

An important phenomenon and one that is key to plant transformation is that whole plants can be regenerated from single cells. When a plant is wounded mechanically, a patch of soft cells called callus grows over the wound. If a piece of young callus is removed and placed in a liquid culture medium containing the appropriate nutrients and plant growth hormones, the cells continue to grow and divide as a suspension culture. These cells can be planted out onto solid media and will grow to form new calli. The callus will then redifferentiate into shoots and roots, and ultimately a whole plant will be formed. The differentiation of the cells in a callus depends on the relative concentrations of the plant hormones, auxins and cytokinins. If the ratio of auxins to cytokinins is high, then roots develop, shoots develop if the ratio is low. Transformed plants can thus be obtained by introducing DNA into calli or wounded plant tissues such as leaf disks from which callus can be grown and regenerated Fig 4.1.

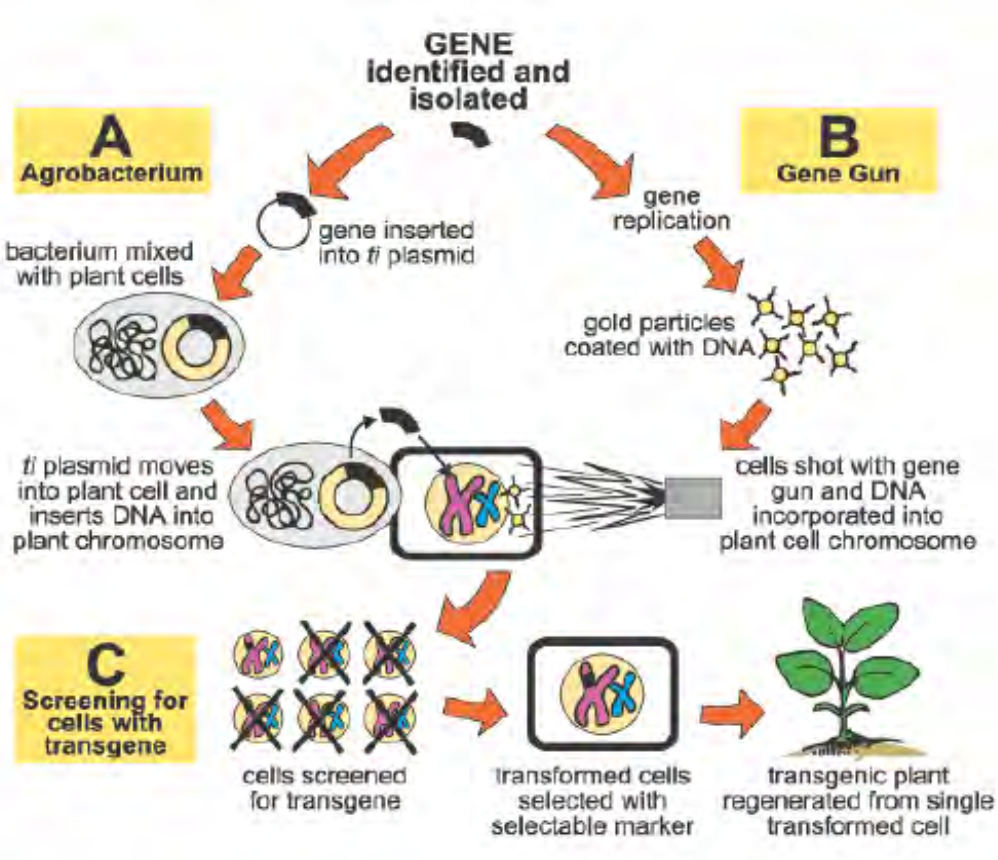

Fig 4.1. Steps involved in the generation of genetically transformed plants using either the *Agrobacterium* of microprojectile bombardment approaches

Plant Transformation Techniques

There is an expanding repertoire of plant transformation approached available, ranging from well proven techniques to highly experimental methodologies. In Table 4.2 these alternative approaches to gene delivery are listed with brief comments on their application, efficiency and limitations. The most widely used techniques are the *Agrobacterium,* microprojectile bombardment ('gene gun' or biolistic) and direct gene transfer to protoplasts. The biolistics technique has been especially useful in transforming monocot species like maize and rice whereas transformation via *Agrobacterium* has been successfully practiced in dicots, but only recently has it been effective in monocots (grasses and their relatives). In general, the *Agrobacterium* method is considered preferable to the gene gun, because of the greater frequency of single-site insertions of the foreign DNA, making it easier to monitor. These techniques are briefly described in the following sections.

Microprojectile bombardment

The technique uses high velocity particles or microprojectiles coated with DNA to deliver exogenous genetic material into the target cell or tissue, which is then cultured *in vitro* and regenerated to produce mature transformed plants.

The particles, either tungsten or gold, are of small size (0.5-5μm) but big enough to have the necessary mass to be accelerated and able to penetrate the cell wall carrying the coated DNA on its surface which once integrated into the cell nucleus can be expressed. Gold particles are chemically inert, although rather costly, and present more uniformity. Tungsten particles, although with some phytotoxicity and of more variable size, are adequate for most studies. Furthermore, the chosen microprojectile should also have good DNA affinity but, at the same time, be able to release it once it has hit the target. DNA coating of surface sterilized particles can be accomplished by binding the DNA, using for instance the calcium chloride method, with the addition of spermidine to protect the DNA. However, recently a report describes the novel use of *Agrobacterium* as coating material for the microprojectiles which are then shot into the target tissue. Once coated the particles are ready for shooting and in some cases macrocarriers are employed to support and accelerate the particles. The macrocarrier is retained by a screen or stopping plate and the particles continue traveling and collide with the target. The DNA, delivered utilizing this direct gene strategy, can be expressed after reaching the nucleus.

Agrobacterium-mediated Plant Transformation

Agrobacterium tumefaciens is a remarkable species of soil-dwelling bacteria that has the ability to infect plant cells with a piece of its DNA. When the bacterial DNA is integrated into a plant chromosome, it effectively hijacks the plant's cellular machinery and uses it to ensure the proliferation of the bacterial population, causing crown gall disease.

The DNA in an A *tumefaciens* cell is contained in the bacterial chromosome as well as in a plasmid known as a Ti (tumor-inducing) plasmid. The Ti plasmid contains a series of *vir*

(virulence) genes that direct the infection process and a stretch of DNA termed T-DNA (~20kb long) that is transferred to the plant cell in the infection process (Fig. 4.2).

Agrobacterium can only infect plant through wounds. When a plant root or stem is wounded it gives off certain chemical signals. In response to those signals, *vir* genes become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant cell through the wound. It is not clear how the bacterial DNA moves from the cytoplasm to the nucleus of the plant cell, nor how the T-DNA becomes integrated into the plant chromosome.

Table 4.2. Status of alternative plant transformation techniques.

Gene delivery method	Characteristics
Agrobacterium	Well-established transformation vector for many dicots and a promising vector for gymnosperms. A wide range of oncogenic and disarmed Ti- or Ri-derived plasmid vectors are available. Restricted use with monocots, but valuable for delivery of viral genomes to graminaceous hosts by agroinfection.
Direct gene transfer to protoplasts	Well-established transformation technique with no host range limitation. Plasmalemma permeabilized to DNA by chemical agents or electroporation. Alternatively, genes can be delivered to protoplasts by fusion with DNA in encapsulated liposomes.
Microprojectile Bombardment	A widely authenticated technique for accelerating DNA coated particles into walled cells. No intrinsic host-range limitation. Gene transfer to *in situ* chloroplasts has been achieved.
Microinjection	Effective gene delivery technique offering visual targeting to cell type and intracellular compartment. Labor-intensive to process relatively few cell, and requiring specialist skills and equipment.
Macroinjection of inflorescence	Technically simple approach to deliver DNA to developing floral tissue by hypodermic needle. Germline transformation has not proved reproducible.
Impregnation by whiskers	Suspension cells mixed with DNA and micron-sized whiskers exhibit transient expression and stable transformation.
Laser perforations	Transient expression from cells targeted with a laser microbeam in DNA solution.
Imbition of tissues	Transient and stable expression from tissue bathed in DNA solution of tissues or infiltrated under vacuum.
Floral dip	Stable integration and expression following diping of floral buds in DNA solution.
Pollen tube pathway	Claims of germline transformation by treating pollen or carpels with DNA remain controversial.
Ultrasonication	Stable transformation reported by ultrasonication of explants with DNA. Molecular confirmation is required.

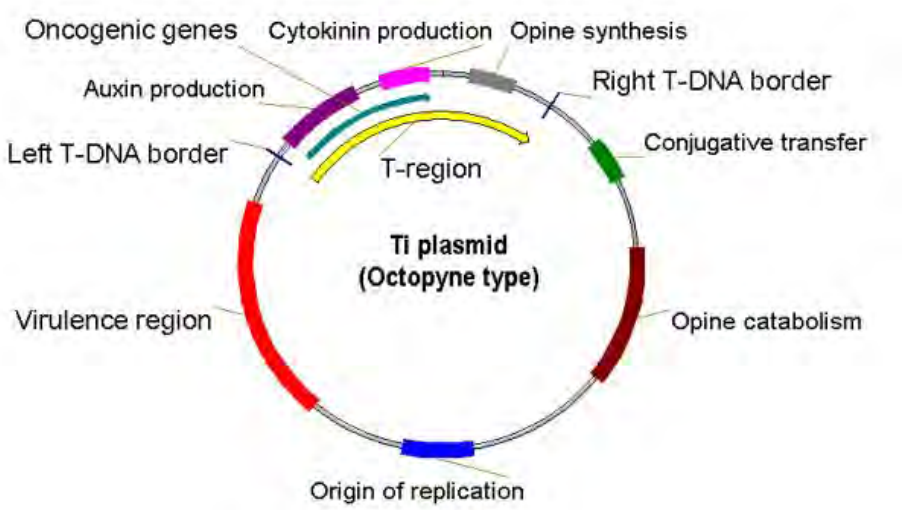

Fig 4.2. Wild type Ti plasmid of *Agrobacterium tumefaciens*

To harness *A tumefaciens* as a transgene vector, scientists have removed the tumor-inducing section of T-DNA, while retaining the T-DNA border regions and the vir genes. The transgene is inserted between the T-DNA border regions, where it is transferred to the plant cell and becomes integrated into the plant's chromosomes. To achieve transformation, *Agrobacterium* cells carrying an appropriately constituted T-DNA based plasmid vector can be inoculated into plants stems, leaf disks etc to facilitate infection and T-DNA transfer to the plant cells. These explants that have been co-cultivated with the *Agrobacterium* can then go through various tissue culture steps leading to the selection and production of transformed cells and plants.

Protoplast Transformation Techniques

Protoplasts are plant cells in which the cell wall has been removed. Therefore protoplasts can behave like animal cells, which have no cell wall barrier. Plant regeneration from single protoplasts is possible due to the totipotency of plant cells. Removal of the cell wall is achieved by treating the plant material (leaves, tissue cultures, suspended cells, etc) with a cocktail of enzymes including pectinases, cellulases, and/or hemicellulases in an appropriate incubation medium of the right osmolality. After removal of the cell wall, the protoplasts must be kept immersed in a solution of the appropriate concentration to prevent them from bursting. Also the protoplasts must be incubated in a culture medium of the correct osmolality until wall formation occurs.

Different approaches exist for the insertion of transgenes into protoplasts through the plasma membrane. These include: chemical, electroporation and microinjection techniques.

Chemical Techniques

The most common methods are polyethylene glycol (PEG), Ca^{2+} -DNA co-precipitation and liposomes. PEG is the most widely used, employing solutions of 10-15% PEG, with high calcium content and high pH. After mixing isolated DNA and protoplasts, followed by different washes, DNA and protoplast fusion takes place. Here PEG alters the plasma membrane properties causing reversible permeabilization enabling exogenous macromolecules to enter the cell cytoplasm.

Ca^{2+} -DNA co-precipitation depends on the formation of a co-precipitate of plasmid DNA and calcium phosphate. On contact with protoplasts under high pH conditions, the co-precipitate trespasses the cell.

Liposomes, these are negatively-charged spheres of lipids, are also employed for DNA transfer. DNA is first encapsulated into the liposomes and these are fused with protoplasts employing PEG as a fusogen.

Electroporation

Electrical pulses are applied to the DNA-protoplast mixture, provoking an aincrease in the protoplast membrane permeability to DNA. It is much simpler than the chemical method, giving attractive results. However, the electrical pulses must be carefully controlled as cell death can occur above a certain threshold. The pulses create the transient formation of micropores in the lipid bilayer which last for a few minutes, allowing for DNA uptake.

Microinjection

This technique was originally designed to transform animal cells, later it gained importance and interest in transforming plant cells. However, in plant cells the existence of a tough cell wall, a natural rigid barrier, as well as the presence of vacuoles which can produce cell death after breakage due to the release of hydrolases and toxic metabolites, and in some instances where vacuoles surround the nucleus make microinjection impossible. Therefore, protoplast rather than intact plant cells are more suitable for microinjection, and thus subsequent genetic modification. Clearly, this method is rather labor intensive and requires specialized microequipment for the manipulation of host protoplasts and DNA. However, some success in transforming both monocotyledonous and dicotyledonous species has been achieved employing this technique.

Selection of Successfully Transformed Tissues

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 in the expression cassette. Selectable markers are those which allow the selection of transformed cells, or tissue explants, by their ability to grow in the presence of the antibiotic or herbicide. Only cell/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

will only use these surviving plants. In addition to selecting for transformants, such markers can be used to follow the inheritance of a foreign gene in a segregating population of plants.

Most transformation cassettes also include screenable markers/reporter genes that encode gene products whose enzyme activity can be easily assayed, allowing not only the detection of transformants but also and estimation of the levels of foreign gene expression in transgenic tissue. Markers such as β-glucuronidase (GUS) and luciferase allow screening for enzyme activity by histochemical staining or fluorimetric assay of individual cells and can be used to study cell-specifics as well as developmentally regulated gene expression.

Selectable marker genes

The selectable functions on most general transformation vectors are prokaryotic antibiotic resistance enzymes which have been engineered to be expressed constitutively in plant cells. In some experiments, enzymes affording protection against specific herbicides have also been used successfully as dominant marker genes. The selective agent concerned must be able to exert stringent selection pressure on the plant tissue concerned.

Neomycin phosphotransferase (NPT-II) Gene

Neomycin phosphotransferase-II (NPT-II) is a small (25kd) bacterial enzyme which catalyses the *ortho*-phosphorylation of a number of aminoglycoside antibiotics including neomycin and kanamycin. The reaction involves transfer of the γ-phosphate group of ATP to the antibiotic molecule, which detoxifies the antibiotic by preventing its interaction with the target site-the ribosome. This transfer reaction has been exploited to develop a sensitive solid phase assay for the enzyme. The total proteins are first extracted from the tissues to be analyzed for the presence of the transgenes.

Chloramphenicol Acetyltransferase Gene

This is the chloramphenicol resistance (*cat)* gene encodes the enzyme chloramphenicol acetyltransferase (CAT) and was the first bacterial gene to be expressed in plants. The enzyme specifically acetylates chloramphenicol antibiotics to the 1 -, 3 -, and 1,3-acetylated derivatives, which are inactive. Although not used as a selection system in plants, the gene is used frequently as a reporter gene in plant promoter work.

β-glucuronidase Gene

The *E. coli* β-glucoronidase gene has been developed as a reporter gene system for the transformation of plants. β-glucuronidase, encoded by the *uidA* locus, is a hydrolase that catalyses the cleavage of a wide variety of β-glucuronides, many of which are available commercially as spectrophotometric, fluorometric and histochemical substrates. There are several useful features of the GUS gene which make it a superior reporter gene for plant studies. Firstly, many plants assayed to date lack detectable glucuronidase activity, providing a null background in plants. Secondly, glucuronidase is easily, sensitively and cheaply

assayed both *in vitro, in situ* in gels and is robust enough to withstand fixation, enabling histochemical localization in cells and tissue sections. The preferred histochemical substrate for tissue localization of GUS is 5-bromo-4chloro-3-indolyl-β-D-glucuronide (X-gluc). The advantage of these substrates is that the indoxyl group produced upon enzymatic cleavage dimerizes to indigo which is virtually insoluble in an aqueous environment. The histochemical assay for GUS consists of soaking tissue in substrate solution and watching for blue color to appear.

Luciferase Gene

The luciferase (*luc)* gene isolated from *Photinus pyralis* (firefly) encodes the enzyme catalyzing the ATP/oxygen-dependent oxidation of the substrate luciferin which produces emission of light (bioluminescence). As a reporter, the gene is the basis of highly sensitive assays for promoter activity and for protein targeting sequences, involving the measurement of light emission using liquid scintillation counter photomultiplers, luminometers, X-ray film exposure or sensitive camera/film.

Molecular Analysis of Transgenic Plants

Analysis for the transgenes at the molecular level is mainly carried out by Polymerase Chain Reaction(Box 7.1) and genomic Southern analysis. Polymerase chain reaction shows the presence of the transgene where as stable integration of the transgene is confirmed by genomic Southern analysis. To analyze DNA where *Agrobacterium* vectors are used for transformation, it is important to prepare plant DNA from sterile tissue, as contamination with *A.tumefaciens* DNA will interfere with the interpretation of the results. Genomic Southern analysis also yields information on the copy number of the integrated DNA sequences, whether any multiple inserts are tandemly linked or dispersed, and on the stability of this DNA in the F_1 progency of the transformed plants.

Biotechnology in Animal Production

Biotechnology has a number of applications in livestock production. It is being used to hasten animal growth, enhance reproductive capacity, improve animal health and develop new animal products. This chapter looks at these biotechnology applications and how they are impacting on animal improvement and production.

Biotechnology in Animal Breeding

Animal breeding is a field related to a whole range of biotechnologies. The impact of a biotechnology can be measured by the influence it has on genetic progress. According to the type of biotechnology considered, different component of genetic progress may be affected: accuracy of prediction, generation interval, intensity of selection and genetic variance.

The first type of biotechnologies affects the efficiency of male and female reproduction: artificial insemination, multiple ovulation, in-vitro-fertilization, ova pick-up, embryo-transfer,

twining, sexing of semen and embryo cloning and selfing. The impact of these technologies is mainly in the enhanced distribution of superior germplasm and the selection intensity, but also in the accuracy obtained when testing animals.

A second group of biotechnologies can improve determination of the genetic merit of animals. These are all the techniques related to quantitative or economical trait loci (QTL), their detection and use. Their main feature is the early availability in life, therefore allowing an earlier and more accurate selection. Two directions of research exists: detection of markers for the unknown QTL and direct use of a potential candidate genes as QTL.

Animal Reproduction

Artificial insemination (AI)

This is the process of semen collection from a desired bull to be used in fertilizing many cows. The semen can be diluted, and preserved through cryopreservation. The technique can enable a single bull to be used simultaneously in several countries up to 100,000 inseminations a year. The high intensity and accuracy of selection arising from AI can lead to a four-fold increase in the rate of genetic improvement in dairy cattle relative to that from natural mating. Since its widespread use in the 1950s, AI has been a very successful biotechnology, enhancing greatly the genetic progress. Use of AI can reduce transmission of venereal diseases in a population and the need for farmers to maintain their own breeding males, facilitate more accurate recording of pedigree and minimize the cost of introducing improved stock. Though widely used in dairy cattle breeding AI had its greatest impact in broiler chicken production. In the 1950s the chicken was a luxury item costing around $30 at today's prices; Harry S Truman won the 1947 US Presidential election with the slogan "a chicken in every pot".

Embryo transfer (ET)

Although not economically feasible for commercial use on small farms at present, embryo technology can greatly contribute to research and genetic improvement in local breeds. There are two procedures presently available for production of embryos from donor females. One consists of superovulation using a range of hormone implants and treatments, followed by AI and then flushing of the uterus to gather the embryos. The other, called *in vitro* fertilization (IVF) consists of recovery of eggs from the ovaries with the aid of the state-of-the-art ultrasound-guided transvaginal oocyte pick-up (OPU) technique. When heifers reach puberty at 11-12 months of age, their oocytes may be retrieved weekly or even twice a week. These are matured and fertilized in vitro and kept until they are ready for implantation into foster females. In this way, high-value female calves can be used for breeding long before they reach their normal breeding age. IVF facilitates recovery of a large number of embryos from a single female at a reduced cost thus making ET techniques economically feasible on a larger scale. Additionally, IVF makes available embryos suitable for cloning. However, embryo transfer is still not widely used despite its potential benefits.

Embryo sexing

Technologies for rapid and reliable sexing of embryos allow the generation of only the desired sex at specific points in a genetic improvement programme, markedly reducing the number of animals required and enabling increased genetic progress. A number of approaches to the sexing of semen have been attempted, and several have been reported as successful. However, the only method of semen sexing that has shown any promise has been the sorting of spermatozoa according to the DNA content, by means of flow cytometry. Embryo sexing has been attempted by a variety of methods, including cytogenetic analysis, assays for X-linked enzyme activity, analysis of differential development rates, detection of male-specific antigens, and the use of Y-chromosome specific DNA sequences.

Animal cloning

Animal cloning may be produced by embryo splitting and nuclear transfer (somatic cell cloning). These offer the possibility for creating large clone families from selected superior genotypes which, in turn, can be used to produce commercial clone lines. The process of somatic cell cloning involves replacing the DNA in an unfertilized oocyte with DNA from a somatic (body) cell. The oocyte has the ability to reprogram the somatic cell DNA so that the unfertilized oocyte can develop as an embryo and, in some cases, give rise to healthy calves which have DNA that is entirely from the somatic cell. Because it is possible to obtain an unlimited number of genetically identical somatic cells from an animal, cloning is a technology that can be used for producing genetically identical calves. However, the somatic cell can also be genetically manipulated prior to being introduced into the oocyte, so cloning is also a convenient method of making transgenic cattle.

Genetic markers and marker-assisted selection

A genetic marker for a trait is a DNA segment which is associated with, and hence segregates in a predictable pattern as the trait. Genetic markers facilitate the “tagging” of individual genes or small chromosome segments containing genes which influence the trait of interest. Availability of large numbers of such markers has enhanced the likelihood of detection of major genes influencing quantitative traits. The process of selection for a particular trait using genetic markers is called marker assisted selection (MAS). MAS can accelerate the rate of genetic progress by increasing accuracy-of selection and by reducing the generation interval. However, the benefit of MAS is greatest for traits with low heritability and when the marker explains a larger proportion of the genetic variance than does the economic trait.

Marker identification and use should enhance future prospects for breeding for such traits as tolerance or resistance to environmental stresses, including diseases.

Two types of marker can be considered. First, markers that are sufficiently close to the trait gene on the chromosome such that, in most cases, alleles at the marker and the trait gene are inherited together. This type of marker is called a linked marker. At the population level

alleles at linked markers cannot be used to predict the phenotype until the association between alleles at the marker and alleles at the trait-gene is known (called 'phase'). To determine phase, inheritance of the marker and trait gene has to be studied in a family. However, information on phase is only valid within that family and may change in subsequent generations through recombination.

The second type of marker is a functional trait. These markers are called 'direct' markers. Once the functional polymorphism is known it is possible to predict the effect of particular alleles in all animals in a population, without first having to determine the phase. Therefore, 'direct' markers are more useful than 'linked' markers for predicting the phenotypic variation of target traits within a population. A further complication is that the mechanisms of genetic control differ between traits. The variation seen in some traits is directly controlled by a single gene (monogenic traits), which may have a limited number of alleles. In the simplest situation a gene will have two alleles: one allele will be associated with one phenotype and another allele with a different phenotype (e.g. black versus brown coat color in cattle: the brown coat color occurs as a result of mutation in the melanocyte hormone receptor gene, which results in the creation of a different allele that alters its function)

However, the traits that are important in livestock production are generally more complex and have a very large range of variation in the observed phenotype. Growth rate and milk yield are examples of two traits that exhibit a continuous phenotypic variation. Such traits are called quantitative traits. The variation in quantitative traits is controlled by several genetic loci (called quantitative trait loci [QTL]), each of which is responsible for a small amount of the overall variation. The behavior of genes (including major genes) that control a trait is likely to be dependent on the genetic background. The myostatin allele responsible for double muscling in Belgian Blue cattle is also found in other breeds, however, the phenotype associated with the allele is variable between the breeds. This suggests that there are genes at other loci in the genome that act to modify the phenotypic expression of the major gene. Thus, information is required not only on the major genes that control a trait, but also on the interactions between genes. It is therefore premature to start using DNA-based selection widely. However, some DNA tests for specific polymorphisms are being offered commercially, e.g. GeneSTAR tests for tenderness (based on variations in the calpastation gene) and marbling (based on variations in the thyfoglobulin gene), and the Ingenity test for fat deposition (based on variations in the leptin gene). These tests can be used by breeders and evaluated in their populations.

Transgenic Animals

The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome. Foreign DNA is introduced into the animal, using recombinant DNA technology, and then must be transmitted through the germ line so that every cell,

including germ cells, of the animal contain the same modified genetic material. The first transgenic animal was a mouse produced in 1982 by microinjection of DNA into the fertilized single cell oocyte. This ground breaking work was published in Nature and the cover of the magazine showed a comparison of the transgenic mice and their non transgenic litter mates. The transgenic mice were huge, twice the size of their litter mates. This image stimulated the imaginations of both the public and scientists and created a tremendous amount of speculation about the potential impact of transgenic technologies for agricultural animals. It was surmised by inserting a single growth regulating gene into an animal of agricultural value at growth rate and feed efficiency could be greatly increased and fat deposition reduced, transforming the entire meat animal industry. Furthermore, many other applications, including, enhanced milk production , production of milk with novel properties, enhanced disease and parasite resistance and increased wool production were imagined. By 1985, transgenics has been produced in pigs, sheep, and cattle with chicken following a little later. Since then there has been a slow, but relatively steady, effort to apply transgenic technologies to agricultural species. Initially, technical limitations, cost and lack of understanding about genes and their regulation severely limited progress, particularly in species such as the cow.

The first transgenic technology has limitations: less than 1% of embryos injected and 10% of animals born are transgenic, genes can only be added, not replaced or deleted, because multiple copies are inserted at random, correct regulation of gene expression is difficult.

To overcome these problems in the mouse, embryonic stem cells (ES cells) have been developed. These cells can be grown stably in culture for many passages and transformed with gene constructs. The constructs not only permit transformed cells to be selected but also gene targeting to be accomplished. Transformed cells are introduced into the blastocoel cavity of an embryo, produce a mosaic (chimaeric) animal and contribute to the germline. After one generation this will produce a germline transgenic animal. This technique, in principle, produces 100% transgenic animals and, by gene targeting a much wider variation of genetic modifications (such as gene knock-outs). For many years, several labs world wide have tried to produce ES cells in farm animals, although some success has been claimed, no robust and repeatable method has been published. Indeed, ES cells can only be produced even in mice from a limited number of inbred strains.

Recently a robust method for gene targeting in cattle, using somatic cell cloning technology, has been developed. Gene targeting is the insertion of a transgene, or any exogenous DNA sequence, into a specific, targeted site in the host DNA. The technique is more complex than random gene insertion but gene targeting is a much more powerful technology because it can be used to inactivate genes, insert new genes into predetermined sites or replace one variation of a gene with another variation. It overcomes many of the limitations of random gene insertion by microinjection. Because the insertion site is predetermined, a series of transgenic founder animals can be made, including both males and females, which can be mated to make homozygous offspring. An even simple approach to

making homozygous transgenic animals is to sequentially insert a copy of the transgene into one member of a pair of chromosomes and then insert a second copy into the other chromosome without germ line transmission of the transgene.

Some research groups have developed a rejuvenation system for bovine fibroblast cells. The system involves making a genetic modification in a fibroblast cell line established from a bovine fetus. Because the cells only grow for a limited number of cell divisions in culture only one genetic modification can be made before the cells become senescent and stop dividing. The cells are then used in a cloning procedure to produce cloned fetuses. Young healthy cell lines can then be made from the fetuses and used for a second round of genetic modification. When the genetic modifications are complete then the final fetal cell line can be used for making calves.

A second advancement in cattle transgenics, which has been accomplished recently, is microchromosome transfer. A microchromosome is different from a typical transgene in a couple of characteristsic. First, a typical transgene consists of a couple of gene sequences and may be up to 25,000 DNA bases long, whereas, a microchromosome typically consists of millions of DNA bases and can contain either very long genes or potentially hundred of genes. Second, a typical transgene must integrate into the host DNA, either randomly or targeted to a specific sequence, to be carried along through cell division. Microchromosomes do not integrate but replicate on their own and are carried along during cell division as independent chromosomes. Human-derived microchromosome have been successfully inserted into cattle. A microchromosome was needed because there was need to transfer the human antibody genes into cows. Antibody genes are very complex and are up to several million DNA bases long, well beyond the capacity of a typical transgenic vector. The microchromosome is stable in cattle and appears to have no harmful effects on the animals.

Since the production of the first transgenic mice, work in cattle has focused primarily on technology development. At this time, many technical hurdles for application of transgenic technology to cattle have been overcome. In fact, transgenic technologies for the cow are comparable to that of the mouse. Two kinds of applications for transgenic technology in cattle are being pursued. One involves genetic modifications that are aimed at improving the efficiency of food (meat or milk) production. The second is the production of novel products, such as pharmaceutical proteins for human health care.

Transgenic Animals for Food Production

There are a few research reports describing the use of transgenic technologies in cattle directed towards a food production application. Brophy *et al*, (2003) introduced additional copies of bovine beta or kappa casein into dairy cattle and evaluated the effect on milk production and composition. Transgenic offspring had an 8 to 20% increase in beta casein and a two-fold increase in kappa casein. In pigs several attempts have been made at improving growth and composition by the addition of transgenes. In one study expression of

an exogenous insulin-like growth factor gene in the muscle of pigs resulted in significant reduction in fat and an increase in lean muscle in gilts but not boars (Pursel *et al,* 1999). In another study, a widely expressed exogenous growth hormone gene tended to increase live weight gain, improve feed efficiency and reduce back fat thickness (Nottle *et al,* 1999).

Although these studies demonstrate the feasibility of improving food production efficiency with transgenics, no attempts have been made to commercialize any transgenic food producing animals. In addition to technology, there are several factors that will impact the use of transgenic animals for food production. The first involves regulatory approval of meat or milk from genetically modified cattle. The authorities regulating genetically modified animals must address three factors;

1. safety of the food product for human consumption
2. environmental impact of the genetically modified animals and
3. welfare of the animals

conceptually, many of the modifications that might be considered to enhance production efficiency would not have any impact on the safety or quality of the food product. Since there are no wild bovine species, the transmission of modified genes into wild species is not a concern with cattle as it is with genetically modified plants, therefore, it is unlikely that genetically modified cattle would have a significant impact on the environment. The welfare of the animal could be a concern with some genetic modifications but could be easily evaluated.

Transgenic Animals for Human Therapeutic Production

A second application for genetically modified cattle is the production of human therapeutic proteins. Humans proteins that have been expressed in milk include human lactoferrin (van Berkel *et al.,* 2002), human alpha lactalbumin (Eyestone, 1999), human serum albumin (Behoodi *et al.,* 2001) and human bile salt stimulated lipase (Chen *et al.,* 2002). The mammary gland in dairy cows is an excellent protein production factory. Large quantities of very complex proteins can be produced and collected at very low cost.

Some research groups are using microchromosome transfer and gene targeting technologies to develop a line of genetically modifies cows that produce human polyclonal antibodies. To get rid of contaminating bovine antibody the bovine antibody genes are targeted with a knock out sequence to prevent expression. Antibodies are currently used for many different human clinical applications, including treatment of infectious disease, cancer, transplanted organ rejection, autoimmune diseases and for use as antitoxins. To make a human antibody product the genetically modified cows are immunized with a vaccine containing the disease agent. For example, a product could be made for treatment of *Staphlococcus aureus* infections acquired following hospitalization by immunizing the genetically modified cow with the *Staphlococcus aureus* bacterium. Following immunization the cow builds up an antibody response to the bacterium. To harvest the antibodies from the

cow, blood plasma is collected using a procedure that is similar to collecting plasma from human donors. The plasma is then processed to remove all contaminating bovine components so the final product is a human antibody that reacts to *Staphlococcus aureus* which can be injected into hospital patients to help them fight and infection.

Biotechnology in Animal Health

One important benefit from biotechnology is the diagnosis of livestock diseases, and genetically transmitted conditions which damage health and productivity. Biological techniques can also produce cheaper and more efficient drugs. In cases where a natural source material is prohibitively expensive, genetic engineering (in microbial of tissue culture systems) can be used to produce drugs of high value for humans or animals. Examples are insulin, human growth hormone and tissue plasminogen activator (used in treating heart disease).

Vaccines

Vaccines are used to stimulate an animal's immune system to produce the antibodies needed to prevent infection. Recombinant DNA technology has provided the means to produce large quantities of inexpensive vaccines, while a better understanding of the immune system has helped produce vaccines that do a better job of boosting the body's immune system. These engineered products are safer than traditional vaccines. Whereas conventional vaccines sometimes revert to virulent (disease causing) forms the new vaccines can be engineered to eliminate this threat.

Biotechnology is also producing an entirely new use for vaccines. They are being used to modulate hormones to increase growth rates, improve the efficiency of feed conversion, stimulate milk production, contribute to improved carcass quality and leaner meat, and enhance or suppress reproductive functions.

Korean scientists have developed a combined vaccine against pleuropheumonia, pneumonic pasteurellosis and enzootic pneumonia in swine. Molecular biology has been used to produce an improved vaccine to protect pigs from swine fever. In the Philippines, it has been used to develop an improved vaccine to protect cattle and water buffalo against hemorrhagic septicemia. This disease is the leading cause of death among these animals. The new vaccine gives improved protection at a very low cost. A field kit has also been developed, to diagnose this disease from nose swabs.

Diagnosis of Disease and Genetic Defects

Successful control of a disease requires accurate diagnosis. The ability to generate highly specific antigens by recombinant DNA techniques has made it possible for an increasing number of enzyme-linked immunosorbent assays (ELISA) to have the capacity to differentiate between immune responses generated by vaccination from those due to infection. This has made it possible to overcome one of the major drawbacks of antibody detection tests: the fact

that, because antibodies can persist in animals for long periods, their presence may not indicate current infection.

The advent of PCR has enhanced the sensitivity of DNA detection tests considerably. For example, PCR used in combination with hybridization analysis, has been shown to provide a sensitive diagnostic assay to detect bovine leukosis virus.

Other diagnostic techniques include nucleic acid hybridization and restriction endonuclease mapping. A good example of the specificity of nucleic acid hybridization is its application in distinguishing infections caused by *peste des ruminants* (PPR) virus from rinderpest, diseases whose symptoms are clinically identical and which cannot be distinguished antigenically with available serological reagents. This technique also allows comparison of virus isolates from different geographical locations.

Molecular epidemiology is a fast growing discipline that enables charaterisation of pathogen isolates (virus, bacteria, parasites) by nucleotide sequencing for the tracing of their origin. This is particularly important for epidemic diseases, where the possibility of pinpointing the source of infection can significantly contribute to improved disease control. Furthermore, the development of genetic probes, which allow the detection of pathogen DNA/RNA (rather than antibodies) in livestock, and the advances in accurate, pen-side diagnostic kits can considerably enhance animal health programmes.

DNA testing is also being used to diagnose hereditary weaknesses of livestock. One test identifies the gene which produces Porcine Stress Syndrome in pigs. Pigs with this gene tend to produce pale, poor-quality meat when they undergo the stress of transport or slaughter. Now that pigs with this gene can be identified, they can be excluded from breeding programs, so the gene will become less common.

DNA is also being used to diagnose a mutation of Holstein cattle that causes leucoyte adhesion deficiency. Cattle with this condition suffer diseases of the gum, tooth loss and stunted growth. They usually die before they are one year old. This test will identify carriers and eliminate them from breeding herds. Bulls used for breeding can be tested to make sure they are not carriers.

DNA technologies in animal nutrition and growth

Nutritional physiology

Applications are being developed for improving the performance of animals through better nutrition. Enzymes can improve the nutrient availability from feedstuffs, lower feed costs and reduce output of waste into the environment. Prebiotics and probiotics or immune supplements can inhibit pathogenic gut microorganisms or make the animal more resistant to them. Administration of recombinant somatotropin (ST) results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows. Immunomodulation can be used for enhancing the activity of endogenous anabolic hormones.

In poultry nutrition, possibilities include the use of feed enzymes, probiotics, single cell protein, and antibiotic feed additives. The production of tailor-made plant products for use as feeds and free from antinutritional factors through recombinant DNA technology is also a possibility.

Plant biotechnology may produce forages with improved nutritional value or incorporate vaccines or antibodies into feeds that may protect the animals against diseases.

Rumen biology

Rumen biology has the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and of limited value for other animal species. Biotechnology can alter the amount and availability of carbohydrate and protein in plants as well as the rate and extent of fermentation and metabolism of these nutrients in the rumen contribution of introduced new strains.

Methods for improving rumen digestion in ruminants include the use of probiotics, which is the supplementation of animal feed with beneficial live microbes to improve the intestinal microbial balance for better utilization of feed and for good health. The added bacteria may improve digestion of feed and absorption of nutrients, stimulate immunity to diseases, or inhibit growth of harmful microbes. Transgenic rumen microbes (chapter 6) could also play a role in the detoxification of plant poisons or inactivation of antinutritional factors. Successful introduction of a caprine rumen inoculum obtained in Hawaii into the bovine rumen in Australia to detoxify 3-hydroxy 4(IH) pyridine (3,4 DHP), a breakdown product of the non-protein amino acid mimosine found in *Leucaena* forage is an example.

Genetic Engineering of Microorganisms of Interest to Agriculture

Introduction

In the context of genetic engineering targeting development of genetically modified organisms (GMOs), micro organisms of interest to agriculture represent a genetic resource. These microbes may find use as gene transfer systems or donors or recipients of desirable genes. Microbes functioning as gene transfer systems and as donors of genes, have already been discussed. The focus of this chapter is therefore on microbial recipients of transgenes.

Microorganisms play important roles in different sectors of agriculture, food processing, pharmaceutical industries and environmental management. Microbial processes are under the control of genes and there is need to continually improve and optimize their specific processes through genetic improvement. Traditionally this largely depended on the identification and selection of mutants with desirable characteristics. Recombinant DNA technology presents a number of benefits to this area, since specific metabolic pathways can be manipulated with more precision and completely new functions can now be engineered into the microbes. The following sections give some examples of microorganisms of economic importance that have been genetically modified through recombinant DNA Technology.

Genetically modified Microbes as Biopesticides and Biofertilizers

Biological control agents are especially targeted for genetic enhancement because of an increasing emphasis given to them in modern agriculture. Biological control represents an alternative to chemical pesticides which have been the object of much criticism due to their adverse impact on the environment and human health. There is therefore a need to develop safer and environmentally amenable control using existing organisms in their habitats. These organisms offer protection against a wide range of plant pests and pathogenic microbial agents without damage to ecosystems.

In order for biological control agents to be effective in plant disease management, they must be effective, reliable and economical. To meet these conditions superior strains are often required. In this case the existing attributes of the biocontrol agents can be genetically manipulated to enhance their biocontrol activity and expand its spectrum.

The foreign genes used for transforming biological control agents can be integrated into the host genome or plasmid. To express a heterologous gene in fungi or bacteria, the regulatory region of this gene must be modulated in promoter and terminator exchange in order to optimize the activity of the inserted gene in the new host. Addition of specific genes known to confer biocontrol activity may enhance or improve biocontrol capacity of organisms deficient in the genes targeted for transfer. The majority of rhizobacterial with biocontrol activity produce chitinases.

Free living bacterial associated with plants have been targeted to enhance their capacity either as soil inoculants or as biocontrol agents of plant pathogens. Studies on microbes capable of enhancing plant growth have concentrated on the rhizosphere whereas those on biocontrol target both rhizosphere and phylloplane. Several important rhizobacteria including *Rhizobium melliloti* and *Pseydomonas putidrii,* both of which are excellent root colonizers lack the ability to synthesize chitinases. Introducing genes encoding chitinases into their genome have enabled them to provide protection against plant pathogenic fungi. These two bacteria are good targets because of unique beneficial characteristics they confer. *Rhizobium* is a symbiotic bacterium which stimulates formation of root nodules in legumes involved in fixing atmospheric nitrogen. Many pseudomonads in the rhizosphere environment produce siderophores which chelate iron ions, thereby increasing iron uptake by plants. The genetically modified commercial strain (RMBPC-2) of *Sinorhizobium melliloti* has added genes that regulate nitrogenase enzyme (nitrogen fixation) from the plant to the bacterium.

Trichoderma species are widely represented in soils and are antagonistic to other fungi. *T. harzianum,* in particular is a strong rhizosphere colonizer which is also able to parasitize plant pathogenic fungi. It establishes tight physical contact with hyphae of target fungi with the aid of binding lectins. Several extracellular enzymes including chitinases, glucanases, lipases and *proteases* produced by *Trichoderma* species has been improved with the transfer of chitinase genes, notably from *Serratia marcessens.*

Agrobacterium radiobacter strain k84 protects plants against crown gall caused by *A. tumefaciens* strains carrying Ti-plasmid of the nopaline type. Protection conferred by *A. radiobacter* strain k84 is due to agrocin 84, an adenine nucleotide derivative, when taken up by *A. tumefaciens,* inhibits DNA synthesis, resulting in cell death. *A radiobacter* has an additional negative effect on soil pathogens by being a very effective rhizosphere colonizer. Although *A. radiobacter* strain 84 has been widely used commercially for a long time, there was concern about its long term effectiveness as a biocontrol agent. This is because the gene encoding agrocin is carried on a transmissible plasmid, which can be transferred by conjugation to *A. tumefaciens.* In the event of agrocin-encoding plasmid transfer, receipient *A. tumefaciens* strains will not be subjected to biocontrol by *A. radiobacter* strain k84. This concern was addressed by modification of the agrocin-encoding plasmid to prevent its transfer to *A. tumefaciens*. The ensuing genetically engineered strain, known as *A radiobacter* strain K1026, is a transgenic organism approved for use as a pesticide.

Bacillus thuringiensis (Bt) has been used as a biopesticide for a long time, however it has the disadvantage of fast degradation in sunlight. Different cry genes encoding the Bt toxin have been cloned and introduced into another bacterium, *Pseudomonas flourescens.* The transgenic *P. flourescens* strains are killed and used as a more stable and persistent biopesticide compared to the *B. thuringiensis* sprays.

Baculoviruses are also being manipulated to be effective biopesticides against insect pests such as corn borer, potato beetle and aphids.

Microbes for Enhancing the Use of Animal Feeds

Animal digestive tracts harbor beneficial microflora that aid in the digestability of various feeds. However, the function of these microbes is easily affected by the unfavorable conditions within the gut, such as acidity and antibiotics used to treat pathogenic microbes. Examples of gut microbes that have been genetically modified include; *Prevotella ruminicola* with a tetracycline resistant gene, cellulolytic rumen bacteria with acid tolerance, hind gut bacteria with cellulose activity, rumen bacteria transformed with genes to improve protein yield and yeast (*Saccharomyces cerevisiae)* containing a transgene from a closely related *Saccharomyces diastaticus,* allowing it to better increase the digestibility of low-quality roughage in conventional feeds. The major limitation to the use of these engineered organisms has been their establishment in the appropriate regions of the gut. Some organisms are being used as beneficial supplements in animal feeds. These are called probiotics and their use aims at improving digestion of feed and absorption of nutrients, stimulate immunity to diseases and inhibit growth of harmful microbes.

In the improvement of silage, strains of the bacteria *Lactobillus planetarium* are being developed which increase the lactate content and reduce the pH and ammonia-N content.

Microbes are being used as bioreactors for the production hormones and other substances that enhance animal size, productivity and growth rates. Synthetic hormone bST (bovine

somatotropin) was among the first innovations available commercially. It can increase milk yield by as much as 10 to 15 percent in lactating cows. Current development efforts are looking at a wide spectrum of genes that affect growth and production within the animal.

Genetically Modified Microbes in Food Processing

Many microbes are being manipulated with the objectives of improving process control, yields and efficiency as well as the quality, safety and consistency of bioprocessed products. Modifications target food enzymes, amino acids, peptides (sweeteners and pharmaceuticals) flavors, organic acids, polysaccharides and vitamins. Classical example is the production of the recombinant cheese making enzyme, chymosin in bacteria. Its use was approved in 1990 in the USA, and now 80% of US cheese is produced using this product.

Genetically Modified Microbes in Bioremediation

Microbes are widely used in cleaning up pollution such as oil spills, agricultural and industrial wastes by degrading them into less toxic compounds. Some bacteria are being used as "bioluminescensors" that give luminescence in response to chemical pollutants e.g. the mercury resistance gene *mer* expressed in some bacteria can light up signaling the presence of even very low levels of mercury in the environment.

A modified bacterium, *Rhodopseudomonas capsulate*, has the ability to grow rapidly in simple synthetic media. It is being used in advanced swine waste treatment plants in both Japan and Korea. Short chain fatty acids, one of the main sources of the bad odor of swine wastes, decreased dramatically after treatment. The residue after treatment can be used as a safe organic fertilizer.

GMOs Detection Methods

Introduction

Different stackholders involved in the development, use and regulation of genetically modified organisms (GMOs) do at some point need to monitor and verify the presence and the amount of GMOs in agricultural crops and in products derived thereof. This need has generated a demand for analytical methods capable of detecting, identifying and quantifying either the unique DNA sequences introduced or the protein(s) expressed in transgenic plants. Thus comprehensive GMO analysis techniques consists of three steps: detection, identification and quantification.

1. ***Detection (screening of GMOs):*** The objective is to determine if a product contains GMO or not. For this purpose, a screening method can be used. The result is a positive/negative statement. Analytical methods for detection must be sensitive and reliable enough to obtain accurate and precise results.

2. ***Identification:*** The purpose of identification is to reveal how many different GMOs are present and if they are authorized or not. Specific information (i.e. details on the molecular make-up of the GMOs) has to be available for the identification of GMOs.
3. ***Quantification:*** If a food product has been shown to contain (one or more) authorized GMOs, then it becomes necessary to assess compliance with the set threshold level regulation by the determination of the amount of each of the GMOs present in the individual ingredients from which the food item has been prepared (e.g. maize flour).

GMO detection by phenotypic characterization

Phenotypic characterization is possible if the gene targeted allows for determination of absence or presence of a specific trait. Detection methods using this approach are referred to as bioassays. This approach can be used to test for the presence or absence of herbicide resistance transgenes. This test involves germinating seeds in the presence of the herbicide of interest. Herbicide assays are considered to be accurate and inexpensive. Controls, including seeds with or without the trait targeted should be included in all samples tested. Herbicide assays are available for Roundup-Ready crops. Typically a test of 400 seeds is used. The accuracy is dependent on the germination: the higher germination the higher is the confidence level of the test. Only viable seed or grain can be tested (no processed products), and each test requires seven days to complete. The potential error of accuracy increases as the germination level of the sample decrease. Furthermore, bioassays require separate tests for each trait in question and at present the tests will not detect non-herbicide tolerance traits. Therefore, the tests are only of limited value for inspection authorities.

Molecular Detection of GMOs

Methods that target the inserted DNA and its expressed proteins have been developed and are widely used for detecting GMOs. This is because the target DNA can be purified and amplified by polymerase chain reaction (PCR) [Box 1]. Another advantage associated with working with DNA is that there is usually a linear relationship between quantity of GMO and the inserted DNA carried in the nucleus, thus it can be used to quantify the amount of GMO material present in a sample.

Protein-based methods rely on a specific binding between the protein and an antibody. The antibody recognizes the foreign molecule, binds to it, and in GMO detection assays the bound complex is successfully detected in a chromogenic (color) 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 GMO itself, or it can be synthesized in a laboratory if the composition of the protein is known in detail.

The Polymerase Chain Reaction (PCR)

The PCR reaction allows the million-fold amplification of a specific target DNA fragment framed by two primers (=synthetic oligonucleotides, complementary to either one of the two strands of the target sequence). In principle, the PCR is a multiple-step process with consecutive cycles of three different temperatures, where the number of target sequences grows exponentially according to the number of cycles. In each cycle the three temperatures correspond to three different steps in the reaction. (*Refer to diagrams in the power point presentation).*

In the first step, the template, i.e. the DNA serving as master-copy for the DNA polymerase is separated into single strands by heat denaturation at ~94^{o}C.

In the second step, the reaction mix is cooled down to a temperature of 50-65^{o}C (depending on the GC content) to allow the annealing of the primers to the target sequence. Primer hybridization is favoured over DNA-DNA hybridization because of the excess of primers molecules. However, the annealing process is uncontrolled and can give rise to a large number of mismatched DNA duplexes,

In the third step, the annealed primers are extended usually by a *Thermus aquaticus* (Taq) polymerase at the optimum temperature of 72^{o}C. With the elongation of the primers, a copy of the target sequence is generated).

The cycle is then repeated 20 to 50 times, depending on the amount of DNA present and the length of amplicon (= amplified DNA fragment).

PCR Based GMO Detection

For routine GMO screening purposed one should focus on target sequences that are characteristic for the group to be screened. Genetic control elements such as cauliflower mosaic virus 35S promoter (P-35S) and the *Agrobacterium tumefaciens* nos terminator (nos3') are present in many GMOs currently on the market. The first GMO screening method is based on the detection of the P-35S and nos3' genetic elements. However, a few approved GMOs are not screenable/detectable with the P-35S or the nos3' primers and additional target sequences are needed to guarantee a complete screening procedure. A further aspect is the choice of primers that allow the detection of as many variants as possible of a GMO marker, e.g. there are at least 8 variants of P-35S used in GM crops. It should be stressed however, that the detection of these generic GMO markers only indicates that the analysed sample contains DNA from a GM plant, but does not provide information on thespecific trait that has been engineered in the plant.

Most PCR-based GMO detection methods include a positive control primer set, which is specific for a gene that is present naturally in all varieties of the applicable species. This reference is a species-specific gene, such as the lectin gene is soybean or the invertase gene in maize. If a strong signal is not obtained with the positive control primer set, then there may be problems with the integrity or purity of the DNA. On the other hand, if the DNA is

detectable, the samples can be screened using the general genetic elements which cater for multiple varieties of GMO DNA. Furthermore, if positive results from this initial screening are obtained, additional confirmation using tests which screen for specific genes or constructs are most common in GM crops is carried out. This three-step approach ensures that results obtained have been confirmed using multiple screening systems. This approach applies to situations where sequence material or reference data are available.

Confirmatory assays

Confirmation/verification of the identity of the amplicon is necessary to ensure that the amplified DNA is really corresponding to the chosen target sequence and is not a by-product of un-specific binding of the primers. Several methods are available for this purpose. Gel electrophoresis is the simplest approach to control if the PCR products have the expected size. However, there is a risk that an artifact having the same size of the target sequence may have been amplified. Therefore, the PCR products should additionally be verified for their restriction enzyme profile. Even more reliable is a Southern blot assay, whereby the amplicon is separated by gel electrophoresis, transferred onto a membrane and hybridized to a specific DNA probe. Another possible control is to subject the PCR product to a second round of PCR cycle in a technique that is called nested PCR. Here, two different sets of primers – an outer and an inner (nested) pair – are being used within the target region in two consecutive rounds of PCR amplifications. This strategy reduces substantially the problem of un-specific amplification, as the probability for the inner pair of primers of finding complementary sequences within the non-specific amplification products of the outer pair is extremely low. The most reliable way to confirm the authenticity of a PCR product is its sequencing. However, only few laboratories are equipped to carry out this approach routinely.

Protein-based methods: Immunoassay

Immunoassay is the current method for detecting and quantifying a target protein associated with genetic modification. It can be used for qualitative and quantitative measurements over a range of concentrations. Different types of immunoassays including enzyme-linked immunosorbent assay (ELISA), dipstick and lateral flow procedures are available for use in the field and in the laboratory.

ELISA

In ELISA, the protein-antibody reaction takes place on a solid support and the protein and antibody react to produce a complex. This complex is usually visualized by adding a second antibody linked to an enzyme. When the substrate for the enzyme is added, a colored product is formed. The intensity of the color can be measured photometrically and used for quantitative assessments.

Some ELISA plates are supplied with calibration or known concentration of target protein in solution and a negative control defined by the absence of the target. The standards

will exhibit distinctively different intensities of a given color at the different concentrations of target molecules provided. By comparing the intensity of color of the sample tested for GMO target molecules with that of the standards, it is possible to work out the concentration range of the target. These immunoassay measurements are qualitative.

Quantitative measurements can however be obtained by using a microplate reader which measures the absorbance of all samples and standards at the same time. The detection limit is less than 0.01 percent.

Lateral flow strips and dipsticks

Paper strips or plastic paddles on which antibody is captured are also used to detect proteins targets of GMOs. The strip is dipped in vials containing solutions of the sample to be tested. Each dip is followed by rinsing. The positive reaction is a color change in the vial. Recent improvements of the 'dip stick' have produced lateral flow strips in which reagents are transported through nylon membrane by capillary action. Antibodies specific to the target protein are coupled to a colored reagent and one incorporated into the lateral flow strip. When the strip is brought into contact with a small amount of the sample containing the target protein, an antibody-antigen complex is formed with some of the antibody. The membrane contains two capture zones, one for the bound protein and the other for the colored reagent. A colored band appears in the capture zone corresponding to the bound antibody-protein complex and colored reagent. Appearance of a single colored band in the membrane is a negative test for the presence of the protein targeted. The presence to two bands represents detection of the target.

PCR Methods for GMO Identification

Following a positive result from a GMO screening exercise the next step is the unequivocal identification of the GM event(s) involved. This can be achieved by one of the following PCR strategies.

Gene specific PCR. This is a PCR system targeting one element of the transgenic element. It is less specific and only useful if the genetic element is present in only one event.

Constructive specific PCR. Sometimes its sufficient to only identify GMOs using PCR system designed for the amplification of the junction between different elements of the transgenic insert.

Event specific PCR. This is the most specific GMO identification strategy. It is designed to amplify the junction between the transgenic insert and the host DNA (the so called fragments)

Thus there is need for a continuous survey of all data available on GMOs – especially the introduced genetic elements and their integration sites, not only for GM products approved for market release but also for any other GMO released for field trials worldwide. This can guarantee a complete comprehensive monitoring, detection and identification of GMOs.

Quantitative PCR

In the event that there is positive test for GMO content in a sample, it is important to quantify the amount to test for compliance with specific threshold levels of GMOs established by biosafety regulations. The typical approach to quantification utilizes one or more of the broad-spectrum primer set that recognizes common transgenic elements in GMOs. However, since different transgenic events contain these common elements in different numbers, accurate determination of GMO content cannot rely on the use of these common sequence elements. Quantification based on event-specific primers is therefore the most accurate means of obtaining quantitative results on GM content.

Use of Conventional PCR quantification

One possibility for DNA quantification using conventional PCR is competitive PCR. In competitive PCR, one primer pair is used to amplify both the target GMO DNA and a synthetic DNA fragment of known concentration in the same reaction mixture. The second fragment, which is a different size to the GMO target DNA, is called the competitor. By conducting a series of experiments with varying amounts of the synthetic DNA, it is possible to determine the amount of target GMO DNA. In situations when the PCR products are equal in intensity as determined visually following gel electrophoresis, the initial amounts of target GMO DNA equal the initial amounts of synthetic DNA. Competitive and double competitive PCR methods are semi-quantitative as a standard is required for comparison. In these cases the standard is the known amount of synthetic DNA. Consequently, the results will only indicate a value below, equal or above a defined concentration of the standard.

Real-time PCR for GMO quantification

Another strategy of the second group that improves accuracy, specificity and throughput of quantitative PCR is "Real-time PCR". This technique was originally developed in 1992 and is rapidly gaining popularity due to the introduction of several complete real-time PCR instruments and easy-to-use PCR assays. A unique feature of this PCR technique is that the amplification of the target DNA sequence can be followed during the whole reaction by indirect monitoring of the product formation. Therefore, the conventional PCR reaction has to be adapted in order to generate a constant measurable signal, whose intensity is directly related to the amount of amplified product. Real-time detection strategies rely on continuous measurements of the increments in fluorescence generated during the PCR reaction. The number of PCR cycles necessary to generate a signal that is significantly and statistically above noise level is taken as a quantitative measure and is called cycle threshold (C_t) (figure 7.1A). As long as the C_t value is measured at the stage of the PCR where the efficiency is still constant, the C_t value is inversely proportional to the log of the initial amount of target molecules (figure 7.1B).

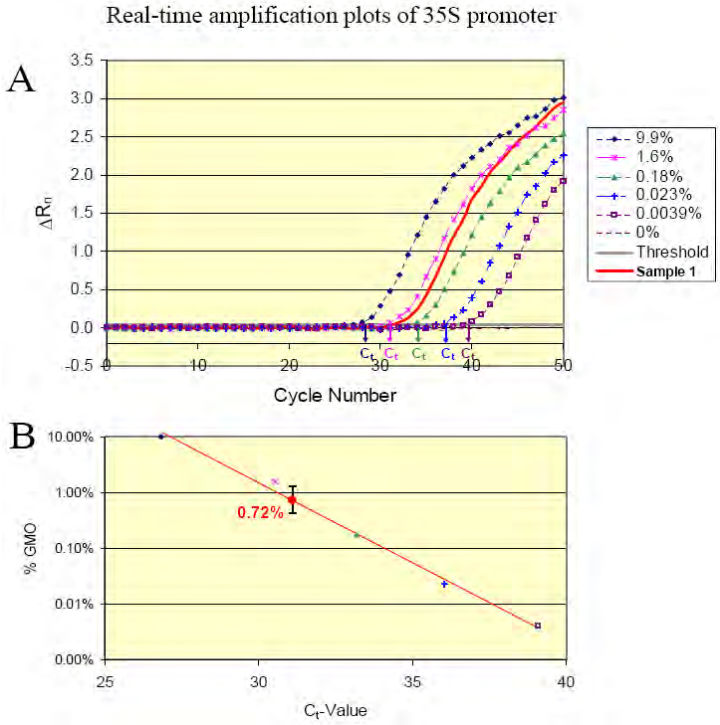

Fig 7.1. Real Time PCR. **A)** Diagram showing the accumulation of the target analyte 35S promoter at six different ratios of GMO/non-GMO material (% w/w). PCR product formation is visualized in real time by taking fluorescence measurements (R_n) at each cycle. The initial template concentration determination is based on the threshold cycle (C_t), i.e. the PCR cycle at which fluorescence is first detected statistically significant above background. C_t is inversely proportional to the log of the number of target copies present in the sample. **B)** Linear regression diagram showing the logaritmic relation between the GMO/non-GMO ratios and the C_t values.

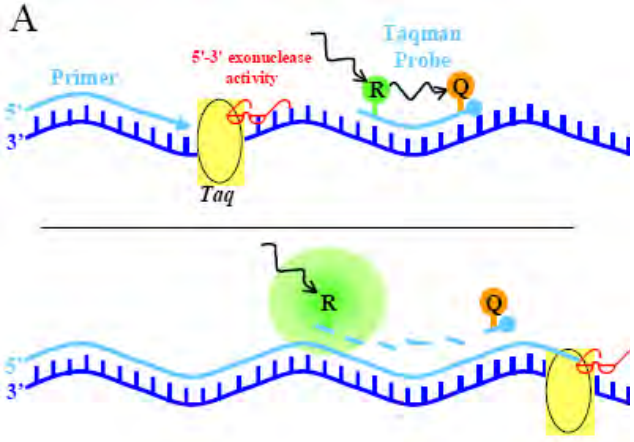

Fig 7.2. Amplicon Specific TaqMan probes used to monitor the PCR product formation in real-time (see text for details)

One of the most popular assays for real-time PCR is the Taqman® or 5'-exonuclease assay, which employs a fluoregenic probe consisting of an oligonucleotide with both a reporter (R) and a quencher (Q) dye attached (TaqMan® probe, see Fig 7.2). when the probe is intact the reporter fluorescence is quenched by the proximity of the quencher dye. Due to its target-specific sequence, the probe anneals specifically to the amplification product (target DNA) between the forward and the reverse primers. If hybridization has occurred, the 5'-3' exonuclease activity of the Taq polymerase cleaves the internal probe during the extension step of amplification. The cleavage reduces the quenching effect and the fluorescent signal of the reporter dye becomes a measure of the amount of amplification product generated (Fig 7.1A). Because the development of the fluorogenic reporter signal takes place only if both the PCR primers and the TaqMan® probe anneal to the target DNA, the specificity of real-time PCR detection is considerably higher than that of conventional PCR. The relative quantification of the target gene is made possible by preparing a standard curve from known quantities of an additional endogenous gene and extrapolation from the linear regression line.

Limits of quantification by PCR and Sampling

Although 0.01 percent is the limit of detection using PCR, quantitative measurement is not possible at this level. This is because the number of GMO targets in preparation derived from a sample containing 0.01 percent GMO material will be in the range 1 to 4 or more. These preparations will produce GMO target measurements that are more influenced by statistical variation associated with sampling than by actual GMO content in the sample. As a result, most laboratories set the limit of quantification by PCR 10 times higher (0.1%) to avoid measurement problems near the limit of detection.

Irrespective of the analytical method selected for GMO detection, correct sampling is critically important. Samples must therefore be taken in a manner that ensures that they are statistically representative of the larger volume or quantum of material. The sample size will therefore depend on the detection level required and what is being detected.

The International Seed Testing Agency (ISTA) has produced sampling guidelines. For example:

- 100 plants give a 95 percent confidence limit for detecting a 3 percent contamination level.
- 200 plants give a 95 percent confidence limit for detecting a 1.5 percent contamination level
- 300 plants give a 95 percent confidence limit for detecting a 1 percent contamination level.

The working sample which is a sub-sample of the overall sample should also be properly determined. According to ISTA methods the working seed sample should contain a minimum of 3000 seeds.

Alternative Techniques for GMO Analysis

Chromatography and Near Infrared Spectroscopy

Where the composition of GMO ingredients, e.g. fatty acids or triglycerides is altered, conventional chemical methods based on chromatography near infrared spectroscopy can be applied for detection of differences in the chemical profile. This has been demonstrated with oils deriving from GM canola for which high performance liquid chromatography (HPLC). Triglyceride patterns and content can be compared between GM and Non GM samples. However, it must be stressed that this methodology is only applicable when significant changes occur in the composition of GM plants or derived products. Moreover, it is a qualitative detection method rather than a quantitative method. Low additions of, e.g., GM canola oil with an altered triglyceride composition to conventional canola oil will most probably not be detected, also considering the natural variation of ingredient patterns.

Microarrays

Microarrays 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. Microarrays technology is based on the classical DNA hybridization 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.

Microarrays allow analysis of expression patterns of thousands of genes within the confines of one experiment. Arrays are direct descendents of DNA gel-blot (Southern) based assays that exploit interactions between complimentary strands of DNA. The addition of a solid glass substrate, precision robotics, and the use of fluorescence provide expression arrays with increased precision, speed and scale over their filter-and radioactivity-based cousins. Although transcript monitoring is currently the most popular use for arrays, they have been successfully utilized in fields ranging from mutation detection to evolutionary sequence analysis. Micro-arrays can be constructed using either PCR-amplified cDNAs or oligonucleotides.

Some GMO chip kit detects species-specific DNA of plants and viruses, generally used genetic construction elements, and specifically introduced genetic modifications for the identification of approved and non-approved plant varieties. For example, the GMO chip version “The European” detects specific DNA from soybean, maize, oilseed rape, rice, CaMV (species) and the following GMOs: RR-soybean, Maximizer Bt 176 maize, Bt11 maize, Yieldgard Mon810 maize and Bt-Xtra maize. In addition, GMO chip allows screening for all GMOs with the CaMV 35S promoter, Nos-terminator, bar-gene and pat-gene.

As microarrays technology has expanded, quantitative comparison of data within and across microarray platforms has proven difficult. The main reasons for this are a lack of universal references and the variety of data analysis methods in use. The microarray, in

principle, enables the detection, identification and quantification of large numbers of GMO varieties present in a sample in one single assay. Furthermore, microarrays are very flexible, as new varieties can be included in the screening procedure by adding additional sequences to the array.

Genes of Interest to Agriculture

Introduction

The field of field production has benefited immensely from recombinant DNA technology. Transgenic crops with novel agronomic and quality traits have developed and are grown in many developed and developing countries. For a detailed account on the nature and extent of utilization of the various GM crops, one can consult online databases such as AGBIOS (http://www.agbios.com) FAOBioDeC (http://www.fao.org/inventory.admin/dep/default.asp). ThAGBIOS website includes details of the transgenes, the science underpinning the traits and environmental and food safety issues. The FAOBioDeC database is an online inventory of biotechnologies in developing countries. It covers both genetically modified and non-GM technologies. Surveying information in these and similar databases it is possible to see what genes have been used in generation transgenic crops and which ones are still being developed.

Genes that seem to be of much interest to both the public and private sectors are those that confer resistance to biotic and abiotic stresses as well as quality traits. Comparisons of attainable and actual yields demonstrate that most crops are at best only reaching 20% of the genetic potential for yield. The reduction in yield are attributed to both biotic (e.g. pests, pathogens and weeds) and abiotic stresses. Out of a $1.3 trillion annual food production capacity worldwide, the biotic stresses caused by insects, diseases and weeds cause 31-42% loss ($500 billion), with an additional 6-20% ($120 billion) lost post harvest to insects and to fungal and bacterial rots. Crop losses due to pathogens are often more severe in developing countries (e.g. cereals 22%) when compared to crop losses in developed countries (e.g. cereals 6%). Weeds are also a major and continuing biotic constraint affecting cropping systems worldwide. Another 6-20% ($120 billion) is estimated to be lost to abiotic causes (drought, flood, frost, nutrient deficiencies, various soil and air toxicities). One of the most significant abiotic stress reducing crop yields is water stress, both water deficit stress (drought) and excess water stress (flooding, anoxia). It is in this context that the need arises to develop crops which are more resistant to biotic and abiotic stresses.

Genes for Resistance to Biotic Stresses

Herbicide Resistance Genes

Glyphosate herbicide tolerance: The genetically modified glyphosate resistant crops contain a gene encoding the enzyme 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS). The EPSPS gene was originally obtained from a strain of the soil inhabiting bacterium.

Agrobacterium tumeraciens. EPSPS allows plants to survive the otherwise lethal effects of the herbicide glyphosate. The EPSPS is an important part of shikimate biochemical pathway which is required to produce aromatic amino acids plants needs to grow and survive. Conventional plants treated with glyphosate cannot produce the aromatic amino acids. Glyphosate binds to and activates EPSPS.

Glufosinate ammonium herbicide tolerance

Glufosinate ammonium is the active ingredient in the phosphinothricin herbicides. Glufosinate chemically resembles amino acid glutamate and functions by inhibiting the enzyme glutamate synthase which converts glutamate to glutamine. Glutamine synthesis is also involved in the ammonia detoxification of glufosinate resulting in reduced glutamine levels and increases in ammonia concentration. Elevated levels of ammonia damage cell membranes and impair photosynthesis. Glufosinate tolerance is the result of introducing a gene encoding the enzyme phosphinothricin-aan-acetyl transferase (PAT). The gene was originally obtained from the soil actinomycete Streptomyces hygroscopiens. The PAT enzyme catalyses detoxification of phosphinothricin.

Sulfonylurea herbicide tolerance

Sulfonyl urea herbicides, such as triasulfuron and metasulfron-methyl target the enzyme acetolactate synthase (ALS), thereby inhibiting the biosynthesis of the branched chain animo acids valine, leucine and isoleucine. It resuts in accumulation of toxic levels of alpha-ketoglutarate. In addition to the native ALS gene, herbicide tolerant crops contain the ALS gene from a tolerant line of Arabadopsis thaliana. This variant ALS gene differs from the wild type by one nucleotide and the resulting ALS enzyme differs by one amino acid from the wild type ALS enzyme.

Oxynil herbicide tolerance

Oxynil herbicides and bromoxynil are effective against broad leaf weeds. Transgenic herbicide resistant crops contain a copy of the bxn gene isolated from the bacterium Klebsiella pneumoniae. The gene encodes a nitrilase which hydrolyzes oxynil herbicides to non-phytotoxic compounds.

Insect Resistance

Among the insect pests, Lepidoptera represent a diverse and important group. Most insect-resistant transgenic crop varieties developed so far for the control of Lepidoptera, predominantly using transgene cassettes including toxin-producing Cry-type genes obtained from strains of the soil bacterium Bacillus thuringiensis (Bt). The proteins bind to specific sites on the gut lining in susceptible insects. The binding disrupts midgut ion balance which eventually leads to paralysis, bacterial sepsis and death. In addition to Bt genes, protease inhibitors, neuropeptides and peptide hormones that control and regulate the physiological

processes of several insect pests have become candidates for developing insect resistant crops. Other biocontrol toxins currently studies are chitinases, lectins, alpha-amylase inhibitors, cystatin and cholesterol-oxidase and glucosidase inhibitors.

Resistance to Pathogens

Among the disease causing organisms, viruses have received a lot of attention in as far ad the development of transgenic crops is concerned. This has been possible since the discovery of pathogen derived resistance, where the expression of a viral protein (e.g. coat protein, replicase, helicase enzyme etc) in a transgenic plant renders that plant resistant to the virus. As a result many viral genes have been cloned and used to transform crops. Genes encoding chitinases and glucaneses have been used to generate plants resistant to fungal and bacterial pathogens respectively. Other strategies for conferring resistance to pathogens in transgenic crops include genes for phytoalexins which are involved in pathogen induced localization or infection and R genes (resistance genes) being identified as responsible for defense mechanisms in plants.

Genes for Resistance to Abiotic Stresses

So far there are no commercialized transgenic crops with resistance to abiotic stresses such as drought, heat, salinity and frost. However, a number of approached are being developed to tackle these stress factors in crops.

Genes for Improved Quality Traits

Modified Flower Colour

Many flowers including carnations, roses, lilies, chrysanthemums and gerberas, all of which are important in global flower trade, do not produce the blue pigment delphinidin. Transgenic carnation lines with unique violet/mauve color have been developed. The genes of interest here include structural and regulatory genes of the flavanoid biosynthetic pathway.

Delayed Fruit Ripening and Increased Shelf life

Genes encoding an enzyme which degrades 1-aminocyclopropane 1-carboxylic acid (ACC), an ethylene precursor, and those encoding polygalacturonase (PG) have been suppressed in some transgenic plants. Suppression is accomplished by inserting a truncated or anti-sense version of the PG gene. Reduced ACC results in delayed fruit ripening while that of PG decreases the level of cell wall breakdown and hence delays fruit softening and rotting.

Modification of oil composition

Oil seed rape and soybean have been modified to increase the content of oleic acid in particular. The modified oils are lower in saturated fat and have greater heat stability than oils from the corresponding unmodified crops. In unmodified crops the FAD2 gene encoding a

desaturase converts C18:1 (oleic acid) to C18:2 and C18:3. In the modified crop a mutant FAD2 gene blocks expression of the active desaturase and as a result there is accumulation of oleic acid.

Modified Vitamin and Mineral Profiles

Vitamins and minerals are essential components of the human diet and dietary deficiencies of these nutrients can have tragic effects. In addition to fortification and supplementation strategies for alleviating these deficiencies, transgenic crops with elevated and bioavailable vitamins and minerals are being developed. Here the strategy is to express the genes responsible for the production or accumulation of the concerned nutrient in the edible parts of the plant. Thus promoters and sequences that target the expression of the genes of interest are equally important. In order to improve vitamin A production in rice the genes encoding phytoene synthase and phytoene dasaturase have been express in the endosperm. To improve iron accumulation and bioavailability in rice, genes such as ferritin synthase from soya (Fe storage), metallothionein (cystein rich storage protein, improve Fe sabsoption) and a heat stable phytase gene (degrades phytic acid which inhibits Fe absorption) have been expressed in the rice endosperm.

In: Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Chowdhury MKA, Hoque MI and Sonnino A (Eds.). pp. 51-105.

Chapter 2: Ecological Aspects of Biosafety

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Introduction

The debate on GMOs is no longer whether they should be released or not; it has moved to how and why they are being put to use. The GMOs are here to stay; the challenge is to use them to our advantage without jeopardizing the safety of the environment and human health. The acreage being planted to GMOs is increasing at exponential rates. In 1999, 11 million ha were planted to GM crops. By 2001, the area had increased to 44 million ha up to 114.3 million ha in 2007. GM crops were grown in 23 countries in 2007 (12 developing countries and 11 industrial countries). The total GM crop area was planted to four main crops: soybeans, maize, cotton and oilseed rape. Size of farm has not been a factor affecting use of the technology. Both large and small farmers have adopted GM crops. Size of operation has not been a barrier to adoption. In 2007, 12 million farmers were using the technology globally, 91% plus of which were resource-poor farmers in developing countries notably Argentina, Brazil, China, India and Paraguay (James, 2007).

GMOs carry exogenous genes or sequences. There is potential for these genes to affect gene expression in the plant and also for the product of this gene expression to interact with compounds in the plant. GMOs are introduced into ecosystems, and they will invariably interact with other organisms. The purpose of this module is to provide the necessary background information on ecology and evolution needed to analyse and understand the consequences of introducing GM organisms into the environment, as well as to show that many areas in ecology can benefit from research tools based on applications of molecular genetics and biotechnology. These tools include investigations into population biology and evolution, and conservation and use of genetic resources for both human requirements and environmental protection.

Introduction to Environmental Biosafety

Meeting the food needs of the world's growing population while reducing poverty and protecting the environment is a major global challenge. It is essential to take environmental concerns into account in order to develop technological solutions that are sustainable in the long run. Meeting these demands will require agricultural productivity increases and product diversification to improve the livelihoods of the poor, protect the environment, and ensure broad-based economic growth (UN Millennium Project 2005). Plant genome sciences, and plant biology as a whole, are vital enterprises that can contribute significantly to human health, agriculture, energy security, and environmental stewardship (The Academies of

Sciences, 2007). Promoting agriculture alone will not be enough to massively reduce poverty, but it can contribute to produce faster growth, reduce poverty, and sustain the environment (World Bank, 2007). Modern biotechnology is an important tool for crop improvement and novel uses of plants, animals, and microorganisms. Biotechnology is not a panacea, but a resource that can be useful when combined with adaptive research capacity (FAO, 2004).

Environmental benefits and concerns related with GM crops

Agriculture of any type – subsistence, organic or intensive – affects the environment, so it is natural to expect that the use of new genetic techniques in agriculture will also affect the environment. Releasing transgenic crops into the environment may have direct or indirect effects including: gene flow -gene transfer to wild relatives or conventional crops-, weediness, trait effects on non-target species, pest resistance, and other unintended effects. These risks are similar for transgenic and conventionally bred crops. Although scientists differ in their views on these risks, they agree that environmental impacts need to be assessed on a case-by-case basis and recommend post-release ecological monitoring to detect any unexpected events (ICSU, 2003). Transgenic crops may also entail positive or negative indirect environmental effects through changes in agricultural practices such as pesticide and herbicide use, and cropping patterns.

Some GM micro-organisms can be used in the environment as biological control agents or for bioremediation of environmental damage (e.g. oil spills), and their environmental effects should be assessed prior to release. One of the most significant environmental benefits of GM crops is the dramatic reduction in pesticide use (varying between crops and the introduced trait). Additionally, no-till, or conservation till cultivation systems are used, contributing to a reduction in fuel use in soil cultivation. The cumulative global impact of GM technology on farm income, pesticide usage, and greenhouse gas emissions among other indicators, has been studied and documented. Among the significant benefits for the farmers derived from the use of GM crops are higher crop yields, reduced farm costs, increased farm profit, and improvement in health and the environment (Brookes & Barfoot, 2008; 2006; NRC-CEI, 2002; Traynor *et al.*, 2002). The studies show that up to 2006 there have been substantial economic benefits at the farm level, amounting to a cumulative total of US$27 billion derived from enhanced productivity. Since 1996, there has been a 24.6% reduction in the environmental impact, and a 22.9% decrease in the volume of insecticides applied; the majority of the environmental benefits associated with lower insecticide and herbicide use have been for developing country farmers. The vast majority of these environmental gains have been from the use of GM IR cotton and GM HT soybeans (Brookes & Barfoot, 2008). Over the period 1996 to 2006 the cumulative permanent reduction in fuel use is estimated at 5,821 million kg of carbon dioxide (arising from reduced fuel use of 2,120 million litres). The carbon dioxide savings from reduced fuel consumption since the introduction of GM crops are equal to removing 2.58 million cars from the road for one year, and the additional probable

soil carbon sequestration gains in 2006 were equivalent to removing nearly 6.02 million cars from the roads (Brookes & Barfoot, 2008).

The “first” generation of GM crops has proven their ability to lower farm-level production costs. Now, research is focused on “second-generation” GM crops that will feature increased nutritional and/or industrial traits. These crops will have more direct benefits to consumers, and include: rice enriched with iron and provitamin A, potatoes with higher starch content, edible vaccines in maize and potatoes, crop varieties able to grow in stress conditions, healthier oils from soybean and canola (Lemaux, 2008; AgBios, 2002). Despite their potential, there is a multitude of concerns about the impact of GM crops on the environment. Key issues in the environmental assessment of GM crops are putative invasiveness, vertical or horizontal gene flow, other ecological impacts, effects on biodiversity and the impact of presence of GM material in other products. These are all highly interdisciplinary and complex issues. A crucial component for a proper assessment is defining the appropriate baseline for comparison and decision. For GM crops, the best and most appropriately defined reference point is the impact of plants developed by traditional breeding, which is an integral and accepted part of agriculture. In many instances, the putative impacts identified for GM crops are very similar to the impacts of new cultivars derived from traditional breeding. When assessing GM crops relative to existing cultivars, the increased knowledge base underpinning the development of GM crops will provide greater confidence in the assurances plant science can give on the risks of releasing such crops.

Where legislation and regulatory institutions are in place, there are elaborate steps to precisely avoid or mitigate these risks. It is the obligation of the technology innovators (i.e., scientists), producers, and the government to assure the public of the safety of the novel foods that they offer as well as their benign effect on the environment. There are also those risks that are neither caused nor preventable by the technology itself. An example of this type of risk is the further widening of the economic gap between developed countries (technology users) versus developing countries (nonusers). These risks, however, can be managed by developing technologies tailor made for the needs of the poor and by instituting measures so that the poor will have access to the new technologies.

Environmental biosafety evaluation

Safety is a relative concept. Agriculture and animal husbandry have inherent dangers, as do the consumption of their products. Any sound evaluation of the safety of genetic engineering must also consider the "safety" of current methods of producing food. Risk is an integral part of everyday life. No activity is without risk. In some cases inaction also entails risk. Agriculture in any form poses risks to farmers, consumers and the environment. Risk analysis consists of three steps: risk assessment, risk management and risk communication. Risk assessment evaluates and compares the scientific evidence regarding the risks associated with alternative activities. Risk management –which develops strategies to prevent and

control risks within acceptable limits– relies on risk assessment and 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 decisions can be made (FAO, 2004).

There is broad consensus that the environmental impacts of transgenic crops and other living modified organisms should be evaluated using science-based risk assessment procedures on a case-by-case basis depending on the particular species, trait and agro-ecosystem. The environmental release of transgenic organisms should be compared with other agricultural practices and technology options (FAO, 2004, Ammann *et al*., 2003). All GM crops are thoroughly evaluated for environmental effects before entering the marketplace. They are assessed by many stakeholders in accordance with principles developed by environmental experts around the world. The evaluation procedures include information about the role of the introduced gene, and the effect that it brings into the recipient plant. Also there are specific questions about unintentional effects such as: impact in non-target organisms, whether the modified crop might persist in the environment longer than usual or invade new habitats, likelihood and consequences of the gene being transferred unintentionally to other species (FAO, 2004). A safety assessment is specific to the product and region and considers the nature of the trait, crop plant biology, farming practices and the ecological community among other aspects. Environmental risk assessment and management include: identification of the possible adverse effect, evaluation of the risk magnitude and its possible occurrence, risk characterization, probability of occurrence, consequences of possible adverse effects and design of strategies for risk management

Regulators in different countries typically require similar types of data for environmental impact assessments, but they differ in their interpretation of these data and of what constitutes an environmental risk or harm. Scientists also differ on what the appropriate basis for comparison should be: with current agricultural systems and/or baseline ecological data, in the value of small-scale laboratory and field trials and their extrapolation to large-scale effects and in the use of modelling approaches that incorporate data from geographical information systems. More research is needed on the post release effects of transgenic crops, as well as more targeted post-release and better methodologies for monitoring (ICSU 2003; FAO, 2004). Risk is often defined as “the probability of harm”. A hazard, by contrast, is anything that might conceivably go wrong. A hazard does not in itself constitute a risk. Thus assessing risk involves answering the following three questions: What might go wrong? How likely is it to happen? What are the consequences? (Risk = hazard × probability × consequences). The seemingly simple concept of risk assessment is in fact quite complex and relies on judgment in addition to science. Risk can be underestimated if some hazards are not identified and properly characterized, if the probability of the hazard occurring is greater than expected or if its consequences are more severe than expected. The probability associated with a hazard also depends, in part, on the management strategy used to control it. People are more likely to

accept the risks associated with familiar and freely chosen activities, even if the risks are large. In risk analysis, the following questions should be kept in mind: Who bears the risk and who stands to benefit? Who evaluates the harm? Who decides what risks are acceptable?

Biotech crops contribute to reducing the environmental impact of productive agriculture, thereby increasing global food security without the need for increased land clearance. Insect resistant crops offer an alternative to chemical inputs on some crops and have allowed development of more targeted, flexible, effective and sustainable integrated pest management programmes. Biotech applications in the R&D pipeline (disease resistant, drought and stress tolerant crops, biofortified) offer additional opportunities to increase global food security while further reducing the environmental footprint of agriculture. All possible paths of action must be compared, including inaction, in respect of improving, in a cost-effective and environmentally sustainable way, human health, nutrition, and the ability to afford an adequate diet. The improvement of agriculture and food security depends on several factors. These include stable political environments, appropriate infrastructures, fair international and national agricultural policies, access to land and water, and improved crop varieties, which are suited to local conditions. In particular cases, GM crops can contribute to substantial progress in improving agriculture, in parallel to the (usually slow) changes at the socio-political level. GM crops have demonstrated the potential to reduce environmental degradation and to address specific health, ecological and agricultural problems which have proved less responsive to the standard tools of plant breeding and organic or conventional agricultural practices.

Evaluations that involve both molecular geneticists and environmentalists could help to identify likely applications of genetic technology and establish the most effective and appropriate methods of using them. Considerable experience exists in the introduction of organisms into the environment and this experience can provide some meaningful guideposts. It is also apparent that new genetic tools will open up new opportunities to obtain additional useful knowledge concerning the relationship among various species in the environment. Such knowledge can provide a rational base to the establishment of prudent procedures for future applications. Science cannot declare any technology completely risk free. GM crops can reduce some environmental risks associated with conventional agriculture, but will also introduce new challenges that must be addressed. Society will have to decide when and where genetic engineering is safe enough. As the world population explodes there is an ever-increasing pressure on the earth's limited resources. Thus, it is appropriate that any intervention into the environment be scrutinized carefully. It is also important in such deliberations to consider the alternatives and to measure the `hidden' cost of inappropriately restrictive regulations, the cost of saying 'No'.

Some regulatory requirements can now be modified to reduce costs and uncertainty without compromising safety. Long accepted plant breeding methods for incorporating new diversity into crop varieties, experience from two decades on research on and

commercialization of transgenic crops, and expanding knowledge of plant genome structure and dynamics all indicate that if a gene or a trait is safe, the genetic engineering process itself presents little potential for unexpected consequences that would not be identified or eliminated in the variety development process before commercialization. As in conventional breeding, regulatory emphasis should be on phenotypic rather than genomic characteristics once a gene or trait has been shown to be safe (Bradford *et al.*, 2005).

Introduction to ecology: basic concepts - definitions

What is ecology?

The word ecology, coined in 1866 by the German biologist Ernst Haeckel, derives from the Greek word “oikos” *oikos* meaning "house" or "dwelling", and *logos* meaning "science" or "study". Thus, ecology is the "study of the household of nature", namely the systematic study of the distribution and abundance of living organisms - plants, animals, micro-organisms- and the interactions with one another and with their natural environment. The environment consists of both a living component, the biotic environment (organisms) and a non-living component, the abiotic environment, e.g. physical factors such as temperature, sunlight, soil, rainfall, winds, and marine streams.

Few fields of study are more relevant to the human condition than the field of ecology. The increasing globalization of our economy and social and political structures has resulted in both intentional and accidental introductions of organisms, including pest and diseases, to all corners of the earth – ecological globalization on a grand scale. All the activities of the human populations affect the natural systems. Ecology today, involves several aspects and concerns: a- Interactions organisms-environment; b- Understanding, conservation, restoration and sustainable use of biodiversity; c- Impact of foreign species in ecosystems and d- Strategies for management and reduction of impacts caused by human activity. Critical considerations for ecological studies are that the natural world is diverse, complex and interconnected; that it is dynamic but also stable and self-replenishing; it is organized by physical and biological processes and that the order of nature is affected by human activity.

Life depends upon the physical world, and also affects it. The organisms must continually exchange materials and energy with the physical environment. Organisms interact with one another, directly or indirectly, through feeding relationships, or trophic interactions. Trophic interactions involve biochemical transformations of energy and the transfer of energy from one individual to the next through the process of consumption. Materials move within ecosystems, and the path ways of such movements are closely associated with the flow of energy (Purves *et al.*, 2004). The flow of energy and its transfer efficiency summarize certain aspects of the structure of an ecosystem: the number of trophic levels, the relative importance of detritus, herbivore, and predatory feeding, the steady-state values for biomass and accumulated detritus, and the turnover rates of organic matter in the community. Unlike

energy, nutrients are retained within the ecosystem and are cycled between its abiotic and biotic components.

Human activities modify the great natural biogeochemical cycles and create cycles of synthetic chemicals –pesticides-. These changes can be large enough to cause serious environmental problems. However, ecosystems have the capacity to recover from many disturbances if the alterations have not been too great and the disturbing forces are reduced or eliminated. Controlling our manipulations of biochemical cycles so that ecosystems can continue to provide the goods and services upon which humanity depends is one of the major challenges facing modern societies (Purves et al., 2004).

Organisation of life: hierarchy of interactions - levels of ecological organization

Individual	It refers to the organism inhabiting the environment as an isolated entity or as a member of a social group.
Species	Is the basic lower unit of classification of closely related similar organisms that have a high level of genetic similarity, are capable of interbreeding freely, and are reproductively isolated from other groups. This definition works well with animals. However, in some plant species fertile crossings can take place among related species.
Population	Group of individuals of the same species living in a particular area. Populations are characterized by several parameters, such as abundance and distribution. The amount of resources available, disease, competition for the limiting resources. Predation, birth and death rates, immigration and emigration affect the size of a population. Populations have age structures and age distributions. They are also characterized by an intrinsic rate of increase, the biotic potential (r). Populations do not have unlimited growth, they are limited by the carrying capacity (K) of their habitat. Density dependent and density independent factors also serve to limit growth.
Community	It is made up of the interacting coexisting populations of different species occupying the same geographical area. Communities are characterized by the numbers of species present, their relative abundance, and their feeding and other ecological relationships. Within the community, there is competition for resources, symbiotic relationships may occur and exchange of genes occurs. Populations and communities include only biotic factors

Ecosystem	It is the complex of a living community (biotic factors) and its abiotic factors (soil, rain, temperatures, etc) in a given area. Ecosystems are further influenced by global phenomena such as climate patterns, nutrient cycles, winds. The communities influence the environment, and the environment influences the community, leading to changes and succession in the ecosystem. Energy flow, geochemical, water and nutrient cycling characterize ecosystems. The flow of energy leads to clearly defined trophic structure, biotic diversity, and material cycles (ie: exchange of materials between living and nonliving parts) within the system is an ecosystem.
Biosphere	The totality of ecosystems constitutes the biosphere, the portion of the earth that contains living species. It includes the atmosphere, oceans, soils and the physical and biological cycles that affect them
Biome	Is another level of interaction placed between the ecosystem and the biosphere. It is a major ecological community or complex of communities, extending over a climatically and geographically defined area. There are two broad categories of biomes: aquatic and terrestrial. Biomes are defined based on factors such as plant structures (such as trees, shrubs, and grasses), leaf types (such as broadleaf and needleleaf), plant spacing (forest, woodland, savanna), and climate. Biomes are often given local names. For example, a Temperate grassland or shrubland biome is known commonly as steppe in central Asia, prairie in North America, and pampas in South America.

Attributes of individuals

The ecology of the individual is mainly concerned with the effects of the abiotic and biotic environment on survival and reproduction. Any shortcomings in the phenotype or genotype of an individual will have a selective pressure exerted on them, and the individuals mostly affected by the environment will be removed from the population. This, of course, can be extended up to the species level, where such stabilising selection determines the range of species according to their environmental requirements and susceptibilities

Attributes of populations

Populations have characteristics that define them. They have characteristic distributions over space, and they differ in age and size; they can be clumped, randomly or uniformly distributed in their environment. They have growth rates, which define their abundance. The number of individuals in a population depends on the birth and death rates, and the difference between immigration and emigration. The population has a spatial structure, which includes features such as the density, spacing, and movement of individuals, the proportion of individuals in different age classes, genetic variation, and the arrangement and size of areas of suitable habitat, all of which may vary in space and time. Population structure also affects the

dynamics of parasites and their hosts, including human diseases (Purves et al., 2004). The structure of populations changes continually because demographic events – births, deaths, immigration (movement of individuals into the area), and emigration (movement of individuals out of the area) – are common occurrences. The study of birth, death and movement rates that give rise to population dynamics is known as demography. Individuals within a population compete with each other for resources such as space, mating partners and food. A population continues to grow until the habitat carrying capacity is reached. However, density independent factors such as storms, floods, weather conditions, and natural disasters - earthquakes, volcanic eruptions.

Genetic differentiation of populations depends far less on the movement of individuals among populations than on the forces of selection, mutation, and random change (genetic drift). Gene flow is the exchange of genetic information among populations resulting from the movement of individuals. The genetic structure of a population describes the distribution of the variation among individuals and among subpopulations, as well as the way in which organisms manage the consequences of genetic variation by means of mating systems. Genetic variation is important to a population because it is the basis of the population's capacity to respond to environmental change through evolution (Ricklefs & Miller, 1999). Genetic variation is also important to individuals: variation among an individual's progeny may increase the likelihood that at least some of them will be well adapted to particular habitat patches or to changed conditions. Genetic variation is maintained primarily by mutation and by gene flow from other localities in which different genes have a selective advantage.

Attributes of communities

Factors that define populations also define communities. Communities are usually defined by the nature of the interactions among the populations in the association or by the place in which the association occurs. Communities are characterized by unique interrelated properties: structure and function. Structure is related to the number of species, called species richness, the types of species present and their relative abundances, the physical characteristics of the vegetation, and the trophic relationships among the interacting populations in the community. Rates of energy flow, properties of community resilience to perturbation, and productivity are examples of community function. (Ricklefs & Miller, 1999). The species composition of ecological communities changes constantly over time.

Organisms interact with one another in different ways in their community:

- Two organisms may mutually harm one another. This type of interaction –competition- is common when organisms use the same resource. Intraspecific competition is competition among individuals of the same species. Competition among species is referred to as inter-specific competition.

- One organism may benefit itself while harming another, as when individuals of one species eat individuals of another (*i.e.* herbivores). The eater is called a predator or parasite, and the eaten is called prey or host. These interactions are known as predator-prey or parasite-host interactions. Predators act as evolutionary agents by selecting for adaptation to protect against them (toxic hairs and bristles, tough spines, noxious chemicals, and mimicry).
- Mutualist interaction is when both participants benefit. Mutualistic interactions occur between members of different groups of organisms (between plants and prokaryotes, between fungi and protests, between animals and protists, between animals and plants and with others animals). If one participant benefits but the other is unaffected, the interaction is a commensalism. If one participant is harmed but the other is unaffected, the interaction is an amensalism**.**

Attributes of ecosystems

Ecosystems have trophic levels called *energy pyramids* or *food pyramids*. The first trophic level is made up of primary producers that utilise light energy to make food. These are referred to as autotrophs and are mostly plants (but include also bacteria and algae). Since only photosynthetic organisms are able to use light energy to produce food, they have a key position in the ecosystem. Any factor that affects plants has implications on the ecosystem. The second level is made up of primary consumers, which are the herbivores; then the next level up is made up of secondary consumers, the carnivores; then top carnivores and decomposers saprophytes. The feeding relationships ensures transfer of energy from one level to the other, with only about 10% of energy at the preceding level being available for use at the next level.

Biodiversity: genetic, species and ecosystems

Biodiversity

Biodiversity is the variation of life at all levels of biological organization -genes, species, and ecosystems-. At the UN Earth Summit in Rio in 1992, it was defined as: “The variability among living organisms from all sources, including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems”. The three most commonly studied levels of diversity are: ecosystem diversity, species diversity, and genetic diversity. If the gene is the fundamental unit of natural selection, the real biodiversity is genetic diversity. For geneticists, biodiversity is the diversity of genes and organisms. They study processes such as mutations, gene exchanges, and genome dynamics that occur at the DNA level and generate evolution.

In ecological indexes, Alpha diversity refers to diversity within a particular area, community or ecosystem, and is measured by counting the number of taxa within the ecosystem (usually species)

Beta diversity is species diversity between ecosystems; this involves comparing the number of taxa that are unique to each of the ecosystems. Gamma diversity is a measure of the overall diversity for different ecosystems within a region. Cultural or anthropological diversity is also involved when studying the regional diversity. Biodiversity is not static; it is constantly changing. It is not evenly distributed on earth, and tends to be richer in the tropics. It varies with climate, altitude, soils and other physical parameters. Hotspots, regions with many endemic species, are usually in areas with limited human impact, while the heavily populated regions tend to have the lowest number of species.

Values of biodiversity

Biodiversity has paramount importance for the social, cultural and economic development of humankind. Some ecosystem services that benefit society are air quality, climate (both global CO2 sequestration and local), water purification, disease control, biological pest control, pollination and prevention of erosion. It plays a part in regulating the chemistry of our atmosphere and water supply. Biodiversity is directly involved in water purification, recycling nutrients and providing fertile soils. There are a multitude of anthropocentric benefits of biodiversity in the areas of agriculture, science and medicine, industrial materials, ecological services, in leisure, and in cultural, aesthetic and intellectual value. The most direct and important use of biodiversity is as a source of food. Although a large number of plant species are edible, only a small percentage is used intensively in the production of food with significant nutritional value. Likewise only a few of the numerous animal species are used for food. Plant biodiversity is the basis of development and the sustainability of agricultural production systems. A reduction in the genetic diversity of crops represents an increase in vulnerability to new pests and diseases. The economic value of the reservoir of genetic traits present in wild varieties and traditionally grown landraces is extremely important in improving crop performance (The Academies of Sciences, 2007). Important crops, such as the potato and coffee, are often derived from only a few genetic strains. Improvements in crop plants over the last 250 years have been largely due to harnessing the genetic diversity present in wild and domestic crop plants.

Ecosystem, species, and genetic diversity

Besides the great diversity of life on Earth, our planet also contains a rich variety of habitats and ecosystems. Biodiversity is determined by both its biotic components, represented by the living organisms, and its abiotic components, represented by the characteristics of the places where the organisms live. In a strict sense, diversity is a measure of the heterogeneity of a system. This concept, when applied to biological systems, refers to

the biological heterogeneity that is defined as the amount and proportion of the different biological elements contained in a system.

Ecosystem diversity comprises the diversity of natural and artificial habitats, plus the species complexes they contain. Species are assembled in distinct ecological systems, such as a tropical forest, a tropical savanna, or a coral reef. However, measuring ecosystem diversity may be difficult because the boundaries among communities and ecosystems are poorly defined. Human influence on natural ecosystems can result in severe consequences, for example, desertification, poor soils, changes in the atmosphere composition, and pest outbursts.

Species diversity results of the relation between the species richness (number of species) and the relative abundance (number of individuals of each species). A more precise concept is taxonomic diversity, which accounts for the diversity of a group of species more or less related among them. One of the major challenges for today biologists is to describe, classify and give a sustainable use to organisms living in poorly known habitats such as those in tropical rain forests, marine ecosystems and soil communities (Ricklefs & Miller, 1999). Species diversity has an important ecological effect on the structure of communities due to the interactions and interdependences among species: the reduction or disappearance of a given species may influence others that depend on it (WCMC, 2002).

Genetic diversity refers to the variation of genes within any population, among populations or within species. This type of diversity can be characterized at the molecular, population, species or ecosystem level. A lot of attention has been paid to genetic diversity because of its practical applications on plant and animal breeding and production, and on evolutionary studies (Purves *et al.*, 2004).

Megadiversity

The term mega-diversity refers to those areas, which account for a high percentage of the world's biodiversity by virtue of containing the most diverse and the greatest number of plant and animal of species.

Species diversity in natural habitats is higher in warm and rainy zones and decreases as latitude and altitude increase. The richest zones of the world are undoubtedly the tropical rain forests, which cover 7% of the world surface and contain 90% of the insect species of the whole world. (CBD, 2002; WCMC, 2002).

Megadiverse Countries: Brazil, Colombia, Indonesia, China, Mexico, South Africa, Venezuela, Ecuador, United States, Papua-New Guinea, Índia, Austrália, Malaysia, Madagascar, Dem. Rep. Congo, Philippines, Peru

Concentrating on geographical areas and not specific countries, 25 hotspots for biodiversity are identified: Polynesia/Micronesia, Flower Province of California, Central America, Choco/Darien/west Ecuador, central Chile, Caribbean, Atlantic forest of Brazil, Brazilian cerrado, forests of West Africa, the karoo (succulents), the Mediterranean basin,

Madagascar, the coastal forests of the eastern arc of Tanzania and Kenya, the Caucasus, Sri Lanka and the Western Ghats, south-central China, Sundaland, the Philippines, Wallacea, southwest Australia, New Zealand and New Caledonia. These zones occupy only about 1.4% of the earth's surface and contain 44% of the known plants and 35% of the known animals. Tropical forests and Mediterranean zones predominate. Three of the zones are of special importance; Madagascar, the Philippines and Sundaland, followed by the Atlantic forests of Brazil and the Caribbean. The tropical Andes and Mediterranean basin are also important for their rich plant diversity

Problems and threats to biodiversity

Extinction has been a naturally occurring phenomenon over millions of years, without any human involvement. However, due to the human activities and their effect on the environment, species and ecosystems have become increasingly threatened in an alarming way (WCMC, 2002), undermining the basis required for sustainable development. Almost all human activities practiced in a regular basis result in a modification of natural environments. These modifications are harmful to the relative abundance of species and may even lead to their extinction. The main causes of environmental modification are: habitat alteration by i.e. pollutants; habitat fragmentation, which can divide a big population into small subpopulations isolated among each other and increasing their risk of extinction if they are excessively reduced in size; introduction of exotic or non-native species; overexploitation of plants and animals; soil, water and atmosphere pollution; alteration of the global climate; and agroindustries, including forest ones (WRI, 2000). Although the loss of biodiversity in the form of crop varieties and domestic animal races has a small significance if compared to the global biodiversity, their genetic erosion is of immediate concern as it has profound implications and consequences for food supply and sustainability of local practices of animal and agricultural production (WCMC, 2002). Genetic erosion in gene banks is difficult to assess quantitatively. It is usually calculated in an indirect way in terms of cultivated surface with genetically uniform varieties which have selected traits.

After 10,000 years of sedentary agriculture and the discovery of 50,000 varieties of edible plants, only 15 crop species represent today 90% of the food of the world. Rice, wheat and maize are the basic food for two thirds of the world population. The continuous genetic erosion of wild species of cereals and other cultivated plants poses a risk for plant breeding programs. Unless the loss of genetic diversity is controlled, by 2025 about 60,000 plant species —a quarter of the total world capital— might be lost (FNUAP, 2001). Fish stocks are also at risk. FAO estimates that 69% of marine commercial fish supplies of the world have been depleted. The greatest threats to biodiversity are destruction and deterioration of habitats, particularly in tropical developing countries (where biodiversity is concentrated), and introduction of exotic species. Many of the factors affecting biodiversity are related to the needs of agricultural production: the increase in population and the limited arable land, have demanded increased agricultural productivity, and have lead to more intensive agricultural

practices, which has negative impacts on natural biodiversity. Habitat loss due to the expansion of human activities is identified as a main threat to 85% of all species described in the IUCN Red List. Main factors are urbanization and the increase in cultivated land surfaces (Amman et al., 2003).

Causes of biodiversity loss

Biodiversity loss has been indicated by the loss in number of genetic resources and species. It has also been inferred from population decline and the degradation of ecosystem functions and processes. Several causes have been suggested; some of them are direct and some others are identified as underlying factors. Among the direct causes are:

- Habitat conversion/fragmentation
- Unsuitable land use and management
- Domestication/ genetic erosion
- Introduction of invasive and exotic species
- Trade
- Pollution
- Natural events

Among the underlying causes of loss are:

1. Demographic changes
2. Poverty and inequality
3. Climate change
4. Public policies and markets
5. Economic policies and structures.

Climate change is also a factor of biodiversity loss. Excessive burning of fossil fuels is altering the balance of gases in the atmosphere; carbon is building up to levels where the natural ecosystems cannot absorb all of it. For one thing, there are fewer plants to utilize the carbon, and the amounts that are being produced are estimated at 3 billion tons per year. The interdependence of ecosystems is amply demonstrated here. For example, deforestation releases carbon dioxide and methane, which increase global temperatures. It also reduces ground cover, which disrupts the water cycle as well as leading to soil erosion. The soil is washed into lakes and rivers, which silt and reduce aquatic biodiversity among other things.

Although deforestation can be controlled at a local level, the massive amount of deforestation is due to over harvesting of trees for economic use, rather than local use. Most of the fossil fuels generating the carbon in the atmosphere are from the industrialized nations, but the effects are felt throughout the less industrialized world.

Commitments and opportunities

Biodiversity makes part of the national patrimony of each country and represents great environmental, cultural and economic values. Conservation and sustainable use of biodiversity concern all the inhabitants of the world, are an enormous potential for diverse countries and require clearly defined strategies and policies for biodiversity management. As population grows, the demand for freshwater, food and energy resources pushes to risk the sustainability of the environment. Technologies and the way in which we should use them are a growing challenge, and problems related to governability, social organization and human rights are of increasing importance in achieving sustainable results (FNUAP, 2001). In order to feed 8,000 million people that are expected to live on Earth by 2025 and to improve their diets, the world will have to improve food production and work on a more equal distribution of food to avoid malnutrition. Given that the available land surface suitable for agriculture is getting reduced, the increase in production will have to be achieved with higher yields instead of more cultivated surface. For example, scientists are working on genes that help plants to efficiently extract nutrients from soil, which would reduce the need for fertilizers; efforts are directed also to the development of drought resistant plants using the genes through which certain species manage to survive drought. (The Academies of Sciences, 2007). Development strategies that are beginning to materialize in several countries, especially developing ones, are based mainly on a wide use of natural resources in a sustainable way, maximizing the potential of the plant genome sciences toward sustainable and environmentally responsible models of production for food, fuel, and fiber, and incorporating them steadily into the productive sector. Biological resources represent a huge potential, insufficiently exploited, that requires strengthening and applying scientific and technological progress aimed to understand, characterize and use these resources for the benefit of local communities: genomics offers unprecedented tools to use these critical resources (Lemaux 2008).

Evolution and speciation

The Development of Evolutionary Theory

Patterns of reproduction, foraging, social interaction, growth and senescence are shaped by natural selection through the interactions of organisms and their environment. Those behavioral, physiological or developmental responses that allow an organism to accommodate or acclimate to the current conditions are called evolutionary adaptations. In biology, evolution is change in the inherited traits of a population of organisms from one generation to the next. These changes are caused by a combination of three main processes: variation, reproduction, and selection. In a biosafety context, evolution is one of the most important concepts from the point of view of the possible ecological impacts. Some of the principal considerations involve concepts related to natural selection pressures and genotype changes (which affect the rate of evolution), phenotypic variance, heritability, response to selection, and inbreeding – out-crossing and genetic variation among others.

Biology became a defined science when the British Charles Darwin published "On the Origin of Species." Darwin's Theory of Evolution is the widely held notion that all life is related and has descended from a common ancestor. Darwin's general theory presumes that complex creatures evolve from more simplistic ancestors naturally over time. In a nutshell, as random genetic mutations occur within an organism's genetic code, the beneficial mutations are preserved because they aid survival -- a process known as "natural selection." These beneficial mutations are passed on to the next generation. Over time, beneficial mutations accumulate and the result is an entirely different organism (not just a variation of the original, but an entirely different creature). Natural selection acts to preserve and accumulate minor advantageous genetic mutations; is the preservation of a functional advantage that enables a species to compete better in the wild. Natural selection is the naturalistic equivalent to domestic breeding. Over the centuries, human breeders have produced dramatic changes in domestic animal populations by selecting individuals to breed. Breeders eliminate undesirable traits gradually over time. Similarly, natural selection eliminates inferior species gradually over time.

In Darwin's theory of natural selection, new variants arise continually within populations. A small percentage of these variants cause their bearers to produce more offspring than others. These variants thrive and supplant their less productive competitors. The effect of numerous instances of selection would lead to a species being modified over time (Purves *et al.*, 2004). Darwin didn't know that the true mode of inheritance was discovered in his lifetime. Gregor Mendel, in his experiments on hybrid peas, showed that genes from a mother and father do not blend. An offspring from a short and a tall parent may be medium sized; but it carries genes for shortness and tallness. The genes remain distinct and can be passed on to subsequent generations. It was a long time until Mendel's ideas were accepted. Mendel studied discrete traits. These traits did not vary continuously. The discrete genes Mendel discovered would exist at some frequency in natural populations. Biologists wondered how and if these frequencies would change. Many thought that the more common versions of genes would increase in frequency simply because they were already at high frequency.

The evolutionary mechanisms of selection and genetic responses are studied in population genetics, developing quantitative predictions of changes in gene frequencies in response to selection. Hardy and Weinberg showed how genetic variation is retained in Mendelian inheritance, and that the frequency of an allele would not change over time simply due to its being rare or common. Their model (Annex 2) assumed the use of large populations in which there is random mating, no selection, no mutations, and no migration to or from the population. Later, R. A. Fisher showed that Mendel's laws could explain continuous traits if the expression of these traits were due to the action of many genes. After this, geneticists accepted Mendel's Laws as the basic rules of genetics.

Evolution is a change in the gene pool of a population over time. The process of evolution can be summarized in three sentences: Genes mutate; individuals are selected; and populations evolve.

Gene is the unit of genetic inheritance that can be passed on unaltered for many generations. Part of the DNA molecule that encodes a given protein.

Genotype is constituted by all the genetic characteristics that determine the structure and functioning of an organism

Phenotype is the physical expression in the organism of the interaction genotype/environment; the outward appearance of the organism.

Gene pool is the set of all genes in a species or population.

Allele is one of several alternative forms of a gene

Locus is the location of a particular gene on a chromosome

Mutation is a permanent change in the genotype –DNA sequence- of an organism, usually applied to changes in genes to new allelic forms

Recombination refers to the mixing of genetic material via sexual reproduction

Gene flow is the transfer of alleles of genes from one population to another

Genetic basis of the evolutionary mechanisms

Evolution, the change in the gene pool of a population over time; it can occur in different ways. Two mechanisms remove alleles: natural selection and genetic drift. Selection removes deleterious alleles from the gene pool, while drift removes alleles randomly from the gene pool. Three mechanisms add new alleles to the gene pool: mutation, recombination and gene flow. The amount of genetic variation found in a population is the balance between the actions of these mechanisms.

Mutation

Mutations, permanent change in the DNA sequences that make up a gene, range in size from a single DNA building block (DNA base) to a large segment of a chromosome. There are many kinds of mutations. A point mutation is a mutation in which one "letter" of the genetic code is changed to another; lengths of DNA can also be deleted or inserted in a gene. Finally, genes or parts of genes can become inverted or duplicated. Most mutations are thought to be neutral with regards to fitness. Mutations that result in amino acid substitutions can change the shape of a protein, potentially changing or eliminating its function. This can lead to inadequacies in biochemical pathways or interfere with the process of development. Only a very small percentage of mutations are beneficial (Purves *et al.*, 2004). A change in environment can cause previously neutral alleles to have selective values; in the short term evolution can run on "stored" variation and thus is independent of mutation rate.

Recombination

Is the process by which a strand of genetic material is broken and then joined to a different DNA molecule. In eukaryotes recombination commonly occurs during meiosis as chromosomal crossover between paired chromosomes. In general, genetic recombination happens during meiosis, a special type of cell division that occurs during formation of sperm and egg cells and gives them the correct number of chromosomes (haploid). Recombination can occur not only between genes, but within genes as well. Recombination within a gene can form a new allele. Recombination adds new alleles and combinations of alleles to the gene pool.

Gene Flow (migration)

New organisms may enter a population by migration from another population. If they mate within the population, they can bring new alleles to the local gene pool. This is called gene flow. Immigrants may add new alleles to the gene pool of the population, or may change the frequencies of alleles already present if they come from a population with different allele frequencies. It operates when there are no spatial barriers. Gene flow has therefore implications on the introduction of GMOs into an environment, and is therefore the subject of a specific attention in this unit.

Natural Selection

Some types of organisms within a population leave more offspring than others. Over time, the frequency of the more prolific type will increase. The difference in reproductive capability is called natural selection. Natural selection is the only mechanism of adaptive evolution; it is defined as differential reproductive success of pre- existing classes of genetic variants in the gene pool. The most common action of natural selection is to remove unfit variants as they arise via mutation. This is called reproductive success. This is what is commonly referred to as “survival of the fittest”. Fitness is a measure of reproductive success and is due to a number of selection factors:

- Survival/mortality selection. Any trait that promotes survival, increases fitness
- Sexual selection. Sexual selection is natural selection operating on factors that contribute to an organism's mating success. Traits that are a liability to survival can evolve when the sexual attractiveness of a trait outweighs the liability incurred for survival. A male who lives a short time, but produces many offspring is much more successful than a long lived one that produces few.
- Fecundity selection (size of offspring). High fecundity is due to the production of mature offspring due to earlier breeding or number of fertilized eggs produced in species that provide litte or no care for their young. The number of offspring gives family size, e.g. in species that take care of their young

Genetic Drift

Allele frequencies can change randomly. Genetic drift, more precisely termed allelic drift, is the process of change in the gene frequencies of a population due to chance events, which determine which alleles will be carried forward while others disappear. It is distinct from natural selection, a non-random process in which the tendency of alleles to become more or less widespread in a population over time is due to the alleles' effects on adaptive and reproductive success. When sampled from a population, the frequency of alleles differs slightly due to chance alone. Alleles can increase or decrease in frequency due to drift. A small percentage of alleles may continually change frequency in a single direction for several generations. A very few new mutant alleles can drift to fixation in this manner (Purves *et al.*, 2004). Both natural selection and genetic drift decrease genetic variation. If they were the only mechanisms of evolution, populations would eventually become homogeneous and further evolution would be impossible. There are, however, mechanisms that replace variation depleted by selection and drift.

Speciation

Speciation is the evolutionary process by which new biological species arise, in others words speciation is a lineage-splitting event that produces two or more separate species. Many biologists think speciation is key to understanding evolution. There are various types of speciation: allopatric, peripatric, parapatric and sympatric speciation, which differ in geographical distribution of the populations in question. Separate species arose when genetic changes (mutations) between relatives no longer allowed for interbreeding, for instance after geographic separation (Ammann *et al.*, 2003).

Types of speciation (Purves et al., 2004)

Allopatric *(allo=other, patric=place)* is thought to be the most common form of speciation. It occurs when a population is split into two (or more) geographically isolated subdivisions. In order for a speciation even to be considered allopatric, gene flow between the soon-to-be species must be greatly reduced, and eventually the two populations' gene pools change independently until they could not interbreed even if they were brought back together.

Peripatric *(peri=near)* new species are formed in isolated, small peripheral populations which are prevented from exchanging genes with the main population. Genetic drift, and perhaps strong selective pressures, would cause rapid genetic change in the small population.

Parapatric *(para=beside)* the zones of two diverging populations are separate but do overlap; there is no specific extrinsic barrier to gene flow. Individuals mate with their geographic neighbors more than with individuals in a different part of the population’s range. In this mode, divergence may happen because of reduced gene flow within the population and varying selection pressures across the population’s range.

Sympatric (sym=same) occurs when two subpopulations become reproductively isolated without first becoming geographically isolated. Insects that live on a single host plant provide a model for sympatric speciation. If a group of insects switched host plants they would not breed with other members of their species still living on their former host plant. The two subpopulations could diverge and speciate.

The key to speciation is the evolution of genetic differences between the incipient species. For a lineage to split once and for all, the two incipient species must have genetic differences that are expressed in some way that causes mating between them to either not happen or to be unsuccessful. These need not be huge genetic differences. A small change in the timing, location, or rituals of mating could be enough. But still, some difference is necessary. This change might evolve by natural selection or genetic drift. Reduced gene flow probably plays a critical role in speciation. Speciation requires that the two incipient species be unable to produce viable offspring together or that they avoid mating with members of the other group. Some of the barriers to gene flow (reproductive Isolation) that may contribute to speciation are the evolution of different mating location, mating time or mating rituals; the lack of fit between sexual organs or the offspring inviability or sterility. In terms of reproduction, plants have a lot more options than animals do. Many plants can reproduce sexually, by fertilizing other individuals or themselves, and asexually, by creating clones of themselves through vegetative reproduction, while most animals only reproduce sexually. Similarly, in terms of speciation, plants have more options than animals do. Two modes of speciation are particularly common in plants: speciation by hybridization or speciation by ploidy changes (Ricklefs & Miller, 1999).

All species, living and extinct, are believed to be descendants of a single ancestral species that lived more than 3 billion years ago. If speciation were a rare event, the biological world would be very different than it is today. The result of speciation processes operating over billions of years is a world in which life is organized into millions of species, each adapted to live in a particular environment and to use environmental resources in a particular way (Purves *et al.,* 2004).

Extinction

Extinction is a natural process in evolution that occurs when every living individual of a species -or group of taxa- disappear. The history of extinctions on Earth includes several mass extinctions during which large numbers of species have disappeared in a rather short term (Purves *et al*., 2004). The main causes of mass extinctions are disturbances such as: volcanic eruptions, impacts of meteorites, fires, floods, species overexploitation, introducing exotic or non-native species, habitat fragmentation, predation, parasitism, and a reduction of mutualism. Extinction depends on many ecological factors or characteristics of populations (size, geographical distribution, age class structure and spatial distribution). Small populations are in higher danger than large populations, and endemic species —those which are limited to

one or very few populations in specific locations and not found anywhere in the world— are at higher risk than widespread species (Ricklefs & Miller, 1999). The rate of extinction is affected by population size, geographic range, age structure, and spatial arrangement, and may result from a decrease in competitive ability.

Despite mass extinctions, speciation processes (new species arising from preexisting species) have allowed a net increment of species number throughout the history of life on Earth. However, current concern arises due to the accelerated rates of extinction. Scientists calculate that during the past 400 years at least 350 vertebrates and 400 invertebrates have gone extinct and several hundreds of plants have disappeared, as a result of anthropogenic extinction. For 2000, the estimated risk of extinction for mammals was of 24 % and for birds, 12 % (WRI, 2000). Several national and international conservationist agencies have developed strategies and programs aimed at the conservation of wild species. For instance, the IUCN has created the Red Lists of species classifying them into categories according to the level or degree of threat: extinct, extinct in the wild, critical, endangered, vulnerable, susceptible, safe/low risk, insufficiently known and not evaluated. These categories are a guide to conservation activities that must be prioritized.

Agricultural Ecology : Centres of Origin / Diversity

Agricultural activities have become the dominant ecological force over nearly one third of the land areas of the earth. Agro-ecosystems incorporate the concepts of ecology into their design and management. After a long history of separation and lack of interaction, ecologists and agronomists are combining forces to study and help solving the problems confronting our food production a system, facing the natural resources threatens, identifying the ecological problems in agriculture. Application of this knowledge can lead to development of more sustainable agricultural ecosystems in harmony with their larger ecosystem and eco-region (NRCS, 2004). Agro-ecosystems are controlled by definition, by the management of ecological processes.

For 4 million years, people procured food by hunting and gathering. Agriculture began in several places more than 10.000 years ago, and was a necessary condition for the development of civilizations. Crops and farm animals were domesticated and selection took place. Identifying the geographic origin of species is very useful when plant breeders attempt to grow any crop in another zone with different environmental conditions to those of its original zone (Chrispeels & Sadava, 2003). Hybridization has played a major role in the development of new crops, in the modification of existing ones and in the evolution of some troublesome weeds. One of the consequences of agriculture is the conversion of natural ecosystems into crop fields and pastures by removal of climax vegetation, controlling succession and exposing the soil to erosion.

Domestication of species

Domestication is simply accelerated evolution and involves relatively few genes. Domestication implies changes in the genetic makeup and the morphological appearance of plants and behavior of animals, such that they fit the needs of the farmer and consumer. For example, in wheat, as in many other grains, a major difference between the wild progenitor and domesticated descendants lies with seed dispersal. Wild plants spontaneously shed their seeds at maturity in order to assure their dispersal. Early farmers, during domestication have selected plants to hold on their seeds, to minimize yield losses (Chrispeels & Sadava, 2003). The evolution of crops is determined by three bottlenecks for genetic diversity: domestication, dispersal from the domestication centres, and crop improvement in the 20th century. Early agricultural society domesticated few plant species, which were the source of carbohydrates, proteins, fats and fibers. For instance, the emergence of Mediterranean and Middle Eastern civilizations was based on the domestication mainly of wheat, barley, lentils, peas, and linen. Later, the number of domesticated species increased and thus new crops appeared: oat, rye, olives, fruits, and others. Human migrations and exchanges among cultures helped to increase the number of plants cultivated in each region. The discovery of the American continent and all the exchanges that came after led to the greatest levels of genetic diversity within agricultural systems. Unfortunately at the same time, the new available lands began to be used for extensive monocultures especially of coffee, sugarcane, cotton, and tobacco in the colonies of the New World. Agriculture began at similar times in different regions of the world. In each of the regions where the centres of origin are located, human populations domesticated different crops with similar uses.

The domestication of plants and animals is related to the use of a reduced fraction of the existing biodiversity in each region and the adaptation of selected species to new environmental conditions suitable to human use. The domestication by artificial selection to new environmental conditions is opposed to the evolutionary mechanisms of adaptation by natural selection, as the environments where domestication take place differ from the natural environments where wild relatives grow and the selective pressures in each location are different. Domestication results in many morphologic and physiological changes in plants that make them difficult to distinguish from their wild relatives. The most noticeable are related to seed dispersal, seed dormancy, growth type, harvest index, photoperiod, organ size, presence of toxic compounds, and pest and disease resistance. Due to the fact that almost all the crops share the same modified traits that distinguish them from their wild relatives, the whole set of new traits is known as domestication syndrome. Domestication is an artificial selection process directed by farmers. It leads to genetic changes and confers adaptive traits for environmental culture conditions, fitting farmers and consumer's needs.

Centres of origin and diversification

Local and geographic distribution of species depends on ecological conditions, both biotic and abiotic factors, and on evolutionary processes (Purves et al., 2004). The

combination of all of these environmental conditions and processes determines the natural vegetation found in a given region as well as the capability of developing certain crops in particular areas. The geographic distribution of wild relatives of a crop provides a general idea where a crop may have originated. Careful botanic explorations are necessary to determine the precise distribution of wild progenitors. Additional genetic studies involving crosses between the crop and presumptive wild ancestors and a comparison of their DNA can identify in more detail a specific region of domestication.

The Centre of Origin is considered a geographical area where a group of organisms, either domesticated or wild, first developed its distinctive properties. Centres of Origin of cultivated plants are identified on the basis of the number and diversity of wild species as well as the number of endemic species of the concerned genus in a given region, while the Centres of Diversity are recognized on the basis of the number and diversity of different varieties, wild and cultivated, of the species. The Centres of Origin and Centres of Diversity of crop plants as known to us are largely based on circumstantial evidence. In the cases of crops that are extensively cultivated over wide geographical ranges, a large number of new varieties were continuously developed, involving a large number of parents, making the issues virtually intangible. For example, IR-64 rice appears to have had more than 100 parents, with consequent extensive genomic rearrangements, some natural and the others induced (Kameswara & Shantharam. 2004).

Centres of origin and centres of diversification

Centres of origin: The geographic locations where a particular domesticated plant species originated.

These areas are the likeliest sources of natural genetic variation, and represent ideal targets for in situ conservation

Centres of diversity: The locations recognized on the basis of the number and diversity of different varieties, wild and cultivated, of the species.

The most important classification of the centres of origin of cultivated plants was established by the Russian geneticist Nikolai Ivanovich Vavilov (1887-1943). Vavilov realized the importance of genetic diversity of crops and their wild relatives for crop improvement. His most important contribution was the identification of eight major geographic zones, known as “centres of diversity”. There are a limited number of zones where crops originated. They are located in the tropical and subtropical zones, at different elevations, wide variety of topographies, and characterized by distinct dry and wet seasons. They also correspond in many cases to the places where important human civilizations established and flourished.

Centres of origin and domestication of cultivated species

Based on the work of Vavilov in 1949 and Bryant in 2001

I. ***Chinese centre:*** soybean (*Glycine max*), odder radish (*Raphanus sativus*), rapeseed (*Brassica rapa* var. *rapa*), pak-choi (*Brassica chinensis*), Chinese cabbage (*Brassica pekinensis*), Japanese shallot (*Allium fistulosum*), rakkyo (*Allium chinense*), cucumber (*Cucumis sativus*), yam (*Dioscorea batatas*), sorghum, millet.

II. ***Indo Malayan centre****:* Burma and Assam: egg plant (*Solanum melongena*), cucumber (*Cucumis sativus*), mung bean (*Phaseolus aureus*), cowpea (*Vigna sinensis*), taro (*Colocasia esculenta*), yam (*Dioscorea batatas*), rice.

III. ***Indochina and Malayan Archipelago****:* banana (*Musa paradisiaca*), breadfruit (*Artocarpus altilis*), coconut, sugarcane.

IV. ***Indo Afgani-Central Asia centre****:* garden pea (*Pisum sativum*), broad bean (*Vicia faba*), mung bean (*Phaseolus aureus*), leaf mustard (*Brassica juncea*), onion (*Allium cepa*), garlic (*Allium sativum*), spinach (*Spinacia oleracea*), carrot (*Daucus carota* var. *sativus*), apple, chickpea, lentil.

V. ***Near East centre****:* lentil (*Lens culinaris*), lupin (*Lupinus albus*), barley, oat, wheat.

VI. ***Mediterranean centre****:* celery (*Apium graveolens*), asparagus (*Asparagus officinalis*), beetroot (*Beta vulgaris* var. *crassa*), oilseed rape (*Brassica rapa* var. *rapa*), cabbage (*Brassica oleracea* var. *capitata*), parsnip (*Pastinaca sativa*), pea (*Pisum sativum*), rhubarb (*Rheum officinalis*), oat, olive, wheat.

VII. ***Abyssinian centre****:* okra (*Abelmoschus esculentus*), watercress (*Lepidium sativum*), cowpea (*Vigna sinensis*), barley, coffee, sorghum.

VIII. ***Mexico-Central America centre****:* sweet pepper, chili (*Capsicum annuum*), alcayota (*Cucurbita ficifolia*), pumpkin (*Cucurbita moschata*), sweet potato (*Ipomoea batatas*), lima bean (*Phaseolus lunatus*), kidney bean (*Phaseolus vulgaris*), maize (*Zea mays*), tomato.

IX. ***South American centre****:*

X. ***Peru-Ecuador-Bolivia****:* sweet pepper, chili, pumpkin, tomato, kidney bean, potato.

XI. ***Chile****:* potato.

XII. ***Brazil-Paraguay:*** peanut, cassava.

XIII. ***North American centre:*** sunflower.

XIV. ***West African centre:*** millet, sorghum.

North European centre: oat, rye

Agro-ecosystem characteristics

Agricultural ecosystems -agro-ecosystems- have been described as domesticated ecosystems, in many ways intermediate between natural ecosystems (such as grasslands and forests) and fabricated ecosystems –cities (ASAP, 2004). Just as natural ecosystems they can be thought of as including the processes of primary production, consumption, and decomposition interacting with abiotic environmental components and resulting in energy flow and nutrient cycling. Economic, social, and environmental factors must be added to this primary concept because of the human element that is so closely involved with agroecosystem creation and maintenance. Any agroecosystem contains some or all of:

- Crops – plants cultivated for the benefit of human kind
- Weeds – plants that are potential competitors
- Pests - animal predators and parasites
- Pathogens - microorganisms causing diseases
- Domestic animals - animal crops
- Beneficial microorganisms - e.g. rhizobia and other nitrogen fixing bacteria, mycorrhizal fungi
- Beneficial arthropods - pollinators, natural enemies of pests
- Soil

Definitions of agro-ecosystems often include the entire support base of energy and material subsidies, seeds, and chemicals, and even a social-political-economic matrix in which management decisions are made. Agro-ecosystems retain most, if not all, the functional properties of natural ecosystems — nutrient conservation mechanisms, energy storage and use patterns, and regulation of biotic diversity.

Agro-ecosystem patterns and processes

Energy and matter flow in agro-ecosystems is altered greatly by human interference. Inputs are derived primarily from human sources and are often not self-sustaining. They become open systems where considerable energy is directed out of the system at the time of harvest, rather than stored in biomass that could accumulate within the system. In an agro-ecosystem, recycling of nutrients is minimal, and considerable quantities are lost with the harvest or as a result of leaching or erosion, because of a great reduction in permanent biomass levels held within the system. Because of the loss of niche diversity and a reduction in trophic interactions, populations are rarely self-regulating.

Agro-ecosystems are solar powered as are natural systems, but differ from natural systems in that (ASAP, 2004):

- There are auxiliary energy sources that are used to enhance productivity; these sources are processed fuels along with animal and human labor;

- Biodiversity is notably reduced by human management in order to maximize yield of specific foodstuffs (plant or animal);
- Dominant plant and animal species are under artificial rather than natural selection; human inputs determine population sizes -linked to the productivity of the ecosystem.
- Control is external and goal-oriented rather than internal via subsystem feedback as in natural ecosystems.

Creation and maintenance of agro-ecosystems is necessarily concerned with the economic goals of production, productivity, and conservation. They are controlled, by definition, by management of ecological processes and they would not persist but for human intervention. It is for this reason that they are sometimes referred to as artificial systems as opposed to natural systems that do not require intervention to persist through space and time. Knowledge of the ecological interactions occurring within an agro-ecosystem and the sustainable functioning of the system as a whole, allows management for the long term. Sustainability can be achieved in an agriculture that is ecologically sound, resource-conserving and not environmentally degrading.

Sustainable Agriculture

Sustainable agriculture is both a philosophy and a system of farming. It has its roots in a set of values that reflects an awareness of both ecological and social realities. Sustainable agriculture systems are designed to maximize advantage of existing soil nutrient and water cycles, energy flows, and soil organisms for food production. An ecologically sustainable agriculture maintains the resource base upon which it depends, relies on a minimum of artificial inputs from outside the farm system, manages pests through internal regulating mechanisms, and is able to recover form the disturbances caused by cultivation and harvest through successional processes minimizing waste and environmental damage, while maintaining or improving farm profitability (ASAP, 2004). In practice such systems have tended to avoid the use of synthetically compounded fertilizers, pesticides, growth regulators, and livestock feed additives. Natural, biological, and cultural controls are used to manage pests, weeds and diseases.

Management of agro-ecosystems for sustainability both influence and are influenced by biodiversity. Sustainable practices leading to increased crop and genetic diversity have resulted in increased agro-ecosystem stability –increasing crop diversity benefits agriculture by reducing insect pests-. Conservation tillage increases habitat and wildlife diversity, and numbers of beneficial insects species.

Sustainable Agriculture (ASAP, 2004)

- Is based on the prudent use of renewable and/or recyclable resources. It uses renewable energy sources such as biological, geothermal, hydroelectric, solar, or wind.

- Protects the integrity of natural systems so that natural resources are continually regenerated. Sustainable agricultural systems should maintain or improve groundwater and surface water quality and regenerate healthy agricultural soils.
- Improves the quality of life of individuals and communities. In order to stem the rural to urban migration, rural communities must offer people a good standard of living including diverse employment opportunities, health care, education, social services and cultural activities.
- Is profitable. Transition to new ways of knowing, doing and being require incentives for all participants.
- Is guided by a land ethic that considers that long- term good of all members of the land community. An agroecosystem should be viewed as a dynamic interdependent community composed of soil, water, air and biotic species, with capacity for self-renewal.

Many of the approaches in conventional agriculture (minimum tillage, chemical banding) would fall into the "efficiency" category. Efforts to substitute safe products and practices (botanical pesticides, bio-control agents, imported manures, rock powders and mechanical weed control) are being used. Despite the reduced negative environmental damage associated with them, they remain problematic. Botanical pesticides also kill benefic organisms, the release of bio-controls does not address the question of why pest outbreaks occurs, dependence on imported fertilizer materials makes the system vulnerable to supply disruptions, and excessive cultivation to control weeds is detrimental to the soil. As in conventional agricultural systems, the success of sustainable approaches is very dependent on the skills and attitudes of the producers (ASAP, 2004). What has become increasingly clear to a growing number of agricultural professionals in the last few years is that good agronomy is based on an understanding of ecology. An "agro-ecological" approach is used increasingly by agricultural professionals to analyze the success of sustainable farming systems, and to identify ways of improving the productivity profitability, and resource efficiency of them.

Agricultural Biodiversity

Agricultural biodiversity is a broad term that includes all components of biological diversity of relevance to food and agriculture, and all components of biological diversity that constitute the agricultural ecosystems, also named agro-ecosystems: the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agro-ecosystem, its structure and processes (CBD, COP decision V/5, appendix). It includes crops and livestock and their wild relatives, but also many other organisms such as soil fauna, weeds, pests and predators. Agricultural biodiversity is the outcome of the interactions among genetic resources, the environment and the management systems and practices used by farmers. This is the result of both natural selection and human inventive developed over millennia.

Dimensions of agricultural biodiversity

1. *Genetic resources for food and agriculture,* which constitute the units of production in agriculture, and include cultivated and domesticated species, managed wild plants and animals, as well as wild relatives of cultivated and domesticated species

- Plant genetic resources, including crops, wild plants harvested and managed for food, trees on farms, pasture and rangeland species
- Animal genetic resources, including domesticated animals, wild animals hunted for food, wild and farmed fish and other aquatic organisms
- Microbial and fungal genetic resources

2. *Components of biodiversity that support ecosystem services* upon which agriculture is based. These include a diverse range of organisms that contribute, at various scales to, *inter alia*, nutrient cycling, pest and disease regulation, pollination, pollution and sediment regulation, maintenance of the hydrological cycle, erosion control, and climate regulation and carbon sequestration

3. *Abiotic factors*, such as local climatic and chemical factors and the physical structure and functioning of ecosystems, which have a determining effect on agricultural biodiversity

4. *Socio-economic and cultural dimensions*. Agricultural biodiversity is largely shaped and maintained by human activities and management practices, and a large number of people depend on agricultural biodiversity for sustainable livelihoods. These dimensions include traditional and local knowledge of agricultural biodiversity, cultural factors and participatory processes, as well as tourism associated with agricultural landscapes

Biodiversity and agriculture are strongly interrelated because while biodiversity is critical for agriculture, agriculture can also contribute to conservation and sustainable use of biodiversity. Indeed, sustainable agriculture both promotes and is enhanced by biodiversity. Maintenance of this biodiversity is essential for the sustainable production of food and other agricultural products and the benefits these provide to humanity, including food security, nutrition and livelihoods.

Conservation of genetic resources

Genetic Resources for Food and Agriculture

Genetic resources for food and agriculture are the basis of global food security. Genetic resources for food and agriculture are the biological basis of world food security and, directly or indirectly, support the livelihoods of every person on Earth. Used either in traditional farming and breeding, or in genetic engineering, they constitute a world patrimony of invaluable usefulness for humankind existence. Plant genetic resources comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species (FAO 2002). Genetic diversity provides farmers and plant breeders with

options to develop, through selection and breeding, new and more productive crops, resistant to virulent pests and diseases and adapted to changing environments.

Genetic diversity of the majority of modern crops is very limited in comparison with their wild ancestors. This reduction in diversity during crop evolution is not recent, as it began with crop domestication. The development of improved '*elite*' varieties during the twentieth century accelerated the rhythm of genetic erosion, which reduces considerably the opportunities for the world community and the small farmers, who depend in many cases on wild species and natural habitats to subsist. The better performance and higher yield obtained with new varieties led farmers to stop using their local varieties and instead to use high-yielding hybrids and new varieties preferred by consumers (Chrispeels & Sadava, 2003). Domestication, artificial selection and constant manipulation of biological diversity by humankind since 10,000 years ago and overall human activities have converted vast forest extensions, savannas and prairies into production fields and industrial complexes. Human societies in the current context are strongly linked to monocultures, the worst condition from the point of view of diversity as it can be attested by the well known devastating consequences of the Irish famine (caused by a potato disease) and the desertification of Sumer in ancient Mesopotamia due to soil salinization (WRI, 2000). Genetic erosion reduces considerably the opportunities for the world community and the small farmers, who depend in many cases on wild species and natural habitats to subsist (Pullin, 2002).

FAO estimates that since 1900, 75% of crop genetic diversity has been lost. Without a constant contribution of new wild genes, plant geneticists and breeders cannot continue improving basic crops. Plants obtained by means of crop selection must be invigorated each 5 to 15 years in order to provide them with new or better traits such as pest and disease resistance, higher yields, or higher tolerance to droughts and saline soils. The most effective way to achieve it is by mixing commercial varieties with wild ones. Many of the local varieties and wild species that are being lost may contain genes with potential utility to plant breeders and biotechnologists for crop improvement (FAO, 2001; WCMC, 2002).

The growing deterioration of the natural and agricultural environments, and concerns for the loss of biodiversity, has resulted in rapid development of the discipline of conservation biology. The origins of gene resource conservation -and the interest of agriculturalists in the origin of domesticated crops and in the use of wild relatives for breeding programs- can be traced to the 1910's. By 1924 the Russian botanist Vavilov founded the All-Union Institute of Applied Botany and New Crops. The number and size of crop gene banks has continued to grow dramatically ever since.

Conservation and restoration

Conservation biology studies the use and management of the biodiversity present in natural and cultivated ecosystems in order to guarantee their renewal, conservation and productivity, thus providing benefits and opportunities for the present and future generations.

The main approaches used today in conservation biology include conservation strategies for undisturbed natural ecosystems, restoration strategies for disturbed ecosystems and sustainable use strategies for transformed ecosystems, which include agroecosystems, urban ecosystems, dams, gardens and recreation areas among others. (WCMC, 2002; WRI, 2000).

When degradation and decline are extreme, and no preservation is possible, restoration ecology studies how to recover and rehabilitate an ecosystem. Restoration involves species reintroduction, the total or partial replacement of extinct populations with the same or similar species having an ecological, social, cultural or economic value. The most effective way to conserve viable populations is to conserve zones which are large enough to allow species and their habitats to exist. An important concept in wildlife conservation is that of biological corridors, which are strips of land connecting fragmented habitats through which species can move from and to different fragments of their natural habitats. Corridors allow the re-colonization of fragments where populations have disappeared and help to avoid inbreeding or endogamy in subpopulations (Pullin, 2002; Ricklefs & Miller, 1999). There is no global consensus as to what constitutes an important species, but species may be singled out for conservation action if they fall into one or more of the following categories: i) Threatened species, ii) Ecologically important species, iii) Species useful to humans, and iv) Species with non-use value.

In situ and ex situ conservation of plant genetic resources (PGR)

As already mentioned, agro-biodiversity is currently threatened by the progressive loss of plant genetic diversity. This problem has increased agriculture vulnerability and has also impoverished food provision for humans (FAO, 2002; Bryant, 2001). The growing concern on genetic erosion has led to the establishment of germplasm conservation programs worldwide. The effort to save biodiversity is directed at both crops and wild relatives. Wild relatives of crops are critical for increasing and improving agricultural production by providing useful genes for resistance against disease and pests, abiotic stress tolerance (drought, salinity, waterlogging), as well as for improving nutritional qualities. They also provide ecosystem services such as pollination, nutrient recycling and water flow management. The effort to conserve the crop wild relatives is happening at national and global level, as this is believed to be one of the most important ways to improve food security. Countries that are richest in genetic diversity are also the poorest in economic terms.

There are two complementary approaches for conservation of plant genetic resources, namely *in situ* and *ex situ*. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of the traditional agricultural systems. *Ex situ* conservation, involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration -stored in centralized banks away from the origin. Approaches to *ex situ* conservation include methods

like seed storage banks, field gene banks, botanical gardens, world heritage sites, research centres and laboratories. DNA and pollen storage also contribute indirectly to *ex situ* conservation of PGR (Rao, 2004). Biodiversity International and the Svalbard Global Seed Vault efforts are directed to genetic resources conservation. As a part of the worldwide work, about 2.5 million accessions are being conserved by 700 seed banks around the world, although there has been limited success when using wild seeds in crop improvement crosses (Bryant, 2001).

In situ conservation of plant genetic resources (PGR)

The aim of ***in situ* conservation** is to protect habitats of target species so that natural evolution processes are assured. It includes establishing protected areas such as national parks, caring for peasant plots containing local varieties, preserving forests to protect medicinal or wild species used by indigenous communities. The vision is for the protected areas to include multiple uses, extractive reserves, and use the systems to preserve rare, endangered and threatened species. In these systems, there is a need for increase ecological related to geographic distribution of target species; population structure, dynamics and genetic variability within and between populations; for Identification of threats to target species in the wild and any mitigation actions and for management of ecosystems, including genetic resources, ecological restoration and species recovery plans. The *in situ* conservation strategies include for natural ecosystems: national natural parks, forests, protected areas, reserves and sanctuaries, and for agrobiodiversity: community and domestic parcels including land races and folk varieties. *In situ* conservation of cultivated species is primarily concerned with the on-farm maintenance of traditional crop varieties (or landraces) and with forage and agroforestry species (Rao, 2004). Active participation by farmers and other users of genetic resources is an important part of *in situ* conservation of cultivated species. Crop resources in landraces are passed from generation to generation of farmers and are subject to different selection pressures to fit specific farming situations.

Ex situ conservation of plant genetic resources (PGR)

Among the various *ex situ* conservation methods (germplasm banks), seed storage is the most convenient for long-term conservation of plant genetic resources. Seeds are dried to low moisture content and stored at subzero temperatures in cold stores or deep freezers. According to FAO, this technique accounts for 90% of the 6 million accessions conserved *ex situ* globally.One of the must important examples is the the Svalbard Global Seed Vault, which is a secure seedbank located on the Norwegian island of Sptisbergen in the remote Arctic. The facility was established to preserve a wide variety of plant seeds from locations worldwide in an underground cavern, and holds duplicate samples, or "spare" copies, of seeds held in genebanks worldwide. The Seed Vault will provide insurance against the loss of seeds in genebanks, as well as a refuge for seeds in the case of large scale regional or global crises.

However, there are a large number of important tropical and sub-tropical plant species which produce recalcitrant seeds that quickly lose viability and do not survive desiccation, hence conventional seed storage strategies are not possible. There are also a number of other important crop species that are sterile or do not easily produce seeds, or seed is highly heterozygous and clonal propagation is preferred to conserve elite genotypes.

Ex situ conservation requires skills in management of resources, development of infrastructure and facilities to accommodate the collections. It should be considered as a tool to ensure survival of wild populations and other diversity, and should be integrated into in situ conservation. The collections include: i) Whole plant/animal collections; ii) Zoological parks and botanic gardens where species can be kept without threats; iii) Wildlife research facilities; iv) Germplasm collections of wild and domesticated taxa in any form including zygotes, gametes and somatic tissue. Strategies used in *ex situ* conservation include: Seed banks and germplasm banks, reproduction propagation (as in clonal orchards) and re-introduction into the wild.

Biotechnology for characterization, conservation and sustainable use of biodiversity

Humans have manipulated the genetic make-up of plants and animals since agriculture began more than 10,000 years ago. This exploitation of the natural variation in biological organisms has given us the crops, plantation trees, farm animals and farmed fish of today, which often differ radically from their early ancestors. Increasing the efficiency of agricultural production can reduce these impacts; biotechnologies can have an important role in this respect. Biotechnology is an important tool for biodiversity conservation and utilization, and is a complement – not a substitute – for many areas of conventional agricultural research. It offers a range of tools to improve our understanding and management of genetic resources for food and agriculture. Modern biotechnologies can help to counteract trends of genetic erosion in all food and agriculture sectors (FAO, 2004). One of the most valuable molecular biology techniques are molecular markers, which are used in identification and characterization of species, populations and genotypes, and are very useful for quantifying the genetic diversity within populations. Molecular marker assisted selection (MAS) is a powerful tool in conventional plant breeding and crop improvement programs, because it facilitates the identification of genes with agronomic importance (pest and disease resistance genes), hybridization ratios, to distinguish variety lines, and enables the purity control and certification of varieties(Henry, 2000). Molecular techniques are also useful tools when studying the influence of plant genetic diversity on ecosystem sustainability, due to the fact that diversity within species may contribute in a significant way to the productivity of an agroecosystem. Modern agricultural biotechnology includes a range of tools that scientists employ to understand and manipulate the genetic make-up of organisms for use in the production or processing of agricultural products. Addressing problems such as diseases and pests, abiotic stresses –drought, salinity-, improving nutritional quality, creation of new

diagnosis tools, measurement, conservation and study of genetic resources, production of vaccines, (FAO, 2004).

Germplasm characterization requires observation, measurement and documentation of heritable plant traits. There is need for identification, classification and confirmation of collections by using descriptors:

- *Morphological descriptors*, which are easy, reliable to use and are cheap, but are limited because of limited polymorphisms that can be visualized. They are also affected by the environment, which affects phenotypic expression. These descriptors can also be highly subjective.
- *Agronomic descriptors/traits*, which are useful for crops, but require large-scale field experiments, and are labour intensive.
- *Molecular descriptors*, which use molecular marker technology to identify polymorphisms. These descriptors have proved to be very useful in accessing genes of interest for use in plant breeding and genetic engineering. There is high throughput of information and most techniques are highly repeatable

Biotechnology is being utilized for collecting and storing genes through seed and tissue culture. It is also being used for detection and elimination of diseases in gene bank collections. Identification of desired genes using molecular techniques ensures that the genotypes of choice are used for downstream operations. Long-term storage using cryopreservation of tissue culture results in safer and more efficient storage as well as distribution of germplasm. Molecular techniques are used to confirm identities of germplasm when it is taken out of the banks for regeneration in addition to screening the accessions for identification of genes of interest.

The aim of modern breeders is the same as that of early farmers – to produce superior crops or animals. Conventional breeding, relying on the application of classic genetic principles based on the phenotype or physical characteristics of the organism concerned, has been very successful in introducing desirable traits into crop cultivars or livestock breeds from domesticated or wild relatives or mutants. Biotechnology can make the application of conventional breeding methods more efficient (FAO, 2004). Progress of molecular techniques and in vitro culture of plant organs, tissues and cells has been increasing during the past 50 years. Traditional plant breeding combined with improved agricultural practices and modern biotechnology techniques has resulted in higher crop yields (Henry, 2000). Recombinant DNA technology has been an invaluable tool in crop improvement.

Biotechnology techniques

The most significant breakthroughs in agricultural biotechnology are coming from research into the structure of genomes and the genetic mechanisms behind economically important traits. The rapidly progressing discipline of genomics is providing information on

the identity, location, impact and function of genes affecting such traits –knowledge that will increasingly drive the application of biotechnology in all agricultural sectors. The use and organization of this information is bioinformatics. Advances in bioinformatics may allow the prediction of gene function from gene sequence data; form a listing of an organism's genes, it will become possible to build a theoretical framework of its biology. The comparison across organisms of physical and genetic maps and DNA sequences will significantly reduce the time needed to identify and select potentially useful genes (FAO, 2004).

Molecular markers are identifiable DNA sequences, found at specific locations of the genome, associated with the inheritance of a trait or linked gene. They can be used for i) marker-assisted breeding, ii) understanding and conserving genetic resources and iii) genotype verification. These activities are critical for the genetic improvement of crops, forest trees, livestock and fish. Reliable information on the distribution of genetic variation is a prerequisite for sound selection, breeding and conservation programmes. Genetic variation of a species or population can be assessed in the field or by studying molecular and other markers in the laboratory. A combination of the two approaches is required for reliable results.

- ***Marker-assisted breeding:*** Genetic linkage maps can be used to locate and select for genes affecting traits of economic importance. The potential benefits of marker-assisted selection (MAS) are higher for traits controlled by many genes, such as fruit yield, wood quality, disease resistance, milk and meat production, or body fat. Markers can also be used to increase efficiency of introducing new genes from one population to another, *i.e.* when wishing to introduce genes from wild relatives into modern plant varieties. Key genes for disease resistance and tolerance to acid soils have been isolated from barley and rye using these techniques. Studies carried out using these technologies in fish and forest tree species have revealed high levels of genetic variation both among and within populations. Livestock species are characterized by a high degree of genetic variation within populations, whereas crops exhibit a higher degree of variation across species.
- ***Genotype verification:*** Molecular markers have been widely used for identifying genotypes and for genetic "fingerprinting" of organisms, which is used in advanced tree breeding programmes in which the correct identification of clones for large scale propagation is essential. Genotype verification is used intensively in parentage testing of domestic animals and for tracing livestock products in the food chain back to the farm and animal of origin.

The molecular method used for characterization/conservation depends on the information required:

- If phylogenetic information is required, e.g. to determine origin and relationships of plants, RFLPs and DNA sequencing are the methods of choice.
- If information on **population genetics** is required, e.g. to compare genetic variation, then micro-satellites, AFLPs, RAPDs and allozyme analyses are used.

Cell and tissue culture and micropropagation: The maintenance of genetic collections is a basic requisite for crop improvement. Cryopreservation, an ultra-low temperature conservation technique that uses liquid nitrogen (-196°C) is the only procedure available at present for long-term and low-cost ex-situ conservation of genetic resources. It is widely used for plant species with recalcitrant seeds or vegetative propagation (Engelmann & Takagi, 2000). Micropropagation involves taking small sections of plant tissue, or entire structures such as buds, and culturing them under artificial conditions to regenerate complete plants. It is particularly useful for maintaining valuable plants, breeding otherwise difficult-to-breed species, speeding up plant breeding and providing abundant plant material for research. For crop, horticultural and forest species, micropropagation is now the basis of a large commercial industry involving hundreds of laboratories around the world. Micropropagation can also be used to generate disease-free planting material, especially if combined with the use of disease-detection diagnostic kits.

Genetic transformation: When one desired trait is found in an organism that is not sexually compatible with the host, it may be transferred using genetic engineering. In plants, the most common method for genetic engineering uses the soil bacterium *Agrobacterium tumefaciens* as a vector. Researchers insert the desired gene or genes into the bacterium and then infect the host plant. The desired genes are transmitted to the host along with the infection. Once the gene has been transferred, the crop must be tested to ensure that the gene is expressed properly and is stable over several generations of breeding. This screening can usually be performed more efficiently than for conventional crosses because the nature of the gene is known, molecular methods are available to determine its localization in the genome and fewer genetic changes are involved. Some transgenic crops and traits of greater potential interest for developing countries have been developed but have not yet been released commercially. Nutritionally enhanced crops could make a significant contribution to the reduction of micronutrient malnutrition in developing countries. The well-known transgenic Golden Rice contains three foreign genes – two from the daffodil and one from a bacterium – that produce provitamin A. Scientists are well on their way to developing transgenic "nutritionally optimized"' rice that would contain additionally to the genes producing provitamin A, trhe ones responsible for iron and more protein (Potrykus, 2003). Other nutritionally enhanced foods are under development, such as oils with reduced levels of undesirable fatty acids. In addition, foods that are commonly allergenic (shrimp, peanuts, soybean, rice, etc.) are being modified to contain lower levels of allergenic compounds. Traits for which genetic modification has been contemplated for forest trees include insect and virus resistance, herbicide tolerance and lignin content.

Diagnostics and epidemiology: Plant and animal diseases are difficult to diagnose because the signs may be misleading or even entirely absent until serious damage has occurred. Advanced biotechnology-based diagnostic tests make it possible to identify disease-causing agents and to monitor the impact of disease control programmes to a degree of precision not

previously possible. Molecular epidemiology characterizes pathogens (viruses, bacteria, parasites and fungi) by nucleotide sequencing, which enables their origin to be traced. Enzyme-linked immunosorbent assay (ELISA) tests have become the standard methodology for the diagnosis and surveillance of many animal and fish diseases worldwide, and the polymerase chain reaction (PCR) technique is especially useful in diagnosing plant diseases and is proving increasingly so also for livestock and fish diseases.

DNA based assays are able to detect genetic diversity at higher levels, and unlike morphological descriptors, they are not affected by the environment. Beyond genetic characterization, biodiversity management involves:

- Collection of information to address key issues of both *ex situ* and *in situ* germplasm management. For *ex situ*, molecular techniques are used for sampling, management and development of core collections as well as utilization of genetic diversity. For *in situ*, for identifying the most representative populations within the gene pool of a landrace, considering as well the gene flows occurring in these populations. More than 60% of all *ex situ* accessions in gene banks are for just five species: wheat, barley, rice, maize and beans
- Assistance in decision-making. Out of the whole genome only about 5% is used for coding information and the remainder serves for regulatory control or structural integrity of the genome. Most of these non-coding regions are used to identify:
  - genetic fingerprinting to clarify relationships of taxa. They have been used for crop identification to prove breeders rights and also to identify endangered species in illegal trade,
  - lineages of high conservation, and
  - variation in genotypes.

Genomes contain microsatellites or simple sequence repeats (SSRs) that differ in length and number of repeats among individuals in a population and these repeats are unique for individuals. These characteristics are useful for identification of accessions.

Biotechnology, biodiversity and sustainable agriculture

Biotechnology has the potential to improve sustainability in several ways and is expected, thereby, to help maintain natural as well as agricultural biodiversity. Agriculture has to respond, additional to the traditional focus on higher yields, addressing the protection of environmental goods, consumer concerns for food safety and quality. Biotechnology can overcome some production constraints difficult or intractable by conventional methods. It can speed conventional breeding programmes, create crops resistant to diseases and pests, reducing the use of toxic chemicals that harm environment and human health, and it can provide diagnostic tools and vaccines that help control devastating animal diseases. It can

improve the nutritional quality of staple foods such as rice and cassava and create new products for health and industrial uses (FAO, 2004).

Developing sustainable agricultural systems with minimal impact on biodiversity will require utilizing all available technologies while simultaneously encouraging appropriate farmer practices. Biotechnology should be part of integrated and comprehensive agricultural research and development programmes that gives priority to the problems of the poor. Biotechnology is not a substitute for research in other areas such as plant breeding, integrated pest and nutrient management and livestock breeding, feeding and management systems (FAO, 2004). A great deal needs to be done so that developing-country producers are empowered to make their own decisions regarding these technologies for their own benefit. Identifying small farmers' constraints to technology access and use continues to be an issue that the development community must address. Investments in biotechnology research capacity for the public sector will only be worthwhile if the current difficulties in delivering conventional technologies to subsistence farmers can be reversed (FAO, 2004). We need a better understanding of the sustainability of crop and animal production systems, as well as promoting the development of integrated crop management systems linked to biotechnology progress, in order to establish production systems more friendly to the environment and thus to guarantee resources to future generations.

Gene flow

Gene flow, also known as gene transfer, is the movement or exchange of genes between different species or between different populations of the same species (adjacent conspecifics). Genes may flow (transfer) from one organism to sexually compatible relatives, in which case it is called vertical gene transfer, or by other means –*i.e.* infection- to totally unrelated species and families of organisms, generally referred to as horizontal gene transfer. Gene flow is a natural process, important in the maintenance of genetic variation in populations, as well as in the spread of new traits among populations and across species boundaries, adding new alleles to the gene pool of the populations, or changing the frequencies of alleles present (Ammann *et al.*, 2003). Gene transfer within species is almost essential to preserve the fitness of most species of plants and animals and many species of crop plants and is the basis for evolution. In crops, gene flow typically involves movement of pollen and is dependent upon wind or animal vectors (pollinators). Gene flow occurs with all species, and thus with all crop species, but the amount of gene flow is a function of crop biology. Given its importance, the processes that affect gene flow have been widely studied and generally are well understood.

Vertical Gene Transfer (VGT)

Vertical gene flow occurs naturally between crops and weeds and from crop-to-crop. It occurs between sexually compatible plants and wild relatives if the appropriate conditions are met. Gene transfer between crops and sexually compatible relatives has occurred since the

domestication of plants began more than 10000 years ago. Over the centuries farmers kept seed from the best plants in their crops that had been formed by mutation or had arisen from natural crosses. Gradually, major differences arose between the domesticated and wild species, so that farmers were keeping plants that contained combinations of genes that improved the domestic attributes of the crops (Ammann *et al*., 2003). Most ecological scientists agree that gene flow is not an environmental problem unless it leads to undesirable consequences. In nature gene flow is through pollen transfer to the ovaries. For plants, gene flow may occur in nature by pollen spreading from one population to another. The pollen may be spread in a variety of ways, e.g. by wind, water or insects and other animals. In self-pollinated plants, pollen transfer can be by gravity. Genes from the resulting offspring can be spread further by pollen or by seeds. The minimum requirements for GM gene flow to occur are thus the presence of a sexually-compatible non-GM population in close proximity to the GM population, the possibility of outcrossing between the two populations and the production of fertile hybrids. The degree of outcrossing varies amongst species: e.g. maize and millet are typically cross-pollinated while rice, wheat and barley are primarily self-pollinated. Important aspect is that gene flow refers to the exchange of genes among populations and not simply to the dispersal of pollen or seeds. Introgression is what defines the stable incorporation of genes from one pool to another, and determines the real gene flow between populations.

Since transfer can happen between crops where GM crops are being introduced, it is important to know the crop progenitors as well as their wild relatives in order to assess the likelihood of gene transfer. There is a likelihood of transgene increasing in frequency following gene flow or establishment of feral populations. The answer to the concern of "Does it occur?" now seems clear: gene flow is inevitable from those crops that naturally outcross both to conventional varieties of the same crop and to a small number of wild relatives, although this latter phenomenon is usually a rare event. However, for ecologists and agronomists the key question is "Does it matter?" More specifically, does outcrossing of transgenes affect fitness of recipient offspring in both natural and agricultural ecosystems? The inherent characteristics of a crop and its proximity to closely related plants are some of the factors that determine the likelihood of gene transfer to other plants. The key to understanding gene flow is knowledge of the sexual compatibility of the crop with other species growing in the same landscape.

Factors affecting vertical gene transfer

Gene escape depends on many ecological and agronomical factors: reproductive biology of the plants, whether or not the crop is allowed to flower, how far its pollen travels, success of fertilization, extent of seed dispersal, seed survival, among others. Even if a gene does "escape", its future may be bleak if it handicaps its new host. The probability of success pollination depends on a great number of interrelated factors, including level of pollen production, rate of self- and cross- fertilization of receptor plant, rate of pollen dispersion,

pollinating agents, spatial distance between donor and recipient population, local density of recipient population, difference in phenology between crop and wild population. There is need to evaluate crop and recipient populations' overlap in space and time; hybridization between different crops; the stable incorporation of the transgene into the population (introgression) depends on the fertility of the hybrid produced; and use of landraces, e.g. in Kenya, farmers frequently cross landraces with the improved varieties; thus crop-to-crop gene flow is already widespread.

A trait with selective advantage and improved fitness has a chance of accumulating in offspring of a population, e.g. if the trait is out-crossed with wild relatives, it has a good chance of accumulating in the wild population, and that trait may be preferentially attained. There must be a benefit associated with the given gene in order for it to persist. If there is, for example by increasing survival or reproduction, it is likely to spread more rapidly through the population. Conversely, if it has a detrimental impact on the fitness of individuals, the rate of gene flow is likely to be reduced and the transgene may eventually be lost.

Key Issues to consider for gene flow in crops

- Sexual compatibility between the plants, presence of wild or domesticated relatives
- Pollen production rate
- Out-cross rate and auto-pollination
- Pollen dispersal rate
- Pollen viability and competitive ability
- Characteristics of the pollinator agents
- Spatial distances between GMOs and recipients
- Environmental factors
- Local density of the population
- Temporal differences in flowering -phenologic isolation-. Synchrony of flowering -timing for pollen shed –anthesis- and receptivity must coincide for the crop and nearby relatives
- The resulting offspring must be viable and fertile

Horizontal gene transfer - HGT

Horizontal gene transfer refers to non-sexual transfer between totally unrelated species and families of organisms. HGT is not new: it has occurred during the history of life in earth. It has been a very important feature in the evolution of species and will continue to be important, but there is no obvious reason why its rate should be enhanced by biotechnology. Horizontal gene transfer is very common for bacteria -where DNA move easily between unrelated bacteria-, not so common between other groups of organisms. HGT is a particularly unusual form of gene transfer in that it is frequent and is frequently an essential component of

the pathogenic relationship between the host and microorganism. Gene transfer from bacteria to plants is a well known natural phenomenon and forms the basis for much plant genetic manipulation. The bacteria concerned, *Agrobacterium* species, have evolved a series of plasmid-borne genes that enable them to attach to exposed cells in wounded plants, transferring genes from the plasmid to apparently random sites within the plant genome (Chrispeels & Sadava, 2003). *Agrobacterium* genes are introduced into plant genomes and this constitutes the basis for *Agrobacterium*-mediated plant genetic transformation. There is no evidence to date that other bacteria have evolved specific methods to transfer genes to plants or animals (Ammann et al., 2003).

Differences and similarities between HGT and transposition

It has been argued that HGT is no different from transposition, a natural process that involves genes moving from one locus to another on a chromosome. The so-called jumping genes, or mobile genetic elements, that are also used as vectors in genetic engineering, that were first discovered in maize.

There are similarities between the two, but there are also major and fundamental differences. *Transposons are endogenous in plants*, whereas *transgene are introduced*. Transposition is a rare event that seldom gives rise to new plants, while transgenic plants are grown in large areas. Both transgenes and transposons can silence genes and activate dormant genes. Both are capable of causing mutations. Activation of the transposase gene in plants is not alien, and normally transposons do not give new information in a plant.

The integration of the transposons is regulated by the plant. There is conflicting evidence about the insertion of transposons, some studies show site-specific insertions, while others show no site preferences. In the case of random insertions, the two are similar. In maize, the frequency of transposition depends on the stage of the plant. Activation and deactivation of genes is controlled by the plant. Transgenes on the other hand are present throughout the development of the plant. Transposons are also known to insert in sequences that have been duplicated before, although it is not clear whether this is a consequence of the jump or a presupposition of insertion.

The integration of transgenes is irreversible, while transposon insertion is reversible, although this reversibility might imply higher risk associated with transposition in terms of side effects because of the mutations they cause

Effects of gene transfer

Gene transfer within species is almost essential to preserve the fitness of most species of animals and many species of crop plants, and is very common among micro-organisms. There is an absolute need for the incoming DNA to be replicated if the genes carried are to be maintained in the new host. If DNA that has entered a cell is to be maintained there is a need

for it to confer a selective advantage on the host, or be very closely linked to a gene of this nature. If it does not, the frequency at which genes are present in populations will remain at the frequency at which the genes are inherited. Thus, although some species are very effective in taking-up DNA from the environment, they remain species because integration of foreign DNA is very infrequent and seldom does such integrated DNA confer a selective advantage on the new host. Other factors that are likely to reduce the frequency with which DNA can be maintained in populations are that the different species can have different regulatory signals controlling the expression of genes, and also gene expression can be affected by different preferences for codon usage (Ammann *et al.*, 2003). In crops, the variable homology of the genomes between related species leads to a wide range of possibilities for the introgression rate of a transgene, or any other gene after the F1 hybrid generation. Meiotic abnormalities caused by the distant relationship between parental genomes decrease rates of introgression into new genotypes, thus the production of initial hybrids does not ensure that transgenes will move into weeds or wild relatives. This may result in higher rates of infertility and decreased rates of seed production. Recombination –important process in the incorporation of foreign DNA- is reduced by the unstable chromosome configuration of hybrids produced by distant relatives (Chrispeels & Sadava, 2003). When crosses between plants result in a stable incorporation of genes from one pool to another, differently composed gene pool, the process is called introgression or introgressive hybridization. It is often difficult to prove with certainty because shared traits may also be result of common ancestors or convergent evolution. The most powerful way to detect introgression is by tracking linked molecular markers.

The consequences of the transfer of transgenes to weeds or wild relatives depend on the nature of the novel gene and the biology and ecology of the recipient plant. Gene flow from GM to wild relatives has two potentially harmful consequences: the evolution of increased invasiveness and persistence, and the likelihood of extinction of wild relatives. The transfer of herbicide tolerance is unlikely to confer any competitive advantages to hybrids outside agricultural areas. On the other side, the transfer of traits such as resistance to particular pests and diseases or stress tolerance could potentially give selective advantages to a given plant (increased fitness). Transgenes related to agricultural practices (herbicide tolerance) won't affect non-agricultural environment. Transgenes that provide fitness enhancing characteristics under natural conditions have the potential to disrupt the balance of established ecosystems. In the case of herbicide tolerance, wild weed species may become super weeds. For insect resistance, wild species may become unpalatable and this would affect non-target invertebrates in field margins if their host plants take up the resistance gene. Crops that are being engineered for attributes such as modified starch, reduced pod shatter; virus resistance, etc, may affect wild relatives and cause ecological imbalances.

Measures to limit gene flow in plants: biological and physical barriers

- Separating distance: where purity of a crop is paramount (transgenics grown in the vicinity of organic crops)
- Barrier crops planted around the transgenic crop and this can capture any pollen drift. Serve to dilute pollen being introduced into the next crop, border rows act as buffers to pollen dispersal.
- Crop isolation zones between the GM crop and non-GM crop neighbour, creating a geographic barrier to ensure purity of non-GM crops.
- Manipulation of flowering time or blocking flowering
- Prevent access of pollinators to the flowers of the transgenic plants:
  - Bag flowering structures to prevent pollen spread by insect vectors, wind, or mechanical transfer, or cover female flowers after pollination to prevent loss or dissemination of GM seed
  - If seed production is not required, remove flower heads before pollen and seed production
- Harvest plant material of experimental interest before sexual maturity
- Locate test plots surrounded by roads or buildings.
- Cleistogamy incorporated into the crop so that flowers remain closed during pollinating e.g. as what happens in wheat and soybeans.
- Hybrid barriers. Interspecific incompatibility at the stigma surface or within the style or post-fertilization barriers that cause seed abortion
- Genetic engineering male sterility so that plant produces infertile anthers
- Seed sterility so that GM produces seed that cannot germinate
- Apomixes, the production of seed without fertilization.

Evaluation of gene transfer

Studies in risk evaluation for gene flow must consider primarily, for each crop in each location, the distinctive characteristics of pollen production, as well as the dispersal and potential out crossing. There are three main types of crops: i) Crops with no sexually compatible wild relatives; ii) Crops with wild relatives but with poor compatibility; but spontaneous hybridisation could still occur, e.g. oilseed rape and wild turnip; iii) Crops with fully compatible wild relatives e.g. sugar beet hybridises readily with wild sea beet. The possible implications of hybridization and introgression between crops and wild plant species are so far unclear because it is difficult to predict how the transgenes will be expressed in a related wild species. The fitness of wild plant species containing introgressed genes from a GM crop will depend on many factors involving both the genes introgressed and the recipient ecosystems. While it is important to determine frequencies of hybridization between crops

and wild relatives, it is more important to determine whether genes will be introgressed into wild populations and establish at levels which will have a significant ecological impact (Eastham & Sweet, 2002). The Information needed to assess potential environmental risks associated with outcrossing from transgenic plants include: biogeographical information of the species involved, reproductive biology of the plant and distribution of sexually compatible relatives, and the impact of the introduced trait, if introgressed into other plant species. Currently there are several useful tools available for evaluation such as geographical information systems GIS, modelling, and data related to geographical origin, and region of cultivation. Considerable information already is available on the biology of all major crops, making it relatively straightforward to characterize the likelihood of gene flow for any given crop using published literature and simple field surveys.

Key questions about crop-wild gene flow

- Does the crop occur near its wild relative and flower at the same time? How far can pollen from the crop travel?
- How easily can crop alleles introgress into wild/weedy populations? Do some crop alleles persist indefinitely?
- What is the baseline fitness of crop-wild hybrids compared to the wild relative? Are there strong interactions? Are later generations more fit than early ones?
- Are transgenic traits associated with fitness benefits and/or fitness costs? Could fitness enhancing traits exacerbate weed problems (spread of herbicide resistance) or harm non-target organisms (pollinators)?
- Considerations related with viability and fertility of the hybrid progeny Are the seeds produced viable? Will the plants be fertile and produce viable seeds?

Overall, the potential impacts of gene flow from GM crops are assessed in two steps: (1) the potential for gene flow to occur (likelihood) between the GM crop and any wild relatives is estimated (the exposure component), and (2) the potential environmental impact of gene flow (the hazard component), if it were to occur, is assessed. Gene flow will be higher from crops possessing characteristics that include high pollen production, an ability to disperse pollen over long distances, pollen production over a long period of time, and/or abundant, out-crossing wild relatives.

The development of effective strategies for the safe use of GM crops will depend on adequate biological and ecological characterization of the systems of interest that can only be achieved through a combination of appropriate field tests conducted in relevant environments and development of appropriate models and monitoring methods (Ammann *et al.*, 1999). The Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants of the National Research Council (NRC-CEI, 2002) found that …"the transgenic

process presents no new categories of risk compared to conventional methods of crop improvement but that specific traits introduced by both approaches can pose unique risks".

Ecology of GM Crops : Environmental Effects

Prior to the advent of genetic engineering, plant breeding was not subject to a great deal of regulation. Seed certification standards ensure the purity and quality of seeds, but little attention has been paid to the possible food safety or environmental impacts of new plant varieties derived from conventional breeding. Conventional plant breeding differs considerably from natural selection. Artificial selection and conventional plant breeding break down the resilience in agroecosystems, thereby creating gene combinations that would rarely survive in nature. Conventional breeding has been responsible for a few cases of negative effects on human health. The concerns associated with genetically transformed crops are equally applicable to conventional crops. Most of the world's major food crops are not native to their major production zones; rather, they originated in a few distinct "centres of origin" and were transferred to new production areas through migration and trade. Highly domesticated plants are grown all over the world and migration outside cultivated areas has only rarely caused a serious problem (FAO, 2004, NRC-CEI, 2002). While there are risks associated with the introduction of any novel organisms into a habitat, the ecology of genetically engineered organisms is exactly the same as the ecology of any other living thing (FAO, 2004). The rules are precisely the same, no matter how the genotype is put together.

Ecological risks of GM crops are considered: persistence -the transgenic plants become serious arable weeds; invasion -the transgenic crops become invasive of natural habitats; gene flow -transfer of introduced genes via pollen (or some other process) to other plant species (such that these then become persistent or invasive); reduction of *in situ* biodiversity; development of pests resistant to GM crops; and effects in non-target organisms. The risks are not currently perceived as being high; transfers of genes resulting from conventional crop breeding into non-crop plants has not created conspicuous problems; nor have traditional crop plants themselves become invasive of natural habitats (FAO, 2004). To date, none of the potential risks has been manifest to any significant extent.

The foremost environmental issue is the presence of sexually cross-compatible relatives, whether domesticated or wild. The wild types may be directly related to a crop as progenitors or they may be indirectly related as neighboring taxa. Domesticated relatives are local, farmer selected cultivars, also called landraces. Both wild and domesticated relatives fulfill important roles as reflections of sociocultural identities, production capital of farmers, and repositories of genetic diversity for plant breeders and farmers alike. An important feature of these domesticated or wild relatives is that they generally cross readily with introduced cultivars. This feature sets the stage for potentially extensive gene flow in domestication centers between transgenic cultivars and their relatives. On the one hand, crops have evolved to increase self-pollination, which would reduce gene flow among crop varieties.

An alternative classification groups the concerns by type of impact:

a) ***Impact on the environment***.

- Persistence of the transgene (better adaptation, invasiveness) or the products of the transgene (cumulative effects).
- Susceptibility of non-target organisms.
- Increased use of agrochemicals.
- Unpredictable expression of the transgene or its instability.

b) ***Impact on agriculture and agricultural production***.

- Development of resistance or tolerance in non-target organisms.
- Development of weeds and superweeds.
- Reduction in nutritive value
- Reduction in number of varieties (increase in susceptibility to pest and diseases) and loss of biodiversity (for preference of GM crops over conventional crops).
- Increased costs of agricultural production
- Lack of capacity for risk evaluation and management
- Ethical aspects, labelling (rights to information).

c) ***Impact due to interactions***

- Genetic contamination through pollen and seed dispersal and horizontal transfer of genes
- Transfer of the transgene to micro-organisms (DNA) or generation of new viruses
- Interaction among different GMOs.

Aims of ecological risk assessment:

- to determine the potential for persistence and spread of transgenic crops in a variety of habitats
- to determine the range of plant species that can cross-pollinate with transgenic crops
- to investigate the ecological performance of hybrid plants produced by such pollination
- to develop protocols that would allow crop breeders to carry out their own ecological risk assessments on new transgenic plants in the future.

The risk assessment studies need to consider the fate of the genetically engineered plants (and their pollen) and the effects of the introduction on the environment (i.e. on subsequent crops in the same fields, on adjacent crops, and in nearby natural habitats), considering:

- Problems concerned with the persistence of the vegetative plant and its propagules in different kinds of environments

- Problems related to the spread of the plant by vegetative growth and by seed in both, arable fields and natural habitats
- Problems involving the risks of lateral spread of the engineered genes, either by pollination of different plant species or by other means

Certain principles guide the safety assessment. First, the safety assessment must be specific to the crop and trait involved, and the region where introduction is going to occur in a case-by-case basis. Because the environmental impact of the product will depend upon local conditions and practices, the environmental safety assessment must consider the nature of local agro-ecosystems and farming practices within these systems. Differences in cropping practices and native flora and fauna must be taken into consideration when identifying potential hazards and prioritizing research needs. Second, it is not possible to demonstrate absolute safety for any technology or activity, all technologies and activities carry some risk. Instead, relative safety compared with alternative technologies is what must be assessed (in this case, typically other pest control practices). A regulator must consider whether the product involves greater risks that comparable technologies. Alternatively, the regulator may compare the net benefit (benefit-risk balance) for the product. Note that this risk-benefit balance will reflect local views on the importance of risk and uncertainty, and thus regulators in different regions may make different decisions based on the same data. The assessment then should consider the relative risks and benefits of the new product relative to current practices, and should include the potentially important ecological impacts of these technologies. For an insect-control product like Bt cotton, current practices typically involve the use of conventional insecticides. For herbicide-tolerant crops, that would be other herbicide regimes. These comparisons must be carried out based on local conditions.

Concerns and potential risks of GMOs to the environment

Persistence of the gene or transgene

This includes existence of volunteer plants, increase in capacity to adapt or invade and cumulative effects of transgenic products. In evaluation of possible impacts of a transgenic plant, one of the fundamental issues is to establish whether the introduced genes (traits) can result in the crop becoming more persistent (weedy) in an agro-ecosystem or more invasive in natural habitats. It is known that the characteristics of a weed are the sum of many different traits and that the addition of a single gene is unlikely to turn a plant into a weed. Special attention should be paid, however, to those crops that already have some weed traits or those in which addition of a gene might increase the competitiveness in agro-ecosystems or their invasiveness in natural ecosystems. For example, crops that have a short history of domestication are closest to this situation as they still have wild genes, conferring competitiveness, that are usually eliminated during selection processes to improve a crop. Those GM crops used to date do not show evidence of having increased in persistence or invasiveness. It is important to consider whether a crop is sown in its centre of origin or

domestication, and the type of environment that is introduced into. For this reason risk must be studied and evaluated on a case-by-case and step-by-step basis.

Gene flow and gene dispersal from transgenic crops

Gene flow and gene dispersal are two separate phenomena and their potential consequences are different. Gene flow refers to exchange of genes (transgenes) among species and results in fertilization, whereas gene dispersal solely refers to movement of pollen. Concerns for gene flow are that there will be genetic pollution of species through creation of 'unnatural' hybrids and that a new super-weed species could be created that would have direct consequences for the environment. If gene dispersal has any effect, it is likely to be short-term, but effects of gene flow could be long-lived. Introduced genes could potentially spread in adjacent populations creating new phenotypes. Investigating this requires insight into ecological impacts of such events, including studies of population sizes, dynamics, spread and development to quantify and predict possible scenarios.

An additional factor in the need to restrict inadvertent gene flow is the possibility of generating feral populations of the crop. Many crops do not survive long off-farm, but under semi-natural conditions seed may remain dormant but viable for long periods and feral populations of the crop might establish themselves. This represents a potential problem among members of the cabbage family where species such as rape have become serious weeds. If weedy species contain herbicide resistance genes, for example, they could pose a particularly serious management problem. If these genes were passed among different species within a genus, or among related genera, hybrid weeds could be created. Similar concerns as have been voiced for herbicide tolerance genes will be heard should genetic use restriction technology (GURT) genes be deployed in crop plants. The major fear in this case is that they could be transferred to non-genetically modified crops of the same or related species. The spread of resistance or tolerance genes to pests and diseases has to be considered in a double sense. There are possibilities for those genes to render related weed species more resistant, but depending on the case, they could represent possibilities for better survival of wild species. In general terms, it is likely that they represent an environmental impact only when a new transgene confers enhanced fitness to a crop or its wild relatives with which it is sexually compatible.

In general, assessing the impacts of introducing new technologies into centers of diversity requires a special degree of care for several reasons. There is widespread consensus among scientists and policy makers that the biological and genetic diversity of these regions needs to be preserved, and may be vulnerable to ecological disturbances. Centers of diversity, and centers of origin for crop species, represent areas where many potentially-impacted wild species may exist, including wild relatives of crop species that may be recipients of gene flow, as well as many non-target species that could be directly or indirectly impacted by changes in agro-ecosystems (Lemaux, 2008).

Susceptibility of non-target organisms

Toxicity to living organisms refers to inadvertent effects caused by GMOs to benign organisms in the environment. This can be the case if a GM crop carries resistances to pests and diseases. The ideal situation is to identify a resistance gene to a pest or disease and introduce it such that its product is expressed solely in the tissues where needed. Only then is it likely only to have an effect on the target organism and not on non-target organisms. To do this is not easy however. There are currently advances in this area and there are commercial cultivars with tissue-specific gene expression. For example, there are numerous maize lines that express toxins from *Bacillus thuringiensis* (Bt) specifically to combat insect infestation, others with increased expression of genes for lysine in the grain, canola that expresses male sterility in the pollen, maize with higher oil content in the grain and others with a changed fatty acid profile and polysaccharide reserve structure.

The most studied examples of genetically manipulated resistance in crops are those employing the Bt delta endotoxins. This soil bacterium is abundant under natural conditions and produces a toxin that is lethal to certain insect pests with specific characteristics. One of the most discussed experiments was that involving Bt toxins and the monarch butterfly in the USA. The results of a laboratory study published in 1999 suggested that Bt maize represented a danger to the monarch larvae that consumed *Asclepias spp*. that were covered in transgenic maize pollen. The study did not determine the ecological consequences of the results and the tests were done under laboratory conditions that did not equate with natural conditions. A publication based on results of the experiments generated global interest and stimulated setting up a cooperative research programme in the same year. The research centred on the effects of the supposedly toxic transgenic maize pollen on monarch larvae feeding on pollen-dusted leaves of their food plant. The authors concluded that although the Bt pollen could be toxic at certain high concentrations, under field conditions there was little risk to the monarch larvae as such high concentrations of pollen would be unlikely to occur in nature. The ideal resistance mechanism for pest control would be one with no unwanted adverse effects on other organisms or the ecosystem. It is necessary to evaluate and predict the possibilities for release of a GM crop and pest control in a niche causing a secondary, more competitive, organism to invade.

Ladybirds are generally considered to be beneficial organisms; many eat aphids that are capable of damaging crops through direct feeding and vectoring viruses. Recent studies on the effect of the Bt toxin Cry1Ab from transgenic maize on the biology of the ladybird *Stethorus punctillium* indicated that the toxin had no effect on its fitness. It was shown that the ladybird lacked the mid-gut receptors for the active toxin to bind to. This research indicates that there is a long way to go before the effects of transgenic crops on non-target organisms are sufficiently well understood.

Unforeseen gene expression and instability of transgenes

This potential risk relates to concern over wide crosses in conventional crop breeding. In conventional breeding techniques it is not possible to determine a priori which genes will be introduced. This means a long process of targeted selection after crossing to remove unwanted genes and traits. With GM crops however, it is known with almost certainty which genes are introduced and it is the subsequent laboratory work that determines which will be expressed and will be stable. In general terms, given that there is ample knowledge of the genes and DNA sequences used in genetic transformation, the number of genes introduced into a GM plant is smaller than in a conventional cross. Technical developments mean that a transgene insertion can be specifically located and its expression quite accurately controlled. What distinguishes this technology from the conventional technology is the improved precision in introduction of a small number of well known genes to make for a much better controlled process. To date there appears to be no evidence for such a phenomenon in the GM crops studied and evaluated.

Weeds

Weeds fall into two major classes, parasitic and non-parasitic. Weed control is a major component of crop management programmes. Biotechnology has been less successfully applied to weed management than to management of other biotic stresses. For non-parasitic weeds, biotechnology has been applied to develop herbicide resistance, an indirect control strategy where the crop is the target of the transgenes and not the weed. Species of two parasitic weed genera, *Striga* and *Orobanche*, represent important weeds of the tropics and Mediterranean areas. They are currently managed through various strategies including manual weeding, rotations, chemical control and biocontrol. Biotechnology has potential to transform crops to allow herbicide application for weed control and to alter gene action controlling the stimuli that trigger germination and development of parasitic weed seed. More knowledge of the host-parasite relationship at the molecular level will allow more environmentally sound management methods to be developed.

Parasitic weeds represent a very specific management challenge. Each plant of *Striga hermontheca*, a major problem of cereal crops in the tropics, is able to release 100,000 seeds into the soil, each of which can remain viable for up to fifteen years. There is variation in resistance of some crops, including sorghum, which appears to be under genetic control. This can be selected for using traditional plant breeding methods, but can probably be enhanced in the future using methods from molecular biology such as marker assisted selection (MAS). For crops including maize, there is no naturally occurring host-plant resistance and the only possibilities of obtaining any, though this has not been done yet, would be to induce it or transfer non-host resistance, which occurs in many grass species. Unfortunately, very little is known about the mechanisms of non-host resistance.

Transforming crops to tolerate contact herbicides would not be effective in managing parasitic weeds as they have already done their damage before they appear above the soil surface. Transforming the crop for application of systemic herbicides, as has been done for non-parasitic weed management, is unlikely to be effective as the crop breaks down the herbicide into harmless chemicals that do not consequently reach the parasite, which is intimately linked with the crop via its roots. Enzymes in the crop that are associated with herbicide uptake could be modified to prevent herbicide binding and promote build-up of the herbicide in the parasite. Glyphosate resistance works in this way and it is termed target-site-resistance. This represents the most feasible form of control and has been effective in controlling *Striga* and *Orobanche* infestations in several crops sprayed with several herbicide formulations. Seed dressings that rely on this mechanism can also be used.

One issue constantly being raised is that of the development of a *superweed* which, created through flow of herbicide tolerance transgenes would become impossible to control using standard herbicides. To date such a weed has not developed, but serious weed problems have arisen through deliberate introduction of new ornamental plants and inadvertent introduction of exotics. Some of these have literally become some of the world's worst weeds and yet have been relatively unnoticed by environmental lobby groups. They represent introductions of entire new genomes, and are not merely the result of (trans) gene flow. Perhaps in the future there will generally be a better understanding of the relative level of risk posed by the flow of ethically contentious genes.

Some questions related to the release of genetically engineered organisms can be answered only with practical experience. Realistic, small-scale field tests are the way to evaluate potential risks from commercial scale uses of genetically engineered organisms. However, these short-term studies are only appropriate to risk assessments on annual crop plants. At the end of a three year study of the population biology of transgenic and non-transgenic annual crop plants, one should be in a position to:

- Provide data on persistence and invasion in natural and arable habitats.
- Show how (and if) genetic engineering alters these parameters.
- Describe pollen spread by insect vectors and by other means.
- Show how (and if) genetic engineering alters the production, spread, or compatibility of pollen.
- Catalogue the wild plants that share insect pollinators with the crop.
- Provide quantitative data on successful cross-pollination between the crop and its wild relatives.
- Provide data on the persistence and invasiveness of any transgenic hybrid plants produced by crossing experiments.
- Potential benefits of GMOs

Agrochemicals reduction

The use of industrial agrochemicals has a substantial bearing on the sustainability of agro-ecological systems. Pesticides have not only had direct negative impacts on the quality of the environment, but have also adversely affected biodiversity through removing beneficial and inoffensive organisms. Interestingly, glyphosate, which several crops have been transformed to tolerate, is much less toxic than some of the herbicides (e.g. atrazine) it replaced. There is concern that GMO use in the field of herbicide resistance will result in increased use of herbicides. Evidence suggests that this has not been the case, but that herbicide use has been reduced at the commercial level. Reduction in pesticides can be obtained by identifying, developing and deploying durable host-plant resistance to pests and diseases. Insect pests (9,000 species), plant pathogens (50,000 species) and weeds (8,000 species) account for the greatest crop losses, and their control requires the greatest use of agrochemical crop protection. The advantages of host-plant resistance are numerous and include: It is relatively inexpensive for the farmer in comparison with chemical control; It is always present.It has no effect on organisms other than the target ones; It can be extremely durable; It can employ a diversity of resistance genes; It does not interfere negatively with other forms of control; It has no negative effects on yield. There are also countless possibilities to improve crop production through breeding for adaptation to a range of abiotic stresses, including drought, salt and heat, and more efficient use of nitrogen and water.

Biotechnology applications to date have focussed on engineered traits such as herbicide resistance for some of the major commodity crops, but there is considerable potential for expanding the methods to include a broader range of crops and genetically more complex traits. Many disease resistances are governed by few genes and represent relatively easy targets for the molecular breeder. Resistance to some diseases is controlled by many genes, each of small effect. Using modern methods, including QTL (Quantitative Trait Loci) analysis, important areas of the genome in resistant lines can be identified, located and ultimately cloned for inclusion into susceptible, but otherwise adapted, germplasm.

GURTs - Genetic use restriction technology

GURTs (genetic use restriction technology) are molecular switches that can be inserted into plants to control seed fertility. GURT technology is very far from being fully developed and can only be used in plants that can be successfully transformed. There are fundamentally two classes.

- V-GURTs are variety use restriction types that work in various ways, but seed sterility results from disrupted embryo formation. A disrupter gene is inserted into the plant that can be induced through chemical seed treatment. Alternatively, the default can be that the seed is rendered sterile through incorporation of a gene that is inactivated during the breeding process by chemical application that restores seed fertility. A similar mechanism is potentially feasible for application in crops that are reproduced vegetatively, whereby

plant growth is inhibited or restored through activation and deactivation of transgenes. This would theoretically extend shelf-life of the crop.

- T-GURTs, which relies on restriction of a specific trait, which is nearer application. T-GURTs rely on a trait being controlled through inducible promoters that silence genes, or through physical gene removal using enzymes

The nature of the impacts of deployment of GURT technology will differ according to country infrastructure and farming system. Intensive agriculture is the norm in developed countries and farmers are used to relying on private industry for a whole range of agricultural inputs, including seed. There is little reliance on the public sector for such inputs and saved seed is not a major feature of the farming systems, although it is still practised.

For very large numbers of farmers in developing countries however, seed is saved from one season to the next to perpetuate the crop. For these farmers GURT technology would certainly restrict their operations were it to be introduced into their crops. The social structure of many farming communities could be disrupted by such a technology as GURT. Germplasm exchange between farmers would almost certainly be limited by GURTs, thereby reducing agro-biodiversity. Many of the consequences of introductions of GURTs might parallel those of Green Revolution technologies, with changes in patterns of land ownership, reduced reliance on the public sector for inputs, a widening gap between resource-rich and resource-poor farmers. There would be serious consequences for developing country agriculture if seed monopolies developed, or worse if companies collapsed and farmers were unable to purchase alternative varieties.

GURTs are ethically contentious, and have potentially negative socio-economic and environmental consequences, particularly for farmers and ecosystems in developing countries. They are not however, without some potential advantages and could conceivably be a useful additional or alternative regulatory tool in specific circumstances. If research and development of GURTs continues, their applications will probably have to be considered on a case-by-case basis.

Potential benefits

One of proposed benefits of GURTs is that they can be used to restrict the spread of transgenes into the environment by rendering sterile any seed produced by accidental transgenes.

Potential risks

There is a potentially very serious consequence for farmers, particularly those in developing countries, of not being able to save seed from GURT-transformed crops for sowing in subsequent seasons. Much in the same way that hybrid seed results in increased reliance of farmers on seed companies, seed of GURT-transformed crops would have to be purchased each season.

The greatest potential threat of GURTs probably comes from the possibility of transgenic pollen from out-crossing crops spreading to neighbouring fields of non-transgenic crops, and the use of chemicals to trigger or switch-off gene expression.

Although GURTs are purported to be useful in restricting potentially deleterious dissemination of transgenes, it is possible that the very molecular switching mechanism that controls seed sterility could enter the ecosystem via release of transgenic pollen or transfer by vectors, such that crops, crop relatives and wild species could be rendered sterile. The same fears over biosafety of transgenic crops in general apply to GURT-transformed species. In addition, inducer chemicals used to inactivate and activate the molecular switches include some, such as steroids and antibiotics, which could harm the environment and human health.

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Chapter 3: Risk Analysis for GMOs: Concepts, Methods and Issues

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Introduction

Modern biotechnology[1] or genetic engineering has numerous applications in medicine, industry and agriculture. It is well recognized that modern biotechnology has significant potential to contribute to increased agricultural productivity, enhanced food security and national development particularly for developing countries where agriculture is important. Applications of genetic engineering in agriculture have led to the introduction of new traits into plants, animals and micro-organisms and the creation of genetically modified organisms (GMO)[2], which are then used to grow/produce and manufacture GM foods. In 2007, commercial GM crops are grown in 114.3 million hectares in 23 countries with traits introduced primarily to protect the crops from pests and diseases (James, 2007). With increasing adoption, and more diverse products in the pipeline, GMOs are expected to become widespread.

Despite the enormous potential benefits of modern biotechnology, there is considerable international concern that products of modern biotechnology may pose potential risks to human health and the environment. With genetic engineering, it is now possible to manipulate the genetic materials of unrelated species that can result in new gene combinations not previously seen before. This led some to who are concerned with the environment to fear that the resulting GMOs will become invasive, harm other non-target and endangered species, result in loss of biodiversity, etc. From the perspective of human health, some believe that GM foods produced through modern biotechnology are distinctly new and different from conventional foods. Thus, they are potentially dangerous (i.e. toxic) and must be avoided. Beyond these safety considerations, there are also concerns that GMO may result in seed monopoly, are detrimental to farmer's rights to save seeds; and ethically wrong ("playing God"), etc.

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[1] The definition of modern biotechnology from the Cartagena Protocol on Biosafety is adopted: "Modern biotechnology means the application of: *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers that are not techniques used in traditional breeding and selection."

[2] In this document, the term 'genetically modified organism' (GMO) is meant to be the same as the 'living modified organism' (LMO) of the Cartagena Protocol on Biosafety. Other terminologies used for "genetically modified " are "genetically engineered", "transgenic", "bioengineered" and "products of modern biotechnology"

Because of the potential human and environmental risks, real or perceived, posed by GMOs, it is widely recognized that there is a need for each country to establish a regulatory regime specifically to assess the safety of products of modern biotechnology. There are several options available[3] that each country may choose to enable them to explore the benefits of modern biotechnology while at the same time address concerns about potential adverse effects of the introduction of GMOs to human health and the environment. The options are related to the design and objectives of the regulatory system, implementation mechanism and regulatory structures, and other considerations that includes public participation, stand alone or integration into other national objectives and harmonization with other regional and international obligations. Whatever option the country selects, a biosafety framework typically includes four important elements: a national biosafety policy instrument (e.g law, act or decree), a regulatory system, a system for monitoring and compliance and procedures for ensuring transparency, public participation and accountability.

The Cartagena Protocol on Biosafety (CPB), an international multilateral agreement on biosafety, was adopted in 2000 and came into force in 2002. It has been signed by more than 100 countries in recognition of the need to ensure biosafety through national systems of risk assessment. Its main objectives are: to set up the procedures for the safe transboundary movement of living modified organism, harmonize principles and methodology for risk assessment and establish a mechanism for information sharing through the Biosafety Clearing House (BCH). In addition to environmental safety, the Codex Alimentarius Commission has also adopted safety principles and guidelines for risk assessment of GM foods and food products derived from modern biotechnology.

The Government of Bangladesh, through the Bangladesh Department of Environment officially released the 'National Biosafety Framework 2007 (NBF)' along with the revised 'Biosafety Guidelines of Bangladesh 2006 (Biosafety Guidelines) on May 10, 2008. Currently, these are non-statutory instruments that outline the regulatory framework specifically applied to regulate the conduct of activities of modern biotechnology and assess the safety of products to the environmental and human health. As a Party to the Cartagena Protocol on Biosafety (CPB), a new act or law is expected to be promulgated to meet the country's obligation as a Party to the Protocol and harmonize it with other regional and international obligations that address biotechnology-related concerns.

The Bangladesh NBF provides the regulatory framework for biosafety that includes a mechanism for risk analysis and decision-making in regard to authorization and issuance of licenses/permits for all dealings[4] with GMOs. The components of the risk analysis process are: risk assessment, risk management and risk communication. The Biosafety Guidelines

[3] World Bank Agricultural and Rural Development Department Report No. 26028. Biosafety Regulation A Review of International Approaches April 2003

[4] 'Deal with GMOs' means " (a) conduct experiments with GMOs; (b) make, develop, produce or manufacture GMOs; (c) breed GMOs; (d) propagate GMOs; (e) use GMOs in the course of development or manufacture of a thing that is not GMOs; and (f) grow, raise or culture GMOs etc." Definition is adopted from the Bangladesh National Biosafety Framework (Chapter 3.5.3).

contain standards and codes that cover aspects of risk assessment and safety requirements and risk management needed for undertaking (a)Laboratory work, (b) Field trial and (c) Commercial use, involving i) Microorganisms ii) plants and iii) Animals.

Using the Guidelines, the Technical Committee on Crop Biotechnology in the Ministry of Agriculture has approved the import for contained trials of GM crops (Golden Rice, Bt eggplant, late blight resistant potato, Bt chickpea, and ring spot virus resistant Papaya). Bt. eggplant has completed the limited field trials and is waiting for the next stage of approval for multi-locational trials in farmers' fields. However, Bangladesh has not yet allowed commercial propagation and imports of GM food and agricultural products.

This Lecture Module was prepared provide the participants with information on the basic concepts of biological risks, concepts, principles and methodologies of risk assessment, risk management (except monitoring which is addressed in another lecture module) and risk communication. It focuses on crop biotechnology and environmental risk assessment of GM crops since these are the GMOs that are of immediate interest to Bangaladesh. Food safety assessment is not addressed specifically because it is not within the scope of this Training Workshop. This lecture does not cover risk assessment of GM animals and GM micro-organisms.

Biological Risk: Basic Concepts and Classifications

Biological Risk

Biological risk refers to naturally occurring or human made risk caused by exposure to biological agent or microorganism. The following terminologies associated with biological risks are defined or described:

Risk - likelihood that under particular conditions of exposure an intrinsic hazard will represent a threat or harm; risk is a function of hazard and exposure.

Risk = hazard x exposure

Hazard – intrinsic (it is there or it is not) *potential* of a material to cause harm to human health and/or the environment; also synonymous to threat

Exposure – the extent and the duration of or the frequency with which the operator is exposed to the hazard

Biological agent - a micro-organism, cell culture, or human endoparasite, whether or not genetically modified, and products derived from them which may cause infection, allergy, toxicity or otherwise create a hazard to human health and the environment; also synonymous with biohazard

Microorganisms - a microbiological entity, cellular or non-cellular, which is capable of replication or of transferring genetic material

Naturally Occurring vs. Biotechnological Risks

Biological risk can be classified into two broad categories: naturally occurring or human-caused.

Naturally occurring biological risks – includes (1) the emergence of antibiotic resistant bacterial infections (tuberculosis, pneumonia, flu epidemic); (2) naturally emerging pathogens attributed to deforestation (monkey pox, Ebola, Lassa fever); (3) spreading of a zoonosis i.e. infected animal population conveying the disease to humans via direct contact, vector or water/foodstuffs; (4) toxins arising from certain molds and fungi (deoxynivalenol, alflatoxins, ochratoxin a); (5) parasitic infection outbreaks in humans; (6) invasive alien species (plants, animals and microorganism)

Human caused or related biological risks – can be further classified into: (1) deliberately induced risks such as the use of harmful biological agents through warfare or terrorism; and (2) biotechnological risks such as products of traditional cross breeding and selection, mutation and modern biotechnology.

It is noteworthy that many of the biological risks that threaten society are natural in origin. In plants for example, introduction of invasive species has caused direct economic and environmental damage (e.g. in the US, cost of damage of 50,000 non-indigenous species is $137 billion per year, Pimentel et al., 2000) or presents risks to public health (e.g West Nile virus). Arguably, the risks posed by GM crops compared to invasive species are minimal. However, it is widely acknowledged that risks present with biotech crops are no different than those present from traditional breeding (NRC, 1989, Tiedje 1989; OECD, 1992; NRC, 1993; NRC, 2002).

Biological Agents and Risk Groups

Another way to classify biological risks is based on the risk posed by biological agents to the health of the laboratory workers and to human health and the environment upon accidental or intentional release. Biological agents are typically used in research or biomedical laboratories. These include the full range of micro-organisms: bacteria, viruses, fungi, protozoa and multi-cellular parasites. Laboratory associated infections (LAI) has been documented since the beginning of the 20th century. The historical accounts of LAIs, though relatively infrequent and the advent of modern biotechnology raised awareness about the hazards of infectious microorganisms and the risks these posed to laboratory workers who handle them and the community if they escaped from the laboratory.

There are three ways that will bring workers into contact with materials that may pose a biological risk. These are:

1. *exposure as a result of working with biological agents* – areas of work include a microbiology laboratory; greenhouse, animal house; activities include isolation, identification and culture of microorganisms or cells including materials used for genetic

modification, intentional contact with animals and plants and materials that originate from animals and plants as part of planned experimental work.

2. *exposure which does not result from the work itself but is incidental to it, mainly because biological agents are present as contaminants* - areas and activities include farming, refuse collection, sewage treatment, handling human body fluids and excreta; handling materials that may be contaminated by these materials such as hypodermic needles or sewage treatment plant.
3. *exposure which is not a result of the work that you do* – unintentional contact with animals and animal and plant materials or people in the workplace.

The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on the risk criteria/factors described below.

- *Pathogenicity of the agent or its product* - inherent risks of a pathogen are based on factors such as the severity of the disease it causes, its virulence and infectivity; 'disease' caused by agent's products include toxicity, allergenicity, physiological activity (e.g. anti-nutritional).
- *Mode of transmission and host range of the agent* – these are influenced by existing levels of immunity, density and movement of the host population, presence of appropriate vectors and standards of environmental hygiene.
- *Availability of effective preventive measures* - measures may include: prophylaxis by vaccination or antisera; sanitary measures, e.g., food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; and the importation of infected animals or animal products.
- *Availability of effective treatment* - includes passive immunization and post-exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of emergence of resistant strains.

Other considerations that maybe taken to account in classifying biological agents include

- *Origin/source* – indigenous (native, local) or exotic (foreign, alien); e.g. exotic agents posed higher risks to human health because it may cause more severe infection with no available treatment
- *Ability of the organism to survive* – dormancy or resting period; duration
- *Number/concentration of microorganism* – the higher the number the greater the possibility of infection
- *Nature and route of transmission* – inhalation (dust, aerosol), ingestion (food, drink, saliva), contact (cuts, bites, injection)

The *National Institute of Health, USA (NIH) Guidelines*[5] established a comparable classification of genetically modified hazard agents into a particular risk group using the same criteria indicated above. Many countries, including Bangladesh (*see Annex 1 of the Bangladesh Biosafety Guidelines 2006*), have adopted the WHO and NIH risk group classifications and criteria. The descriptions of the WHO and NIH risk groups are presented in Table 1 below.

Table 1. Classification of Infectious Microorganisms By Risk Group ***(Source: BMBL[6] 5th Edition 2007)***

Risk Group Classification	NIH Guidelines For Research Involving Recombinant DNA Molecules 2002	World Health Organization Laboratory Biosafety Manual 3rd Edition 2004
Risk Group I	Agents that are not associated with disease in healthy adult humans	(No or low individual and community risk). A microorganism that is unlikely to cause human disease or animal disease
Risk Group II	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited.
Risk Group III	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group IV	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

The four risk group classification of biological agents is widely recognized but disagreements exist in allocating agents to a particular risk group. WHO recommends to each country to draw up its own classification by risk group of the agents encountered in that country based on the criteria and other considerations enumerated in points 14 and 15.

Containment and Biosafety Levels

Containment refers to the ability to reduce or eliminate exposure of workers, other persons, and the outside environment to potentially hazardous agents. It is also used to

[5] NIH Guidelines For Research Involving Recombinant DNA Molecules (NIH Guidelines)

[6] Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services Public Health Service, Centers for Disease Control and Prevention

describe safe methods, facilities and equipment for managing infectious materials in the environment where they are being handled or maintained. For experimental plants and animals and microorganisms that are restricted within a chosen outdoor environmental zone of control, the term confinement has been used.

The fundamental elements of containment include the following:

- *Safe laboratory practices and techniques*- the most important element of containment; It refers to strict adherence to standard microbiological practices and techniques which require properly trained personnel, a biosafety or operations manual that identifies the hazards and specifies practices and procedures designed to minimize or eliminate exposures to the hazards.
- *Safety equipment (primary physical barrier or personal protective equipment)* – primary barrier includes biological safety cabinets (BSCs), enclosed containers (e.g. centrifuge cups), and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.
- *Facility design and construction (secondary physical barrier)* - provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. Special design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory.
- *Biological barriers* - *natural barriers* that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment; also include *design of facilities* and *special practices* for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location; used for experiments involving recombinant DNA technology to replace hazardous agents and decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the experimental area.

Various combinations of physical and/or biological barriers along with a constant use of standard practices were used to establish different levels of containment for organisms within laboratories (BSL1 through BSL4) and large scale uses (BL1-LS through BL4-LS), plants (BL1-P through BL4-P) and animals (BL1-N though BL4-N). In all cases, four biosafety levels were established, which provide increasing level of protection to personnel,

environment and the community. Categories of containment and biosafety levels are considered separately and detailed descriptions are found in the NIH Guidelines 2002, BMBL 2007 and Bangladesh Biosafety Guidelines 2006.

It is important to note that the risk groups correlate with but do not equate to biosafety levels. The risk group of an agent should be one factor, to be considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted. A summary of the characteristics of the four BSLs within laboratory is shown in Table 2 below.

Table 2. Summary of Recommended Biosafety Levels For Infectious Agents *(Source: BMBL 5th Edition 2007)*

BSL	Agents	Practices	Primary barriers and safety equipment	Facilities (Secondary barriers)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	• Agents associated with human disease • Routes of transmission include precutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infections materials. PPEs*: • Laboratory coasts; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents • PPEs: • Protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

*PPE = Personal Protective Equipment

Good Laboratory Practice (GLP)

Effective containment and many testing procedures are based on sound laboratory management practices. Many guidance documents refer to these practices in general terms as good laboratory practice ("lower case glp") and more specifically as GLP ("upper case GLP"). The former refers to a set of standards used to accredit testing and calibration laboratories (e.g. ISO/IEC 17025). The latter refers to the OECD Principles of Good Laboratory Practice[7], which sets the standards for specific tests studies. Some countries issue their own versions of the GLP Principles based on the OECD Principles of GLP, incorporated as part of national legislations.

[7] Organization for Economic Co-operation and Development; published by the OECD's Environment Directorate, and most recently revised in 1998

The OECD Principles of GLP describe a "quality system concerned with the *organizational process* and the conditions under which *non-clinical studies* are planned, performed, recorded, archived and reported" (OECD definition). It is concerned with assurance of data quality (sufficient, rigorous, reproducible) rather than the technical validity of the studies undertaken.

Data generated under GLP are designed for product registration, mutual acceptance of data among OECD member countries, and to contribute to protection of human health and the environment. Non-clinical studies include physico-chemical testing, toxicity, mutagenicity, environmental toxicity, bioaccumulation and residue studies; studies of effect on mesocosms[8] and ecosystems, and the analytical chemistry associated with such studies. Test items include synthetic chemicals, items biological in origin and living organisms. A study covers work done in a laboratory, in animal houses, in greenhouses, and in the field.

The GLP Principles prescribes a set of guidelines for the following: test facility organization and personnel, quality assurance program, facilities, apparatus, material, and reagents, test systems, test and reference items, Standard Operating Procedures (SOPs), performance of the study, reporting of study results, storage and retention of records and materials. The elements unique to GLP are as follows: Study Director (and any Principal Investigators), Quality Assurance unit, Standard Operating Procedures (SOPs), Study plans (protocols) and reports and Data archive.

GLP compliance monitoring is required for mutual acceptance of data. Periodic inspection of test facilities and/or auditing of studies are conducted for the purpose of verifying adherence to GLP principles. Compliance and monitoring are conducted by international, regional or national accreditation bodies e.g International Laboratory Accreditation Cooperation (ILAC), Asia Pacific Laboratory Accreditation Cooperation (APLAC), Australia's National Association of Testing Authorities (NATA). Different countries may require different proofs of compliance with regard to GLP requirements.

Risk Analysis[9]: Concepts and Issues

In this document, risk analysis is used in its broadest sense as an integrated process consisting of three major components: **risk assessment, risk management and risk communication.** The individual components are distinct, but are linked to achieve a well-functioning risk analysis process that forms the basis for decision making on any operation or

[8] An experimental system that simulates real-life conditions as closely as possible. For example, a sediment sample contained in an opened vessel and placed on a riverbed or in a flow-through system in a laboratory (not temperature controlled as it should simulate outdoor temperatures). Environment Canada St Lawrence Glossary URL: http://www.qc.ec.gc.ca/csl/glo/glo006_e.html

[9] Information on this section were abstracted from the following references: Bangladesh NBF 2007 and Biosafety Guidelines 2007; Australia Risk Analysis Framework, 2005; FAO/WHO, 1997 and Codex Alimentarius Commission, 2003; Cartagena Protocol on Biosafety 2000; PRRI Guide For Notifications And Risk Assessments For Releases Into The Environment Of Genetically Modified Organisms Module 1: Genetically Modified Crop Plants URL: http://www.pubresreg.org

dealing of GMOs. The use of risk analysis in this manner generally conforms to Bangladesh's proposed NBF and Biosafaty Guidelines, and other national and international principles and guidelines to protect human health and the environment from risks posed by or as a result of modern biotechnology.

The terminologies associated with risk analysis in this section are:

Risk - likelihood that under particular conditions of exposure an intrinsic hazard will represent a threat or harm; risk is a function of hazard and exposure.

Risk = hazard x exposure

Hazard – intrinsic (it is there or it is not) *potential* of a material to cause harm to human health and/or the environment; also synonymous to threat

Exposure – the extent and the duration of or the frequency with which the operator is exposed to the hazard

Likelihood – probability of something happening

Consequence – adverse effects or outcome caused by an event

Stakeholders – individual, groups and institutions who may affect, be affected by, or perceive themselves to be affected by the decision, activity or risk; synonymous with 'interested parties'

Components of Risk Analysis

Risk assessment is the first and the *scientific component* of risk analysis. It is a rigorous science-driven process used to identify a hazard and obtain qualitative or quantitative estimate of the levels of risk posed by a hazard including possible adverse effects to human health and the environment. It typically consists of four steps: (1) hazard analysis (identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (5) risk estimation. A more detailed discussion of risk assessment is presented in Section and some examples for GM crops are presented in Section 5.

Risk management is the second and *decision-making component* of the process of risk analysis. It is primarily supported by risk assessment but is informed by other risk considerations. Risk management is concerned with evaluating whether the risks identified by the risk assessment process are acceptable and manageable, then selecting and implementing the control measures as appropriate to ensure that risks are minimized or controlled. A more detailed discussion on the methodology of risk management and other considerations is presented in Section 6. A more thorough treatment of the risk management for various uses of GMO and the post-monitoring aspects are presented in a separate Lecture Module.

Risk communications is recognized as the third component that underpins the risk assessment and risk management processes. It is the process of exchange of information and opinions concerning risk and risk-related factors among various stakeholders concerned with risk (Codex definition). It strengthens the over-all process of risk analysis by helping to

define the issues and providing the link and the feedback mechanism that informs the two processes. The principles, structure and process of risk communication presented in Section 7.

Risk analysis applied in the broad sense separates the risk assessment from risk management. The reasons are: to maintain the scientific integrity of the risk assessment, to avoid confusion over the functions to be performed by risk assessors and risk managers, and to minimize any conflict of interest. In practice, however, this separation is rarely clear-cut and variation in its implementation exists among countries and across regulatory institutions.

Principles of risk analysis: general aspects

While regulatory frameworks for risk analysis vary among countries, the underlying general principles in assessing safety from risks posed by GMOs to human health and the environment share many similarities. These include:

Science-based – Risk should be assessed using information obtained through application of science and the scientific method i.e. rigorous and systematic, reproducible, with testable null hypothesis, qualitative and/or quantitative. Methods used should be appropriate and data generated of high quality to withstand scientific scrutiny and peer review.

Open, transparent and documented – All aspects of the process of risk analysis should be documented fully in a transparent manner. Documentation should be accessible to all interested parties, while respecting legitimate concerns to preserve confidentiality. This principle also refers to the selection of experts who will conduct the risk assessment. Experts responsible for risk assessment should be selected on the basis of their expertise, experience, and their independence with regard to the interests involved.

Case-by-case -Risk should be assessed on a case-by-case basis. This means that for each case, the risk assessment methodology and required information may vary in nature and level of detail, depending GMO concerned, its intended use (e.g. laboratory, field , market) and the likely potential receiving environment (e.g. presence of wild relatives, non-target species, endangered species.

Comparative - Risks should be compared to background risks i.e. risks is considered in the context of the risks posed by the non-modified recipients or parental organisms within the context of the intended use. This requires appropriate comparator and well-established baseline information.

Systematic - The risk analysis should follow a structured, step-by-step approach. The key steps are: establish the purpose, scope and boundaries of the risk assessment, assess the risk, and manage the risks.

Iterative - Risks should be evaluated and reviewed as appropriate in the light of newly generated scientific data. Conclusions and assumptions should be examined relative to new information.

Inclusive – The process of risk analysis should be all-encompassing. The three components of risk analysis should be applied within an overarching framework for management of food related risks to human health and the environment. It should draw information from a wide range of credible sources and could also take into account expert advice of, and guidelines developed by, relevant international organizations. Effective communication and consultation with all interested parties should be ensured in all aspect and stages of the process of risk analysis.

The Methodology of Risk Assessment and Risk Management: Key Steps

General guidance on the methodology of risk assessment and risk management exists and they share many similarities. Annex III 8 of the Cartagena Protocol on Biosafety is a good guide and the steps typically followed are enumerated below.

1. *Hazard analysis* - An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;
2. *Likelihood estimation* - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;
3. *Consequence evaluation* - An evaluation of the consequences should these adverse effects be realized;
4. *Risk estimation* - An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;
5. *Risk management* – A recommendation as to whether or not the overall risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks, including monitoring.

It should be noted that the level of details and sequence of some of the steps indicated above also vary across countries. More detailed discussion of the methodology of risk assessment and risk management are presented in succeeding sections. (see Section 4-6)

Concepts and Issues in Risk Analysis

There are a number of concepts and issues that are very important in gaining a better understanding of the process of risk analysis. These include:

Concept of Familiarity

Risk assessment of GMOs requires information on the identity, characteristics and history of safe use of the organism that is subjected to genetic modification. Most genetically

modified organisms to date have been developed from organisms that are "familiar" i.e. there is sufficient available information about the organism's attributes, and long history and experience of its safe use.

The concept of familiarity provides a way to recognize the potential risks by using already available information on the attributes of the organism. Because of familiarity, effective methods can de devised to avoid or manage the risks to acceptable levels. For example, it will be possible to determine the potential for invasiveness of the crop based on knowledge of the biology (e.g. presence of traits that are associated with invasiveness) and the presence of wild compatible relatives. Likewise, it will be possible to identify the potential allergenicity of the GMO if knowledge and history of safe use of the origin/source of the gene used in genetic modification is available. In this context, the concept of familiarity is not a risk assessment by itself but a useful tool for identifying, evaluating and managing risks.

Concept of Substantial Equivalence

In assessing the risks posed by GMOs to human health and the environment, the concept of familiarity is used together with the concept of substantial equivalence. Substantial equivalence is based on the principle that GMOs can be compared with their conventional counterparts[10] that have an established history of safe use. The concept is used to identify the similarities and differences (includes intended changes and unintended changes)[11] between the GMO and its conventional counterpart to be able to determine if the GMO is 'as-safe-as' or presents any new or greater risks than its conventional counterpart. The concept of substantial equivalence does not establish absolute level of safety, but relative level of safety.

Internationally, the concept of substantial equivalence is recognized as one of the principles for environmental risk assessment by the Cartagena Protocol on Biosafety, and in food safety assessment by the Codex Alimentarius Commission. The relevant texts (italics provided) are as follows:

Cartagena Protocol on Biosafety (2000)

Annex III 5 –Risk Assessment

Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern

[10] 'Conventional counterpart' means a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food; (Codex Alimentarius Commission, 2003); synonymous with 'traditional counterpart' or' non-transformed counterpart'; 'non-modified recipient or parental organisms'

[11] Intended changes refer to the inserted genes and their related substances and traits; Unintended changes –refer to the pre-differences in pre-determined parameters between the GM plant and its appropriate non-GM comparator(s) e.g. changes in "phenotype" - yield, plant morphology, flowering time, day degrees to maturity, duration of pollen viability, response to plant pathogens and insect pests, sensitivity to abiotic stress

biotechnology, *should be considered in the context of the risks posed by the non-modified recipients or parental organisms* in the likely potential receiving environment.

Codex Alimentarius Commission Principles and Guidelines on Foods Derived from Biotechnology (2003)

Section 3.10 –Principles

Risk assessment includes a safety assessment,…The safety assessment *should include a comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences.* If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

As an approach, it should be noted that the concept of substantial equivalence is considered a *starting* point for the safety assessment to structure the safety assessment procedure, and focus on the identified differences that may require further testing. Its application is limited by the choice of an appropriate comparator and availability of sufficient scientific information relevant to the risk assessment. These points are illustrated in the three cases presented below.

1. *GMOs that are shown to be substantially equivalent to the conventional counterparts* are regarded as being 'as safe as' their counterpart. No further safety considerations other than those for the counterpart are necessary.
2. *GMOs that are substantially equivalent to the conventional counterpart except for defined differences* need further safety assessment which should focus only on the defined differences. Typically, the defined differences will result from the intended effect of the genetic modification that may, or may not, change the endogenous traits, or produce new traits in the host organism.
3. *GMOs that are not substantially equivalent to the conventional counterpart***.** Up to now and probably for the near future, there have been few examples of these GMOs. Nevertheless, it is conceivable that with future developments in biotechnology, these kinds of GMOs will be produced. In these cases, the concept of substantial equivalency cannot be applied.

As a final note, in addition to the limitations mentioned above, the use of the concept of substantial equivalence in risk assessment has been criticized as subjective, inconsistent and pseudo-scientific (Millstone et al). However, despite its limitations and criticisms, there is wide recognition that the concept of substantial equivalence remains to be the most practical approach currently available to framing the risk assessment process.

The Precautionary Approach

Principle 15 of the Rio Declaration on Environment and Development (UNCED, Rio de Janeiro, June 1992)

"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

There are a number of important points to keep in mind about Principle 15 of the Rio Declaration in conducting risk analysis.

1. The term 'precautionary approach' is specifically used to differentiate it from the legal connotation of the term 'precautionary principle'. The latter is compulsory or legally binding while the former maybe binding in some cases but normally does not have the same force as a law (Recuerda, 2008). Because it is an 'approach' and not a 'principle', Principle 15 allows for discrimination between countries in applying the approach based on their capability, which a law or principle will not allow. Furthermore, Principle 15 allows other costs (e.g. social or economic) to be considered in order to be cost-effective in applying the approach. In view of these, the 'precautionary approach' is viewed as softening of the 'precautionary principle' (Garcia, 1995).
2. The precautionary principle in the context of Principle 15 explains the idea that scientific uncertainty (i.e. source or form of doubt) should not prohibit using preventive measures to protect the environment; and use of "cost-effective" measures indicates that costs can be considered when applying the approach.
3. Principle 15 identifies the triggers to propose a precautionary approach: 1) a scientifically sound identified threat of damage and 2) there is scientific uncertainty about the extent of the potential adverse effects (PRRI, www.pubresreg.org)
4. Finally, Principle 15 refers to potentially irreversible harm to be the most important application of the precautionary approach. Where risk are irreversible, decision makers will act from the perspectives of prudence and precaution.

Many countries have adopted the same phrasing of Principle 15 of the Rio Declaration in their regulatory systems and have established risk assessment mechanisms based on the precautionary approach. The interpretation and implementation of the precautionary approach vary across countries because they differ in their opinions on thresholds of risk and degree of scientific uncertainty allowed in the process of risk analysis. Many regulatory approaches recognize the imperfect nature of evidence when making decisions. In conformity with the precautionary approach, preventive measures are built in their risk management approaches to allow certain activities with limitations when appropriate.

It is disconcerting to note that over the last years, there seems to be a tendency to interpret the precautionary approach in the excessively conservative way as the precautionary principle i.e. irrespective of possible benefits, a new technology should never be introduced unless there is guarantee that no risk will arise. As Nuffield Council of Ethics (2004) observed, such interpretation is impractical and invokes the fallacy of thinking that the option of doing nothing is itself without risk. In addition to foregone benefit, the members of the

Public Research and Regulation Initiative (PRRI) are concerned that any question about GMO is deemed sufficient to stop research in this field regardless whether any risks (let alone threats of serious or irreversible damage) have been identified (PRRI, www.pubresreg.org).

Uncertainty

Uncertainty is an inherent property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communications (Hayes, 2004). Simply defined, uncertainty is a form or source of doubt. There are five different types of uncertainty that can be applied to risk analysis, which is enumerated below *(Please refer to Appendix D of the Risk Analysis Framework (2005) for more detailed explanation).*

- **epistemic** - uncertainty of knowledge, its acquisition and validation. The most common examples are statistical errors, use of surrogate data (e.g. extrapolation from animal models to humans), incomplete or ambiguous, contested data or unreliable data. Epistemic uncertainty could be reduced by designing more rigorous experiments, applying more powerful statistical analyses and GLP.
- **descriptive** - uncertainty of descriptions that may be in the form of words (linguistic uncertainty), models, figures, pictures or symbols (such as those used in formal logic, geometry and mathematics). Usually associated with qualitative measurements; inconsistent and incomplete definition and application of words. For example the word 'low' may be ambiguously applied to likelihood of harm, magnitude of a harmful outcome and to the overall estimate of risk. Descriptive uncertainty could be reduced by using accurate and consistent definitions and providing clear parameters, scope and boundaries.
- **cognitive** (including bias, perception and sensory uncertainty) Cognitive unreliability can be viewed as guesswork, speculation, wishful thinking, arbitrariness, date, or changeability. One way to reduce cognitive uncertainty is through effective communication strategies.
- **entropic (complexity)** - uncertainty that is associated with the complex nature of dynamic systems such as a cell, an organism, the ecosystem, or physical systems (e.g. the weather). Complexity and incomplete knowledge contribute to inability to establish the complete causal pathway in the system. Consequently, a deterministic system can have unpredictable outcomes because the initial conditions cannot be perfectly specified. Complexity could be reduced by generating more information about the various components and relationships in the system.
- **intrinsic** - uncertainty that expresses the inherent randomness, variability or indeterminacy of a thing, quality or process. Randomness can arise for example from genetic difference. A critical feature of intrinsic **uncertainty** is that it cannot be reduced

by more effort such as more data or more accurate data. In risk management, safety factors and other protective measures are used to cover this type of uncertainty.

The following are examples of uncertainty within the elements of risk analysis (RAF, 2005)

Risk assessment

- uncertainty in the nature of the GMO, such as the lack of knowledge of biochemical properties of the introduced genes, environment- specific performance of the GMO, its interaction with other biological entities and processes, or landscape changes over long time periods;
- uncertainty of the calculations within the risk assessment process, including assessment of hazards, likelihood and consequences;
- uncertainty in descriptions used in qualitative risk assessments due to insufficient explanations of terminology, use of related terms that are not fully congruent or the use of the same term in different contexts.

Risk management

- balancing the sufficiency of protective measures against their effectiveness;
- decision making in the presence of incomplete knowledge and conflicting values.

Risk communication

- uncertainty of communication effectiveness due to difference in knowledge, language, culture, traditions, morals, values and beliefs.

There are a number of ways to address uncertainty in risk analysis of GMOs

1. Request further information on the specific issues of concern. Where there is uncertainty more experiments can be required in order to answer the question. However, it must be recognized that the effort and resources required to acquire greater knowledge increases exponentially with each demand for greater precision or detail. In many instances, these may not be technically (e.g. no valid protocol) or practically (e.g. unaffordable cost) possible.
2. Implement appropriate risk management strategies and/or monitoring the GMO in the receiving environment.
3. In cases where further experimentation may not provide the necessary information, the 'worst case' scenario approach can be applied where the focus is less on determining the likelihood of an occurrence, but rather evaluating what the consequences of the occurrence would be.

Food safety aspects vs environmental aspects

The concepts and issues in risk analysis discussed above apply to risks related to food safety and risk related to the environment. Toxicity and allergenicity are key issues to risks in food safety and risks to human health safety as component of the environment. There are differences between them and it is important to draw these distinctions more clearly to avoid

confusion particularly when addressing the language of the CPB where it mentions "'taking also into account human health". The PRRI Guide (www.pubresreg.org) summarizes these distinctions follows

1. In evaluating the risks of toxicity and allergenicity, here is a difference between looking at toxicity in terms of food safety, where it is assumed that large quantities may be consumed frequently (i.e. scenarios in which even low levels of toxicity may have a consequence) and toxicity in the context of environmental safety, where the focus is on effects of minor consumption (e.g. GMO accidentally eaten during field trial).
2. In evaluating the risks of toxicity and allergenicity as a consequence of exposure, the type of application is taken into account in the case of environmental safety but not in food safety. For example, for small scale field trials, in which the material resulting from the field trial is not consumed by humans or animals, toxicity and allergenicity would generally be of no consequence. For large-scale and market releases, toxicity and allergenicity would be of consequence and therefore needs to be addressed and usually the results of toxicity and allergenicity assessments are included in risk assessment.
4. In looking at toxicity as a result of genetic modification, two aspects need to be distinguished: possible toxicity of the gene product, and, in the specific case of food safety, possible insertion effects that may cause changes in pathways in the plant, including pathways that are related to toxicity. Although the latter case can be compared with the normal effects of genomic rearrangements that happen during plant breeding, it is practice that any such insertion effects, that are applicable to the specific event only, and bear no relation to the transgene, be checked in a food safety assessment, before the crop is placed on the market.

The Process of Risk Analysis: Risk Assessment

Risk assessment is the primary and the *scientific* component of risk analysis. It is a science-driven process of identifying the potential hazards and obtaining qualitative and quantitative estimates of risk by assessing its two components, likelihood and magnitude of any adverse outcome that may arise including the degree of uncertainties in those estimates. Risk assessment can also be described as answering the following questions: *What are the hazards? What are the chances (likelihood) that hazard will occur? What are the potential adverse effects (consequences) if the hazard does occur?* (RAF, 2005)

Risk assessment is a scientific process conducted by experts. It focuses on asking empirical questions about potential risks. It gives more weight to evidence derived from experimental data of testable risks with well formulated hypotheses. It takes care of insufficient information and scientific uncertainty by asking for more evidence, developing better analytical methods and making provisional decisions based on prudence and precaution.

Risk assessment does not deal with speculations. It is not concerned with answering questions on economic, ethical and cultural impacts. These are addressed in the risk management component (see Section 6) of the risk assessment process.

Risk assessment typically consists of four steps: (1) hazard analysis (identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (5) risk estimation described below.

The Methodology of Risk Assessment

General guidelines and many papers exist on the methodology for assessing risks to people and environment[12]. In this section, Annex III 8 (a-d) of the Cartagena Protocol on Biosafey (2000) is used as guide to enumerate the steps typically followed in risk assessment whether for food or environment. The additional information to help explain each step was abstracted primarily from the Risk Analysis Framework (2005) and PRRI draft guide for risk assessment (www.pubresreg.org).

(1) Hazard analysis (identification and characterization) - An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health (CPB, Annex III 8 (a))

Hazard identification establishes the intrinsic or 'built-in' potential of the biological agent (e.g. GMO or GM foods) to cause harm. Hazard characterization aims to evaluate in qualitative and quantitative terms the nature of the identified intrinsic hazard. Quantitative and qualitative techniques are used in hazard identification (Hayes et al, 2004). Qualitative techniques include checklist, brainstorming, expert consultation, fault and event trees. Quantitative techniques include HAZOP analysis, hierarchical holographic model (HHM), SWOT analysis, Dephi analysis, etc. Approaches to hazard analysis may be inductive (top down) or deductive (bottom up). Checklist and the inductive approach appear to be the status quo of hazard analysis. Evidentiary support could range from unsubstantiated statements (weak evidence) to experimental data (strong evidence).

Hazard analysis also involves establishing the causal link and pathway or route of exposure between hazard and an adverse outcome. It also involves identifying the measurable properties of the hazard in order to accurately assess that harm has occurred (Table 3).

(2) Likelihood estimation- An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism (CPB, Annex III 8 (b))

Likelihood is the probability that the harm will occur. It is expressed as relative measure of frequency (the number of occurrences per unit time) and probability (from zero to one,

[12] As of May 2008, a total of 155 citations on risk assessment and risk management is available through the Biosafety Information Resources Centre of the Biosafety Clearing House

where zero is an impossible outcome and one is a certain outcome). It is important to remember that likelihood estimation is a predictive process. The accuracy of prediction is directly proportional to time of occurrence i.e. short term outcome is more accurately assessed than long term outcome.

Table 3. Examples of potential harm identified and their measurable properties

Hazard	Measurement Attributes
Reduced fitness, increased persistence, invasion	Occurrence and biological properties – traits for weediness and invasiveness;
Toxicity to non-target organisms	Mortality; survival; population morbidity, species richness
Habitat modification- altered bio/geo-chemical cycles	Carbon, nitrogen, phosphorus flux; frequency of floods, fire; pollutant concentration
Loss of biodiversity and extinction of species	Diversity indices; species richness;
Creation of new viruses	Occurrence, number. severity, host range
Human toxicity and allergenicity	Biological, physiological and physical abnormalities; mortality; frequency and age of morticity

Here the term 'estimation' is chosen, because exact numbers of the frequency with which something will happen in nature cannot always be measured. It is possible in certain risk calculations such as non-target risks but more frequently the risk finding is qualitative on the basis of a weight-of-evidence analysis.

Likelihood assessment maybe qualitatively described as follows:

- *Highly likely* - is expected to occur in most circumstances
- *Likely* - could occur in many circumstances
- *Unlikely (Negligible or Effectively zero)* - could occur in some circumstances
- *Highly unlikely* - may occur only in very rare circumstances

For GMOs, the most important factors that contribute to the likelihood that harm will occur are the survival, reproduction, persistence of GMO; and the receiving environment, including its biotic and abiotic attributes.

(3) Consequence evaluation- An evaluation of the consequences should these adverse effects be realized (CPB, Annex III 8 (c))

Consequence evaluation involves characterizing the significance of the adverse outcome if the hazard occurs. The following criteria should be taken into consideration:

- Severity – number, magnitude, scale
- Spatial extent – geographical (local, national, global); organism (individual, population, community, ecosystem)
- Temporal extent – duration and frequency
- Cumulative and Reversibility
- Background risk – risk that may occur in the absence of the stressor (e.g. GMO)

Descriptors of consequence assessment

- *Marginal* - Minimal or no injury except to a few individuals that may require first aid; Minimal or no degradation of the environment
- *Minor* - Slight injury of some people that may require medical treatment; Disruption to biological communities that is reversible and limited in time and space or number of individuals/populations affected
- *Intermediate* - Injury to some people that requires significant medical treatment; Disruption to biological communities that is widespread but reversible or of limited severity
- *Major* - Severe injury to some people that may require hospitalization or may result in death**;** Extensive biological and physical disruption of whole ecosystems, communities or an entire species that persists over time or is not readily reversible.

(4) Risk estimation - An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized (CPB, Annex III 8 (d))

Risk estimation combines the information on likelihood and consequence of the identified hazard to come up with the risk estimate matrix shown below. As a general rule, risks with moderate and high estimates will invoke the corresponding risk management treatments or control measures.

Descriptors of Risk Estimate

- Negligible - risk is insubstantial and there is no present need to invoke actions for mitigation
- Low - risk is minimal, but may invoke actions for mitigation beyond normal practices
- Moderate - risk is of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective
- High - risk is unacceptable unless actions for mitigation are highly feasible and effective

Finally, in conducting the steps outlined above, the characteristics of the following, depending on the dealing of GMO, could be taken into consideration::

- Recipient, host or parental organisms.
- Inserted genes, sequences and related information about the donor(s) and the transformation system
- The resulting GMO,
- Detection and identification of the GMO
- The intended use (e.g. the scale of the activity - field trial or commercial use)
- The receiving environment.

Information requirement for risk assessment

Risk assessment for the release of GMOs typically takes into consideration the points enumerated above obtained from Annex 9 of the Cartagena Protocol on Biosafety. A more detailed discussion of the various points is presented below. These information were abstracted from the paper of Konig *et al.* (2004) and PRRI Guide of Risk Assessment (www.pubresreg.org)

1. Information on the recipient or parent organism

The type of information on the parent crop that should be gathered at the outset include

a. *identity , phenotypic and agronomic performance* – taxonomic identity (including complete name, family name, genus, species, subspecies, cultivar/ breed/race/isolate, common name, sexually compatible wild relatives); chemical proximate composition and key nutrients and anti-nutrients

b. *geographical distribution/source or origin* – area of cultivation, center of origin and centers of diversity

c. *history of safe use* – any known nutritional, anti nutritional, toxicological, allergenic characteristics or intolerance; importance in the diet including information on preparation, processing, cooking

d. *compositional analysis* – key nutrients, toxins, allergens, anti nutrients, biologically active substances associated with parent and sexually compatible relatives; information both from the literature and from analytical data

The recipient or parent organism refers to the organism into which the genes are introduced through genetic modification methods. The characteristics of the recipient organism guide the choice of test parameters for comparison of the GMO with its non-modified counterpart i.e. it serves as the reference point. Knowledge of the natural variation of the traits in the recipient is essential in interpreting data when comparing the GMO to its non-modified counterpart under different receiving environment. The history of safe use of the parent can provide additional information to help plan the risk assessment strategy e.g. identifying what should be the focus of further assessment

The OECD has been compiling consensus documents on the (1) biological attributes and (2) compositional characteristics for certain crops species. These documents provide excellent sources of relevant information of the parent or recipient crop. Information from these OECD consensus documents have been accepted by biosafety regulatory authorities in some countries.

2. Information on the inserted genes and sequences and related information about the donor(s) and the transformation system.

The information required includes:

a. *Description of donor (s)* – includes classification and taxonomy, evidence of potential toxicity, allergenicity or pathogenicity, history of use and exposure to the donor; where possible, function of any recombinant DNA sequences used in the transformation study

b. *Description of vector DNA* – includes information on source of all genetic elements used to construct and amplify the vector, functions of all genetic elements including coding sequences, promoters and termination signals, vector map with relevant restriction site; proof of absence of vector fragments not intended to be transferred, nucleotide sequence information

c. *Transgene delivery process* – For *Agrobacterium*-mediated transformation the information requirement includes donor strain and any plasmid contained in that strain; for direct transformation method, such as particle gun include absence of contaminating sequences of bacterial chromosomal DNA or other plasmid DNA, vector sequences.

d. *Characterization of introduced DNA* – includes information on number of insertion sites, copy number of the introduced DNA, ends of inserts adjacent to plant genomic DNA; genomic library of each transformed plant line (under discussion), absence of vector backbone; stability of transgene insertion verified over five or more generations

e. *Characterization of insertion site* – information on the junction of the inserted recombinant DNA and the plant genome,

With regard to transformation method, it has been argued that in using *Agrobacterium*, the risk of transfer of random DNA to the plants is relatively small (Gelvin, 2000); Hellens and Mullineux, 2000). The vector with the recombinant DNA is separate from the vector with transfer function and has a recognition site for the transfer-mediating gene products.

With regard to characteristics of the introduced DNA, all inserted *functional genes* are, in principle, relevant to the risk assessment, regardless of whether they are the 'genes of interest' or genes that have 'traveled along' in the process, such as selectable markers. The underlying reason is the possibility of unintended effects due to the presence of the DNA sequence. For example, a gene with a prokaryotic origin of replication (*ori*) will not be expressed in a plant cell but will also be considered in the risk assessment because it may facilitate replication of genes in the – unlikely – event that they are taken up and recovered in a replicable form by a bacterium. *Oris* are abundant in the bacteria found in the digestive tract of humans and animals, for example, and in bacteria present on plants and animals.

Finally, the level of detail required should depend on the nature of the dealing. For example, in the early stages of research and development of the GM product, full molecular characterization are not yet conducted so it can be assumed that the entire construct may have been integrated into the recipient plant. Hence, the risk assessment is conducted on that basis

and risk is managed by strict containment measures (see Section 2). When the activity has moved to confined field trials, more detailed characterization is requested, leading to a full characterization as a required for large scale field trial or commercial/market release. This is all part of the 'case-by-case' and 'step by step' approach of risk analysis.

3. Information on the gene products; recombinant protein and/or metabolites

With certain exceptions like anti-sense DNA, all inserted functional genes transferred to the recipient organism are translated into primary (protein) and secondary (metabolites) gene products. Hence, both are relevant to the risk assessment process. The information required for the gene products are:

a. *Structure, identity and characterization* – includes molecular weight, amino acid sequence, post-translational modification (e.g. level of glycosylation and phosphorylation), immuno-equivalence, activity and specificity of reaction (if gene product is an enzyme), expression levels (recombinant proteins in various plant tissues; changes in levels of inherent crop micro- or macro- nutrients (e.g. Vit A in Golden rice), significant unexpected changes in the levels of substances detected during compositional analysis)

b. *Mode of action/specificity* - mechanism of action (e.g. Bt class of proteins which are toxic to insect but not humans), overview of all relevant metabolic pathways that could be affected by the enzymes' presence or altered levels or substance specificity (e.g. CP4 EPSPS enzyme that confers tolerance to herbicide glyphosate but not affect the biosynthesis of the specific aromatic amino acids of all plants and microorganisms)

c. *Toxicity* – information on documented exposure and history of safe use; results of previous toxicity testing programs; for novel protein/metabolite, information on structure and function and toxicity tests are required;

d. *Allergenicity* – changes in the characteristics or levels of expression of endogenous allergenic proteins

Toxicity and allergenicity of the gene products are the primary concerns and focus of risk assessment particulary for GMOs that will be used as food/feed. From the perspective of food/feed safety, it is widely recognized that proteins are not generally toxic when consumed orally as it is largely part of human and animal diet. However, almost all allergens are protein. With regard to toxicity, safety concerns and the amount of new data that will be required should be carefully considered in the light of existing information on the protein/metabolite prevalence, similarity to proteins/metabolites that are routinely used by humans and animals, and history of exposure. Safety concerns and new data requirement should be lower in the case of proteins that have no history of adverse effects to humans and animals. With regard to allergenicity, the amount of new data required should take into account the following key considerations: (a) Is the recombinant protein derived from an allergenic source or known

allergen; able to induce de novo sensitization; cross-reactive with IgE antibodies raised by known allergens?; (b) Has transformation altered the allergenic properties of the product derived from the GMO?.

4. Information on the resulting GMO

Information requirement for the resulting GMO includes: (1) Identity, phenotypic and agronomic analysis; (2) Compositional analysis and (3) Safety analysis (animal studies). The information from these analyses is obtained in comparison with the non-Gm counterpart. These analyses focus on detecting any indicative differences in test parameters such as agronomic performance, compositional and nutritional values, and dietary sub-chronic response in animal feeding studies.

Sources of data to enable detailed comparison can come from a variety of sources. Data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Field trials are usually undertaken under a diversity of environmental conditions representative of those typical for planned commercial growing. Another major source of data are databases on existing food composition, chemical analyses, toxicology tests. Data can also be obtained from the Biosafety Clearing House for information on field and commercial releases of the same GMOs in various locations.

Detection and identification methods are important in hazard identification, characterization. In various stages of research, development and release of a GMO, molecular characterization and toxicological tests are conducted to generate information on the characteristics of the inserted DNA sequences, the gene products, and the resulting GMO. This means that detection, identification and test methods focusing on the inserted DNA, the resulting proteins and the resulting GMO are crucial.

Examples of currently available DNA based studies widely used include

- Southern blot
- Qualitative PCR
- Quantitative end-point PCR

Protein based testing methods include:

- Western blot
- ELISA
- Lateral flow strip
- Magnetic particles
- Protein chips

Toxicology test methods include

- in vivo and in vitro test systems
- chronic toxicity, carcinogenicity and reproduction studies
- acute animal toxicity studies

Each of these methods has its own advantages and disadvantages in terms of targets, ease of use, specificity, sensitivity, costs, etc. Existing methods have proved to be adequate for the safety of the GMOs that are currently available in the market. Development in the area of detection and testing are being pursued to improve existing techniques and address safety of next generation products of modern biotechnology.

Note: For more detailed discussion on DNA detection techniques, please refer to Lecture Module: Agricultural Biotechnology.

5. Information relating to the intended use

In this document, intended use refers to the definition of 'Deal with GMOs' adopted from the Bangladesh National Biosafety Framework (Chapter 3.5.3). Deal or intended use encompasses a wide range of activities and application. These include: (a) conduct experiments with GMOs; (b) make, develop, produce or manufacture GMOs; (c) breed GMOs; (d) propagate GMOs; (e) use GMOs in the course of development or manufacture of a thing that is not GMOs; and (f) grow, raise or culture GMOs.

These activities and application can be classified also into two categories: (1) 'contained use'; and (2) 'release to the environment'. "Contained use" means any operation undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment (*CPB definition*).

'Release into the environment', in this document, refers to non-contained use activities with GMOs. In many regulatory systems, this means any trial conducted in the field irrespective of scale and availability of confinement measures and commercial release (e.g. seed production). The major distinction between commercial release and field trials is that with field trials, the GMO involved is still under various degrees of control, whereas after placing GMO on the market for commercial production, its use is in principle unrestricted except for specific product-use conditions, such as labeling or monitoring.

6. Receiving environment

The characteristics of the receiving environment are crucial for the risk assessment. For field trials, the information requirement includes the specific physical location of the trial taking into consideration the following relevant characteristics:

- comparison between the normal growing environment with proposed environment for release
- specific environmental factors influencing survival and distribution (e.g. climate, soil conditions)
- presence of sexually compatible crops
- presence of sexually compatible wild relatives

Environmental Risk Assessment of GM Crops: Some Examples

Potential Environmental Risks of GM Crops

GM crops and foods derived from them are the most widely used GMO in commercial use. Both have provoked fierce debates because of concerns on the potential risk they pose to human health and the environment. Many papers and reviews[13] have been written on the potential risks of GM crops and GM foods. In general, the public are primarily concerned with the possible health effects of consuming GM foods, but are also concerned about non-risk issues such as ethics of genetic modification and labeling of foods with GM ingredients.

Issues of concern to the environment include: gene flow risks i.e. reduction in ecological fitness, invasion; persistence; loss of genetic diversity; loss of biodiversity; creation of new viruses; toxicity to non-target organisms; increased use of chemicals in agriculture; food web-modification; altered farming practices; habitat modification etc. The potential risk issues posed by GM crops vary depending on the local conditions.

Some of the currently debated potential environmental risks of GM crops are discussed in the PRRI guide (www.pubresreg.org) and outlined below.

(1) ***Invasiveness /Weediness*****:** Can the inserted gene/sequence cause changes in the weedy characteristics of the recipient plant, i.e. can the recipient – due to the genetic modification - become more persistent in agricultural habitats or more invasive in natural habitat? This could be the case when the inserted gene or sequences confer a selective advantage or changes in fitness or dispersal. Weediness of a plant depends on many different characteristics, such as persistence, outcrossing, dispersal, etc. and other factors such as the receiving environment and its climate.

(2) ***Effects on non-target organisms***. Can the inserted gene/sequence cause adverse effects on populations of non-target organisms, for example by indirect effects on population level of other insects than the target insect or, where applicable, predators, competitors, herbivores, pollinators, symbionts, parasites and pathogens?

(3) ***Unintended effects on the target organisms***: Can the inserted gene/sequence cause unintended adverse effects on the target organisms, such as resistance development? Resistance development is not an adverse effect in itself, unless it impairs other types of treatments such as spraying with microbial pesticides.

(4) ***Toxicity****:* This focuses on the question of whether the expressed product of inserted gene/sequence can result in toxic effects in the recipient plant, and thus become a risk in case of incidental (or insignificant) consumption by humans or animals e.g. example in

[13] P. Lemmaux (2008) reviews some general and food issues raised regarding GE crops and foods and cite peer-reviewed scientific literature, where possible in response to the issues. Annu. Rev. Plant Biol. 2008. 59:771–812. Issues related to environmental and socioeconomic aspects of GE crops and foods will be covered in another review to be published in 2009.

the case when someone has taken by accident a maize cob from a test field. The exposure in the case of incidental consumption will be very low.

(5) ***Allergenicity****:* Similarly to the consideration of toxicity, this focuses on the question of whether the inserted gene/sequence can result in allergenic effects arising from cases of incidental consumption of the GMO by humans or animals, or in case of exposure to parts of the plants, such as pollen.

(6) ***Altered farming practice***. Can the inserted gene/sequence result in a change in management of the genetically modified crop plant that has a negative impact on the environment. For example, can the inserted gene/sequence cause adverse *changes in biogeochemical processes*, such as changes in the nitrogen cycle?

(7) ***Other unintended adverse effects.*** For example, can the inserted gene/sequence reduce effectiveness of an antibiotic used in medicine as result of horizontal transfer of antibiotic-resistance genes

(8) ***Creation of new viruses***. Can the inserted gene/sequence result in development of new virus strains due to the introduction of viral sequences in a plant genome and possible recombination of genetic material?

Environmental risk assessment and food/feed safety assessment

A comparison between environmental risk assessment and food/feed safety assessment is summarized below.

Similarities: The methodology and information requirements for food safety and environmental risk assessments discussed above are basically similar. In terms of information requirement, both assessments give importance to the relevant characteristics of host and insert, the intended changes from new gene product and/or resulting trait and possible unintended changes. With regard to process, both assessments are done in a step-by-step fashion; early steps in the assessment indicate whether or not additional information or testing is required and uses the substantial equivalence approach to compare any changes and effects of the GM with the appropriate non-GM comparator(s).

Differences: The safety assessment of GM foods is conducted only in the final product. Food safety assessment is descriptive (i.e. what changes happened). Safety assessment generally investigates toxicity, allegenicity, compositional changes in substance with nutritional and toxic properties, stability of the inserted gene, nutritional effects associated with genetic modification and unintended effects which could result from the gene insertion.

Environmental risk assessment (ERA) covers both the GMO and the receiving environment. The assessment process focuses on evaluation of the genotypic and phenotypic characteristics of the GMO and its effect and stability in the environment. The ecological characteristics of the environment where the GMO will be introduced are also evaluated.

Environmental risk assessments are conducted in all stages of development of the GMO. These ERAs involve a stage approach consisting of

- Laboratory/Greenhouse –with stringent physical containment conditions
- Confined field trial - with conditions that ensure the release is limited and controlled in space and time
- Large scale field trial /Commercial release - with or without specific controls
- Post-commercial release – post-release monitoring requirement

At every stage of this process, scientific knowledge is generated and experiences are gained. This staged approach combined with the appropriate protective measure enable the process to build up the body of evidence if the GMO poses any risks while at same time minimizes exposure to harm of people and the environment.

Environmental risk assessment: some examples

Example 1. PRRI draft guide on environmental risk assessment (www.pubresreg.com)

As indicated in the Section 4, the PRRI Guide on environmental risk assessment methodology typically follows the following steps:

- Hazard identification
- Likelihood estimation
- Consequence evaluation, including a baseline assessment
- Risk estimation

These steps are executed in a phased approach

Phase 1: Consideration of each of the inserted genes and sequences individually

Phase 2: Consideration of the whole plant, including potential synergistic and of possible insertion effects and including available empirical information on the resulting GMO

Phase 1: Consideration of the inserted genes and sequences individually

Step 1. Hazard identification

- Hazard identification step addresses explicitly three closely related topics:
  - the ‘triggers’, i.e. which new genotypic or phenotypic characteristics of the GM plant may cause adverse effects on the environment,
  - the scientifically conceivable scenarios that – in theory - could lead to those adverse effects,
  - a clear description of those adverse effects. For example, it is not helpful to just refer to ‘potential impacts on biodiversity’, because that as such doesn’t clarify the issue at hand.

Step 2. Estimation of likelihood

The next step in the risk assessment is an estimation of the likelihood of a certain inserted gene or sequence actually having a potential adverse effect is influenced by many different factors, such as:

- The characteristics of the inserted gene
- The characteristics of the recipient organism
- The characteristics or the scale of the activity: For example, the likelihood of a genetically modified plant with a certain 'built-in' pesticide resulting in significant impact on insects or other organisms other than the target pest, is negligible in a small-scale confined field trial, but may be likely in wide spread commercial use

In cases where the estimation of likelihood does not result in a clear conclusion, it is sometimes advisable to proceed to the next step of the assessment, by assuming as a 'worst case scenario' that a certain event will occur. The attention is then focused on the next step in the risk assessment, i.e. what are the potential consequences

Step 3. Evaluation of the consequences

This step evaluates the severity of a certain effect in a particular situation and environment. Something that may be of no significant consequence in one environment may be of significant consequence in another.

Evaluating the consequences that the introduction of a genetically modified plant may have on the environment is less straightforward because

1 types of effects that may have to be considered differ strongly from each other, such as weediness, effects on non-target organisms, etc.
2 ecosystems in general are very dynamic systems in which many changes occur constantly.
3 severity of a certain effect has to be compared with the effects of using the non-modified host organism.
4 in the case of introducing a GM variety, it should also be considered that every agricultural activity has an impact on the environment in which it takes place.

In order to evaluate the possible consequences of the introduction of a GMO in the context of these dynamic processes, the concept of "base line" plays an important role.

Step 4. Estimation of risk.

The next step in the risk assessment is the evaluation of risk, for each of the identified potential adverse effects. As explained in Section 4, risk follows from the combination of the severity of a potential adverse effect (i.e. consequence) and the likelihood of it occurring. In

the absence of quantitative descriptions of likelihood, terms often used in this step of the risk assessment are: high, moderate, low, negligible.

It is strongly recommended that steps 1 – 4 above be carried out in a systematic way for each inserted gene or sequence.

To facilitate a systematic approach, matrices such as the one shown below can be used. The use of such matrices helps to focus the assessment, and once a matrix is filled in properly, it can be used to formulate the text into the section 'risk assessment' of the notification.

Annex II - worksheet (risk assessment per gene)

Dossier/Applicant:
Plant:
Type of use:
Gene:

Identified potential adverse effect(s)	Estimation of likelihood	Evaluation of consequence	Estimation of the risk
	Terms used: Highly likely Likely Unlikely Highly unlikely	Terms used: Major Intermediate Minor Marginal	Term used: High Moderate Low Negligible
Potential adverse effect 1			
Potential adverse effect 2			
Potential adverse effect 3			
Etc			

Phase 2: Consideration of the GM plant 'as a whole.

After the systematic 'gene by gene approach', the risk assessment moves to a more 'holistic' phase by looking at the plant 'as a whole'. In this phase, the risk assessment looks at:

1. ***Potential synergistic effects of the inserted genes*** – the introduced traits confer characteristics that may enhance or reduce the effect of the GM plant in the environment; certain combinations of traits may enhance the potential for adverse effects, whereas other combinations may reduce the likelihood of adverse effects e.g two different Bt genes, for example, is sometimes applied to reduce the likelihood of resistance development in the target organism

2. ***Available data of the GMO itself, including data on insertion effects*** - data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Possible insertion effects as a result of insertion of a sequence within a gene, which could interfere with the pathways in the plant is checked before the crop is placed on the market.

   The example shown below was abstracted from Annex 4 of the PRRI Guide (http://wwwpubresreg.org**)** to illustrate the steps in the risk assessment methodology described above and in Section 4. In this example, two potential adverse effects of the environmental release of GM crop with Bt gene are identified and assessed using the risk assessment methodology outlined in Section 4.

Example of risk assessment considerations for releases of GM crop plant with a Bt gene (e.g. CRY1AB, CRY1AC, CRY1FA, CRY2AB)

Identification of potential adverse effect	Estimation of likelihood	Evaluation of consequence	Estimation of the risk
Potential effects on non target organisms			
Potential unintended effects on the target organism			
Etc.			

The different considerations for two different cases are discussed below:

1. Potential effects on non-target organisms

- ***Hazard identification***
  - Trigger: the inserted genes code for insecticidal toxins; risk assessment should include question of potential effects on non-target organisms
  - Scenarios that would be considered are
    - 1) direct effects in the case of other insects or other animals eating the GM plants with the Bt gene, and
    - 2) indirect effects in the case of other animals consuming the target insects.

In the latter case there may be different types of effects, either because a) those other organisms could ingest indirectly the Bt toxin, or b) because those other organisms would have – if the Bt toxin is effective – fewer insects to prey on. Point of debate under scenario b) is the fact that large numbers of insects caused by crop fields are not a natural situation.

- ***Estimation of likelihood:*** In GM crops with Bt genes to date, the gene products are well known to be highly specific and limited to a small group of Lepidoptera. The likelihood of those Lepidoptera insects being directly affected by the Bt toxin depends first of all on the type of activity,
    - Small scale field trials, any impact on the population level of those Lepidoptera insects is very unlikely
    - Large scale commercial use, the estimation of likelihood considers the presence and feeding behaviour of those Lepidoptera insects, which depends on those insects and on the crops involved. When those insects are not present in the area of planting or do not use the crop involved as main source of food, then an impact on the population level of those insects is very unlikely. When they are present and do use the crop involved as main source of food, then additional testing may be required.
- ***Evaluation of consequence*****:** If the empirical testing results show a significant impact on the population level of those other Lepidoptera insects when exposed to GM crops with Bt Toxin, then an evaluation of the consequence will follow. Impact of growing GM crops will be
    - Intermediate or major –if those other insects are threatened species
    - Minor or marginal –if those insects are widely available in the country.
    - Any impact may even be welcome - if the other insects are also pest insects,

The results of any testing this stage needs to be compared with a proper baseline, derived from growing the unmodified recipient plant.

- ***Estimation of risk.*** If the evaluation of the consequences shows that the consequences are not marginal, then the estimation of risk will follow, and will depend on the outcome of the estimation of the likelihood and the evaluation of the consequence. What that estimation finally will be, will vary from case to case.

If for example a certain crop is normally grown on a very large scale in a country, then the conclusion may be different then when a crop is only marginally grown. This part of the risk assessment can also make use of available data resulting from growing Bt crops on a commercial scale. To date, no verifiable reports have been produced of direct effects on non-target organisms in areas where Bt crops are grown.[14]

The conclusion of the risk assessment may be high, moderate, low or negligible.

[14] Note: The October 2005 issue of Environmental Entomology introduces a new section "Transgenic Plants and Insects" with 13 papers on the longer-term assessment of potential non target effects of transgenic Bt cotton and corn active against lepidopteran and coleopteran pests.
http://titania.esa.catchword.org/vl=3626685/cl=31/nw=1/rpsv/cw/vhosts/esa/0046225x/latest.htm.
The articles are listed in endnote of this Guide (www.pubresreg.org)

- ***Consideration of risk management.*** As discussed under 'likelihood',
    - in cases of small-scale confined releases, the risk management applied by confinement is usually sufficient to address the issue of effects on non-target insects.
    - In case of large scale, commercial use whereby the estimation of the risk of effects on certain non-target organisms is not negligible, the next step is normally to consider risk management strategies. However, it is obvious that in the case of commercial use of a crop risk management aimed at preventing certain insects to forage on the crop is practically not feasible. In those cases, the risk assessment moves ahead to the next stage

2. Potential unintended effects on the target organism-resistance development against Bt

- ***Hazard identification***

- ***Trigger:*** Resistance development against Bt is in itself not an adverse environmental effect but an adverse agronomic and commercial effect. However, it can also be an environmental effect, in case it impairs other treatments such as spraying with microbial pesticides. Whether or not this may be the case depends on the pest insect and crop involved. For example in the case of the European Corn Borer in maize, microbial treatments are not widely used and therefore there would be no trigger for further examination from a biosafety point of view, whereas in potato and rice microbial pesticides are used.

- ***Estimation of likelihood:*** in the case of small scale field trials, the likelihood of resistance development is very, very low. In the case of commercial use, the likelihood can be high in cases whereby a certain Bt crop would be grown for long periods on large areas without effective resistance development strategies.
- ***Evaluation of consequence:*** The severity of the environmental consequence of resistant development will depend on the extent to which treatments such as microbial pesticides are used. The larger the area of microbial pesticide use, the more sever the consequence.
- *Estimation of risk.* The estimation of the risk of resistance development will depend on the likelihood of resistance development (which may be different for each type of Bt) and which depends on the availability of resistance management strategies.

Example 2. James Hancock 2003. A framework for assessing the risk of transgenic crops Bioscience 53:512-519

Underlying assumption:

- Potential invasiveness of a plant species can be predicted if we have knowledge about its biology, its distribution and the likely fitness impact of a transgene

Features of the approach

- Scientifically sound—approach supported by Tiedje et al. (1989); U.S. National Research Council (2000)
- Makes use of already-existing scientific information
- Flora of specific regions
- Existing information about the mode of action of the transgenes
- Identifies data gaps that must be filled by new research submitted by an applicant

Assessment Steps

- **Step 1.** Classify species according to invasive biology. The six species categories are shown in Table 1
- **Step 2.** Assess relative fitness impact of transgenes. The five transgene categories are presented in Table 2.
- **Step 3.** Combine species category with transgene category to determine if the crops are safe for release without further experimentation (Table 3) or need additional experiments (Table 4)

Table 1. Invasive biology of crop species and theor compatible relatives in North America

Category	Biology	Examples of crops in North America
S-1.	No compatible relatives. Crop carries only a few weediness traits and does not escape or persist	Broccoli, cabbage, cauliflower, citrus, cucumber, cotton, eggplant, pea, potato, soybean, sugarcane, tomato, and watermelon
S-2	No compatible relatives. Crop carries an intermediate number of weedy traits but rarely escapes and does not persist.	Peanut and beans
S-3	No compatible wild relatives. Crop carries many weediness traits and can escape and persist.	Barley and wheat
S-4	Has compatible relative. Crop or relative carries only a few weediness traits. Crop can escape but does not persist. Native relative does not aggressively spread.	Celery, lettuce, maize, melon, pepper, squash, and tobacco
S-5	Has compatible relative. Crop or relative carries intermediate numbers of weedy traits. Crop can escape and persist. Native relative does not aggressively spread.	Apple, asparagus, beet, blueberry, carrot, cranberry, onion, pear, poplar, plum, radish, spruce, and strawberry
S-6	Has compatible wild relative. Crop or relative carries many weediness traits. Crop can escape and persist. Native relative spreads aggressively.	Oats, rapeseed, rice, sorghum, and sunflower

Table 2. Relative fitness impact of transgenes

Category	Fitness Impact	Examples of transgene use
T-A.	Neutral in the native environment	Marker genes
T-B	Detrimental in the native environment	Male sterility, altered fiber quality, altered fruit ripening, and storage
T-C	Variable, depending on invasiveness of crop or native	Herbicide resistance
T-D	Variable, depending on level of biological control	Viral, fungal, and pest resistance
T-E	Potentially advantageous in the native environment	Cold, drought, and metal tolerance; improved nutrient uptake; altered development

(a) Crops safe to release without further experimentation

Table 3. Transgenic crops safe to release without further experiment

Crop Category	Transgene Category	Examples of crops in North America
S-1.	T-A, T-B, T-C, T-D, T-E	There are no compatible relatives, and the crop has so few weediness traits that even the most dramatic changes in phenotype are highly unlikely to make it invasive. The crop is easy to control without herbicides.
S-2	T-A, T-B, T-C	There are no compatible relatives, but the crop has enough weediness traits that a dramatic change in its adaptations could make it invasive. A lack of pest resistance is unlikely to be the change in its adaptations could make it invasive. The crop is easy to control without herbicides.
S-3	T-A, T-B	There are no compatible relatives, but the crop itself is weedy, so any change in its fitness might make it more invasive. Herbicides are also needed for the crop's control.
S-4	T-A, T-C	The crop or native relative has so few weediness traits that even the most dramatic changes in phenotype are highly unlikely to make it invasive in agronomic systems; however, advantageous and detrimental transgenes could escape into natural populations and significantly alter their fitness. It is relatively easy to control the crop and its relatives without herbicides.
S-5	T-A, T-B, T-C	The crop or native relative has enough weediness traits that a dramatic change in their adaptations could make it invasive; however, advantageous transgenes could escape into natural populations and positively alter their fitness. The escape of detrimental traits is unlikely to have long-term influences on native populations, because population sizes are large. It is relatively easy to control the crop and its relatives without herbicides.
S-6	T-A, T-B	The crop is already invasive, so any positive change in its fitness might make it a greater pest. Beneficial transgenes associated with environmental tolerances and pest resistance could escape into natural populations and alter their fitness. The escape of detrimental traits is unlikely to have long-term influences on native populations, because population sizes are large. In addition, herbicides are needed for the control of the crop and its relatives.

(b) More data needed

Table 4. Various crop-transgene combinations that require further experimentation

Transgene Category	Crop type	Further research needed
T-A.	None	None
T-B	S-4, S-5, S-6	Document that wild recipient species is not endangered.
T-C	S-3, S-6	Document that any wild recipient species can still be controlled as agronomic weed.
T-D	S-1, S-2	Show that a similar phenotype exists in wild populations. If not, measure levels of native biological control. If levels are significant, test fitness of transgenic crop and hybrids in representative native environments.
T-E	S-1	Show that a similar phenotype exists in wild populations. If not, test fitness of transgenic crop and hybrids in representative native environments.

The Process of Risk Analysis: Risk Management

Risk management is the second and the decision-making component of the process of risk analysis. Risk management is defined as "the process of weighing policy alternatives to mitigate risks in the light of risk assessment, and, if required, selecting and implementing appropriate control options, including regulatory measures (FAO/WHO, 1995; 1997). Its objective is to determine which risks require management and how these risks can be effectively managed or controlled so that its goal of ensuring adequate protection for people and environment is attained.

Risk management is primarily supported by the results of the risk assessment process but may consider risks in a wider context. This allows the risk manager or designated national competent authorit(ies) to take into consideration other inputs e.g. socio-economic considerations (if allowed by regulation) from other interested parties concerned with risks, in the final decision on any dealing of GMOs. This makes risk assessment essentially a political process.

To maintain the scientific integrity of the risk assessment process, it is important to keep the conceptual separation between risk assessment and risk management.

The Key Steps in Risk Management

Risk management is also a step-by-step process which consists of:

(1) ***Risk evaluation***. This step decides whether the identified risk is manageable i.e. a consideration of appropriate risk management strategies

As discussed in Section 4, the rigorous scientific process of risk assessment process ends in a risk estimate. Risk evaluation starts from the result of the risk estimation step. In cases whereby, on the basis of the risk estimation step, the risks involved are not deemed to be 'negligible' or 'marginal', the risk assessment continues with this step, which is a consideration of whether the identified risk is manageable or acceptable. The question to

address is whether the identified risks require specific risk management measures. If the answer is 'yes', then a risk management strategy is defined in the next step. For example, risks with estimates of high or moderate would generally invoke a requirement for management.

Risk evaluation serves as the vital link between risk assessment and risk management. In practice, the functional separation between risk management and risk assessment is less clear in this step.

(2) ***Risk mitigation***. This step is central to the risk management process. It determines the options and plans to reduce or avoid the risks. For cases where a risk management strategy has been defined, the risk assessment 'loops back' to the earlier steps in the risk assessment to determine whether the proposed risk management strategies sufficiently reduce the likelihood or the consequence. This is one reason why risk assessment is often called an "iterative process". Availability of new data, derived for instance from a field confined, 'risk managed', field experiment may also be a reason to revisit and possibly revise a risk assessment

Depending on the case, risk mitigation measures or options may include

- specifying the appropriate containment facilities and biosafety levels (please see Section 2), the conditions for use, handling, storage, transport and disposal; for genetically modified plants: reproductive isolation; by removing of flowers, use of isolation distances or border rows, temporal isolation etc., reduction of the size or duration of an application special design features such as male sterility
- requiring submission of contingency or emergency plans;
- monitoring and surveillance
- GMO detection (for details, please see Lecture Module 1, DNA detection)
- labelling (voluntary or mandatory)

NOTE: *Detailed information on all aspects of monitoring, surveillance, emergency planning are presented in Lecture Module: Use of GMOs Under Containment, Confined and Limited Field Trials and Post Release Monitoring of GMOs*

Countries have put up their own guidelines for dealings on GMOs but there are still no internationally agreed guidelines, except for containment, on exactly how these risk management measure are designed and implemented. Efforts are underway to standardize and harmonize the guidelines on these various risk management measures.

3. Selecting and implementing the most appropriate option(s) and actions.

This step refers to the final decision making process that will ultimately lead to authorization and issuance, or not, of the license required for any dealing of GMO. The risk mitigation measures identified are included as part of the license conditions.

Final decisions are based primarily on the results of the scientific process of risk assessment. However, in this step, the risk management process may take into account other non-risk issues (e.g. socio-economic considerations) and other risk-related factors (e.g. risk perceptions) from various stakeholders to inspire confidence and lead to wider acceptance of the decision. These stakeholders have diverse views and may have conflicting interests. Decision makers need to balance the individual rights of different stakeholders with the need to protect human health and the environment from the adverse effects of unacceptable risks. This step is makes the risk management process essentially a political process.

Risk Management and Socio-economic Considerations

Attitudes towards food and the environment vary widely across societies. In addition to providing the basic need of sustenance, clothing and shelter, food and nature have economic, cultural, ethical and religious meanings and importance to people. Perceived risks to food and environment, including those posed by GMO, is hardly tolerated unless understood and acceptable levels of safety are assured. Scientific assessment of safety undertaken by experts is important, but decision-makers are expected to take into account inputs of various stakeholders concerned with risks, which include socio-economic considerations. Socio-economic considerations cover a wide range of issues and concerns such as advantages vs disadvantages, risk-benefit, costs including cost of doing nothing, respect for cultural diversity, etc.

There are two relevant international documents for considering socio-economic considerations in decision making with regard to potential risks of GMOs to people and environment. These are: (a) Cartagena Protocol on Biosafety of the Convention on Biological Diversity; and (b) Codex Alimentarius (international food code).

Article 26 of the Cartagena Protocol on Biosafety, in particular paragraph 1 states that

"1. The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities."

It is clear in Article 26 of the CPB that countries may to take into account socio-economic considerations in making decisions with regards to GMOs. Paragraph 1 of Article 26 defined the limits and conditions when applying socio-economic considerations in decision-making on risk posed by GMOs to the environment. The definition implies that not all socio-economic considerations can be considered, but only those where GMO directly impacts biodiversity. It also specifies the condition that when countries decide to take into account socioeconomic conditions in decisions on GMOs, it "must be done in a manner that is consistent with other international obligations" which includes the World Trade Organization (WTO).

Codex Alimentarious guidance documents also states that socio-economic considerations may be taken into account in decisions on GMOs. Unlike the CPB, Codex principles are not legally binding to national legislations. However, Codex principles are referred to specifically in the Sanitary and Phytosanitary Agreement (SPS) of the WTO, which is a legally binding international treaty also signed by many countries.

Codex principles on risk management particularly relevant to socio-economic consideration include Section 3.16 of Codex Alimentarius for food derived from modern biotechnology (2003), which states that

> "Risk management measures for foods derived from modern biotechnology should be proportional to the risk, based on the outcome of the risk assessment and, where relevant, taking into account other legitimate factors in accordance with the general decisions of the Codex Alimentarius Commission as well as the Codex Working Principles for Risk Analysis."

Appendix IV of the Codex Working Principles for Risk Analysis on human health (Codex, 2003) and the Criteria for the Consideration of the Other Factors Referred to in the Second Statement of Principles (13) outlines the points and criteria relevant to socio-economic considerations. These include

- other legitimate factors relevant for the health protection of consumers and for the promotion of fair practices in food trade based on the following criteria (Point 28)
  - *other factors should not affect the scientific basis of risk analysis*
  - *other factors which can be accepted on a world-wide basis, or on a regional basis*
  - *specific other factors should be determined on a case-by-case basis*
  - *other factor should consider the feasibility of risk management options concerns related to economic interests and trade issues*
  - *other factors should not create unjustified barriers to trade*

- ***Risk management process should:***
  - take into account an assessment of their potential advantages and disadvantages (Point 34)
  - consider the economic consequences and feasibility of risk management options, giving particular attention to the circumstances of developing countries (Point 35)

As can be noted in the above, the existing guidance documents treat socio-economic considerations in general terms. To date, there are is still no international agreed definition and scope of socio-economic considerations and methodologies for analysis and incorporating socio-economic considerations into the decision-making process. Even at the national level

and for what maybe considered as 'legitimate factor' like economic risk- benefit analysis, there are no biosafety regulatory systems that have formally included a benefits assessment within their regulatory structure. It will take resources and resolve before consensus is reached to effectively incorporate socio-economic considerations in biosafety regulations in many countries.

The Process of Risk Analysis: Risk Communication

Risk communication is the third component of the risk analysis process. Risk communication is defined as "the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among individuals, groups, institutions concerned with risk, including the explanation of risk assessment findings and the basis of risk management decisions." (Codex Alimentarius Commission, 2003). Risk communication in this sense is also addressed in Article 23 of the Cartagena Procotol on Biosafety on public awareness and public participation which states that

1. The Parties shall: (a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate, as appropriate, with other States and international bodies; (b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.
2. The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of such decisions available to the public, while respecting confidential information in accordance with Article 21.
3. Each Party shall endeavour to inform its public about the means of public access to the Biosafety Clearing-House.

There is wide agreement that effective risk communication is essential at all phases of risk assessment and risk management. It is also recognized that risk communication involves not only risk assessors and risk managers, but also other interested parties like government, industry, academia, consumers, public interest groups and individuals concerned with risk.

Risk communication is also recognized to be an integral part of the risk analysis process. It is embedded throughout the risk assessment and decision making process; two key steps – hazard identification and selection of risk management measures – require effective risk communication to help build trust, reduce conflicts and achieve desired outcomes. In hazard identification, the views and opinions of interested parties about the potential hazards can help define the issues of concern and reduce potential points of conflicts. During the selection of

risk management options, the risk managers may need to consider factors in addition to science in the evaluation of a risk. This should involve active participation of stakeholders and other interested parties. Finding a common language that will be clearly understood by all parties is needed in explaining the results and the processes of the risk assessment and risk management processes.

In the context of this document, the goals of risk communication are: (1) to improve the knowledge and understanding on all aspects of the risk analysis process by all interested parties concerned with risk; and (2) to promote interactive communication between risk assessors, risk managers and other interested parties concerned with risks in order to achieve the desired outcomes.

Understanding Risk Perception

GMOs are oftentimes controversial because the public have different perceptions of the potential risks that GMOs pose to people and environment. Different perceptions of risk pose present one of the most important challenges to risk communication and must be dealt with.

It must be remembered that in risk communication, perception equals reality i.e. perceived risk is real risk; perceived risk has real consequences. The following theories will help explain what happens to a person's ability to assimilate information when they feel threatened.

Risk = hazard + outrage

A deceptively simple formula developed by Dr. Peter Sandman (www.psandman.com), risk includes both hazard and outrage. Hazard includes the objective, technical and measurable component of risk; outrage includes cultural, emotional, and personal factors, including all levels of fear, anger, and general upset.

Public perception of risk is highly influenced by a large number of outrage or risk perception factors. Risks are perceived either as low or high depending on whether some or all of the outrage factors shown in Table 5 are involved. For example, a high risk hazard that is familiar and voluntarily used may be perceived to be less risky.

In addition to the risk or outrage factors, there are other factors that also contribute to differences in perceptions of risk. These are: (1) the language used to describe the risk i.e. statistical language of scientists, and the intuitive language of the public. For example, in describing the safety of GMO, the public language uses 'safe' when what it means is "as safe as" in the language of a scientist; (2) transparency or access to information; and (3) individual/group values, beliefs and interest (e.g. business, environment).

Trust Determination Theory. People who are upset or feel threatened are often distrustful of others. Trust and credibility are two of the cornerstones of effective risk communications. Without them nobody will listen to your message, people will not make informed decisions and action, and problems can get worse.

Table 5. Risk perception or outrage factors (Source: V.T. Covello. www.centre4riskman.com/downloads/rc_slides_2002.ppt)

Lower perceived risk	Higher perceived risk
Trustworthy sources	Untrustworthy sources
Substantial benefits	Few benefits
Voluntary (personal control)	Involuntary or mandatory (no personal control
Natural origin	Human origin (man made)
Familiar	Unfamiliar
Fair/equitable	Unfair/inequitable
Certain	Uncertain
Moral/ethical	Immoral/unethical
Immediate effects	Delayed effects
Effects reversible	Effect irreversible
Scientifically well understood	Not scientifically well understood
Random/scattered	Catastrophic
Little media attention	More media attention
Victim statistical	Victim identifiable

The factors that build trust and credibility are -

- empathy and caring - 50%
- honesty and openness – 15-20%
- competence and expertise – 15-20%
- dedication and commitment. – 15-20%

In high concern/low trust situations, people want to know that you care before they care what you know. Over 50% of your credibility will depend on whether or not you are caring and compassionate. The higher the level of distrust, the more compassion and sympathy is needed.

Credibility transference. You can build trust and credibility by using support from and working with credible third party (other) sources. The idea is to increase your credibility to the same level as a source with high credibility by enlisting its support to your position on the issue. You can also build up your credibility by getting many highly credible sources to support your position on an issue.

Credibility reversal. In cases of attack on credibility, the source of information with lower credibility end up lowering even more its credibility when it attacks a source with higher credibility. Remember, the only information source that can effectively attack the credibility of another source is one of equal or higher credibility.

Credibility ranking of information sources:

- High: health professionals, scientists, educators, advisory groups
- Medium: Media, activist groups
- Low: Industry, paid external consultants

***Mental Noise Theory*:** When people are upset they have difficulty hearing, understanding, and remembering information. This implies limited attention to information and limited ability to process and retain information.

A highly effective verbally-communicated message should

- limit the number of messages: 3 key messages
- be clear: 6th grade level or 12 year-old language
- be brief or concise: 10 seconds or 27 words
- be repeated 3X: Tell them what your going to tell them. Tell them. Tell them what you told them.
- be assisted by visual graphics, slides
- be aware that it takes three (3) positive messages to balance one (1) negative statement 3N=1P
- not contain negative words: No, Not, Never, Nothing, None

In case of non-verbal message or body language, it is important to remember that body language often overrides verbal communication. It can provide up to 75% of message content. It is noticed intensely and is easily negatively interpreted. For example, poor eye contact can leave an audience feeling that you are dishonest, unconcerned or nervous; or a raised voice can send the message that you are hostile, nervous, or deceitful.

Applying Risk Communication Principles in Risk Analysis

The joint FAO/WHO expert consultation on the application of risk communication to food standards and safety matters identified the elements, principles, barriers and strategies for effective risk communication (FAO, 1999). The principles, applied to risk assessment and risk management processes, are illustrated below:

- *Know the audience.* In the risk analysis process, the different types of audience are: risk assessors, risk managers, government, interest groups and the general public. It is important to listen to and understand their motivations, opinions, concerns and feelings. These are important in the development and delivery of credible information on the risk identified, the decisions made, and the processes used. Understanding the audience's perception of risk can be done through surveys, interviews, focus groups
- *Involve the scientific experts.* Scientific experts are primarily involved in the risk analysis process in their capacity as risk assessors. They work very closely with the risk managers in arriving at the final decision on any dealing with GMO. These experts must be able to explain clearly the results of their assessment including the assumptions and subjective judgments so that risk managers can clearly and fully understand the risks and consequently inform their decision.

- *Establish expertise in communication.* The risk analysis process generates enormous amount of information of interest to a wide range of audience. Developing credible information and delivering them effectively require communication expertise. Risk communication experts have to be involved as early as possible. Communication expertise of risk managers and risk assessors has to be improved by training and experience.
- *Be a credible source of information.* In the risk analysis process, the sources of information are risk assessors, risk managers, applicant, and other interested parties. Information from a credible source will likely be accepted. For example, information of Codex Alimentarius Commission on food safety assessment will more likely be accepted than information from a company consultant. Consistent messages from multiple sources lend more credibility to the risk assessment. Results of safety assessment by regulatory bodies of many counties of a particular GMO will likely be more accepted. To be credible, the source of information should be perceived as genuinely concerned with their views and opinions on the risk issues, trustworthy, competent, committed and consistent. Timeliness in delivery and up-to-date information to address current issues and problems adds to the credibility of the source.
- *Share responsibility:* There are multiple actors involved in the risk analysis process. These include risk assessors, risk managers, other interested parties and the media. Each has a specific role to play, but have joint responsibility for the outcome. Since science must be the primary basis for decision making, all parties involved in the communication process should know the basic principles and data supporting the risk assessment and the policies underlying the resulting risk management decisions.
- *Differentiate between science and value judgment* It is essential to separate "facts" from "values" in reporting the results of the risk assessment and decisions made in the risk management process.
- *Assure transparency.* For the public to accept the risk analysis process and its outcomes, the process must be transparent. This means the process and results of risk assessment and risk management are accessible and available for examination by interested parties, but giving due regard to confidentiality of information (if allowed by regulation)
- *Put the risk in perspective.* In the process of risk analysis, this can be done by emphasizing the information about the risk that are relevant to help the target audience makes up its mind. For example, in the decision-making step, the risk manager may examine the risk in the context of the benefits associated with the technology. Risk comparison that underestimates the concern should be avoided.

Facilitating Public Engagement in the Risk Analysis Process

Risk communications not only aims at informing and educating the public i.e. improving the understanding of risk issues but also at dealing with conflicting views and interest of the regulators, other interested parties and the general public on all aspects of the risk analysis process. Engaging all parties in a responsive and interactive dialogue will may not change their individual positions but will lead to a better understanding of and increased level of acceptance in the decisions made.

The need to engage the public in decision-making processes concerning safety of GMO to people and environment is increasingly being recognized. This trend is clearly presented in the results and background document of the FAO Biotechnology Forum[15]. One of the decision-making processes identified where public engagement is needed is in the risk assessment and risk management, particularly in the approval of GM products. However, there are still no internationally agreed guidelines as to what extent and how public input can be integrated into the risk analysis process.

The joint FAO/WHO expert consultation on application of risk communication to food standards and safety matters (1999) identified steps in the risk analysis process where public input maybe considered. The most important is in the risk management step specifically in the identification and weighting of policy and decision alternatives by risk managers. It was suggested that interested parties, whenever practical and reasonable, should be involved in identifying management options, developing criteria for selecting those options and providing input to the implementation and evaluation strategy.

IDS (2003) also considered some of the choices regarding at which point the public could be involved in decision-making process in the implementation of regulatory framework. In the context of the risk analysis process, some of the choices identified are: (1) identification or risk issues (what do citizens know, what they are concerned about); (2) roles, duties and powers of responsible agencies; (3) mechanisms of reporting, public scrutiny and accountability; (4) location and design of biosafety trials. The kinds of processes that then maybe used include: (1) engaging with areas of public concern (rather than assuming what people need to know); (2) ensuring openness about applications for biosafety review and commercialization; (3) ensuring openness about the purpose, location and design of biosafety trials; (4) ensuring opportunities for public comment. The kinds of tools which maybe considered include stakeholder forums that are accessible and widely advertised and public registers of applications under review, with routine opportunities for public comment and obligations to respond to public comments.

As a final note, IDS emphasized that public participation is highly contextual. While the concerns are similar, there is no ‘one-size-fits-all’ formula for public participation and awareness-raising. What works in some places or in some circumstances will not work

[15] Please see Ruanne J and A Sonnino. 2006. Results from the FAO Biotechnology Forum Background and dialogue on selected issues.

everywhere. Appropriate forms of public participation and consultation need to take into account the different situations, capabilities, and stages of development of each country.

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Chapter 4: Use of GMOs Under Containment, Confined and Limited Field Trials and Post-Release Monitoring of GMOs

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Introduction

The Genetically Modified Organisms (GMOs) in the plant world are those plants which are produced in the laboratory by incorporating into the native DNA of the plant, a small DNA portion carrying a gene that is foreign to the native species. This foreign gene is a recombinant DNA construct (rDNA) with all other regulatory switches to help the foreign gene express in its new genetic environment. This expression can be different from its original expression to the extent of expression which may make the GMO overproduce, underproduce, differently produce or not produce the protein product it is known to produce. But, when rDNA is produced it is within the confines of a highly specialized laboratory with skilled scientists and people handling the product who are generally trained to deal with the positive output as well as the negative ones and the unperceived consequences which comprise the major amount of risk involved. However, when it gets out of the laboratory, the element of risk associated with it passes into the hands of those who may not be associated with the technology or who would not understand the technology at all. The commercial activities about the technology makes the exposure still wider confounded with the risk involved in its release into an environment.

The direct use of DNA either from unrelated organisms or from synthetic sources through molecular biological techniques to manipulate the genetic make-up of organisms has provided a large number of alternative strategies in making agriculture productive. The potential is vast beyond the realms of the conventional (though scientific) approaches the breeders have been following ever since agriculture was domesticated as source of sustenance and livelihood.

The genetically modified organism (GMO, also referred to as living modified organism or LMO or popularly, transgenic organism) follows the same evolution cycle like that followed by any other technology that a evolving society invents, develops, produces, markets a commodity followed by R & D by the producer involving the inventor after consumer feed-back. In our context, GMO mostly concerns the transgenic crops to a large extent and micro-organisms to a lesser extent.

Ever since the discovery of the scientific fact that genes from a totally unrelated organism (virus, bacterium, plants or animals) to plant in mid 1980s came to light, when a gene from Bacillus thuringiensis was cloned into a plasmid vector and transferred into tobacco (a process known as genetic engineering), the brightness of the discovery enlightened scientists from public and private domains alike to the limitless scope the technology possessed. Since then,

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genetic engineering of plants has gone from a new and largely untested technique to a common agricultural phenomenon in most developed countries and some developing countries like China and India.

The first field trial of a GM organism went ahead in 1986. Frostban was a spray containing genetically modified bacteria. In the trials, Frostban was sprayed over a strawberry crop to protect them from frost damage. Frostban was designed to stop the growth of other bacteria that catalyse the formation of ice. Frostban was tested at a site in Brentwood, California. But this open-air experiment didn't please the local population, who formed a protest group called The Strawberry Liberation Front – the first pressure group opposed to genetic modification. But in the US, opposition to GM agriculture was short-lived and small-scale. In 1993 the US Food and Drug Administration declared GM food was 'not inherently dangerous', clearing the way for biotech companies to begin marketing modified seed.

Within a decade after the first of the commercial transgenic crops became available to farmers to cultivate the area, more than 70 million acres of transgenic crops are grown in 2002. In the case of crops such as soybean, cotton and canola put together, nearly 60% of the cultivars are transgenic in origin. Such has been the pace with which commercial agriculture adopted the new technology. Of these crops under transgenics, about 40% are those which carry the *Bt* toxin (crystal protein gene – *cry* in its variant forms) that provides the crop an ability to kill the lepidopteran (sap-sucking) insects, followed by about 25% carrying the herbicide resistance genes (RR gene) which can withstand a particular type of herbicide while the weeds cannot. The other transgenics are those that have been given resistance to particular viruses by having a gene from the virus inserted into their DNA. In nutshell, the transgenics have arrived and are more likely to stay than otherwise, despite the disfavors among consumers and ecological concerns of nature watching scientists.

Rationale for post-release monitoring*:* In 1995, the Conference of the Parties (COP) to the Convention on Biological Diversity set up an open-ended ad hoc Working Group on Biosafety to draft a protocol. After several years of talks, the COP adopted the Cartagena Protocol on Biosafety in Montreal on 29 January 2000. The Protocol is named to honour the city of Cartagena, Colombia, which had hosted the COP's first extraordinary meeting intended to finalize and adopt the Protocol in 1999.The Biosafety Protocol was finally adopted in January 2000 with a stated aim to "contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms 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" . The need for a protocol to be followed before and after an introduction of a GMO came into focus as it was also realized that there could be unintended hazards and risks from the use of GMOs and products thereof, if the new technology was not properly assessed before use. A gene construct comprising a host compatible promoter, a gene of interest and a terminator sequence or a polyadenylation

sequence is integrated in a stable manner into the genome of the organism/cell line of the target gene to be expressed and stably inherited. A genetically modified (GM) organism can be safe but this can be unsafe too. This will depend upon the trans-genes, the host organism and the environment where the GMO is being tested. In case of GM plants, in laboratory experiment, viral disease resistant transgenic plants have given rise to newer viruses by recombination. Transgenic rape seed plants containing bar genes transferred the transgenic trait to near relatives of Brassica spp. Insect resistant Bt plants coding for specific Bt proteins developed bt protein resistant insects in laboratory experiments. Transgenic soybean genetically modified to increase its sulfur containing amino acids by incorporating Brazillian nut 2S gene was allergenic to serum of people who were allergenic to Brazillian nut 2S protein. Potatoes genetically modified with specific lectin genes protected attack from insects but such portatoes were not safe to rodents when they were fed with such potatoes. The transgenic pollens of corn coding for Bt proteins killed the monarch butterfly larvae when they were forcibly fed with such pollens. It is expected that transgenic pollens coding for Bt porteins would affect the silkworm larvae, as these are insects that are susceptible to Bt proteins. There are examples of microoganisms, especially genetically modified viruses that turned virulent after modification.

Monitoring plays a central role in environmental risk assessment and management and is undertaken to gain continuous or periodic information about aspects of an intention before it starts, during its lifetime and after its completion. Information generated from monitoring programmes is integrated into environmental risk assessment and management in various ways:

- as the baseline against which to compare actual and predicted impacts;
- as an input to models, forecasts and quantification stages;
- to provide information to feed back into the risk assessment in an iterative process;
- to confirm that risk assessments and management options are meeting their desired aims; and
- as an alert mechanism if adverse impacts are found

The magnifying effect any possible post-release disaster may lead us to is not something that has not happened with the non-GMOs in the past. The effect could be as drastic as that is perceived as that can be caused due to the commercialization of a GM crop. Ever since the occurrence of such epidemics and ecological calamities, scientists have been very cautious by monitoring the varietal composition in geographical area, monitoring releases through multi-locational trials advising breeding communities to ensure diversity in the genotypic composition of the material being released for commercial cultivation. Organized pre-commercial release testing under monitored experimental conditions is a pre-requisite at least for three years, which actually is nothing but a post-release monitoring of the material in a given target environment.

History, the guide: Thus, obviously, post-release monitoring is not new to organized agriculture. Agricultural scientists have been constantly monitoring crop ecologies for spotting any new pest, pathogen or any reduction/invasion of naturally existing vegetation in the region. This type of monitoring is also assisted by farmers who notice and observe anything that is unusual. Apart from such ad-hoc monitoring which often ended up in a research experimentation on created conditions, the recorded abnormal effects observed also led to the development of a scientific approach to systematic monitoring where collection of data to build information databank on the crop. This began from public investments because, each of such abnormalities observed had consequences of public funds required in their amelioration. Quickly, the governments realized the value of such data collection on crop plants as the sole authenticated information that could distinguish the "rumour" from "fact". This also proved valuable in policy formulation, which in turn led to directed development which could be properly planned and executed.

The same experience works good to necessitate the monitoring of GM crops which have the novelty their predecessors never possessed, and an intensive investment, the sort of which was never seen before, once the crops are released in an environment. The proportion of investment in the development of GMOs also exceeds the search for novelty which the mutation based crop breeding involved in the early decades of 1950s-1970s, world over. Obvious reason is the fact that the conventional breeding is largely an imprecise process because of the involved unknown number of genes, selection for several generations done not only to bring in desired characters but also eliminate the undesirable gene combinations that might have deleterious effects. This lengthy, winding process measured through several eyes, hands, locations and seasons has basically contributed to the safe history and low risk image of conventional plant breeding unlike the molecular breeding involving the GMOs .

Present, in the real world of GMOs: The first concerns which consolidated the indispensability of post-release monitoring of the GMOs came with the observations on observed and/or perceived occurrence of the death of the Monarch butterflies in the vicinity of Bt maize transgenics in the late 1990s (Jesse and Obrycki, 2000) and the movement of the herbicide resistant transgene into related wild weedy species in the case of canola in US and Canada. That this scare is widely recognized as unfounded extrapolation from limited research should give us a hope in the wake of several such "uninformed or ill-informed" perceptions being aired by opponents of this most useful technology.

With the developing countries like China and India making inroads into developing and cultivating GM crops, the exposure to a different production system than that exists in North American countries like the prairies and vast agricultural stretches. In the developing countries, the size of holdings will be much smaller which means potential mosaic of same crop but different cultivars and same cultivars from different seed sources in a concentrated sympatric existence. Needless to say, the emphasis on the concerns on the ill-effects as a consequence of release of the GM into an environment is of extremely relevant to us in this

part of the world. The value of monitoring that uses the Precautionary Principle into consideration is obvious when we look into the cases such as the Brazil nut case in soybean. In 1997, US scientists inserted a gene from a Brazil nut into soybeans to make them more nutritious. But the gene also passed on the property of nuts that causes allergies in people. Luckily, the risk was perceived, assessed, analyzed and monitored. The monitoring detected the allergy transferred in about 75% of the events being tested and the project was abandoned. One is not really aware if such cases of allergenic responses have been passed into cultivated varieties from wild species in conventional transfers done by breeders. Under no circumstances we can presume that such a potential never existed in conventional mode of gene transfers through interspecific hybridization as these were never monitored for such traits!

Monitoring GMOs Under Containment

The activity of monitoring the GMOs under containment specifically refers to the physical structure of the containment specificity seen together with the degree of risk involved with the specific GMO. Yet, there is a need to consider both general inspection of the facility for compliance to GM containment needs irrespective of the extent of risk involved in any particular GMO being monitored. This is simply because, containment facilities cannot be built for each GMO and therefore, the basic structure of the containment facility must meet minimum standards set for each category of risk. With the basic minimum structure in place and met with in general, the monitoring should then be specific to the transgenic event involved. Thus, the "What should be structurally in place" for a particular level of risk classification becomes the check list to be verified for its compliance and proper commissioning. It is suggested therefore, that the following should be taken as standard to be met for clearing the execution of the experiment under containment as "conducted in compliance to biosafety regulation" by the monitoring agency.

Monitoring GM Plants Under Containment

The GM plants need to be first classified into groups based on the extent of safety the material has to be accorded in terms of hazard to human health, crop ecology and environment concerned for effective monitoring of the contained use being applied. The monitoring team should always consider the specific categories for appropriate monitoring indicators of generic nature apart from those specific to the event or trait while recommending the containment trial or evaluating the experiment for furthering to field trial.

As an example, the categories of transgenic plants are as follows:

CATEGORY I

This category includes routine cloning of defined genes, defined non-coding stretches of DNA and open reading frames in defined genes in E_ coli or other bacterial and fungal hosts which are GENERALLY CONSIDERED AS SAFE (GAS) to human, animals and plants. A list of such microorganisms will be prepared by the RCGM and shall be made available to the RI. on request.

This category involves experiments in the lab in contained environment and includes the following.

i. Routine cloning of defined DNA fragments of microbial, animal and plant origin in GRAS organisms.
ii. Transfer of defined cloned genes into Agrobacterium;
iii. Use of defined reporter genes to study transient expression in plant cells to study genetic transformation conditions;
iv. Molecular analysis of transgenic plants grown in-vitro.

Category I experiment need only intimation to the Institutional Biosafety committee the prescribed proforma.

CATEGORY II

This category includes lab and green house/net house experiments in contained environment where defined DNA fragments non-pathogenic to human and animals are used for genetic transformation of plants, both model species and crop species and the plants are grown in green house/net house for molecular and phenotypic evaluation.

This category includes the experiments described below:

i. Transgenics with constitutive, tissue specific and chimeric promoters used for experimenting expression of defined DNA fragments
ii. Marker genes extensively used in genetic transformation of plants in lab and green house/net house experiments.
iii. Lab and green house/net house experiments with plants with herbicide resistance conferring genes;
iv. Lab and green house/net house experiments with plants using heterologous genes which confer resistance to biotic and abiotic stresses (i.e. genes like chalcone synthase, heat shock proteins, chitinase, protease inhibitors etc.);
v. Lab and green house/net house experiments with genes from plants, animals and micrcúial sources that would confer resistance to plant pathogens.
vi. Lab and green house/net house experiments on transgenics with genes for the production of antibodies.

vii. Green house/net house experiments with transgenics with transposable elements for gene tagging in crop species or model species.

In India, the permission for performing Category II experiments will be provided by IBSC. The decision of the IBSC would be intimated to a broad based committee that is independent of the Institution which developed the transgenic (in India, RCGM) before execution of the experiments and RCGM would put this information on record.

CATEGORY III & ABOVE

This category pertains to high risk experiments where the escape of transgenic traits into the open environment could cause significant alterations in the biosphere, the ecosystem, the plants and animals by dispersing new genetic traits, the effects of which can not be judged precisely. All experiments conducted in green house and open field conditions not belonging to the above Category I and Category II types, would fall under Category III risks. Such experiments could be conducted only after clearance from RCGM and notified by the Department of Biotechnology.

Based on the category of risk and nature of the transgene/s, the containments are physically built with varying degrees of Biosafety levels, lowest being BL1 and highest Biosafety at BL4 levels. Most of the transgenic plants can be safely experimented within BL-1 and BL -2 when they fall in the category of I and II. Those plants or microbes in category I and most plant analyses that do not involve any reproductive phase analysis of plants or subjectivity to spread through insects (such as viruses) can be effectively managed in BL1.

The brief descriptions of the greenhouses rated to BL1-BL4 are as follows (NIH, 1999):

Biosafety Level 1 (BL 1): Biosafety level is suitable for work involving agents of unknown or minimal hazard to laboratory personnel and the environment. The work is conducted on open bench tops. Special containment equipment is not required or generally used. The greenhouse floor need not necessarily be impervious. But the walk ways must be of impervious material.

Biosafety Level 2 (BL 2): The access to the greenhouse is restricted to individuals directly involved with the experiments in progress. Decontamination of runoff water is not necessarily required. The floor of wall of the greenhouse should be periodically treated to eliminate any entrapped organisms in the gravel or grooves. The floor is composed of an impervious material. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any barrier against pollen or microbes. But screens are required to prevent insects, birds and animals.

Biosafety Level 3 (BL 3): Prior to entering the greenhouse, the personnel shall be required to pass through a set of two self closing locking doors. Disposable clothing like aprons shall be worn before entering into the experimental area. The hallway leading to the green house is

also a part of the containment. The need is to maintain negative pressure. The floor should be composed of concrete or other impervious material with a provision for collection and decontamination of run off. Windows shall be sealed and all glazing shall be resistant to breakage. The plumbing and utilities need to be sealed. The supply and exhaust airflow shall be interlocked to assure inward or zero airflow at all times.

Biosafety level 4 (BL 4): BL 4 is known as maximum containment level. The personnel shall enter the greenhouse facility only through the clothing change and shower rooms and shall shower each time they leave the facility. An outer and inner change rooms separated by a shower shall be provided for personnel entering and exiting the facility. Windows shall be sealed to permit fumigation. Every plant material leaving the green house has to be autoclaved. The material shall not be brought through the change rooms but through ventilated airlock. The supply and exhaust airflow shall be interlocked to assure inward air flow at all times. HEPA filters shall be provided to treat air supplied to the facility. All liquid effluents shall be decontaminated. Other biological containment options like cover reproductive structures, remove the same if seed not required and ensure that plants flower at that time when cross fertile plants are not flowering within the normal pollen dispersal range of the experimental plant or ensure that cross fertile plants are not growing within the known dispersal range.

Monitoring indicators

The monitoring indicators basically have to be developed on a case by case basis with reference to the gene involved, the category of risk as well as the Biosafety level the material is contained in. The application for approval by the Institutional Biosafety committee should contain the details on which basis the IBSC grants the permit under intimation to the next higher regulatory body, the RCGM which may not involve any one on its body from the Institution, whereas, two of the representatives in the IBSC will necessarily be from the Biotechnology Department, in Indian context. This removes any bias or institutional priority which may lead to Biosafety oversight.

It is required to fill the form with full information the monitoring team can utilize for generating a checklist that identifies the oversight if any in following the Biosafety regulations. The questionnaire should therefore be related to the agreement to the prescribed standards mentioned in the table. All the stipulated regulations if are followed will in general meet the containment characteristics which in other words would suggest that the experimentation has been contained. However, precaution is to be taken to include the items related to the training available with the users and the managers of the containment facility.

The Monitoring team can develop a series of questions which relate to adherence of maintenance of protocols for greenhouse maintenance at appropriate level when they contain the transgenic plants or plants tested with transgenic microbes. Complete details of protocols to be followed in a greenhouse maintenance including biosafety aspects that are followed in

the greenhouse at the National Phytotron Facility, in IARI, New Delhi are presented in a schedule-wise list of activities by Romer et al. 2000. The summary of the schedules which are appropriate are detailed in Table 2. Activities need to be monitored by the members of the IBSC or an institutional committee so that containment is ensured.

Containment of Genetically Modified Animals

The very first activity the monitoring agency should consider in the case of GM animal based activities is whether the experimenter, Institution or the Organization has the approval of the LOCAL ANIMAL ETHICS COMMITTEE OR ANIMAL WELFARE COMMITTEE for dealing with the animal species and the attempted trait modification. If this is not there, then however important the research or objective is, should be kept in abeyance. The Biosafety cannot overlook the ethical consideration and animal welfare.

The current level of transgenic developments in animals is generically divisible into two levels synonymous with the BL1 and BL2 levels of the plants. There are requirements for higher category of biosafety which are not with reference to the GM status of the animals, but with the hazardous products being developed from these like vaccines, sera, antibodies, etc. For our purposes restricted to the GM animals, the two categories are,

1. Containment A representing the minimum, or basic, recommended level of containment consistent with good practice.
2. Containment B represents a higher category of containment.

GM Animal Containment A

Containment A is recommended for GM animals which exhibit any of the following traits or properties:

- they are incapable of surviving in the environment in the location or
- they have limited ability to transfer genetic material to local animal species, or
- female farm animals which are easily recalled, for example transgenic sheep, or
- the genetic modification does not increase the level of risk to human health or the
- environment above that of the non-modified parental organisms;

  and
- the animals have not been inoculated with GMMs or other pathogens.

In all cases, Containment A is only suitable if the risk assessment, taking into account the animal, modification, activity and containment, is shown to be low or effectively zero. Examples of the types of GM animals for which this containment is appropriate are likely to include "knockout" mice; "nude" mice; tropical fish that are unlikely to survive or large mammals expressing pharmacologically active proteins in their milk, etc.

The recommended procedures to be followed in Containment A

The following procedures and containment are recommended as minimum standards of good practice. They will need to be supplemented by measures for specific animal types (Annex I). The specific measures must be chosen in accordance with the risk assessment.

Minimum/baseline measures for Containment A

- The containment areas for vertebrates should be in accordance with the Animal Ethics Committee or Animal Welfare Committee.
- Animals should be kept in appropriate containment, such as in animal rooms, or securely fenced areas, to minimize the possibility of accidental escape or theft as per the animal type and risk involved with the trait.
- All potential routes of escape should be identified, and appropriate measures put in place to prevent egress. Mesh covering should be used to cover drains. The mesh size should be suitable to prevent the smallest animals escaping.
- The containment area should be kept locked, where appropriate, and monitored at frequent intervals.
- Animal barriers should be placed on exits from animal rooms to corridor areas when rooms or cages are being cleaned.
- A barrier should separate male and female animals, unless reproductive studies are part of the experiment, or unless other measures are taken to avoid sexual reproduction. Animals should be separated as soon as possible after weaning. Where it is difficult to determine the sex of an animal until sexual maturity, this should be carried out as early as possible. The use of reproductive incapacitation such as induction of triploidy in fish, may be used and if so must be covered in the risk assessment.
- A written record should be maintained of the experimental use and disposal of each animal or group of animals. The permanent marking of GM animals may be appropriate (depending on size), or alternatively the cages should be clearly labeled. For work with vertebrates, records required to be kept as part of a Home Office license should provide sufficient detail.
- Animals should be transported to and from the facility in appropriate animal containers.
- Access to the containment facility should be restricted.
- A set of local rules should be produced, and these should be read by all staff using the facility.
- Staff should be given appropriate training and instruction on the procedures to be carried out.

GM Animal Containment B

Containment B is recommended for GM animals which have any of the following characteristics:

- the animals could cause harm to humans or the environment if they escaped from the containment facility, **and** they have the ability to transfer novel genetic materials to local animal species,

  or

- the animals could establish outside of the containment facility,

  or

- the genetic modification increases the level of risk to human health or the environment above that of the non-modified parental organisms;

  and

- the animals have not been inoculated with GMMs or other pathogens.

In all cases Containment B must be used when Containment A is insufficient to reduce all risks to low or effectively zero.

The following procedures and containment are recommended standards of good practice and should be applied in addition to the provisions of Animal Containment A. They will need to be supplemented by measures for specific animal types (Annex I).

In many cases the measures will be a more rigorous implementation of the requirements for Containment A, such as additional barriers, or more tightly controlled access. In addition to the measures required for Containment A, the following procedures should be applied:

- Where small animals are being kept, floor drains should be avoided if possible. For older animal houses this may not be practicable, and double mesh barriers on drains should be used. These should be checked on a regular basis.
- Written operating procedures should be produced for all routine operations carried out within the facility.
- Staff should be given appropriate training and instructions on the procedures to be carried out, and written records of training should be kept.
- Written records of any accidents or escapes from cages or primary containment should be kept.
- The containment area must be kept locked, and access tightly restricted. Security measures must be implemented to prevent theft or intentional release of animals through vandalism. Regular security patrols, and/or the use of closed circuit television may be appropriate.
- Discharge of water from tanks holding aquatic animals must not be direct to drain, but should pass through several filters. Regular checks should be made to ensure that filters

are kept clean. If discharge into rivers or the marine environment is considered, additional protective measures should be implemented.

General features to be monitored for their GM compliance of the building

The facility should be lockable and isolated generally from other area of human and animal inhabitation.

There should be separate rooms for each of the following: staff offices, staff tea room, staff showers and toilets, animal housing, manipulative procedures (injection, bleeding, surgery, testing, and euthanasia), food storage rooms and quarantine. Separate areas should also be provided for cage cleaning/washing and garbage handling/storage. There should be separate delivery access and lifts to avoid mixing human and animal traffic. All restricted areas should be clearly signposted. Doors should have vision panels.

All surfaces should be impervious, including wall/ floor/ceiling surfaces. Rodents and wild birds should not have access. False ceilings are not recommended as they create a haven for cockroaches. Walls should be smooth or rendered to facilitate cleaning. Hand basins should be provided in a convenient location. Drainage should be adequate and in accordance with treatment for the effluents, waste materials and fecal discharge.

A 'contamination barrier' should be provided at the entrance/exit to the animal house. This area should include shower and laundry (footbaths are not considered sufficiently effective) and clothing storage space.

The pressure gradient at which animal rooms are ventilated has implications for human and animal health. Where possible, non-animal areas (e.g. corridors) should not receive contaminated air from animal areas. A negative pressure gradient inside animal rooms will minimise this but may compromise animal health. On the other hand, specific pathogen free rooms and operating theatres should have positive pressure to avoid contaminants entering these rooms. Users of animal houses should be consulted to determine the pressure gradients required for each area. Where practicable, exhaust ducts should be close to floor level where the concentration of animal residue is highest. The exhaust air should be pre-filtered, then subjected to HEPA (high efficiency particle arresting) filtration, before being discharged. Rooms which do not have HEPA filtration should not be used for potentially infected animals until filtration has been upgraded. Exhaust ducts for effluent air should have removable grills so that they can be maintained free of fur and other particulates. Exhaust ducts should not be placed in populated or enclosed areas, such as a frequently used footpath or courtyard (IT IS THE DUTY OF THE MONITORING AGENCY TO BE AWARE OF THESE INDICATORS AND DOCUMENT THEM AT EVERY INSPECTION OF THE FACILITY).

Local exhaust ventilation must be provided in operating theatres to exhaust anaesthetic gases at the source of generation. Local exhaust ventilation should also be provided in areas

where there is a high production of dusts, aerosols, etc. (e.g. emptying feed bags, cleaning cages, handling sawdust). Where this is not practicable a charcoal filter mask or airstream helmet should be used. In some cases, e.g. handling sawdust, both exhaust ventilation and respiratory equipment may be required. Work with animals involving volatile anaesthetics, particularly in an open system, should be done in a fume cupboard, or using local exhaust ventilation. Where practicable, vapouriser-style anaesthetic apparatus should be used or anaesthetic machines should be fitted with scavenging units. Beakers of soaked cotton wool are not appropriate. A reduction in stock density also reduces levels of airborne allergens and exposure to animal byproducts

Annex 1 - Additional Recommendations For Containment Of Specific GM Animal Types

a. Small mammals (rodents e.g. mice etc)

- Animals should be kept in appropriate cages. Cage sizes and minimum space requirements should be in accordance with Animal Ethics Committee. Cleaning procedures should be instituted which minimize the likelihood of escape.
- Floor drains and low level ventilation should be avoided, or made escape proof through the use of wire mesh or similar barriers.
- Appropriate rodent traps should be used.
- All animals should be tagged or marked, to allow individuals to be identified. Cages should be clearly marked.
- Experimental procedures, such as the administration of drugs and the bleeding of animals should be carried out in a way that minimizes chance of escape.

b. Large mammals

- Animals should be kept in appropriate pens or fenced areas. Double fencing may be appropriate, dependent on the level of risk.
- Where the fenced area is in a remote location, or some distance from the main buildings, security measures should be taken to prevent theft or vandalism. Regular monitoring of the perimeter fencing, and/or the use of closed circuit television may be appropriate.
- In the case of animals that might burrow, the fencing should go down to a sufficient depth to minimize or prevent escape.
- Fencing should be of sufficient height and mesh size to prevent egress.
- Written records should be kept for all animals, including births, deaths and movement to the incinerator.

c. Aquatic animals

- Fish, including developing fertilized eggs should be kept in appropriate tanks. Tanks should have filters of sufficient mesh size to retain the smallest organism likely to be

present. At the early stage of embryo development, the mesh size should be determined through knowledge of the variation in egg size.

- Secondary filters should be used to retain any eggs that may be detached from the hatching trays and also to prevent escape of fish throughout the life cycle; the number of secondary filters required will depend on the risk assessment.
- A cleaning regime should be instituted to prevent filter clogging. Filters should be checked regularly, particularly if water supply is from a river, or the sea, to prevent build up of algae or other material. Consideration should be given to the (sand) filtration of supply if build up of algae is identified as a major problem.
- High water alarms may be required depending on the risk assessment. It is likely that low water alarms may be used to protect stocks.
- A Secondary containment may be required in case of overflow or rupture of vessels or tanks. Rupture or other damage may occur where people have to enter the tank to clean filters. Such procedures should be avoided where possible - for example, the use of long handled brushes may provide an alternative.
- The containment area may need to be bunded to prevent overflow to outside. This would depend on the risk assessment and geographical considerations, such as proximity to watercourses or sea. (In several countries, GM fish cannot be handled in any other water bodies than those which are completely land locked within a limit of less than 10 X 10 m with sub tanks for each event separation)
- Consideration should be given to the use of high water alarms at floor level, so that flooding is detected early. The alarm system should be audible and visible throughout the site and operate 24 hours a day.
- The use of a "soak away" outside the facility should be considered if the facility is close to a river or the sea. Such an area may consist of a graveled area which allows water to soak through whilst retaining any escaped fish on the surface.
- Where an outlet pipe from a contained facility discharges directly into a river or the sea, extra care will need to be taken. Consideration should be given to the use of a filter barrier on the pipe into the final holding tank. An electrical kill system typical of the kind used in the aquaculture industry may provide a suitable final barrier before final discharge.

c. Invertebrates

- Appropriate cages or culture vessels should be used.
- The use of secondary containment measures, such as muslin "tents" should be considered if indicated by the risk assessment.
- Measures should be taken to enable escaped invertebrates to be detected and recaptured or destroyed. For ticks and mites, containers should be kept over trays of oil.

- All experimental cages/pens must be numbered and documented.
- Used culture vessels must be decontaminated before disposal or thoroughly cleaned before re-use.
- Flying or crawling arthropods should be handled on white trays to facilitate the detection of escape.
- The use of an electric insect control unit should be considered. - The activity of the arthropod and the risk of accidental escape can be reduced by chilling, or keeping cages in cold rooms. The use of chilled corridors surrounding an insectory also helps prevent egress.

Important

Till a larger body of information is made available, the GM animals should not be used for other hazardous of infectious disease related vaccine production activities. The monitoring of this activity has to be locally devised by physically marking, segregating and labeling individual animals so that there is no risk of a GM based protein product or serum gets into a disease or industry or food processing industry.

- In the event of a GM animal developing an accidental infection, its treatment should be done under isolation within the containment
- In the event of death of a GM animal due to unnatural causes, the autopsy has to be carried out in a BL3 level containment as done in the case of diseased animals which are examined through autopsy.

This means that at least ONE BL3 level animal room is required when dealing with large animals irrespective of the containment level of the GM animal of A or B.

*[**Source**: abstracted from the ACGM Compendium of Guidance, March 2000 issue AND Guidelines to Animal Containment Facilities (revised),OH & S Unit, University of Queensland, Australia. 2003*]

Monitoring Field Trials of GM Plants

Issues in transgenic plant experiments and methods for proceeding step by step (Ghosh, 2002)

The issues that are taken into consideration before authorizing field trials under contained conditions using GM plants include the potential of the transgenic plants for dissemination into the open environment such as through cross pollination, the dispersal mechanism of the pollens as well as the seeds, the presence of wild members of the species in the eco-system and the presence of other non-transgenic planting materials in the vicinity. While designing field experiments efforts are made to maintain appropriate reproductive isolation so as to prevent the likely-hood of seed setting outside the experimental plot. The transgenic plants are isolated from the gene pool represented by sexually compatible plants to prevent the escape of transgenes. Conditions are also introduced in certain cases to prevent flowering of plants.

It is ensured that the genes or the genetically modified plants are not released into the environment beyond the experimental sites. Only such plants are taken into the open environment for experimentation, which have the minimum chance of unintended and uncontrolled adverse affects. The time of sowing, flowering and planting are also taken note of. Only those plants have been used in Indian trials for open field experiments under contained conditions, where the transgenes are considered to be safe or where the pollens are linked with imparting male sterility properties. Experiments have also been designed to study the potential for gene transfer and the consequence of transferring transgenic properties to weeds or other near relatives. The probability of pollen transfer and the natural mutation rate have been made conditions for computation in the experimental designs. The transgenic traits that have been looked at in such experiments in India include Bt-insect resistance, Bar resistance, Bar-barnace as well as Bar-barstar systems, Bar-Bt systems, antibiotic resistance, altered nutritional properties and abiotic stress resistance properties. As indicated earlier, data for submission by the applicants include mating systems in plants comparison of germination rate, invasiveness, toxicity and allergenicity or alterations in the anti-nutritional properties of the plants due to the transgenes including the marker genes etc.

The monitoring of the field trial where there should be no cause for any gene spread to higher organisms, the soil and airborne pests or organisms might be influenced. This factor should be kept in mind while recording data from a field trial release while monitoring.

The Indian model : Considering its topography and agroecological climate, the Indian model for field testing may be appropriate with modifications that suit case by case requirement of the concerned transgenic. In India, a detailed format for submitting information has been devised comprising nine chapters, and applicants are required to provide such information to the Government of India seeking permission for commercial release of target transgenic plants under Rules 7,8,9,10 or 11 of the above Notification (Enclosed separately). A few experimental designs have been evolved and approved by the RCGM for conducting trials using GM plants in the open environment.

The designs are for monitoring and studying pollen dispersal, the comparison of cross-ability of non-transgenic plants with the transgenic is and evaluation of their comparative competitiveness or invasiveness potential in unmanaged and managed land. The experimental result from two studies has shown that the pollen escape was real phenomenon. The cross-ability studies conducted for example on transgenic Indian mustard has shown that their existed pre and post fertilization barriers and the results corroborated the classical literature, confirming that escape of transgenes from same crops like the Indian mustard crop was not favoured in nature; however, viable F1 seeds could be produced by manual cross-pollination with related cultivated as well as wild species, which observation was consistent with similar studies made with Brassica napus. It was observed, while studying the Bt. Cotton plants that their pollen also traveled to some distance with the help of insects. It can be stated from these that gene transfer shall be taking place in open environment when transgenic plants are

cultivated. By appropriate management practices it might be possible to reduce the extent of pollen transfer into the open environment for all crops, but it cannot be fully contained. Therefore, the consequence of gene transfer is an issue, which is real. The implications of this issue have not yet been satisfactory resolved. A decision has to be taken by the Indian Government on this to decide to what extent transgene flow can be allowed and what are the consequential risks, taking also into consideration the agronomic benefits expected from the use of transgenic plants. In March, 2002, the Indian Government finalized its decision on the commercialization of insect resistant Bt. Cotton containing Cry 1 Ac gene. Three cotton hybrids containing the gene were approved for commercial cultivation in India, subject to certain conditions. The conditions were worked out based on the experimental results of Bt cotton, conducted in India. These have been discussed in detail later on. In addition to these experiments, major chunks of data are required to be generated on food safety in accordance with the latest Indian guidelines. The information emphasizes quantitative production of transgenic proteins and their effects on as-is-where-is basis on experimental animals in the context of determining the toxicity allergenicity and anitnutritional properties etc., The data generated in Indian experiments for Bt cotton at Industrial Toxicology Research Centre, Lucknow using goat as the ruminant modes, and for transgenic Indian mustard assessed on rat, rabbit, guinea pig and hen model (at Shriram Industrial Research, Delhi) as well as on goats model (at Fredrick Institute of Plant Protection and Toxicology, Tamilnadu) did not show any additional food safety risks.

The transgenic field experiments conducted in India has enabled the country to have hands on experience on several genetically modified plants. Most important among them are transgenic Bt cotton, Bar-Barnace and Bar-Barstar mustard and Bt tomato. Data generated in India has demonstrated substantial agronomic benefits from transgenic plants over the corresponding non-transgenic controls.

Monitoring and evaluation mechanisms for green house / net house experiments and limited field trials in the open environment in India

The RCGM can bring out manuals of Guidelines specifying procedures for regulatory process with respect to activities involving genetically engineered organisms in research and applications to ensure environmental safety. To monitor, over a period of time, the impact of transgenic plants on the environment, a special Monitoring cum Evaluation Committee of the following constitution will be set up by the RCGM. The Committee shall have the following constitution.

a) Chairman of the Committee : Secretary, DBT & Secretary, DARE shall jointly discuss and elect a leader of the Committee.
b) Eminent Plant Biotechnologists 3-4 scientists nominated by ICAR
c) Seed Technologists 2-3 scientists nominated by ICAR

d) Plant Breeders 2-3 scientists nominated by ICAR

e) Plant Ecologist/Environmentalist 2-3 scientists nominated by GEAC

f) Nominee of NBPGR

g) Nominee of MoE&F

h) Member-Secretary nominated by RCGM

This committee will undertake field visits at the experimental site/s. The committee shall be guided by the RCGM on the design of field experiments and on the preparation of formats for collecting scientific information on plants in green house / net hoi.3se conditions as well as in limited field trials. Based on the on-the-spot situation the committee can suggest remedial measures to adjust the original trial design and assist the RCGM in collecting, consolidating and analyzing the field data for evaluating the environmental risks emanating from the transgenic plants. This committee shall also collect or cause to collect the information on the comparative agronomic advantages of the transgenic plants. From time to time, the committee shall advise the RCGM on the risks and benefits from the use of the transgenic plants put into evaluation. Trials will be done for at least one year with minimum four replications and ten locations in the agroecological zone for which the material is intended. The biological advantage of transgenics will have to be clearly enumerated by the applicant, the Institution, the University or the Industry. The latter would recommend those transgenics, which would be found to be environmentally safe and economically viable by the RCGM, to the Genetic Engineering Approval Committee for consideration for release into the environment

Biosafety aspects of the transgenic plants

Field experiments are designed to systematically identify the hazards, to access to risks and to take step to manage the risks by applying logically valid strategies, to systematically identify the hazards and to assess the risks; the information on the following aspects would be required to be generated.

I. Characteristics of the donor organisms providing the target nucleic acids. These may include the following which can be observed during the field trial

a. Name of the donor organisms with its identification characteristics with relevant reference to published information if any.

b. Pathogenicity and toxicity characteristics to plants and animals.

c. Allergenicity characteristics to human along with of the allergenic substances, wherever possible.

d. The geographical origin of the organism, its distribution pattern and survival mechanism.

e. The method of transfer of its genetic materials to other organisms, especially with lower organisms, insect pests, and microbes such as beneficial microbes should be assessed. Organized samples of microbe sampling can be done and analyzed for the presence of

the gene through one of the tests mentioned in the Chapter on monitoring microorganisms

f. Any odd behavior shown the GM towards known responses to both biotic factors and abiotic factors.

II. Characteristics of the vectors used: These may include the following,

a. The origin, identity and habitat of the vectors used.
b. The sequence, frequency of mobilization, specificity and marker genes if any, present in the vectors.
c. The abilities of the vectors to get established in other hosts; the hosts are also to be specified (relevant with reference to the details on I (d) above).

III. Characteristics of the transgenic inserts : These may include the following,

a. the specific functions coded by the inserted nucleic stretches including the marker gene inserts.
b. The expression of the nucleic acid products and their activities/properties.
c. The toxicity of the expression products on the host plant, if any.
d. The toxicity and allergenicity of the nucleic acid products to human and animals.

IV. Characteristics of the transgenic plants : These may include the following,

a. Methods of detection of the transgenic plant in the environment.
b. Methods of detection and characterization of the escaped transgenic traits in the environment.
c. Toxicity and pathogenicity of the transgenic plants and their fruits to other plants in the ecosystem and the environment.
d. Possibility of and the extent of transgenic pollen escape and pollen transfer to wild near relatives, and the consequences to the environment despite the isolation provided for which random seed samples from single plant, single seed to be collected and progeny analyzed through molecular tests.
e. Pathogenicity, toxicity and allergenicity of the transgenic plants and their fruits to human and animals.

Information on many of the above questions may already be available. Many questions may however be required to be investigated and answers found out, for which appropriate new experiments would have to be designed to gather data. For generating toxicity and allergenicity data, standard protocols devised by international agencies could be used. The Indian national toxicological laboratory like the Industrial Toxicology Research Centre, Lucknow could be consulted to generate appropriate protocol for these purposes.

For minimizing the risk arising from the limited release of transgenic plants, the following may be taken into consideration :

a. Special separation for isolation, for preventing reproduction/fertilization and seed setting.
b. Biological prevention of flowing by making use of sterility properties etc.
c. Human intervention for the removal of reproductive structures of flowers.
d. Controlling the reproductive structures of transgenic plants like the seeds and the plant propagules from unaccounted spread.
e. Controlling and destroying volunteer plants from the experimental field.
f. To take into account the proximity to human activity in case the transgenic plants have allergenic properties to human and animals.
g. Appropriate training of field personnel responsible for handling the transgenic plants.
h. Plans for handling unexpected events.
i. Documentation of previous published information, if any, including any documented evidence of effects of release to ecosystem.

Thorough comparison with national checks for productivity and susceptibility/resistance to biotic and abiotic stresses will have to be made.

Experimental designs used in the field trial and their analysis

The experiment has to be properly designed in the field to evaluate the potential addition to the value of the variety over its non-transformed type. The data should be analyzed in replicated design with statistical analysis and quantified expression of the trait. Any deviation from the approved design should not be considered unless approved by the MEC/RCGM.

All the information as above are to be available in the form of a document which would be called the registration document. An outline of the ingredients of a registration document is available at the web site : http;//www.dbtindia.nic.in

Post-harvest treatment of the site

It is important for the monitoring agency to be satisfied with the post harvest treatment of the left over debris after the economic product harvest of the field trial. What was the type of the waste and how was it treated to eliminate any spread of the volunteers is to be ensured.

Summary of monitoring indicators in a limited field trial , an example

1. *Conduct of the trial :* Is the trial conducted as per the approved field design with the replications and plot size mentioned ?

2. *Isolation:* Is the isolation distance of 50 mts (as in a oilseed mustard crop) around the experimental area maintained with no related species or varieties of the same species in the area?
3. *Toxicity/allergenicity data :* Is the impact of the transgene product for its likelihood of causing any allergies or toxicity symptoms in lab animals as well as any adverse effects on their immune system analyzed ? (If no, the second year trial cannot be conducted on multiple locations). For this purpose, the guidelines are explicit with reference to the kinds of allergies to be tested depending on the kind of gene product (whether part of food chain or otherwise) and toxicity data with reference to consumption of the product (DBT guidelines, 2003 modified, http//:www.dbtindia.nic.in)
4. Are three photographs clearly documenting the isolation of the crop right through planting to harvesting and post harvest management of crop debris appended with the results? If no, again the experiment cannot be taken for another year under multiplication during which period, till the next season, an evaluation of the possibility of volunteers left behind is to be done in the original land.
5. Safe storage of harvested seed and salvaging any spill in the field : Is sufficient care taken to harvest as much of seed as possible and no seed is spilled and left behind/ What measures were taken?
6. *Maintenance of field data :* Entire experimental data are to be maintained and recorded with the monitoring agency.
7. Are all the safety guidelines with respect to the personnel working with the experimenters taken care of?
8. Were any accidents encountered? How was the IBSC and accident emergency attended to by the concerned authorities designated for the purpose by the Institute ? Appropriate proof must be attached in the event of an accident.
9. Were the concerned state government authorities informed of the trial?
10. *Maintenance of the logbook:* The experiment conducted in a fenced area maintained under lock was to be allowed for entry only to the listed persons mentioned in the permit letter or their representatives sufficiently well informed. Was each entry visibly maintained each day when ever there was a field activity?
11. Was any unknown pest, insect or pathogen harmful or otherwise noted on the transgenic crop ? If so, was it brought to the notice of IBSC? What was the action taken about the observation?

Monitoring Commercial Release GM Plants

There is a striking difference between the field trial referred above under confinement or limited conditions and the commercial release of the GM crop in the basic purposes behind the release. The first one is to primarily assess if the material can be actually released into

environment safely and beneficially for the utility of the embedded transgene in the variety. The latter one is purely for commercial viability of the material. While the former one is collectively not going to cover an area like 10 acres at the maximum under isolation, the latter will potentially grown freely in 100s of acres. The former is with an isolation from its own kind of species as well as other related weed species., the latter one is completely free to be grown beside the same crop grown to a normal variety in the open. Therefore, the concerns are different, the impact is different as much as the likely long term effects on the ecology and environment in which a large area is grown. Some concerns which have to be definitely viewed with reference to the gene, crop, geographic region and area of cultivation in a holistic manner specific to the situation than with a general view point. However, there are certain common concerns which occur in varying degrees that need to be addressed while considering a commercial release of the GM.

a. *Gene dispersal and persistence* *:* The ecological spread of the transgene is accomplished by horizontal transfer, dispersal of whole organisms and into new environments and organisms. The persistence of the transgenes depends largely on how these affect the organism's evolutionary fitness. Gene dispersal can occur actively from pollen,. seeds and propagules. Passive dispersal can occur through trade transit, spillage during and after harvest while in transport. Hundreds of invasive species have successfully colonized new regions after unintentional or deliberate dispersal over thousands of miles (Mack and Eisenberg, 2002). Gamete dispersal provides an opportunity for sexual transfer of the transgenes to wild or domesticated relatives as has been observed in the past (BANR and BLS, 2004).

Generally, if an allele confers a fitness advantage to the individual possessing it, it is expected to increase in frequency while, if it is detrimental or competitively less superior to the alternate wild type allele, it is bound to get extinct over a period of time, provided there is no recurrent immigration of the new allele. Experimental field studies have shown drastically different fitness impacts due to different pest resistance transgenes (Burke and Rieseberg, 2003 and Snow et al 2003.

b. *Weediness or invasiveness* *:* This is the most publicized concern in transgene spread phenomenon. The weediness or invasiveness is expected to be a result of sexual transfer of crop alleles to wild relatives. This is not exclusive to transgenes alone, though. Sometimes, the transgenic plant itself may become an environmental problem if the traits expressed altered its performance such that it becomes an invasive or nuisance species. The factors that limit invasiveness of populations are still to be understood clearly for theorization and conclusive evidence.

c. *Extinction of wild taxa* *.:* Due to overwhelming growth of one crop, other related taxa are rendered near extinct which again is not a new phenomenon to traditionally developed new plant types which when introduced tend to eliminate the local land races.

d. *Gene flow to other domesticated organisms* *:* There could be gene flow among transgenics for different genes causing a transgenic stacking that turns a transgenic into double or triple gene line for transgenes (Hall et al. 2000).

e. ***Effect on non-target species****:* Another major concern voiced by ecologists and evolutionists where there are varied interpretations of Bt corn causing lethality to monarch butterfly (Losey et al. 1999 and Sears et al. 2001).

f. ***Delaying the evolution of resistance:*** Although the evolution of resistance is a continuous process, the evolution of resistant pest to the transgene is considered potentially a greater hazard to environment over the normal evolution in nature because more environmentally damaging alternative treatments would be needed for continued control of the broken down resistance.

g. ***Detection of the transgenes from other samples:*** The use of molecular tools such as PCR or ELISA to detect either the gene or protein in all the potential recipients is a quick strategy with appropriate population genetical inputs to generalize the spread.

The measures to reduce in-field contamination have to be clearly emphasized by the seed marketers to the farmers because more often than not, the contamination is from seed impurity of the GM seed that contaminates a normal seed where harvest handling and post harvest handling is done in large-scale by unattended machines. Some of the techniques that minimize the spread through contamination are,

a. efficient weed control by herbicides

b. decreasing the importance of farm-saved seed

c. managing populations in field borders

d. Fallow

e. Isolation in seed production plots

f. lengthening the rotation by introducing a spring crop in which the volunteers cannot flower

g. Staggering the sowing of GM and non-GM by sharing the information among neighbor farm units

In the case of biotic stress resistances such as insect or bacterial resistance, there is a necessity to use refugia, which is recommended to be 20% of the total GM in a given area. This is to be monitored if the commercial farm producer has followed it and the seed marketer has stipulated the same.

The specific factors to consider when assessing /monitoring potential hazards posed by GM plants released in an environment commercially :

1. Purpose of the release
2. Site of the release, if it was different from the natural habitat of the crop
3. Geographical location : a. Release area proposed, b. Actual area where released
4. Proximity of the release area to recognized protected areas such as water reservoirs, bioparks, and sanctuaries. Any reported variation within an year of the release.

5. Adverse effects on plants:
   - direct toxicity to other plants (e.g. root exudates)
   - increased weediness characteristics in the agricultural environment (e.g. increased number of small potato tubers - problems with volunteers in subsequent years)
   - increased invasiveness of natural habitats is exhibiting selective advantage under specific selection pressures (e.g. invasion of salt marsh SSSI due to increased salt tolerance)
   - altered susceptibility to pests and disease, thereby providing new reservoirs for pests and diseases with potentially altered dissemination routes (e.g. by inactivation of endogenous resistance genes)
   - creation of novel pathogens in the modified plant (e.g. virus recombination/t ranscapsidation etc)
6. Adverse effects on animals (e.g. herbivores, pests and predators of pests):
   - direct toxicity to herbivores/plant pests
   - indirect toxicity effects on predators of herbivores/plant pests (i.e. non-target effects)
   - increased allergenicity/toxicity of plant pollen/plant sap
7. Adverse effects on humans (both those working with, coming into contact with and in the vicinity of the receiving environment):
   - increased allergenicity/toxicity of plant sap (assuming people will not be eating plants)
   - increased allergenicity/toxicity of plant pollen
8. Other adverse effects on the environment
   - effect on biogeochemical cycling from decaying GM plant material (e.g. Nitrogen cycling)
   - effect on soil flora and fauna from decaying GM plant material
   - effect on soil micro-organisms from expression of inserted genes
   - altered management practices/compromised plant protection strategies
   - negative economic effects of transgene contamination of organic crops/non-GM crops for sale to non-GM markets
9. Potential for transfer of genetic material between the GMO and other organisms

Once the hazard assessments have been made for the GM plant being modified, assess more indirect/delayed hazards caused by the capacity of GM plant to transfer transgenes to other organisms in the receiving environment e.g. by pollen transfer to sexually compatible crop species/sexually compatible wild relatives or by horizontal gene transfer to other organisms via micro-organisms in the soil rhizosphere. If such gene transfer is likely to occur, revisit the hazards specified above for the recipients of the transgene.

Table: Some examples of wild species and crops where introgressive hybridization may be important for gene transfer of herbicide resistance

Crop	Weed species
barley (*Hordeum vulgare* L.)	wild barleys (*Hordeum* spp.)
canola (*Brassica napus* L.)	numerous wild mustards
carrot (*Daucus carota* L.)	wild carrot (*Daucus carota* L.)
corn (*Zea mays* L.)	teosinte (*Zea mays* spp. *mexicana*)
foxtail millet (*Setaria italica* L.)	green foxtail (*Setaria viridis* L.)
poplar (*Populus* spp.)	cottonwood (*Populus* spp.)
lettuce (*Lactuca sativa* L.)	prickly lettuce (*Lactuca serriola* L.)
oat (*Avena sativa* L.)	wild oat (*Avena fatua* L.)
radish (*Raphanus sativus* L.)	wild radish (*Raphanus sativus* L.)
rice (*Oryza sativa* L.)	red rice (*Oryza sativa* L.)
sorghum (*Sorghum bicolor* (L.)	johnsongrass (*Sorghum hulepense* (L.) Pers)
squash/pumpkin (*Curcbita* spp.)	wild cucurbit species (*Cucubita* spp.)
sugarbeet (*Beta vulgaris*. vulgaris)	wild beet (*Beta vulgaris* spp. *maritima*)
sunflower (*Helianthus annuus* L.)	wild sunflower species (*Helianthussp.)*
wheat (*Trielcum aestivum* L.)	jointed goatgrass (*Aegilops cylindrica* Host)

Beyond the opportunity for gene flow, the consequence of that gene flow must be evaluated. Hybrids are known to occur between the crop and the weed species; however, not all hybrids are fertile. Some hybrids are fertile and display hybrid

10. Protocols of field trials performed in the previous years with the genetically modified and control crops must be specified and documented with respect to:

Agronomic traits

Compositional analysis represents a key component of the risk assessment process. However, unintended effects may also manifest themselves through, for example, changes in susceptibility to important pests and diseases, through morphological and developmental changes or through modified responses to agronomic and crop management regimes.

Environmental risk assessment

Environmental risk assessments are carried out on a case-by-case basis taking into account the biology of the recipient plant, the characteristics of the introduced genetic material, the properties and consequences of the genetic modification, the scale of release and

the evaluation of any risk to the receiving environment that might arise from the release of the GMO. Variable expression or even gene silencing can cause a serious threat to the economic value of the GM product. If a herbicide tolerant gene does not express due to a silencing event, spray of the herbicide would kill the transgenic plant too and cause mass destruction of the crop in the field. While this may not cause a direct environmental risk, but it may lead to a secondary level due to proliferation of saprophytes.

Examples of possible interactions between the GM plant and its environment including potential impact on other organisms are:

– effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations;
– altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;
– compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine;
– effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material.

11. ***Geographical relevance of data***

Data should be provided from field experiments in areas representative of those geographical regions where the GM plant will be grown commercially in order to reflect relevant meteorological, soil and agronomic conditions. Where data from field studies on other continents are supplied, the notifier should submit a reasoned argument that the data is applicable to specific conditions.

12. ***Impact on wild plants***

The potential consequence arising from out-crossing to compatible wild species should be considered and assessed for environmental risk. This will depend on sexually compatible plants being present and available outside the crop to receive pollen and produce fertile hybrids. Selection pressure in non-crop habitats that is required to maintain the selective advantage of any transferred trait should be identified. For example, transferred herbicide tolerance may not be an advantageous trait in habitats where the herbicide is not applied.

13. ***Impact on non-modified crops***

The potential consequence arising from out-crossing to other crop cultivars should be considered and assessed for environmental risk. This will vary with crop. For example, the release of GM oilseed rape raises the issue of gene transfer, since this crop will readily cross pollinate with nearby oilseed rape crops and may spontaneously hybridize with some wild

relatives. In cases where gene transfer cannot be prevented between certain adjacent crops of, for example, oilseed rape or maize, the risk assessment should focus on the consequences of cross pollination even at very low frequency.

14. ***Impact on organisms and ecological processes***

Risk assessments should be carried out for each of the different functional environmental compartments that are exposed to the GM plant. Whether any parts of it will remain in the environment after harvest, will depend on the specific crop and its management regime or agronomic practices. Soil fertility strongly influences the growth and productivity of plants. As rhizosphere and soil microbial communities perform the vital biotransformation that underpins soil fertility any negative impact(s) on microbial participants in this key compartment would have to be carefully evaluated. This should be assessed on a case-by-case basis with particular reference to the nature of the introduced trait and the consequences of the genetic modification/alteration in the GM plant. The risk assessment should aim to establish if direct or indirect effect(s) of the genetic modification in the GM plant have any long-term or sustainable deleterious effect on the recognized soil microbial communities and the associated functional activities that are responsible for maintaining the agronomically relevant processes of soil fertility and plant productivity. The assessment should also address the fate of any (newly) expressed substance(s) in those environmental compartments where they are introduced and which result in exposure of non-target organisms (e.g. in soil after the incorporation of plant material). Risk assessment should also include an analysis to determine if a shift occurs in populations of deleterious organisms in the presence of the modified plant. Exposure should also be estimated to soil organisms and decomposition function (e.g. earthworms, micro-organisms, leaf litter breakdown) in relation to potential transfer to soil micro-fauna and impact on degradation.

Impact should be assessed on non-target arthropods (including pollinators, beneficial and predatory arthropods), grazing birds and mammals or, if appropriate, the aquatic environment. Such studies should include laboratory, greenhouse or field exposure experiments set up in such a way that enough statistical power is obtained to be able to observe possible negative impacts on non-target organisms. This risk assessment should take account of where in the plant and to what degree the inserted genes are expressed and therefore the extent to which non-target organisms are exposed either directly or indirectly.

Data on the comparative susceptibility of the GM plant to pests and diseases compared with that of the non-modified plants are useful indicators of effects together with observations on agronomic performance during greenhouse and experimental field trials.

An assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches. The notifier should describe the appropriate commercial management regime for the crop including changes in pesticide applications, rotations and other crop protection measures where different from the

equivalent non-GM crop under representative conditions. The notifier should aim to assess the direct and indirect, immediate and delayed effects, of the management of the GM crop on all affected habitats. This should include the biodiversity within the GM crop and adjacent non-crop habitats. The necessary scale of such studies will depend on the level of risk associated with a gene in question and the impact.

15. ***Methods for monitoring the GMOs***

a. Detection of the GMO as contaminants
b. Gene movement through pollen spread
c. Ecosystem effects (other non-target organisms, plants, insects, pests)
d. Any non-targeted pest incidence assessment
e. Any report of a allergy response or toxicity effect that does not match with the previous year's data. Ideally, a second year data also should be generated from the fresh end product of the GMO on human health aspects of allergenicity and toxicity induced by large scale exposure to human beings and animals

Monitoring GMO Imports

World wide, plant quarantine is a legal enforcement measure aimed to prevent pests and pathogens from spreading or to prevent these from multiplying further, in case these have already found entry and have established in the new restricted area. The same is extended to the imported GMOs with insured biosafety measures followed while undertaking the quarantine processing of imported seeds/planting materials of germplasm of GMOs meant for release in the entered country from a foreign source. There is a need for controlled testing of transgenic planting material, in containment greenhouse prior to release to the environment so as to avoid their potential risk to environment or health. Therefore, monitoring the import, the quarantine procedure and post quarantine handling/movement of the GMOs is very crucial to regulate proper application of the GMOs and prevent any form of unintended biosafety regulation violations or oversight.

The typical steps involved in monitoring the import of GMOs

a. ***Adequate information on the nature of the transgene, its expression level in the host environment***

The importing Institution/Organization should be fully aware of the nature of the transgene, its source of origin(bacterial, animal/insect, plant), hazard/risk involved and the product of the transgene in the specific host plant material the gene is integrated.

b. ***Clearance from GM regulatory authority in the GM receiving country***

The statutory GM regulatory authority is required to clear the import proposal of the material after assessing,

- the purpose behind the import,
- the product of the transgene with reference to the targeted ecological area,
- detection methodology already employed for detecting the presence of the transgene in the host material,
- known information on regarding the toxicity/allergenicity of the transgene product
- extent of the transgene expression,
- biochemical/physiological consequence or output of the transgene product in a host material,
- the targeted host if the material is in the form of gene-construct vis-à-vis the presence of the product of the proposed transgene,
- research/commercial permit that the material is granted in the host country,
- any IPR regulations connected with the transgene or material for implied restriction of application in the importing country and
- potential utility of the transgene characteristics vis a vis target crop/crops or microbe

c. ***Award of import permit and authorized import accompanied by a phytosanitary certification***

For convenient monitoring, it is necessary that a single nodal agency (with multiple terminals in the case of geographically distributed or large countries) is authorized to award an import license by specifically documenting the existence of an already earlier import of the material with its accession number so that the quarantine unit of the importing country is simultaneously made aware of such a pending import. If there are multiple agencies authorized to award import license, documentation of the incoming material, its reference accession identity can become redundant making monitoring a difficult as well as expensive exercise. While the material is actually being imported, it is required to be accompanied with the original import permit and phytosanitary certificate on the material from the country of export.

d. ***Documentation of the national accession number after entry into the importing country***

A proper databank of all imports has to be maintained with complete information documentation in respect of the material being imported. This is necessary before the material is processed in quarantine set up for monitoring and perceived risk assessment if similar material has a history of import and quarantine processing. The perceived risks can be directly with reference to the foreign transgene or with reference to the crop (if seed or planting

material is imported) or both. The range of risk can be from suspected pests, pathogens, allergens, contamination of adventitious materials, and transgene product in the specific material if already imported earlier, etc.

Assigning specific accession number to every entry which has to be done carefully, by eliminating any duplication, if already imported earlier. An accession number to the material, then becomes the reference number of the specific material for every utilization of the material in deployment, enhancement or commercial utilization subsequently in the country of import.

e. ***Quarantine processing***

Once the material is identified with an accession number by the importing country, GM material is passed through proper quarantine filters taking into consideration the recommendation of the GM regulatory authority on the material while granting the clearance of the import proposal.

After passing through the routine quarantine processes suitable to every planting material, the material has to be necessarily grown in a containment if it is not a bacterial culture with a transgene plasmid vector for one season.

Sampling from bulk imports (ISTA standards)

The sampling procedure is designed to collect a sample that is representative of the consignment as a whole. Several facts are assumed including:

- Individual seeds are either GM or not GM
- Any GM seeds present are randomly dispersed throughout the consignment.
- The sample will be ground and analyzed as a whole, seeds will not be analyzed individually.
- The laboratory will correctly identify the presence or absence of GM material in 99% of samples.

It is required to keep a high level of confidence (95%) that the inadvertent presence of 1 GM seed in 1000 seeds will be detected. In order to achieve this a sample (weight basis) drawn from a consignment for testing must contain at least 3200 seeds (The no. of grains required to detect 0.1% contamination with 95% probability). The weight of the sample size can be calculated by multiplying the standard 100 seed weight by 32 and rounding up to the nearest 5 grams.

Testing laboratories must have validated PCR methods capable of detecting GM seed in the seed sample.

The sample will be collected using either the standard International Seed Testing Association (ISTA) or Association of Official Seed Analysts (AOSA) methodology. The ISTA methodology is summarized in the following tables:

Consignments up to 100kg:

Number of bags or containers per consignment	1-4	5-8	9-15	16-30	31-59	more
Number of sub-samples	3 from each bag or container	2 from each bag or container	1 from each bag or container	15 total each bag or container	20 total each bag or container	30 total each bag or container

Consignments exceeding 100kg:

Weight of line	100-500 kg	501-3,000 kg	3001-20,000 kg	20,001 kg & above
Number of sub-samples	5	1 per 300kg but not less than 5	1 per 500kg but not less than 10	1 per 700kg but not less than 40

Combine the sub-samples evenly to form one uniform collection then reduce it to get a sample

In the case of research samples, where there may not be more than 50 seeds, it would be most desirable not to sample but to grow the plants and have leaf sample of equal tissue from all the plants bulked together to collect one bulk sample

During the contained growth, the GM material is subjected to

- detection of the transgene that it is documented to be carrying (as per the regulatory authority clearance),
- tested for any obvious non-target trait expression of unusual or hazardous nature including harboring seed borne pathological indications
- harvested seed tested for genetic use restriction technologies such as terminator gene and
- analyzed for disease free phytosanitary aspects before it is released to the indenter institution.

f. Storage of each accession imported

It is desirous to develop a “gene bank” with a facility to keep planting material or microbe being imported in a long term storage condition as a referral sample of the material. The sample should be both as planting material and referral DNA of the transgene as extracted from the imported material.

Post-quarantine handling and import monitoring of the GMO

The monitoring agency is required to review the import both at the site of import during its quarantine and post-quarantine handling by the indenting institution. The indicators for monitoring based on the set of information made available would be to assess,

a. The imported material permitted for its legal entry
b. The accompanying phytosanitary certification from the source country
c. Detection of the transgene in the original material imported during quarantine
d. Evaluation of the imported material for quarantine processes under containment for at least one season
e. Progeny analysis for the imported material to possess any known genetic use restricting technologies like the presence of the terminator gene
f. Marker genes the material is known to possess as a source of information for consideration during its subsequent release and commercialization
g. Handling of the material and status of the quarantine containment facility during its contained growth
h. Documentation and maintenance of the DNA from the imported material with reference to the transgene detected as the national referral sample. This referral sample would be of immense utility in the post-release monitoring of the imported material subsequently.
i. Post-quarantine storage material under medium and long term storage

An example of monitoring of imported GM materials in India:

In India, the import of GMOs is done on a single window entry of all imported GM materials through a National containment/Quarantine facility for transgenic planting material at the National Bureau of Plant Genetic Resources (NBPGR, New Delhi). The main objectives of the National Facility are: -

1. Facilitating the processing of the transgenic material from quarantine aspects.
2. Development of probes/markers as required for the containment and for evaluationof the transgenic planting material.
3. Training of the Human Resource in the area.

Presently, the existing quarantine facilities, glasshouses and molecular biology laboratories of NBPGR and National Research Centre on DNA Fingerprinting are being utilized for testing of the transgenic planting material. In addition, a medium term storage module of the NBPGR-National Gene Bank has been allocated for storing the transgenic planting material. An environmentally safe containment facility meeting the necessary Biosafety regulations of BL-3 level is in place for the purpose of testing and quarantine of transgenic planting material has been finalized in consultation with various national and overseas experts. The containment facility (app. 300 sq. mt. area) is completely sealed to

eliminate the ingress or egress of pathogens/pollens or viable plant materials. In this facility in four bays with controlled atmosphere: air temperature 20 -30 ˚C (+/- 1 ˚C) and relative humidity 50-80% (+/- 5%) for pot cultures and one of the bays with ground soil-based plants growing systems are designed. Provision has also been kept for four chambers for undertaking molecular work of the transgenic material with controlled conditions

For developing molecular probes/markers required for evaluation and molecular characterization of transgenic material, methods for DNA extraction, quantification of DNA, polymerase chain reactions (Pars) etc. have been standardized. Extracted DNA of all the transgenic lines grown in the greenhouse will be kept as referral sample and will be used for undertaking different experiments.

The monitoring processes undertaken by the Monitoring and Evaluation Committee of the RCGM involves

a. Periodic analysis of report of the Institutional Biosafety Committee on all imported GMOs (The IBSC of the NBPGR meets once in three months to monitor the activities of quarantine handling of already licensed imports cleared by the RCGM)
b. Evaluation of the documentation procedure of the import license of every GMO imported and its subsequent reporting to the regulatory authority
c. Inspection of the quarantine handling of the GMOs during quarantine
d. Inspection of the post-quarantine handling of the material (seed and molecular probe sample maintenance)
e. Inspection of the preparedness for any accidents/ physical facility failure/unintended accidental release
f. Feed-back monitoring system of the utility handling of the GMO by the indenter
g. Inactivation of spills and ensured destruction of the GMO debris as per perceived risks and adherence to the treatment protocol for all effluents and growth media materials in the containment.
i. The RCGM seeks information before granting a clearance to the indenter for import of the material with documentary evidence regarding the known risks, benefits, expression levels of the transgene material, hazard to environment and human health in the country of origin before granting the clearance. The composition of the RCGM, as explained earlier, therefore involves competent scientists in the concerned areas of interest from genetic, molecular biological, ecological and environmental aspects.

   Ref: http://www.nbpgr.nic.in for more detailed information

Information as an Essential Component of Post-Release Monitoring

The important aspect of developing a strategy or a program of a PRM in an environment is the availability of information on the GMO in question and the target environment. Although a universal mode of monitoring protocol is not possible, it is possible to develop a

document with every possible information item that is normally not historically known on the crop and on the gene as well as its product. The information should normally be able to provide answers to

a. What do we monitor ?
b. How do we monitor?
c. When do we monitor?
d. Why do we need to monitor?
e. How long and much do we monitor?

What do we monitor

The questions are basic in nature and each question is multi-dimensional in its perspective. For example, when we ask what do we monitor, what actually we seek as information that can provide answers are items such as the crop, the target organism (if the gene is pest or pathogen resistant), the gene product or expression, non-target organisms. These items of information were only related directly to the gene. This does not mean other seemingly unrelated information set dealing with the less obvious data with reference to the GMO and the environment is any less important.

Depending on the gene, habitat, cultural practices, traits like type of grain produced, the plant behaviour in competition during stress, the root exudates, etc., even if the gene in question was tolerance to herbicide can become important. The insects and pathogens which normally inhabited the weeds the herbicide would have killed now leaving only the transgenic plants in the vast area will by nature try to domesticate themselves on their non-host GMO. This has always happened even before GMOs hit fields when new plant types were introduced with a totally different pattern of vegetative and reproductive phases. The insects which inhabited on the old type crop at flowering stage looked for inhabiting on a crop the pest was never known to inhabit. This may not happen in the case of genetically regulated host-pathogen interactions but not uncommon. When oilseed Brassica fields were sown early and sprayed with the insecticides, the black aphids which are normally not known to prey on wheat would do so. The selection pressure on the insect population could throw up a new strain of aphid which would equally proliferate on wheat. Such pressures will be more with the GMO. In order to look for this type of "indirect" impacts one has to include data on the possible pests that prevailed in the area on the weeds and other vegetations on the field borders in the information bank. Only then can the question "What to monitor" can be expanded to many possible entities and traits.

How and when to monitor

Base line: A base line is that information which needs to be obtained from the environment on aspects one is likely to monitor (answers to "what"). Without a base line

information, there is no way one can monitor if there is any change at all. The questions of "how" and "when" do we monitor are the difficult questions that cannot be answered globally because a single ecosystem can also vary greatly in space and time. If the ecosystems were uniform, it would have been possible to enter into data sheet information on a certain trait and the monitoring team could measure or spot any effect the transgenic crop would have caused to it in contrast to the non-transgenic crop. Therefore, information on what the ecosystem was like before the transgenic was introduced commercially needs to be generated for at least last three to five years. To monitor any expected trait, one has to record many data points on a comparative plane to see if there is any trend of change due to the transgenic. Developing that base standard data set is therefore very crucial and has to be kept dynamic. That is, more than just one data on a ecosystem at one moment. For example, unless the monitoring team understands how much the population of a particular insect normally varies from year to year as the crop progresses, it would be impossible to know how to interpret a 30% drop in the insect number the year after a crop of Bt transgenic was planted (National Academy of Sciences, 2000). To know this, there is a relationship that has to be given to the weather parameters, its conduciveness during the current year for the insect population build up in comparison to the previous few seasons. The impact as solely due to the transgene should not get confounded by a different form of temporal and spatial variability in the region.

Thus, the most important tasks for PRM is to develop databank to establish what hazards can be posed in the environments including details on what the risk assessors have identified as less known information. Once an awareness of such directly relevant and /or less relevant or less known information is available, the risk assessors can decide which areas are more or less likely to involve a particular risk by preliminary observation at sample site and accordingly intensify the monitoring of that aspect for a fruitful PRM.

Why do we monitor

Do we monitor only for the presence of the gene? Or we looking for any degree of expression of the gene? Or are we concerned about what are the reasons which compel a monitoring? Is the gene product very crucial for the economy of the country but the product is perceived to be dangerous to human and animal population or the ecology? Are we interested in merely detecting the gene? Do we care what has the transgene done to the variety and its surroundings as well as its progeny? Is it likely to cause any allergic, toxic or hazardous to human beings?

At different times, at different locations, different purposes have to be looked into, which makes it necessary to see that monitoring has to rely on information and the purpose of information. In addition, if the transgenic has resulted in a different management of a crop agronomy, then we need to have data on which to base the alterations of the management. For example, if the expression of the Bt gene against an insect is to be monitored without the

usual practice of pesticide spray on the crop, it is necessary to develop the data set on the "refugia" crop again with reference to a base line, and monitor the pest to detect any signs of pressure on the insect to develop resistance to the Bt toxin.

How do we monitor

Finally, to do a PRM effectively, there should be a designed data entries which can detect both unexpected and unpredicted events and the events that are expected. Like the information on how long did the Bt corn take to decompose in the soil compared to the available information on refugia and base data on non-Bt corn is an example which one would not be looking for when the transgenic is with reference to pest resistance. In the first few years, such instances needs to be recorded extremely carefully to be prepared for the unexpected. This is real time monitoring when the crop is put into the field right from its first year to its commercialization. This on-course monitoring in the life of a transgenic provides inputs to take immediate decisions on the life of the transgenic or any agroecological production technology modification for eliminating any obvious risk elements observed.

The PRM, that is not quantifiable is the long-term strategy of monitoring. One cannot fix any generic yardsticks nor any exhaustive model on the information data that is good enough for monitoring the long-term effect of the release of a transgenic organism. A sui-generis system that facilitates a case by case analysis of the transgenic has to be developed in each situation.

In India, there is a four tier system of PRM, all of which have to be passed by the transgenic group step by step. Each step introduces an inbuilt monitoring aspect of multidimensional scanning of the input information vis-à-vis real data being generated. The steps are:

Step one : Approval by the Institutional Biosafety committee (IBSC)

Step two: Approval by the Review Committee on Genetic Modification (RCGM)

Step three : Approval by the Monitoring and Evaluation Committee (MEC) and

Step four : Approval by the Genetic Engineering Advisory Committee (Comrising of four Ministries)

Table 6.1 summarizes the extent of information the transgenic producing group needs to have before considering even a limited isolated field trial. It may look like the onus is entirely on the producer to provide information on the transgenic. But, it definitely is the base data about which the monitoring team can develop its own strategies on expecting any ill-consequences and biosafety as well as the unexpectedly unknown benefits from its release in the system. The focus is therefore on a set of key information that needs to be made available for assessment and evaluation of the biosafety with reference to a GMO during monitoring process. This set of information provides the base for the agency monitoring the biosafety before, during and after the GMO is released into an environment including the pre-release containment experiment after the GMO is developed.

Table 6.1: Basic information that needs to be generated for facilitation of monitoring the GMOs release related to biosafety assessment.

Particulars	**Information Sought**
Rationale for the development	Economic agronomic and other benefits,
Details of the molecular biology of GMOs (microorganisms, cell lines, plants, animals etc)	. Description of the host organisms . Source and sequence of transgene . Sequential block diagram of all trans-nucleic acid stretches inserted . Cloning strategy . Characteristics of inserted genes with details of sequences . Characteristics of promoters . Genetic analysis including copy number of inserts, stability, level of expression of transgenes, biochemistry of expressed gene products etc. . Transformation/cloning methods and propagation strategy (Microorganisms, plants and animals)
Laboratory, Green House Trials (for plants) and contained enclosure trials (for animals)	. Back-crossing methods for plants . Seed setting charateristics of plants . Germination rates of seeds . Phenotypic characteristics of transgenics . Organisms challenge tests where ever applicable . Effects of chemical herbicies for all herbicide resistant plants . Growth characteristics and general health of animals, measured through specific scientific parameters . Toxicity and allergenicity implications to human if any during handling of GMOs
Field trials in open environment	. For GM Plants, comparison of germination rates and phenotypic characteristics, using non-transgenics controls. . Study of gene flow of plants . Possibility of weed formation for GM plants . Invasiveness studies of plants and animals compared to non-transgenics used as controls . Possibility of transfer of transgenes to near relatives through out crossing./cross-fertilization . Implications of out crossing/cross-fertilization . Comparative evaluation of susceptibility to diseases and pests for plants and animals . For human food/animal feed, elaborate determination of composition and assessment of quality of transformed plants/fruits/seeds as well as animals as the case may be, with appropriate controls. Compositional analysis shall include near equivalence studies of all the major ingredients in GMOs so as to assess substantial equivalence with reference to non-transgenics. Change in the levels of allergenes, toxicants if any, beyond acceptable limits is a matter of food safety concern and such substances are

	unsuitable for commercial release. . Toxicity and allergenicity implications of transformed GMOs. This include micoorganisms, plants/fruits/seeds as well as animals, lab animal studies for food/feed safety evaluation is a requisite. . Handling procedures for allergenic substances . Agronomic evaluation for GM Plants . Economic evaluation for GM animals

Statutory regulation on provision of information to monitoring agencies

The power of monitoring agency should be in its responsibility in putting a stop to the release and if required, destroy the material if it is feasible. Such a responsibility therefore relies on the research output that produces the transgenic organism for providing the information relevant in addition to those relevant neither to the crop nor the gene, but the ecosystem. Then, it also becomes essential that the producer of the GMO link itself with other capabilities that provides data on those not directly associated with the gene or the GMO. In India, at least four sets of documents which are entirely information bank on the GMO being addressed to by the researching or commercializing agency. The documents are to be provided to different monitoring and evaluating agencies in specific formats (separately enclosed as Annexure) on

a. Information to IBSC/ RCGM for Import/ Exchange of GMOs and Products Thereof for Research Purpose
b. Information to IBSC/ RCGM to Carry Out Research for Development of r-DNA Products
c. Information to IBSC/ RCGM to Carry out Research for Development of Transgenic Plants
d. Half Yearly Report of the Institutional Biosafety Committee

It should be understood that no amount of information is "ENOUGH" to be assess 100% biosafety of the GMO being released or released. However, an honest disclosure of information and any other perceived information has to be recorded in the interest of human and environmental safety. An example of such information is, as under:

An example of information bank that needs to be generated project-wise by the monitoring agency (*Source: Nap et al,2003.)*

A. ***General information***

1. The name and address of the Researcher/Farmer/Agency
2. The title of the project
3. The area and location where the specific crop is sought to be gown:

B. ***Information about the normal crop/organism***

1. Scientific name :

   Genus :_____ species_____

   sub-species:

   Family :

   Particular variety targeted

   Or culture of the microorganism

2. Mode of reproduction : Sexual/Asexual/Vegetative

   Mating system : Self/cross/often cross/often self/sterile

   Generation time seed to seed or completion of one cycle (organism):

   _____days

   Seed to flowering :_____days

   Flowering to maturity _____days

   Sexual compatibility with other cultivated or wild plant species

3. Information on the survivability of the crop:

   Seed type

   Dormancy if any, etc

   Resting stage of the organism (in case of micro-organism)

4. Information concerning dissemination and seed dispersal of plant: type, extent and factors affecting dissemination

5. The geographic distribution of the plant and centre of origin

6. Natural habitat of the plant or organism

7. Common weeds recorded as problem weed with the crop in the region

8. Information on the weed (1-n)

   family

   mating system

   habit

   known ideal habitat

   maturiry and days to flowering

   seed-seed

   type of seed

9. Diseases of the crop known prevailing over last 5 years and the maximum and minimum distribution (disease-wise /tabulated year wise)

10. Insect pest information along with maximum and minimum infestation data over the last five years along with the plant part targeted by the insect (insect wise data – tabulated year wise)

11. Predator pests with known affinity to the crop including birds and grazing animals
12. Any known allergenicity or toxicity naturally existing as a product of the on any significant interactions of the plant with organisms other than plants in the ecosystem where it is usually grown, including toxicity to humans, animals and other organisms

C. ***Information about the transformation tool and materials***

13. A description of methods used for genetic modification
14. The nature and source of the vector used
15. The size, function and donor organism(s) of each DNA sequence inserted

Information relating to the genetically modified organism

16. A description of the trait(s) and characteristics of the GM plant which have been modified
17. Information on sequences inserted or deleted: size/structure, copy number of insert, information on any vector sequences or foreign DNA remaining in the GM plant. The size/function of any deleted regions. Cellular location of insertion (eg. chromosomal, mitochondria, chloroplast etc.)
18. Information on the expression of the insert and its genetic stabiliy: expression and parts of the plant where expressed
19. How does the GM plant differ from the recipient plant in mode/rate of reproduction, dissemination, survivability
20. The potential for transfer of genetic material from the GM plants to other organisms
21. Information on any toxic/harmful effects on human health and the environment arising from the genetic modification
22. The mechanism of interaction between the GM plants and target organisms
23. Any potential significant interactions with non-target organisms
24. A description of detection and identification techniques for the genetically modified plants
25. Information about previous releases of the GM plants

Information relating to the site of release

26. The location and size of the release site or sites
27. A description of the release site ecosystem, including climate, flora and fauna
26. Details of any sexually compatible wild relatives or cultivated plants present at the release sites
27. The proximity of the release sites to officially recognised biotopes or protected areas

Information relating to the release

28. The purpose of the release
29. The foreseen dates and duration of the release
30. The method by which the GM plants will be released
31. The method for preparing and managing the release site, prior to, during, and after the release
32. The approximate number of GM plants (or plants per m2) to be released

Information on the control, monitoring, post-release plans and waste treatment plans

33. A description of any precautions to minimise or prevent pollen or seed dispersal from the GM plant
34. A description of the methods for post-release treatment of the site or sites
35. A description of post-release treatment methods for the GM plant material including wastes
36. A description of monitoring plans and techniques
37. A description of any emergency plans

Information on potential environmental impact of the release of the genetically modified plants

38. The likelihood of any GM plant becoming more persistent or invasive than recipient plants
39. Any selective advantage or disadvantage conferred to other sexually compatible plant species, which may result from genetic transfer from the genetically modified plant
40. Potential environmental impact of the interaction between the GM plant and target organisms
41. Any possible environmental impact resulting from potential interactions with non-target organisms

Monitoring Release 0f GM Microorganisms

Genetically modified microorganisms (GMMs) are promising for many environmental and agricultural applications, including bioremediation of toxic chemicals and bio control of plant diseases. It is important to ensure that when these organisms are released into nature that they do not harm the environment or human health. Therefore, new GMM products are thoroughly assessed for potential risks before they are approved for widespread application. One important aspect of risk assessment is the actual monitoring of the fate of the GMM in nature (i.e. survival, dispersal, etc.). Specific methods are required to monitor the GMM apart from the natural microorganisms present in the environment. For example, a single gram of

soil contains billions of microbial cells comprising thousands of distinct genotypes. In addition, the monitoring methods should be sensitive to enable low numbers of cells to be counted, since the GMM population could increase in number should the environmental conditions prove more favorable. Considerable research efforts have been directed towards development of sensitive and specific tools for environmental monitoring of GMMs.

Before the microorganism is released, it is essential that it is monitored through its developmental and testing procedures where, the microbes are subjected to 'contained use'. Examples of typical contained use situations are laboratories, animal houses used, for example, for breeding GM mice, plant growth rooms and glasshouses, industrial fermenters used for large scale production, e.g. enzymes for washing powders. Contained use is defined as any activity in which organisms are genetically modified, or in which GMOs are cultured, stored, used, transported, destroyed or disposed of and there are barriers in place to limit contact with humans and the environment, so as to provide a high level of protection. These barriers can be physical, biological or chemical, or a combination of these. Separate regulations cover deliberate release into the environment, food safety and other product approval issues. Thus, the definition does not keep "physical structure" as an essential containment feature where, any activity in which micro-organisms are genetically modified …and for which specific containment measures are used to limit their contact with the general population and the environment (CEC, 2000). This is also reflected in the newly released open book entitled "Biological confinement of genetically engineered organisms" by the Board on Agriculture and Natural Resources, USA (2004). When the quantity of the microbial culture does not exceed 10 lts and is non-commercial in nature, the GMM that is then for a purpose of training or research or non-industrial usage is categorized at a relatively lower grade of A while rest of the GMM is categorized as B. In the case of microbes, any unintended release which may have any bearing on human or animal health is for the monitoring purposes is to be regarded as an "Accident" so that appropriate measures are adopted to inactivate the GMM and the community is cautioned on an alert.

Classification of the GMMs

How are the risks of contained use assessed and classified?

Preparing a scientific assessment of risk to human health and the environment can be a substantial workload. CUR 2000 lays down the steps to be taken, which in simplified form include:

a. identification of any harmful properties of the organisms donating and receiving the genetic material, the intermediary or vector, and the inserted genetic material itself, and any harmful properties arising from any alteration made to the receiving organism's properties by the genetic modification
b. consideration of other relevant legislation, particularly in the way that it classifies the risks of the organisms to be used

c. consideration of the activity (for GMMs, in the context of the relevant environment), particularly non-standard aspects requiring individual attention

d. selection of appropriate containment measures from a table which arranges them in numbered columns. For GMMs, classification to the appropriate risk level, which equals the highest column number corresponding to any individual containment measure selected

Requirements of hygiene & protective measures

1. For genetically modified micro-organisms in Category I, the principles of good microbiological practice, and the following principles of good occupation safety and hygiene, shall apply which involves

(i) keeping workplace and environmental exposure to any physical, chemical or biological agent to the lowest practicable level;

(ii) engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary;

(iii) testing adequately and maintain control measures and equipment;

(iv) testing, when necessary for the presence of viable process organisms outside the primary physical containment and provide training to personnel working in the containment.

2. In addition to these principles, the containment measures shall be applied, as appropriate, to contained uses of genetically modified micro-organisms in Group II so as to ensure a high level of safety.

3. The containment measures applied shall be periodically reviewed by the user to take into account new scientific or technical knowledge relative to risk management and treatment and disposal of wastes.

Re-notification of all Premises/Activities:

When undertaking GM procedures for the first time each centre must register with the IBSC specifying their first activity. For those centres which will only undertake Category 1 activities at containment level 1 this may be the end of their contact with the IBSC. Only activities of Categories 2 and above need to notify the IBSC of each new activity.

Monitoring the GMM in environment

The GMM can be effectively monitored against the parameters given below as information generated on the GMM:

A. Characteristics of the donor, recipient or (where appropriate) parental organism(s)

B. Characteristics of the modified micro-organism

C. Health considerations

D. Environmental considerations

A. Characteristics of the donor, recipient or (where appropriate parental organism(s)

- names and designation;
- degree of relatedness;
- sources of the organism(s);
- information on reproductive cycles (sexual/asexual) of the parental organism(s) or, where applicable, of the recipient micro- organism;
- history of prior genetic manipulations;
- stability of parental or of recipient organism in terms of relevant genetic traits;
- nature of pathogenicity and virulence, infectivity, toxicity and vectors of disease transmission;
- nature of indigenous vectors: sequence,frequency of mobilization,specificity,presence of genes which confer resistance;
- host range;
- other potentially significant physiological traits;
- stability of these traits;
- natural habitat and geographic distribution. Climatic characteristics of original habitats;
- significant involvement in environmental processes (such as nitrogen fixation or pH regulation);
- interaction with, and effects on, other organisms in the environment (including likely competitive or symbiotic properties);
- ability to form survival structures (such as spores or sclerotia).

B. Characteristics of the modified micro-organism

- the description of the modification including the method for introducing the vector-insert onto the recipient organism or the method used for achieving the genetic modification involved;
- the function of the genetic manipulation and/or of the new nucleic acid;
- nature and source of the vector;
- structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified micro-organism;
- stability of the micro-organism in terms of genetic traits;
- frequency of mobilization of inserted vector and/or genetic transfer capability;

- rate and level of expression of the new genetic material. Method and sensitivity of measurement;
- activity of the expressed protein.

C. Health considerations

- toxic or allergenic effects of non-viable organisms and/or their metabolic products;
- product hazards;
- comparison of the modified micro-organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
- capacity for colonization;
- if the micro-organism is pathogenic to humans who are immunocompetent:

(a) diseases caused and mechanism of pathogenicity including invasiveness and virulence;
(b) communicability;
(c) infective dose;
(d) host range, possibility of alteration;
(e) possibility of survival outside of human host;
(f) presence of vectors or means of dissemination;
(g) biological stability;
(h) antibiotic-resistance patterns;
(i) allergenicity;
(j) availability of appropriate therapies.

D. Environmental considerations

- factors affecting survival, multiplication and dissemination of the modified microorganism in the environment;
- available techniques for detection, identification and monitoring of the modified microorganism;
- available techniques for detecting transfer of the new genetic material to other organisms;
- know and predicted habitats of the modified micro-organism;
- description of ecosystems to which the micro-organism could be accidentally disseminated;
- anticipated mechanism and result of interaction between the modified micro-organism and the organisms or micro-organisms which might be exposed in case of release into the environment;
- known or predicted effects on plants and animals such as pathogenicity, infectivity, toxicity, virulence, vector of pathogen, allergenicity, colonization;

- known or predicted involvement in biogeochemical processes;
- availability of methods for decontamination of the area in case of release to the environment.

A brief description on methods to detect microbes for GM from environment

Specific methods are required to monitor the GMM apart from the natural microorganisms present in the environment. For example, a single gram of soil contains billions of microbial cells comprising thousands of distinct genotypes. In addition, the monitoring methods should be sensitive to enable low numbers of cells to be counted, since the GMM population could increase in number should the environmental conditions prove more favorable. Considerable research efforts have been directed towards development of sensitive and specific tools for environmental monitoring of GMMs. According to Jason et al. 2003, the methods are divided into the following categories of desired information below:

1. Number of culturable GMM
2. Number of total GMM cells, regardless of their activity or culturability
3. In situ visualization of GMM distribution
4. GMM activity
5. Presence of genetically modified DNA
6. Plasmid transfer

1. Enumeration of culturable GMMs

Selective plate counting: A simple, sensitive and cost effective method for quantification of culturable cells is selective plate counting. This technique relies on the selective growth of the GMM on agar medium that contains a compound that inhibits growth of the natural microbial population. This compound could be, for example, an antibiotic or a heavy metal. Due to concern about the spread of antibiotic resistance to pathogenic microorganisms, while it is strongly recommended that a GMM with antibiotic resistance selectable marker not be released into the environment, many microorganisms are intrinsically resistant to some antibiotics. Since intrinsic resistance is a natural trait, we do not object to the use of intrinsic antibiotic resistance markers for tracking of GMMs on selective medium, so long as the antibiotics are not important for clinical or veterinary use. For example, rifampicin, and kanamycin are useful antibiotics for selection purposes. The reason that we recommend continued use of selective plating for tracking of GMMs is that the methods are routinely used in most microbiology laboratories and they are cheap and sensitive. While opinions of the kind that kanamycin as an antibiotic does not create any utility concerns of biosafety as most of the animal and human pathogenic bacteria are already resistant to the same and are anyway not prescribed as antibiotic treatment.

An alternative to antibiotic selection is the use of heavy metals in the medium, such as mercury. However, this requires handling of hazardous compounds (i.e. mercury) in the laboratory and should be avoided. In some cases semi-selective plating may be used. This involves for example using media where the GMM is able to grow but only a small portion of the indigenous microorganisms are capable of growth. For example, if the GMM has been modified to degrade and to be able to use an environmental pollutant as sole carbon and energy source, this compound can be added to minimal plates used for bacterial enumeration. Depending on the compound the amount of background varies. Additional identification methods (see below) can be used to distinguish between GMMs and background.

Non-selective plate counting*:* In some cases it may be preferential to use non-selective plate counting to enumerate GMMs in environmental samples. The difference with this method, compared to that above, is that it does not rely on incorporation of an inhibitory compound into the agar medium. Instead the GMM is distinguished on agar plates on the basis of a unique phenotype. This phenotype could be a metabolic property or the ability to luminesce or fluoresce (Table 7.1). The principles are otherwise similar to that of selective plate counting above. The primary disadvantage with non-selective plate counting is that there is usually a large background growth of colonies of the natural microbial population, since these cells are not inhibited by incorporation of a selective compound. However, careful design of cultivation media and use of sophisticated screening techniques can in some cases alleviate this problem. In general, non-selective plate counting is most useful for counting of GMMs when they are relatively abundant and extreme sensitivity is not required.

In both the procedures, the monitoring laboratory needs to keep a repository of the original cultures for comparison of the colony forming unit (cfu) values of the standard cultures. The investment is worth the effort as the infrastructure required is not prohibitively expensive. The sampling itself has to be done in periodic intervals from potential areas near the industrial units or agricultural farms which employ biologicals for control of pests, pathogens or for processed products. Representative samples of a given environment in the suspected or routinely selected region likely to be containing the gene constructs or the GMMs needs to be taken and the sample source location and identity recorded. A sample size can be very small like 10g soil, 10g plant tissue, 10 ml water or 10g of a known agri-produce.

2. Enumeration of total number of GMMs, regardless of their activity or culturability

Nature is comprised of a variety of ecosystems, varying in complexity. Many ecosystems, such as most soil ecosystems, are harsh environments and introduced GMMs may become stressed when released to these ecosystems. Many microorganisms are known to react drammatically to different stress conditions by turning off synthesis of some proteins and initiating synthesis of others. This could partly explain the phenomenon of viable-but non-culturable (VBNC) cells that has been observed for certain bacteria in natural ecosystems. When bacteria are stressed, or in a VBNC state, it is difficult to get them to grow on

traditional laboratory media. Therefore, although these cells may still be present and viable and thus able to exert an effect on their local environment, they may not be counted by traditional methods based on cultivation, such as plate counting methods. A related problem is that starved cells have repressed metabolic activity and low energy reserves. Therefore, methods are necessary to enumerate cells, independently of their culturability or activity.

Table 7.1. Examples of non-selective biomarkers and their corresponding colony phenotypes (source : Jansson et al. 2003)

Marker gene methods (encoded protein)	Substrate/Requirement	Colony phenotype	Detection
LacZ, β-galactosidase)	5-bromo-4-chloro-3-indoyl-β-Dgalactopyranoside (X-gal)	Blue color	Visual
lacZ	Fluorescein digalactoside+UV	Fluorescence	CCD camera
Gfp (Green fluorescent protein)	Blue light*	Fluorescence	CCD camera, visual
luc (eukaryotic luciferase)	Luciferin	Luminescence	CCD camera, Visual, Photographic film
luxAB (bacterial luciferase)	*n*-decanal *luxCD & luxE* genes	Luminescence	CCD camera, Visual, Photographic film
xylE (catechol 2,3- dioxygenase)	Catechol	Yellow color	Visual
gusA β-glucuronidase)	X-GlcA (5-bromo-4-chloro-3-indoyl-β-D-glucuronide)	Blue color	Visual

*UV light can also be employed, but due to the potential for UV-induced mutations, the exposed colonies should not be used in subsequent studies. Bacteria which are known to readily express GFP encoded by a multicopy number *gfp*-vector easily form bright green colonies that can often be seen as yellowish-green colonies under normal illumination (daylight). In some cases a "position effect" of *gfp* integration into the bacterial chromosome is observed leading to unusually bright fluorescence, although the reasons for this effect are currently not known.

An aspect that the monitoring agency may seek to be adopted is that the GMMs should be tagged with GFP genes as the marker system especially on microbes known to be broad host ranged or saprophytic in nature. This regulation would enable the enumeration of the GFP tagged contaminants from the samples by flow cytometry which does not require the need for culturing and staining (Tombolini and Jansson, 1998). Can such a regulatory rule be made mandatory right at the time of approving research on the microbe by the regulatory authority?

A caution with such techniques in monitoring that is actually done to decide the future course of action on the gene or the GMM in question is to carefully analyze the samples using population statistics in sampling and general statistics in working out the background absorption by non-target particles and cells. However, the process can be standardized and is a very fast technique. Another important aspect of this method is the ability to detect the size and shape of the fluorescing particles which can be classified through software. This allows multiple contaminant microbe detection

How do we enable monitoring for the GMM or other non-targeted microbes by adopting this technique? Two monitoring indicators need to be introduced to enable proper verification of the results documented during review by the monitoring agency. One is with respect to proper statistical analysis of the data on escapes or horizontal spread. The other one is survey of available information regarding the physical shape and size of the GMM being released and also the other known microbes that could have the transgene passed into them upon its release into the environment.

3. ***In-situ* enumeration of the bacterial cells using scanning confocal and stereo fluorescence microscope** :

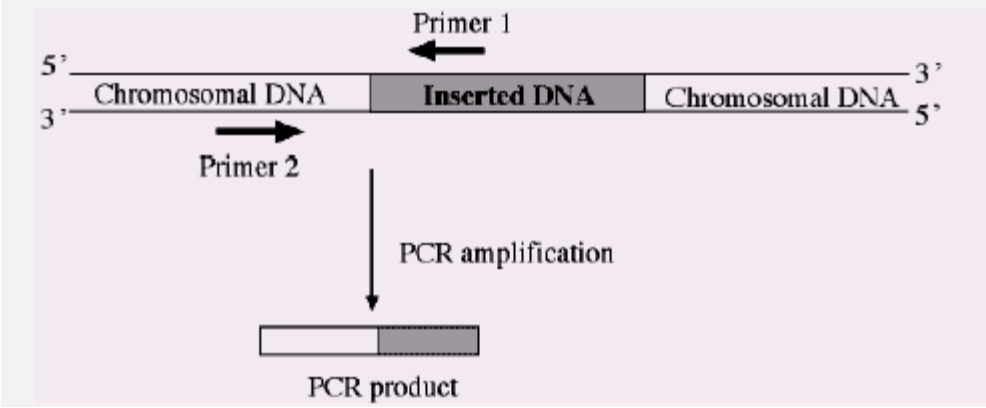
Scanning confocal laser microscopy (SCLM) is a particularly useful non-invasive technique for the detection and localization of fluorescently-tagged GMMs *in situ*. Microbes synthesizing the green fluorescent protein can be readily detected using SCLM. The technique can be as usefully adopted as the flow cytometric technique. Again a statistical interpretation of data is a necessity using microscopic fields and collected samples as units of sampling variability. The technique also does not require any culturing of the microbe. The method can be used for visualizing bacteria from other environments as well. Plant leaf associated bacteria can be divided into epiphytic (surface associated) and total cells. Of these the epiphytic cells can be easily separated from the leaves avoiding any interfering plant cells. The endophytic contaminants can be visualized through SCLM from plant tissue extracts or smears. Fluorescence stereomicroscopy is a relatively new technique for gross visualization of the pattern of colonization of fluorescent (GFP-tagged) microorganisms on samples such as plant tissues. The stereomicroscope can also be equipped with a sensitive CCD camera for *in situ* visualization of luminescent cells on the sample.

4. GMM activity

Often it is the metabolically active fraction of a GMM population that is of the most interest, considering that the metabolically active cells have potential to exert an effect on their surrounding environment. It is also the metabolically active cells that are best equipped to perform the intended function for which they were designed and released (for example, bioremediation or biocontrol). However, bacteria in nature are not always metabolically active since many environments, such as sandy soil, are harsh with few available nutrients.

Therefore, the cells can expend their energy reserves and become quiescent (i.e. in a resting state) until nutrients become available. By contrast, other ecosystems, such as a plant root, may leak nutrients that can be utilized for metabolism by the released GMMs. With appropriate modification of the sample of the environment one can monitor the marker genes such as luminescent marker for luciferase activity (*luc*, or *luxAB*-tagged cells) by luminometry or CCD camera and image analysis. The activity can be enhanced by pretreatment of the samples for which depending on the marker gene, different protocols are available (Jasson et al. 2000). Quenching is a problem that is often encountered when measuring light output in an environmental sample. For example, soil particles and humic acids can quench light emitted from cells resulting in a decrease in the light yield measured by the instrumentation. A pretreatment protocol needs to be followed to enhance the probability of expression of light emission signals. Alternatively, light-emitting cells can be detected directly on plant surfaces, for example, using a sensitive photon counting camera or a CCD camera.

5. Presence of genetically modified DNA



A GMM by definition has a genome that has been altered by genetic manipulation. Sometimes the change in the genome is sufficient as a target for specific monitoring of the GMM using nucleic acid-based approaches. If a novel DNA sequence has been inserted into the GMM, this sequence can be detected by use of DNA probes. Alternatively, the novel sequence can be detected by PCR amplification. Often it is advantageous to design primers for PCR amplification that amplify across junctions of introduced DNA fragments to increase specificity of detection as below:

6. Plasmid transfer

A GMM may be constructed by engineering of the chromosome or by introduction of a plasmid containing genes encoding desired traits. Plasmids are extrachromosomal genetic elements, and some plasmids can be transferred to other microorganisms. The potential for

plasmid transfer from the GMM to known recipients or to members of the indigenous microbial community can be monitored directly by tagging the mobile DNA molecules with appropriate marker genes. For example, different GFP variants, with different excitation and emission spectra, may be used to follow donor, recipient and transconjugants in environmental samples, simultaneously. This can be achieved by tagging the conjugative plasmid with a cassette comprising one constitutively expressed fluorescent marker, and the recipient being chromosomally tagged with another fluorescent marker gene. In this case the donor cells will appear as emitting light in one color, the recipient cells in another, and the transconjugants as a combination of both colors. These methods, while developed specifically for plasmid conjugation studies may be modified with little effort to also be useful in studies of transduction or transformation. For example, the -galactosidase promoter, P*lac*, can be fused to the *gfp* gene on the plasmid of interest. The GMM should also contain the *lacI* gene, encoding the lac repressor protein, LacI, integrated into its chromosome. In this case, the P*lac* promoter will be repressed in the GMM and no GFP will be produced. However, should the plasmid be transferred to an indigenous microorganism lacking the *lacI* gene, the *lac* promoter will be active and the GFP protein synthesized. Green fluorescent transconjugants can then be detected by microscopic methods as described above.

Additional information

The European Commission Biotechnology Programme, DGXII, has recently sponsored the publication of a series of booklets related to the use of marker and reporter genes in microbial ecology that have direct relevance to the methods presented in this manual. The books were written by the MAREP Concerted Action of scientists, consisting of 26 scientific experts from 11 different countries. More information can be found on the MAREP website.

(Website: http://www.sh.se/marep/marep.html)

Booklet titles:

- Marker genes as tags for monitoring microorganisms in nature
- Reporter genes for monitoring microbial activity and/or the environment
- Monitoring methods for specific microorganisms and microbial communities in nature
- Biosafety aspects of marker and reporter genes

5. Surveillance and Emergency Planning

The aspect of surveillance and emergency planning generally a review phase. The action aspect of the planning comes into force only during accidents. Therefore, surveillance is basically that part of monitoring where a generic view point of the institutional mechanism is incorporated for improved performance of the enforcement of the biosafety regulations..

What requirements must be met while the activity is being carried out?

A. ***Under Containment***

Standards of containment and control must be commensurate with, and determined by, the risk assessment. The appropriate standards can be identified from Schedule 8 to CUR 2000 together with the risk classification (1 to 4) arrived at in the risk assessment of a contained use activity. These standards must be maintained. Good microbiological practice and good occupational safety and hygiene (GOSH) should be taken into account.

CUR 2000 referred earlier (EU guidelines, 2000) requires all GMMs to be inactivated before their disposal. The required level of inactivation is related to the risk. For the more hazardous GMMs, 100% 'kill' is required. In intermediate cases, chemical disinfection, typically giving a 10 fold reduction in viability (99.999% kill), may be adequate. For low risk activities, inactivation as part of another processing step, such as the extraction of a product, may be sufficient - provided that the required level of inactivation is shown to be met consistently. In the lowest risk cases, it may be enough that the organisms are biologically incapable of survival after discharge from a contained use facility, so that no harm can result to humans or the environment. However, to use this passive inactivation approach, permission must be obtained from the Institution's Biosafety Committee, which will need to carry out an evaluation. After inactivation, the waste remains subject to waste and pollution law that applies to any waste - see the ACGM Compendium of Guidance, Part 1, paragraph 76, for relevant legislation.

The principles of good microbiological practice and good occupational safety and hygiene (GOSH) are:

- keeping workplace and environmental exposure to any GMMs to the lowest reasonably practicable level
- exercising engineering control measures at source and supplementing these with appropriate personal protective clothing and equipment where necessary
- testing adequately and maintaining control measures and equipment
- testing, where necessary, for the presence of viable process organisms outside the primary physical containment
- providing appropriate training of personnel
- formulating and implementing local codes of practice for the safety of personnel, as required
- displaying biohazard signs where appropriate
- providing washing and decontamination facilities for personnel
- keeping adequate records
- prohibiting in the work area eating, drinking, smoking, applying cosmetics or the storing of food for human consumption

- prohibiting mouth pipetting
- providing written standard operating procedures where appropriate to ensure safety
- having effective disinfectants and specified disinfection procedures available in case of spillage of GMOs
- providing safe storage for contaminated laboratory equipment and materials where appropriate.

B. In open environment

For the appropriate regulation of biosafety, the key issue to resolve has been, and will remain, 'when is safe sufficiently safe?' This requires appropriate science for determining what is meant by 'safe' and judgement for deciding the meaning of 'sufficiently'. The current era of genomics, proteomics, etc. is delivering technologies that will allow the measurement of gene expression at the RNA and protein levels, as well as molecules of each specific metabolite in a plant. Future regulation aimed at 'absolute safety' may eventually demand such measurements as a routine requirement based on the premise that everything that can be measured should be measured, irrespective of its potential (ir)relevance. The baseline for 'safe' should be comparison and the judgement of 'sufficiently' should take the comparative risk into account. The judgements made during a comparative assessment should represent the concerns of the public. However, caution should be observed when items in regulatory procedures are put in place solely for the purpose of enhancing public confidence. The role of regulators must be to recognize when impacts of GM crops might become unacceptable and to require changes to existing or GM farming practices to obtain the balance that society demands. But, what a given society wants and how much it might be willing to pay for additional assurances is unknown.

In this context, the impact of regulation is going to be a crucial issue that must not be forgotten. It is important to emphasize that the regulation of risk is currently turning into a risk of regulation. The regulatory process itself may already cause one of the greatest risks (Brown, 2001). The level of scrutiny imposed is unprecedented for the products of plant breeding. As regulations become impractical, compliance with them becomes less controllable and they are likely to become considerably more costly than anticipated. The plant breeding industry, in general, does not have the resources for GM crop material to be assessed in the same detail as a pharmaceutical. The cost of meeting regulatory requirements is currently a significant negative impact on the release of GM crops compared to the release of cultivars from traditional breeding. Excessive regulatory reviews will frustrate and curtail research and application to such an extent that only a few large multinational companies can afford to make progress. In this manner, over-regulation will help to promote a situation that is a concern of many: corporate control of agriculture (Dawkins, 2002). This trend is already clearly apparent and may result in the creation of a single (or a few) companies dominating

world food production and increasing world dependence (Dawkins, 2002; Josling and Nelson, 2001).

A potentially even larger danger of the trend toward zero-risk in current regulation is that a similar risk scrutiny will be imposed on the activity of traditional, non-GM plant breeding. The results of a recent National Academy of Sciences survey (NAS, 2002) already suggests that conventional crops may pose undesired environmental risks and should be monitored (Gewin, 2002). This would basically be the end of plant breeding as we know it, and dramatically affect the future of plant science. Such ends do not seem to justify the means. Plants, crops and innovation in crops and crop growing will remain essential for global well being in the future. After release of a GM crop plant, therefore, the surveillance should use the basic information we discussed earlier in commercial release permission related proforma and field trial release proforma. Built on that should be to generate the information on the following aspects:

1. The Reference No. of the GM released as per the National Permit (NBC, Bangladesh for example)
2. Applicant's address, date of release and date when the field release monitoring report was produced
3. Dates when monitoring was carried out:

   (Here, the dates when the monitoring was carried out during and after the trials. If monitoring was carried out at frequent intervals, each item should be mentioned with summarized outputs between the dates)
4. Report on actual monitoring of the release

   Report on the outcome of environmental and biosafety monitoring carried out during the release. The report should focus on whether the release progressed as planned and if not, the reasons for this any environmental effects observed. Some details about how the monitoring was carried out should be provided. The statements should also indicate whether risk mentioned in the original application were analyzed and data provided.
5. Report on post-release monitoring

   Report on the post release monitoring describing the effects of the release on the environment, implications for the assessment of damage to the environment being caused by subsequent releases of the same GMO or the marketing of a product consisting of or including such GMO.
6. New information on risks of damage to the environment

   Describe any new information with regard to any risks to damage to the environment which has become available since the release and how this information affects the previous risk assessment.

7. Investigations for the predicted behavior of the genetically modified maize

   Data on the possible effects on other cohabiting plants and animals in the crop ecosystem has to be recorded as a routine to keep surveillance of any ill effects such as the monarch butterfly case in maize.

8. Origin and function of each constituent part of the insert in the GMO

- As a matter of survey, data on the plasmid used as the binary vector of the transgene and its promoter should be maintained for any possible consequence of the plasmid sequences expressing or integrating in the plant.

9. Potential for genetic transfer and exchange with other organisms

   Maize has no sexually compatible wild or weedy relatives in Europe. Sexually compatible plants (genera *Zea* and *Tripsacum*) are present only in Mexico and Guatemala.

   Therefore, maize can not exchange genetic material via pollen with other plants in Europe.

10. Marker genes and their removal

Gene constructs are linked to marker genes both for the direct transformation of protoplasts and for the transformation by means of *Agrobacterium tumefaciens* in order to be able to identify safely and rapidly, after having passed partial steps of the transformation, those cells (in the direct transformation of protoplasts the plant cells; in the transformation with the aid of *Agrobacterium tumefaciens* at first E. coli, subsequently *Agrobacterium tumefaciens* and later on the plant cells) into whose genome the DNA construct has been inserted. Certain antibiotic resistance genes of bacterial origin (in most cases the nptII or hph gene) are commonly used as selection marker genes for the last step which will be inserted into suitable Ti plasmid vectors together with the target gene construct. However, in the transformation procedure with *Agrobacterium tumefaciens*, such antibiotic resistance genes from vector segments may be occasionally transmitted to the plant genome which are located outside the DNA comprising target gene construct and selection marker gene and inserted into the Ti segment and possibly have served the selection of bacteria in the first and second steps.

Antibiotic resistance genes used as markers for genetically modified plants have reached public awareness. They have raised the concern whether horizontal gene transfer from the plant material to micro-organisms may lead to an increased level of resistance to micro-organisms of medical and veterinary use and, therefore, compromising the therapeutically use of antibiotics. This concern is fuelled by the experience that the extensive use of antibiotics for medical and veterinary purposes and as growth promoters for farm animals has lead to increased spreading of antibiotic resistance genes in the microbial population.

Thus, there is the need for science based assessment of possible adverse effects of antibiotic resistance marker genes in genetically modified plants.

11. Evaluation of the biological safety of the antibiotic resistance genes in the genome of gm plants

In the evaluation of the biological safety of antibiotic resistance genes in the genome of transgenic plants, it is of principal importance to relate the probability of transformation of soil and enteric bacteria by the antibiotic resistance genes released from the genome of transgenic plants to the probability of transfer of such antibiotic resistance genes by conjugation from one bacterium to the other.

12. **Administrative tasks during contained use activities in surveillance and emergency handling**

The risk assessment must be reviewed and revised as necessary, and records must be kept. A risk assessment should be reviewed if there is any reason to suspect that the initial assessment is no longer valid because of a significant change in the activity (CUR 2000), such as a change of scale of operation, containment measures, waste treatment procedures, or the availability of new information concerning the organism. Where new information, which may have significant consequences for the risks of a notified activity, becomes known, the IBSC must be informed .

The plan and its revisions must be made known to the emergency services and any body liable to be affected, and made available to the public. Any accidents resulting in significant and unintended release of GMOs must be notified immediately to IBSC, including the details.

Records of risk assessments should be kept for at least 10 years from the date that the work covered by the risk assessments finished.

Accidents and Emergency plans

Notifying accidents

1 Regulations place the responsibility on centres to immediately notify the Competent Authority of accidents, as defined in the regulations, involving genetically modified organisms. There may be situations where as well as notifying the Competent Authority under GMO regulation, the IBSC also requires notification , for example if a person were to require hospital treatment. This guidance aims to explain what should be considered to be an accident for the purposes of the GMO(CU) legislation; what information should be included when notifying an accident to the competent authority; who to contact; and what the CA will do with the information provided in the accident notification.

What is an accident?

An accident with reference to GMO is an incident involving a significant and unintended release of genetically modified organisms in the course of an activity involving genetic modification which presents an immediate or delayed hazard to human health or the

environment. Therefore, an accident is where a GMM is released in such a way that it poses an immediate or delayed risk to human health or the environment, or where a GMO (other than GMM) is released in such a way that it poses an immediate or delayed risk to human health. Accidents which result in release of GMMs and GMOs from primary containment, but not the laboratory or building may therefore constitute an accident, depending on the nature of the GMM or GMO.

Situations, which might constitute an accident, depending on the organisms involved, their mode of transmission and the nature of the accident, might include:

- the spillage of any Category III GMM outside of a microbiological safety cabinet (MSC) or other primary container;
- a major spillage of a Class 3 GMM within a MSC;
- the spillage of any category II and III GMM outside of a MSC or other primary container, where it is thought likely that an individual or the environment could have been exposed during the spill or during decontamination;
- the release or escape of a GMO, other than a GMM which could cause harm to human health, for example by acting as a novel disease reservoir;
- infection (classical) of a person with a (replication competent) GMM, as this constitutes a significant and unintended release.

Spillage or release of Category I GMM is unlikely to count as an accident as class 1 GMMs are unable to pose a risk to human health or the environment. If you are in any doubt as to whether you need to notify the Competent Authority, please contact the inspection team. Please note that any intention to release a GMO is subject to the Genetically Modified Organisms (Deliberate Release) Regulations 2002.

What information do you have to provide?

Where an accident occurs, the person undertaking the activity involving genetic modification shall forthwith notify the competent authority of the accident and shall provide the following information, which should subsequently be kept in public information :

- the circumstances of the accident;
- the identity and quantity of genetically modified organisms concerned;
- any information necessary to assess the effects of the accident on the health of the general population and on the environment; and
- any measures taken in response to the accident.'

What will the CA do with the information?

The Notification Officer will circulate the information provided to all members of the IBSC. and also places duties on the Competent Authority where it is informed of an accident. After taking remedial measures, the IBSC should deliberate on the -

- information on the circumstances of the accident; the identity and quantity of GMOs concerned and the measures taken in response to the accident;
- information on the effectiveness of the measures taken in response to the accident; and
- an analysis of the accident, including recommendations to limit its effect and to avoid similar accidents in future.

This means that concerned inspectors will need to investigate the accident to obtain all the information to make recommendations to avoid similar accidents and to share the lessons learnt in public. Therefore, IBSC should regard any attempt to avoid notification of an accident as a serious matter.

Emergency Plans

An emergency plan must be drawn up where the risk assessment indicates that, as a result of any foreseeable accident, the health and safety of persons outside the premises may be seriously affected or if there is risk of serious damage to the environment. Such a plan is unlikely to be necessary for most small scale activities or those involving low risk organisms.

The plan must specify

a. Methods specific to the GMO for procedures to control the GMO in case of unexpected spread.
b. Method to decontaminate or eliminate the effects of an accident
c. Method for disposal of sanitation of plants, animals, soils etc. that were exposed during the accident or spread

Health Surveillance

All workers undertaking genetic modification activities must register with the Institutional health services or any linked medical services agency as a matter of right. Following registration, health surveillance should be undertaken where this is considered appropriate.

Records

Departments must maintain various records relating to genetic modification work, history of accidents with respect to the GMO. The records must be kept whilst the work is being carried out and thereafter for at least 10 years from the date the work ceases. Records must be made available to the authorities when requested.

Training and Supervision

Supervisors must ensure that all workers for whom they are responsible are competent to carry out their work safely and that they receive an appropriate level of supervision.

Deputizing arrangements must be made to cover times when the supervisor is not available. Procedures should be in place to ensure new workers are familiar with the local codes of practice and the correct use of laboratory equipment. On the job training is important. Each new worker should be trained by staff familiar with the microbiological techniques involved as soon as possible and before the worker starts. Familiarization by means of individual discussion with each new entrant to the laboratory is advised. Training must specifically address safety issues and include discussion of the risk assessment for the project.

No external personnel may enter a containment laboratory for cleaning, servicing of equipment, repairs etc, unless a responsible member of staff has been informed and appropriate arrangements have been made for them to undertake their work safely.

References

General Articles, Reports, Workshops and Reviews that Include Environmental Benefits

An excellent detail on procedures of surveillance and emergency planning is described on the site https://www.hse.gov.uk.htm

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Paper shows that sowing herbicide resistant crops facilitates minimum tillage, which favors conservation and biodiversity of soils.

Pappu HR. 1999. Biosafety issues of genetically engineered virus-resistant plants. In: Biosafety Issues of Genetically Engineered Herbicide-Tolerant Plants-Agriculture and Agri-Food Canada's Perspective. Shantharam S and Montgomery JF (editors). Biotechnology, Biosafety, and Biodiversity:Scientific and Ethical Issues for Sustainable Development.

Pham-Delègue MH, Girard C, Le Métayer M, Sandoz G, Picard-Nizou AL, Hennequet C, Pons O and Jouanin L. 2000. Long-term effects of soybean proteinase inhibitors on digestive enzymes, survival and learning abilities of honeybees. Entomologia Experimentalis et Applicata **92**: 21-29.

Pilcher CD, Obrycki JJ, Rice ME and Lewis LC. 1997. Preimaginal development, survival, field abundance of insect predators on transgenic Bacillus thuringiensis corn. Environmental Entomology **26**: 446-454.

Pimentel DS and Raven PH. 2000. Bt corn pollen impacts on non-target Lepidoptera: Assessment of effects in nature. Proceedings of the National Academy of Sciences (USA) **97**: 8198-8199.

Pleasants JM, Hellmich RL, Dively GP, Sears MK, Stanley-Horn DE, Mattila HR, Foster JE, Clark P and Jones GD. 2001. Corn pollen deposition on milkweeds in and near cornfields. Proceedings of the National Academy of Sciences (USA) **98**: 11919-11924.

Report focuses on the benefits of biotechnology for developing countries and discusses potential risks and the downside of reducing choices for poor countries. Discusses environmental aspects.

Report of a workshop held to assess and discuss what is known and not known about the environmental impact of pest resistant crops. Report includes plenary papers and reports from subgroups organized by type of crop.

Report reviewing the evidence that Bt 176 corn pollen is toxic to Monarch larvae. Report concluded that on average, pollen counts on milkweed leaves were lower that those demonstrated to be toxic to larval neonates and that pollen counts only five meters from the field were close to zero. Most pollen falls within 5 meters of the field; more milkweed occurs in conservation areas compared to cultivated areas, excluding roadsides. Report concludes that data do not provide evidence that monarch larval stages and peak pollen shed in Ontario strongly overlap.

Reviews development of virus-resistant plants and discusses issues of possible concern. Highlights the need to balance risks with benefits and compare products of biotechnology with those of traditional methods.

Schuler TH, Denholm I, Jouanin L, Clark SJ, Clark AJ and Poppy GM. 2001. Population-scale laboratory studies of the effect of transgenic plants on non-target insects. Molecular Ecology **10**: 1845-1853.

Scott SE and Wilkinson MJ. 1998. Transgene risk is low. Nature **393**: 320. (Editorial)

Sears et al 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. PNAS. **98**: 11937-11942.

Sears MK, Hellmich RL, Stanley-Horn DE, Oberhauser KS, Pleasants JM, Mattila HR, Siegfried BD and Dively GP. 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. Proceedings of the National Academy of Sciences (USA) **98**: 11937-11942.

Sears MK, Stanley-Horn DE and Mattila HR. 2000. Preliminary Report on the Ecological Impact of BT Corn Pollen on the Monarch Butterfly in Ontario. Canadian Food Inspection Agency and Environment Canada, Ottawa Ontario Canada. Accessible at: http://www.agbios.com/articles/searsreport.pdf

Showed that a genotype with inferior seed production invaded natural habitats as successfully as did the wild genotype. This result can occur whenever population size is limited by factors other than seed production.

Siegel J P. 2001. The mammalian safety of Bacillus thuringiensis -based insecticides. Journal of Invertebrate Pathology **77**: 13-21.

Snap shots of populations of organisms do not necessarily enable realistic predictions about long term effects on populations.

Stanley-Horn, DE, Dively, GP, Hellmich, RL, Mattila, HR, Sears, MK, Rose, R, Jesse LCH, Losey, JE, Obrycki, JJ, and Lewis, L. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proceedings of the National Academy of Sciences (USA) **98**: 11931-11936.

Study comparing Bt transgenic corn with isogenic corn found no significant differences in distribution, size and parasitism of egg masses of the European corn borer. Predator densities and parasitism of corn borer larvae did not differ between the two plots. The authors concluded that all observed differences in natural enemy population parameters under other study conditions were in the direction opposite to that expected if transgenic plants had an adverse impact.

Study in which Bt176 corn pollen and control corn pollen were fed to lady beetle larvae and survival of larvae was equivalent in both groups.

Study on the effect of Bt corn on aphids and their predators, green lacewings, showed no significant effect on aphid larvae development or lacewing mortality.

Study showed adverse effects of Bt corn pollen on Monarch larvae when pollen was present in very high quantities.

Study is a good example of out-crossing to relatives from conventional crops. Weedy rye most probably arose from hybridization between cultivated and wild plants. This study estimated genetic variation by isozyme analysis and multilocus out-crossing using the private-alleles method; results indicated little genetic differentiation had occurred among these 11 weedy rye populations whereas out-crossing was high.

Study showed multiple herbicide resistant canola volunteers as expected on the basis of the ability of canola to out-cross. Volunteers were easily controlled by alternative herbicides.

Sullivan DS and Sullivan TP. 2000. Non-target impacts of the herbicide glyphosate: A compendium of references and abstracts. 5th edition. Applied Mammal Research Institute, Summerland, British Columbia, Canada. 1-251.

Sun M and Corke H. 1992. Population genetics of colonizing success of weedy rye in northern California. Theoretical and Applied Genetics **83**: 321-329.

The authors measured transgene flow from transgenic oilseed rape to two wild species across 15,000 km2 in southeast England in populations where the wild plants were found. The following year new plants were screened for hybrid status. One hybrid was observed from the 505 plants screened in the B. rapa populations but none of the nine B. oleracea recruits were hybrids. Measures to minimize gene flow are suggested, and a procedure for the post-release evaluation and containment of GM cultivars is proposed.

Tombolini, R. and J.K. Jansson. 1998. Monitoring of GFP tagged bacterial cells. Met. Molecul. Biol. **102**: 285- 298.

Watkinson AR, Frecklelton RP, Robinson RA and Sutherland WJ. 2000. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. Science **289**: 1554-1557. [See editorial comment by Firbank and Forcella]

Westwood JH and Traynor P. 1999. Ecological Effects of Pest Resistance Genes in Managed Ecosystems. ISB Workshop, Jan. 31-Feb. 3. Information Systems for Biotechnology, Blacksburg, VA. Available at: http://www.nbiap.vt.edu/ index.html

Wilkinson MJ, Davenport IJ, Charters YM, Jones AE, Allainguillaume J, Butler HT, Mason DC and Raybould AF. 2000. A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. Molecular Ecology **9**: 983-991.

Wraight CL, Zangerl AR, Carroll MJ and Berenbaum MR. 2000. Absence of toxicity of Bacillus thuringiensis pollen to black swallowtails under field conditions. Proceedings of the National Academy of Sciences (USA) **97**: 7700–7703.

Zangerl AR, McKenna D, Wraight CL, Carroll M, Ficarello P, Warner R and Berenbaum MR. 2001. Effects of exposure to Event 176 Bacillus thuringiensis corn pollen on Monarch and Black Swallowtail caterpillars under field conditions. Proceedings of the National Academy of Sciences (USA) **98**: 11908-11912.

Zuo JR, Nui Q-W, Møller SG and Chua N-H. 2001. Chemical-regulated, site-specific DNA excision in transgenic plants. Nature Biotechnology **19**: 157-161.

In: Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Chowdhury MKA, Hoque MI and Sonnino A (Eds.). pp. 221-284. © FAO 2009.

Chapter 5: The International Framework: WTO and IPRs

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Introduction

World Trade Organization (WTO) is a multilateral system outside the UN with mandate to develop, promote and oversee rule-based, fair and transparent global trade to help producers of goods and services, exporters, and importers for ushering in a more prosperous, peaceful and accountable economic world. Historically, WTO is the new international trade framework erected on the edifice of UN sponsored General Agreement on Tariff and Trade (GATT), which was founded in 1948 to oversee and promote trade in goods. The important differences between the WTO and the GATT are that the former comprehensively covers all aspects of trade, going beyond trade in goods, and binding of Members to all agreements governing the WTO. WTO was established under the Marrakesh Agreement concluded in 1994 on culmination of lengthy GATT trade negotiations held in Uruguay (called Uruguay Round) during 1986 to 1994 and came in to effect from 1 January 1995. GATT continues to be the principal rule-book for trade in goods under the WTO. The Uruguay Round also encompassed trade in services, trade related aspects of intellectual property, dispute settlement, and trade policy reviews.

Main principles of the trading system promoted by the WTO are non-discrimination, reciprocity, market access and fair competition. Non-discrimination is sought to be achieved by the 'most-favoured nation (MFN) status and national treatment to every Member country. MFN status provides all Member countries equal treatment in trade. For example, if a special favour such as a lower customs duty rate for one of the products is offered to an exporting Member by the importing Member, the latter has to extend similar treatment on the same product to all exporting WTO Members. There can, however, be exemption to this status arising from free trade agreements. National treatment means that a Member country market should treat locally-produced and imported goods, after the legal entry of latter through customs port, domestic and foreign services as well as intellectual property rights on equal terms. In other words national treatment means giving others the same treatment as one's own nationals. Reciprocity principle allows that favours or benefits such as concessional terms (include penalties) that are granted by one Member or its legal entities, are returned in kind. For example, low or zero tariff trade is allowed in regional, bilateral or pluri-lateral trade groupings such as the North American Free Trade Area (NAFTA), South American Free Trade Area (SAFTA), Asia-Pacific Economic Cooperation (APEC), South Asian Free Trade Area (SAFTA), Bangladesh, India, Myanmar, Sri Lanka and Thailand Economic Cooperation

(BIMSTEC), US-Singapore Free Trade Agreement, etc. Trade flow is usually hindered by several trade barriers. Lowering or removal of these barriers is one of aims of WTO to promote international trade. The more common barriers include customs duties (or tariffs) and measures such as import bans or quotas that selectively restrict quantity imported or high phytosanitary standards. Member countries are encouraged to "bind" their commitments to open their markets for goods or services. Market access is promoted through tariffication of non-tariff barriers and transparency in cross border trade. Thus the WTO trading system tries to improve predictability and stability in trade, promotes fair and competitive trade by minimizing or removing unfair trade distorting practices, such as export or farm subsidies, dumping, etc. Dumping means exporting products and services at cost below the actual production cost to gain market share.

WTO, started with 123 countries is currently participated by 153 Member and 30 observer countries. It oversees about 95 % of world trade of goods and services. All decisions are taken by the General Council (GC), which is represented by ambassadors or head of official delegations from each Member country. Decisions are invariably made on consensus by all Members. The Trade Policy Review Body and the Dispute Settlement Body are constituted by members nominated from the GC. The top level decision-making body of the WTO is Ministerial Conference (MC), which meets at least once in every two years.

Secretariat of WTO is in Geneva and headed by Director-General. The current annual budget of WTO is about 184.9 million Swiss Francs. Its functions include, administering WTO trade agreements; acting as forum for trade negotiations; deciding on trade disputes through dispute settlement mechanism; monitoring national trade policies; helping capacity building of developing countries through technical assistance and training; and cooperating with other international organizations to promote its goals. The WTO agreements are lengthy legal texts often complex, covering a wide range of activities. The complete set consists of about 30 agreements and separate commitments (called schedules) made by individual members in specific areas such as customs duty rates and services market-opening. The agreements deal with agriculture, textiles and clothing, banking, telecommunications, government purchases, industrial standards and product safety, food sanitation regulations, intellectual property, etc. The basic principles of WTO described above run throughout all of these agreements and constitute the foundation of this multilateral trading system. The developing and least developed countries and countries in economic transition are offered special and differential treatment special safeguard mechanisms in the application of most of the agreements. Broadly, these agreements may be clubbed under major WTO domains, such as trade in goods (GATT), trade in services (GATS), trade related aspects of intellectual property rights (TRIPS), trade related investment measures (TRIMS), dispute settlement mechanism (DSM), and trade policy review mechanism (TPRM).

Among the major agreements bound to the WTO, the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) is briefly discussed to understand the role of intellectual property in the trade.

Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS)

Basic Principles

This agreement has 73 articles packed in seven parts. The first part containing general provisions and basic principles, comprising eight articles, set the overall principles of this agreement and the minimal level of legal protection Members are bound to provide on each of the eight kinds of intellectual property rights. These basic principles offer fair flexibility to Members in establishing and enforcing a *de minimus* intellectual property rights regime in their countries. TRIPS harmonises all earlier intellectual property conventions and treaties such as the Paris Convention, the Berne Convention, the Rome Convention, the Treaty on Intellectual Property in Respect of Integrated Circuits and to some extent the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The basic principles include national and MFN treatments, rights of priority and independence of patent. Rights of priority allows any inventor after making a first application on an invention in one Member country to make any number of applications in as many other Member countries on same invention within a specified period time and to gain the benefit of date of filing in all countries as has been done in the first filed country. This period is 12 months in case of patents and 6 months in the case of trademark. Independence of patent means that the fact that a patent has been granted for an invention in a given country does not exclusively constitute a ground for getting patent for the same invention in another country. The stated aim of the TRIPS agreement is promotion of technological innovation and the transfer and dissemination of technology, to the mutual advantage of producers and users of technological knowledge in a manner conducive to social and economic welfare, and to a balance of rights and obligations. While formulating or amending domestic laws and regulations in compliance with this Agreement, Members are allowed to use flexibility to adopt measures necessary to protect public health and nutrition, promote the public interest in sectors of vital importance to their socio-economic and technological development.

The Standards on different kinds of Intellectual Property Rights (IPRs)

The TRIPS agreement under part II, across articles 9 to 40, specifies the minimal standards and scopes to be enacted and enforced under eight kinds of IPRs. These IPRs are:

1. Copyright and Related Rights;
2. Trademarks;
3. Geographical Indications;

4. Industrial Designs;
5. Patents;
6. Layout-Designs (Topographies) of Integrated Circuits;
7. Protection of Undisclosed Information; and
8. Control of Anti-Competitive Practices in Contractual Licences.

Copyright and Related Rights

Copyright protection is brought in conformity with Berne Convention (1971). It offers exclusive ownership right to expressions and not to ideas. The protection is from unauthorized copying, translation, adaptation or sale of creative works such as literary, artistic or musical works, lectures, plays, art reproductions, models, photographic work, cinematographic works including films, sound recordings, broadcast, phonograms, any other work of applied art, computer programmes, compiled data (machine readable or other forms), etc. A copy right is automatically established when ever a work of this nature is created or performed. The right accrued is global. However, registration of a copy right is important for asserting the legal right during action against infringements. The mandated duration of protection of a work, other than a photographic work or a work of applied art, shall be no less than 50 years from the end of the calendar year of authorized publication over and above the life span of the person who created the work. In case the work is not published within 50 years from the making of the work, 50 years from the end of the calendar year of making is the period of protection. The term of the protection to performers and producers of phonograms shall be a minimum of 50 years computed from the end of the calendar year in which the fixation was made or the performance took place. Where Members grant rights to broadcasting organizations, the term of protection shall be a minimum of 20 years from the end of the calendar year in which the broadcast took place.

Trademarks

Any sign, or any combination of signs, words including personal names, letters, numerals, figurative elements and combinations of colours as well as any combination of such signs, which are visually perceptible and capable of distinguishing the goods or services of one undertaking from those of other undertakings, shall be eligible for registration as trademarks. The duration of protection of a trademark after initial registration, and after every renewal of registration shall be for a minimum term of seven years. The renewal of a trademark shall be indefinite.

An application for trade mark shall not be refused solely on the ground that intended use has not taken place before the expiry of a period of three years from the date of application or the nature of the goods or services to which the trademark is intended. The owner of a registered trademark shall have the exclusive right to prevent all third parties not having the owner’s consent from using in the course of trade identical or similar signs for goods or

services which are identical or similar to those in respect of which the trademark is registered where such use would result in a likelihood of confusion.

Normally continuous disuse of a trademark, without valid reason, exceeding three years may qualify for cancellation of its registration. Trademarks could be licensed or assigned with or without con-joined business.

Geographical Indications

Geographical indications identify a good as originating in the territory of a Member, or a region or locality in that territory, where a given quality, reputation or other characteristic of the good is essentially attributable to its geographical origin.

Unlike other types of IPRs, GI is a collective mark owned by all concerned within an indicated region and not exclusively exercised either by single individual or firm. Examples are Champagne, Scotch whisky, Basmati rice, Florida Oranges, Darjeeling tea, Prosciutto di Parma, New Zealand Lamb, Malabar black pepper, Feta cheese, Czech crystal, Swiss watches, Indian carpets and many more.

GIs have long been common in Europe, where there is a tradition of associating certain food products with particular regions. The Lisbon Agreement, 1958 provided elaborate provision on the protection of Appellations of Origin (AO) and their registration. An AO is a special kind of GI used on products that have a specific quality that is exclusively or essentially due to the geographical environment in which the products are produced. The concept of GI is inclusive of AO. Some, however, view that what is considered a very specific term for a well-known local specialty in one country may constitute a generic term or genericized trademark for that 'type of' product in another. For example, 'Parmigiano' cheese in Italy is generically known as *Parmesan* cheese in Australia and the United States.

TRIPS take into account these variations and require that Member countries interested to protect their GI have to provide the legal mean to prevent misuse of geographical name in a manner to mislead the public ('passing off'). It specify that Members in their national legislation could refuse or invalidate the registration of a trademark which contains a GI with respect to goods not originating in the indicated territory, if use of such indication in the trademark for such goods in their country could mislead the public as to the true place of origin of the goods concerned. However, TRIPS offers no obligation to protect GIs which are not or cease to be protected in their country of origin, or have fallen into disuse. In other words protection under GI should start from the country of origin supported by appropriate legislation.

Where a trademark has been applied for or registered in good faith, or where rights to a trademark have been acquired through use in good faith either before the date of these provisions taking effect in a Member country; or before the GI is protected in its country of origin; measures adopted to implement the GI shall not prejudice eligibility for or the validity of the registration of a trademark, or the right to use a trademark, on the basis that such a

trademark is identical with, or similar to a GI. TRIPS also safeguards the right of any person to use, in the course of trade, that person's name or the name of that person's predecessor in business, except where such name is used in such a manner as to mislead the public.

Members have the right to enter into negotiations for expanding the protection of individual geographical indications and other Members shall cooperate to conduct such negotiations or to conclude bilateral or multilateral agreements. The Council for TRIPS at the request of a Member, shall consult with any Member or Members to facilitate negotiations and to address matters affecting the compliance with the obligations under the provisions of GI.

TRIPS offer special protection to the GI of wines. Accordingly, every Member is required to provide the legal means to prevent 'passing off' for wines not originating in the place indicated by the GI in question or the GI is used in translation or accompanied by expressions such as "kind", "type", "style", "imitation" or the like. It also requires refusal or invalidation of registration of a trademark for wines which contains or consists of a GI identifying wines or for spirits which contains or consists of a GI identifying spirits. In the case of homonymous GIs for wines, protection shall be accorded to each indication, after determining the practical conditions under which the homonymous indications in question is distinguishable from each other, subject that such determinations receive equitable treatment of the producers and that consumers are not misled.

Industrial Design

Industrial design (ID) is the product of professional process of creating and developing concepts and specifications that optimize the function, value and appearance of products and systems for the mutual benefit of both user and manufacturer. It involves a shape, configuration, pattern, ornament, composition of lines or colours applied to any article in a manner to provide a visual appeal and judgement. The role of an industrial design is to provide design solutions towards problems of form, usability, user ergonomics, engineering, marketing, brand development and sales. TRIPS require Members to provide for the protection of independently created industrial designs that are new or original, but not to those which are not new or original and do not significantly differ from known designs or combinations of known design features. However, such protection should not extend to designs dictated essentially by technical or functional considerations. ID may occasionally overlap with copyright. For example, the textile designs may be protected through either industrial design law or copyright law. Like other IP forms, ID offers the owner of a protected industrial design to have exclusive right to prevent third parties from making, selling or importing articles bearing or embodying a design which is a copy, or substantially a copy, of the protected design, without owner's consent when such acts are undertaken for commercial purposes. The minimum period of protection stipulated is 10 years. This can be subsequently extended for equivalent period.

Patent

An important aspect of TRIPS is that it provides for patent for any invention, whether products or processes, in all fields of technology, provided that they are eligible otherwise (Art.27). The three major eligibility criteria to receive patent for an invention are novelty, non-obviousness or involving an inventive step and utility or amenability for industrial application. Further, patents shall be available and patent rights enjoyable without discrimination as to the place of invention, the field of technology and whether products are imported or locally produced.

TRIPS agreement offer three exemptions to its affirmation that patents shall be available for any invention, whether products or processes, in all fields of technology. These are:

1. Members may exclude from patentability inventions, the prevention within their territory of the commercial exploitation of which is necessary to protect *ordre public* or morality, including to protect human, animal or plant life or health or to avoid serious prejudice to the environment, provided that such exclusion is not made merely because the exploitation is prohibited by their law (Art. 27.2);
2. Members may also exclude from patentability diagnostic, therapeutic and surgical methods for the treatment of humans or animals (Art. 27.3 a);
3. Members may also exclude from patentability plants and animals *other than micro-organisms*, and essentially biological processes for the production of plants or animals *other than non-biological and microbiological processes*. However, Members shall provide for the *protection of plant varieties either by patents or by an effective sui generis system or by any combination thereof (Art. 27.3b).*

Patent is a legal grant conferring exclusive rights to the patent owner. This right excludes third parties not having the owner's consent, in the case of a product, from the acts of making, using, offering for sale, selling, or importing that product for these purposes; and in the case of a process, from the act of using the process, and from the acts of using, offering for sale, selling, or importing for these purposes the product obtained directly by that process. The patentee, however, shall have the right to assign, or transfer by succession, the patent and to conclude licensing contracts.

One important condition for the grant of patent is disclosure of the invention in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art. It may also require the applicant to indicate the best mode for carrying out the invention known to the inventor at the priority date of the application. A patent grant is applicable within the geographic territory of the country of grant for a period not less than 20 years from the date of filing of application.

Art. 27.3.(b) is important from the point of view of biotechnology research. It requires all Members to grant patent to qualified inventions related to microorganisms, microbiological and non-biological processes. Novel biotechnology process is also eligible for patenting.

When the product patent principle is applied on a product derived from a novel biotechnology process, such product may or may not be patentable subject to the policy of the Member on the patentability of plants and animals. Apart from biotechnology, the product and process patent have implications to innovations in chemical, pharma, biochemical and food processing areas. While a process patent largely specifies to a chemical/biochemical pathway or an operational or production process leading to the product, a product patent allows enhanced monopoly to the inventor on larger technological domain to exclude others from developing alternate novel pathways or processes to produce the same or equivalent product.

It is important to appreciate that a patent established in one country is of no concern in another where it is not established. In fact, there is no international patent, unless a patent is established in all countries according to their independent laws. Extension of a patent from the first country of application to other countries has to be completed within 12 months. (The Patent Cooperation Treaty-PCT- administered by the World Intellectual Property Organisation facilitates patenting in multiple countries at lower cost and lesser time). Grant of patent to a given invention by a country shall be subject to independence of patent. A patent may automatically lapse after the specified period of protection or earlier when it is abandoned by the patentee.

Notwithstanding a Member's policy on granting or not granting patent to plants and animals, TRIPS make protection of plant variety either by patents or by an effective *sui generis* system or by any combination of these two mandatory. *Sui generis* is a very special kind of IP protection having major difference from patent. Latin words *sui generis* mean 'self generated' or 'unique by itself'. One familiar *sui generis* system used for protection of plant varieties is the plant breeder's right (PBR) in the International Union for the Protection of New Varieties of Plants (UPOV). PBR is a right granted to the breeder to exclude third parties from producing, selling, marketing, distributing, importing or exporting the propagating material of the protected variety. This right, like in the case of patent, is assignable or transferable by succession or licensable. It is important to note that the term *sui generis* system in TRIPS does not essentially imply to the UPOV and Members have flexibility to evolve their own effective *sui generis* system. PBR, unlike patent, is not an absolute right; it is inclusive of farmers' rights (so called farmers' privilege in UPOV) and researchers' rights (what UPOV allows as acts done for experimental purposes including breeding new varieties). The FAO Seed Treaty (the International Treaty on Plant Genetic resources for Food and Agriculture) defines farmers' rights as the right to save, use, exchange and sell farm-saved seed/ propagating material of varieties including protected varieties; right for the protection of traditional knowledge relevant to PGRs and to equitably participate in sharing benefits arising from the utilisation of PGRs; and right to participate in making decisions on matters related to conservation and sustainable use of PGRs.

A patent is liable for compulsory licensing under certain extra-ordinary situation. TRIPS stipulate that such licensing should be done only with authorization of the right holder (Art.

31). Members have the right to exercise TRIPS flexibilities in the healthcare sector to protect public health by facilitating affordable access to medicine in poor economies, particularly during public health emergencies (pandemic) by resorting to compulsory licenses in case the patentee is failing to meet the national requirements either in terms of volume of production or in reasonable cost. Under the compulsory license, the exclusive right granted to the patentee is temporarily suspended to allow third parties to work the patent or its generic equivalents for a determined period.

An important provision in TRIPS in respect of civil proceedings arising from suspected infringement of the rights of the owner of a process patent for obtaining a product is that the judicial authorities are empowered to order the defendant to prove that the process to obtain an identical product is different from the patented process. Here the burden of proof on an impugned infringement lies with the defendant. The legal procedure, however, safeguard the legitimate interests of defendants in protecting their manufacturing and business secrets in case the alleged infringement proves to the contrary.

Layout Designs (Topographies) of Integrated Circuits

Topography of an integrated circuit is a spatial geometric arrangement, fixed on a material carrier, of all the components of an integrated circuit and the connections there between. Integrated circuit is a microelectronic product, designed to carry out the function of the electronic circuit.

TRIPS deem that importing, selling, or otherwise distributing for commercial purposes a protected layout-design, an integrated circuit in which a protected layout-design is incorporated, or an article incorporating such an integrated circuit without the authorization of the right holder as unlawful. Member countries are required enforce domestic law to make such act unlawful and to provide prompt legal relief to the right holder including a sum equivalent to a reasonable royalty such as would be payable under a freely negotiated license in respect of such a layout-design. It also lays out conditions and compensations on non-voluntary licensing. The term of protection of layout-designs shall be at least a period of 10 years counted from the date of filing an application for registration or from the first commercial exploitation wherever is earlier.

Protection of Undisclosed Information

Although undisclosed information has often been referred to as “trade secrets” or “know-how”, TRIPS do not use these terms. It also does it provide a definition of “undisclosed information”. Such a neutral terminology does not characterize the contents of the information, but only its “undisclosed” nature. “Undisclosed information” covers any secret information of commercial value, including technical know-how, such as design, process, formula and other technological knowledge often resulting from experience and intellectual ability; data of commercial value, such as marketing plans, customers lists and other business-

related information that provides an advantage over competitors; and test and other data submitted for the approval of pharmaceutical and chemical products for agriculture. The obligation established is limited to the protection of undisclosed information "against unfair competition as provided in Article 10*bis* of the Paris Convention".

The unfair competition provides a remedy against acts of competition contrary to honest business practices, such as confusing or misleading the customer and discrediting the competitor. An act of unfair competition may be defined as "any act that a competitor or another market participant undertakes with the intention of directly exploiting another person's industrial or commercial achievement for his own business purposes without substantially departing from the original achievement." TRIPS stipulate that an "undisclosed information" may be qualified for protection if it is secret, possess a commercial value and be subject to reasonable steps, under the circumstances, to be kept secret. Members shall protect such data against disclosure, except where necessary to protect the public, or unless steps are taken to ensure that the data are protected against unfair commercial use. The undisclosed information shall remain so as long it is protected by the owner, the legal system of the Member and not deciphered by honest practice by a third party.

Control of Anti-Competitive Practices in Contractual Licenses

One of the important objectives of IPRs is promotion of innovations in technology development, its dissemination to promote competition and public benefit with economic growth and better quality of life. Licensing is a common method in the transfer of IP protected technologies. Therefore those practices, which restrain competition may have adverse effects on trade, transfer and dissemination of technology and public welfare. TRIPS, therefore, allows Members to devise suitable legislative measures to prevent misuse of IPRs to advance monopoly and to prevent competition in a Member's market.

In the event of the IPR owner is a national or domiciliary of one Member and this entity while working of that IPR in another Member country is violating the laws and regulations of the latter, solution to such problems through consultations and cooperation between them are stipulated. Such cooperation may involve supply of publicly available and relevant non-confidential information to the matter in question and of other information available to the Member, subject to domestic law and to the conclusion of mutually satisfactory agreements concerning the safeguarding of its confidentiality by the requesting Member. In the event of nationals or domiciliaries of a Member are subject to proceedings in another Member on alleged violation of the latter Member's laws and regulations, an opportunity for consultations by the other Member, upon request, is to be allowed.

General Obligations

Members, apart from establishing a TRIPS compliant legislative framework for providing a *de minimus* protection to all types of IPRs covered by this Agreement, are required to

establish an administrative and jurisprudential system efficient and effective in enforcement of the IPR laws and regulations. Such system has to permit effective action against any act of infringement of IPRs and expeditious remediation to prevent infringements and deterrent penal action. These procedures, which shall be fair and equitable, are to be applied in such a manner not to create barriers to legitimate trade and to provide for safeguards against their abuse. Also important is that the administrative and judicial process shall not be unnecessarily complicated or costly, or entail unreasonable time-limits or unwarranted delays. Decisions on the merits of a case shall be evidence-based with fair opportunity for the parties to be heard. There should be appellate judicial authority to review the final administrative decisions, subject to jurisdictional provisions in a Member's law concerning the importance of a case. It, however, does not imply that there is any obligation to put in place a judicial system for the enforcement of IPRs distinct from that for the enforcement of law in general.

Transitional arrangements

The transitional period provided to developing countries to bring their IPR regime in compliance with TRIPS, including the product patent, expired on 31 December 2004.

In the case of the least-developed country Members, the time line to bring their domestic laws in compliance with TRIPS in respect of all IPRs embraced by this Agreement was extended just before Hong Kong Ministerial from 1 January 2006 to 1 July 2013. The transition period for these countries for product patents is until 2016. However, all countries, with effect from January 1995 are required to comply with the general principles on Most-Favoured-Nation Treatment, National Treatment and Multilateral Agreements on acquisition or maintenance of protection.

During the transition period and later, the developed country Members are required to provide incentives to enterprises and institutions in their territories for promoting and encouraging technology transfer to least-developed countries to enable them to create a sound and viable technological base.

Dispute Prevention and Settlement

For preventing disputes, Members shall maintain transparency and access to information on the laws and regulations, and final judicial decisions and administrative rulings of general application and those pertaining to availability, scope, acquisition, enforcement and prevention of the abuse of intellectual property rights. This information is either published, or made publicly available in a national language, in such a manner as to enable governments and right holders to become acquainted with them. Any Agreement on the matter which is in force between the governmental agencies of a given Member and others shall also be published. These are also shared with the Council for TRIPS. Such information sharing, however, shall not disclose confidential information which would impede law enforcement or

otherwise be contrary to the public interest or would prejudice the legitimate commercial interests of particular enterprises.

On receipt of a compliant from a Member related to the IPR issues pertaining to another, the Council of TRIPS may examine the scope and modalities for complaints within the stipulated time period and submit its recommendations to the Ministerial Conference for approval. Any decision of the Ministerial Conference to approve such recommendations or to extend the period of decision shall be made by consensus. The approved recommendations shall be effective for all Members without further formal acceptance.

Institutional Arrangements

The Agreement assigns the mandate to monitor its operation, particularly the compliance of Members with their obligations to the Council for TRIPS. It is also obliged for providing any assistance requested by Members in the context of dispute settlement procedures. The Council for TRIPS is required to review the implementation of this Agreement after the expiration of the transitional period. In addition, the Doha Ministerial entrusted the Council for TRIPS a work programme to review of Article 27.3(b), review of the implementation of the TRIPS Agreement and to examine, *inter alia*, the relationship between the TRIPS Agreement and the Convention on Biological Diversity, the protection of traditional knowledge and folklore.

The international framework: The International Treaty on Plant Genetic Resources for Food and Agriculture

The International Treaty on Plant Genetic Resources for Food and Agriculture[1] (IT or ITPGRFA) was adopted on 3 November 2001 under the auspices of the Food and Agriculture Organisation (FAO). As evident from its title, this Treaty relates to plant genetic resources for food and agriculture (PGRFA). The Treaty defines PGRFA as *any genetic material of plant origin of actual or potential value for food and agriculture* [Article 2].

Background of the Treaty

The Treaty had its origin to the historic role FAO had been playing since 1983 with the establishment of the International Undertaking on Plant Genetic Resources (IUPGR) and setting up an independent Commission on Plant Genetic Resources (CPGR)[2]. The Agenda 21 in 1992 called for strengthening the FAO Global System on PGR and its harmonisation in line with the Convention on Biological Diversity (CBD)[3]. The CBD heralded a paradigm change in the global perspective on the PGR from that of "a heritage of humankind" to that with "state exercising sovereign rights over". The CBD adopted in the Nairobi Final Act in May 1992 reaffirmed the sovereign rights of states over their own biological resources. This rights, however, are limited *to the components of biological diversity, in areas within the limits of its*

national jurisdiction (Art. 4), meaning that the said sovereignty did not extend on those PGR which were collected from many States and being kept in *ex situ* gene banks outside their national jurisdiction prior to the adoption of CBD. These PGR were largely constituted by those seed accessions, numbering over 7,00,000, being conserved in 11 International Agricultural Research Centres (IARCs) under the Consultative Group on International Agricultural Research (CGIAR). More than 70% of these accessions comprising landraces and wild materials were collected from farmers and indigenous peoples in the developing countries. Having the rights on these PGRs left outside the scope of CBD, the Nairobi Final Act adopted Resolution 3, with directions to seek solutions to outstanding matters concerning PGR[4].

According to this Resolution the FAO entered into an Agreement with the CGIAR in October 1994 on the PGR collections conserved in IARCs as an interim arrangement. This Agreement placed these PGRs under the politico-legal protection called "trusteeship" of the Commission on Genetic Resources for Food and Agriculture (CGFRA), which is an intergovernmental authority evolved from the CPGR. It was further agreed that: (1) the designated germplasm shall be held "in trust" by the IARCs for the benefit of humanity; (2) this 'in-trust germplasm' shall be maintained properly and shared freely for the purpose of conservation, research and plant breeding only through a model MTA; and (3) none shall be allowed to take out IPR on the "in-trust germplasm in the form received", including third party IPR claims.

Another outstanding issue referred to in the Resolution 3 was the question of Farmer's Rights. The Farmers' Rights (FR) as a concept was brought forth and recognized by the IUPGR of the FAO in 1983. In 1989 FAO recognized the plant breeders' rights (PBR) introduced by the UPOV[5] in 1961 and the FR. According to the IUPGR, FR is "the rights arising from the past, present and future contributions of farmers in conserving, improving and making available plant genetic resources, particularly those in the centers of origin or diversity". These rights are vested with the international community, as the trustees for the present and future generations of farmers, for the purpose of ensuring full benefits to the farmers and supporting continuation of their contributions. Farmers' Rights gained wide socio-political significance in the context of emergence of private seed companies and globalization of PBR by the TRIPS in developing countries where seed system and diversity are strongly associated with farmers' varieties. Crop genetic resources in landraces and farmer varieties share several characteristics with intellectual goods that are protected as intellectual property[6]. However, FR lacked legal sanctity and left excluded in international conventions or agreements like CBD or TRIPS, while PBR gained wider legal status under the *sui generis* system of plant variety protection. Hence the reference to FR in Resolution 3 was a reminder of the unfinished agenda on FR.

Pursuing the Resolution 3, the FAO initiated negotiations at the CGRFA level in November 1994 with following three important mandates: (1) the harmonization of the

IUPGR with the CBD; (2) to decide on the issue of access on mutually agreed terms to PGR, including *ex situ* collections not addressed by the CBD; and (3) the issue of the realization of FR.

The major elements of harmonization between IUPGR and CBD are the paradigm change brought by the CBD conferring "sovereignty of national governments over their PGR" and the facilitated process of access to the PGR through prior informed consent (PIC), mutually agreed terms (MAT), material transfer agreement (MTA), and fair and equitable sharing for the commercial use of these resources. Access on PGR within the national jurisdiction of Members as well as to those deposited in the *ex situ* collections, but not addressed by the CBD was an important negotiation issue. The third issue was on realization of FR to promote the contributions of farmers all over the world, particularly those in centres of origin and diversity, in conserving, improving and making available these resources for the future food and agriculture. On July 2001, the Sixth Session of CGFRA adopted revised IU. On November 2001, the International Treaty was adopted by the FAO Conference and it marked the culmination of a slow and arduous process over seven years. It entered into force on 29 June 2004. As on August 2008, 120 countries have joined as Parties to the Treaty.

Rationale of the Treaty

PGRFA are crucial in feeding the world, human and livestock. It include the land races which farmers have selected and conserved, other varieties and the wild relatives of crop plants. They constitute the raw material of huge value for improving the quality and productivity of all crops through modern plant breeding. The genetic variability of each and every crop is not uniformly distributed in the world. Different regions and countries situated within each region are endowed with variable wealth of PGR in different crops. No single country in the world, however rich it might be for its PGRFA, is self-sufficient in this respect for ensuring its food and feed security, now and in future. This diversity stands as a testimony to the profound contribution made by the farmers world over. They are not only generating a huge genetic variability and conserving them, but also building equally rich traditional knowledge on every component of this variability including the wild relatives. The flow of genetic variability for crop improvement is primarily from the PGR rich regions. Continuous genetic improvement is cardinal for sustaining regional and global food security under increasing population, need for continuous increase in productivity and quality in some cases, mitigating biotic and abiotic pressures including the new challenges from the climate change. Hence, there is an inevitable dependence across countries to sustain agricultural production and global food security. Therefore, collective agricultural future of global community demands a framework for international cooperation in supporting farmers for conservation of PGR and exchange of these resources and their genes. This Treaty devised with this rationale in the CBD context.

The Framework of the Treaty

The Treaty is structured with a Preamble, the text, and two annexure[1]. The text has 35 Articles tucked under seven titles, Introduction; General Provisions; Farmers' Rights; The Multilateral System of Access and Benefit Sharing; Supporting Components; Financial Provisions; and Institutional Provisions. The Annexure I provide the list of crops covered under this Treaty, and Annexure II has Arbitration and Conciliation in two parts. The Treaty is to be administered by a Governing Body (GB) represented by all Contracting Parties (CPs) with equal voting right and decisions taken on consensus.

In the Preamble, the CPs acknowledges the importance of conservation, exploration, collection, characterization, evaluation and documentation of PGRFA for removing contemporary global poverty and ensuring food security of future generations and the need for reinforcing the capacity of developing countries to undertake such tasks in perpetuity. The CPs affirms that the farmers, world over and particularly in the regions of primary and secondary centers of genetic diversity of all crop plants, had been, are and will be playing invaluable role in conserving, improving and making available plant genetic resources and this entitles them for the Farmers' Rights (FRs). This Treaty is the first legally binding international agreement, which has defined the FRs.

Objectives and Scope of the Treaty

The primary objective is to achieve sustainable global agriculture and food security in harmony with CBD by (1) conservation and sustainable use of PGRFA, (2) facilitation of fair and equitable sharing of benefits derived from the commercial use of PGR, and (3) establishment and maintenance of a multilateral system for access to PGRFA and benefit sharing, thereof. The scope of the Treaty is the *in situ* and *ex situ* PGRFA of the crop species listed therein (Art 1).

Treaty mandates

Treaty encourages CPs to establish domestic legislations in conformity with the laws, regulations and procedures spelt out therein. The Treaty mandates that the CPs shall mutually cooperate to promote an integrated approach to explore, conserve and sustainably use PGRFA. This may include survey and inventory of PGRFA, collection of information associated with these genetic resources, their potential use and threat of potential loss, promoting and supporting farmers and local communities to undertake *in situ* conservation of these resources, including wild crop relatives, establishing protected areas of PGRFA by involving indigenous and local communities, promoting *ex situ* conservation along with documentation, characterization, regeneration and evaluation while keeping genetic integrity of collections.

On sustainable use of PGRFA, the Treaty requires the CPs to promote development and maintenance of diverse farming systems, which will support more biological diversity and other natural resources, strengthening research including plant breeding with participation of farmers to promote development of varieties adapted to social economic, ecological and marginal farming conditions, to enhance intra- and inter-specific variation in crops to reduce genetic vulnerability and to prevent genetic erosion, and to encourage increased use of local and locally adapted varieties of crops including underutilized crops.

The Treaty offers international cooperation to establish and strengthen the capabilities of developing countries and countries in economic transition in conservation and sustainable use of PGRFA, strengthening institutional arrangements under Global Plan of Action (GPA), to promote access to and sharing of PGRFA, and to support the CPs in these efforts with funding and technical assistance.

Farmers' Rights

Farmers' Rights find an important place in the Treaty with definition of rights intended thereto. Apart from emphatically declaring the rights of farmers to save, use, exchange and sell farm saved seed and other propagating material, the Treaty adds three more important rights to farmers. These are: (1) the right on the traditional knowledge relevant to the PGRFA, (2) the right to participate in decision making at national level on matters related to conservation and sustainable use of PGRFA, and (3) the right to equitably participate in sharing the benefits arising from utilization of PGRFA. While the Treaty acknowledges that these rights are important to promote the invaluable contributions being made by farmers in the conservation and development of plant genetic resources, it relegates the responsibility of realizing FR to the concerned governments.

Multilateral System of Access and Benefit Sharing

The multilateral system (MLS) provided is the major Treaty instrument and it has three essential aspects: (1) The coverage of PGRFA brought under the MLS (Table 1); (2) The facilitated access provided for this PGRFA; and (3) The system of benefit sharing available to the providers of PGRFA. The MLS is established in conformity with the CBD principles on the sovereignty of states over their PGRFA and their sovereign authority to decide on access to these resources. The Treaty legally binds CPs to place the agreed components of PGRFA under the MLS for facilitated access by other Parties and sharing of the entitled fair and equitable benefits. The PGRFA brought under MLS are listed in Table 1 and these PGRFA are located both under the jurisdiction of the CPs and in the public domain including in the *ex situ* gene banks of IARCs.

The MLS, within the jurisdiction of each CP, seeks to reach out to all PGRFA being held by natural and legal persons, including the public and private sectors, farming and local

communities. The Governing Body is required to review the access to the PGRFA and to decide appropriate measures to achieve this coverage. Out of the 666,000 and odd collections available in the IARC gene banks, only about 532,000 are brought under the MLS of this Treaty and remaining collections belonging to species not listed in Table 1 are left out from MLS. These accessions, according to the Treaty, shall be held in "trusteeship" by the CGIAR and be made available according to the 1994 FAO-CGIAR Agreement. The Treaty further undertakes to make efforts to bring these accessions under the MLS within an indicated timeframe.

Table 1. Crop groups and species brought under the MLS of the FAO Treaty

Crop Group	No. of Crops	PGRFA belonging to
Cereals	10	Rice, Wheat, Maize, Sorghum, Pearl millet, Finger millet, Barley, Oat, Rye, Triticale
Pulses	7	Chick pea, Pigeon pea, Pea, Cowpea, Faba bean, Lentil, Lathyrus
Tubers	5	Potato, Cassava, Sweet potato, Yams, Major aroids*
Oil Crops	3	Sun flower, Brassica complex**, Coconut
Sugar Crops	1	Beet
Fruit crops	5	Banana, Apple, Strawberry, Citrus, Breadfruit
Vegetables	4	Egg plant, Beans, Carrot, Asparagus, (Cabbage, Radish, Turnip)**
Food Crops-Total	**35**	
Legume forages	52 Species	Belonging to 15 genera
Grass forages	26 Species	Belonging to 12 genera
Other forages	3 species	Belonging to 2 genera
* Aroids include four species ** Brassica complex includes few oilseed and vegetable species belonging to 13 listed genera		

The Treaty establishes legal supervision of GB over concerned CGIAR institutions for the purpose of providing policy guidance relating to *ex situ* collections held by them. The GB shall also periodically monitor the access and use of PGRFA from the IARC gene banks, provide for management and administration of these *ex situ* collections in accordance with the internationally accepted scientific and technical standards under the authority of CGIAR. GB is also vested with authority to evacuate and transfer these collections from any IARC, with the approval of host country, in case the maintenance of these collections are impeded or threatened by whatever event.

Access to MLS

Facilitated access through MLS is provided to CPs and the legal and natural persons under their jurisdiction. Legal and natural persons include private and public sectors, farming

and indigenous communities. Access to PGRFA is, however, allowed only for the purpose of use and conservation for research, breeding and training for food and agriculture and not for uses in chemical, pharmaceutical and other non-food or feed industries. Access is to be allowed expeditiously, either free of charge or at a minimal charge. Subject to the national law on the subject, the access to genetic resource may include all available passport data and descriptive information on the accessed material. Access to those PGRFA under development shall be allowed at the discretion of the developer, farmer breeder or professional breeder. The Treaty for the purpose of access discriminates the *ex situ* and *in situ* PGRFA and requires that access to *in situ* material shall be governed by the relevant national legislation or as advised by GB, wherever no national legislation exists. In the case of *ex situ* collections in IARCs, the Treaty requires that each accession has to be identified for its geographic origin and countries have to be allowed access with out MTA to those accessions originated from their jurisdiction.

Another important access requirement is that the Party accessing shall not establish any intellectual property right or such other rights on the PGRFA or its genetic parts of components, in the form received from the MLS and that the accessed material or its products shall be placed in the MLS for continued access by other Parties. Access to PGRFA protected by IPR shall be consistent with the international law applicable thereof. A CP having subjected to a disaster shall have access to appropriate PGRFA through MLS to re-establish the agricultural system lost or damaged in a disaster. Access, according to the Treaty, shall be facilitated through a standard material transfer agreement (SMTA), which was approved by the GB in its Resolution 1/2006 of 16 June 2006. The SMTA is consistent with the relevant provisions of this Treaty. On the obligations of a recipient who may conserve an accessed material, the SMTA requires that the material and the related information shall be made available to the Multilateral System using the SMTA and in case the recipient transfers the material supplied under this Agreement to another person or entity, such transfer shall be only under the terms and conditions of the SMTA, through a new MTA and under notification to the GB. The SMTA has specific provisions on fair and equitable sharing of benefit arising from the commercialization of product that is a PGR**.**

Benefit Sharing under MLS

The Treaty suggests monetary and non-monetary kinds of benefit sharing[7]. The non-monetary benefits include exchange of information, access and transfer of technology, and capacity building. A fixed percentage of monetary benefit sharing is binding on a recipient who commercializes a product that is a PGRFA and that incorporates material accessed through the SMTA and where such product is not available without restriction to others for further research and breeding. In case the commercialization of such product is done in a manner so that it is available to others without restriction for further research and breeding, the recipient is encouraged to make voluntary payments towards benefit sharing. The recipient

is encouraged to share through the MLS non-monetary benefits such as non-confidential information that results from R&D carried out on the Material. Those who obtains IPR on any products developed from the material or its components, obtained from the MLS, and assigns such IPR to a third party, the benefit-sharing obligations thereto shall also be transferred to such third party. The small farmers in developing countries are exempted from paying the benefit share in case they commercialize any PGR accessed from MLS.

The non-monetary benefit sharing like information, may include catalogues and inventories on PGRFA, information on technologies, technical, scientific and socio-economic research outputs including characterization, evaluation and utilization data. The technologies may include techniques for conservation, characterization, evaluation and use of PGRFA, improved varieties bred from accessed PGRFA in conformity with applicable IPR and access laws. The Treaty provides scope for transfer of these technologies under different patterns, depending on the technology absorption capability and the relevant domestic legal framework of the recipient Party. Treaty provides that technology transfer for the benefit of farmers in developing and least developed countries shall be on most favoured terms, including concessional and preferential terms, *albeit* upholding the involved IPRs.

Capacity building is to benefit developing and least developed countries lacking scientific and technical capability in conservation and sustainable use of PGRFA. This may include need-based development of infra-structure and human resource for conservation and sustainable use of PGRFA, and building institutionalized scientific research capability to accrue benefit to the national agriculture.

Treaty is to engage public and private sectors for sharing monetary benefit. The GB is to determine the quantum, form and manner of payment of monetary benefit consistent with the commercial scope, on case-to-case basis. The GB is also responsible to review the process of determining benefit share from time to time to improve fairness and equity. CPs are also encouraged to institute voluntary benefit sharing contributions from food processing industries, because they are beneficiaries of the PGRFA. All monetary payments received, as mandatory or voluntary benefit share will flow to the fund called Global Crop Diversity Trust. These receipts are to be utilized for the agreed activities on conservation and sustainable use of PGRFA by farmers of the CPs, particularly of the developing countries.

Global Plan of Action

The Treaty is essentially a legal framework to implement the global plan of action (GPA)[8] for the conservation and sustainable use of PGRFA detailed in the 1996 Leipzig Declaration. The Treaty provides a forum for the CPs to join hands to undertake national actions assisted by international cooperation. Towards this, the Treaty provides for establishing International Plant Genetic Resources Networks (IPGRN) and a Global Information System (GIS) on PGRFA. The IPGRN is to achieve as complete coverage as possible of PGRFA and relevant institutions dotting across the CPs. The GIS shall cover

scientific, technical and environmental aspects related to PGRFA, with a view to benefit all CPs, to monitor conservation and sustainable use of PGRFA, to serve early warning on the emerging threat to PGRFA, and to generate periodic assessment on the state of the global PGRFA.

Implementation of the Treaty

Each CP is obliged to implement the Treaty within its technical and financial capability. Since all the PGRFA covered by this Treaty are located in the jurisdiction of CPs, particularly the developing countries and the eleven IARCs, their natural or empowered capability will largely determine the effectiveness of the Treaty implementation. The GB shall be chaired by elected Chairperson and Vice-Chairpersons and it shall provide policy directions, approval to plans, programmes and budgets, recommendations, monitoring and do such other functions required for promoting international cooperation, administration of *ex situ* collections in the CGIAR institutions, strengthening financial base, amending the treaty (only with consensus), etc. A regular biennial session of GB is mandatory with scope for as many special sessions as required or requested by at least one-third of the CPs. The day-to-day administration, on behalf of the GB, shall be conducted by the Executive Secretary to the GB, who shall be appointed by the Director General of FAO with the approval of the GB.

Finance for Implementation of Treaty

This Treaty is born with no institutional financial back up. Hence, it seeks to establish an endowment Trust Fund of US $ 260 m with a view to secure an annual yield of interest in perpetuity for its global administration. This fund is to be mobilized from public and private sectors, including national governments, international institutions, non-governmental foundations and a diverse array of industrial sectors. When this Treaty came into force on 29th June 2004, US $ 45 m has been committed and commitment for another US $ 60 m was in progress. Apart from this fund, the GPA on PGRFA has to be supplemented by each CP within their national technical and financial resources. Financial support also needs to flow for the *ex situ* conservation in IARCs and to support special assistance to implement agreed plan and programmes for farmers in developing countries. The GB shall be periodically setting a target for such funding. The monetary benefit share flowing to the CP from Trust Fund shall also be deemed as the funding under the Treaty.

Dispute Settlement Mechanism

Treaty provides mechanism for settlement of disputes arising among the CPs either through mutual negotiation or third party mediation. There are also defined procedures for arbitration and reconciliation.

The international framework: CBD and the Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety (CPB) is the first international agreement to regulate the transboundary movements of living modified organisms (LMOs). The CPB is a subsidiary agreement to the UN Convention on Biological Diversity (CBD), which was signed by 191 governments since its conclusion at the Rio Earth Summit in 1992.

Conceived as a practical tool for translating the principles of Agenda 21 into reality, the CBD recognizes that biological diversity is about more than plants, animals and micro-organisms and their ecosystems – it is about people and our need for food security, medicines, fresh air and water, shelter, and a clean and healthy environment in which to live. A major change from the past brought in by the CBD is the sovereign rights of the States over their biodiversity. The CBD also declared that the States are responsible for conservation and sustainable use of their biological diversity in a manner benefiting the present and future generations, and ensuring fair and equitable sharing of the benefits arising out of the utilization of any component of biological diversity or associated TK. The States also are required to protect the rights of its people who conserve the biodiversity and associated TK**.**

The Background

Article 8(g) of the CBD states that "Each Contracting Party (CP) shall establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms (LMOs) resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health". Article 19(3) further states that "The Parties shall consider the need for and modalities of a protocol setting out appropriate procedures, including, in particular, advance informed agreement, in the field of the safe transfer, handling and use of any LMO resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity." Article 28 of CBD authorizes the CPs to cooperate for formulating and adopting protocols to this Convention. Thus, CPB was spawned by the CBD to prevent or minimize the adverse environmental impacts from LMOs that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to the health human and environment. The CBD, however, did not define the term "living modified organisms" but it is understood to include genetically modified organisms (GMOs), provided they are live.

Agenda 21, which was a precursor to the CBD also sounded the need for environmentally sound management of biotechnology. While acknowledging the promises biotechnology makes for better health care, enhanced food security, improved supplies of potable water, more efficient industrial development processes for transforming raw materials, support for sustainable methods of afforestation and reforestation, and detoxification of hazardous wastes. Agenda 21 stressed that the community at large can only derive full benefit from

biotechnology if it is developed and applied judiciously and safely to prevent the adverse impacts that LMOs may have on the conservation and sustainable use of biological diversity.

Precautionary Principle

All above statements admiring the potential promises of biotechnology had been couched on a precautionary note. Precautionary approach is also evident in Principle 15 of The Rio Declaration on Environment and Development, in the preamble of the CBD, its Art 16 dealing on biotechnology transfer as well as in the proviso 19(3) referred earlier. Rio Declaration is one of the most important international expressions of the precautionary principle, the seminal affirmation that led to the Biosafety Protocol. The concern is amply integrated in the Protocol and its Annex III, where it states "lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk". The precaution is essentially with reference to the regulation of LMOs because of lack of scientific certainty and consensus as to their potential impacts on the environment and human health.

The precautionary principle is said to have its beginnings in the German principle of *Vorsorge*, or foresight. Precaution has three elements: (1) threats of harm; (2) scientific uncertainty; and (3) preventive, precautionary action. The litmus test for knowing when to apply the precautionary principle is the combination of threat of harm and scientific uncertainty. This principle was introduced in 1984 at the First International Conference on Protection of the North Sea. Later, it was integrated into numerous international conventions and agreements, including the Bergen declaration on sustainable development, the Maastricht Treaty on the European Union, and the Global Climate Change Convention. Precautionary is different from preventive approach. When there is certainty about cause and effect, then the approach is preventive and not precautionary. Statutory warning like "Smoking is injurious to health" is precautionary. Precautionary principle serves as a "speed bump" to new technology, ensuring that decisions about new activities are made thoughtfully and in the light of potential consequences. In essence, the precautionary principle provides a rationale for taking action against a practice or substance in the absence of scientific certainty rather than continuing the suspect practice while it is under or without study.

History of the Protocol

Pursuant to Article 19 (3) of the CBD, the Conference of the Parties (CoPs) in 1994 decided to establish an open-ended ad hoc group of experts nominated by Governments. After a meeting, these experts suggested establishment of an Open-ended Ad Hoc Working Group on Biosafety (BSWG). The second CoP meeting established the BSWG to develop a draft protocol on biosafety, specifically focusing on transboundary movement of any LMO resulting from modern biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity. The BSWG in 1999 submitted a draft text of the

Protocol, as well as the outstanding concerns of the Parties, for consideration by CoP. Consideration of the draft at an extraordinary meeting of the CoP on 22 February 1999, in Cartagena, Colombia was incomplete. The session resumed in Montreal from 24 to 29 January 2000, where the Cartagena Protocol on Biosafety to the CBD was adopted. In accordance with its Art. 36, the Protocol was opened for signature at the UN Office at Nairobi till 26 May 2000 and later at UN HQ in New York. On ratification of the Protocol by 50 countries and subsequent elapsing of 90 days, the CPB entered into force on 11 September, 2003. At that time it had received 103 signatures. As of October 2008, 150 countries are Party to the Protocol.

The Protocol

The text of Cartagena Protocol on Biosafety (CPB) has a preamble, 42 Articles with three annexes. The Protocol defines "LMO" as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.

Objective of the Protocol

In accordance with the precautionary approach, the objective of this Protocol is to contribute to ensuring an adequate level of protection during transboundary movement, transit, handling and use of LMOs, that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. CPB does not deal the transboundary movement of LMOs which are pharmaceuticals for humans.

General Provisions of the Protocol require each Party to take appropriate legal, administrative and other measures to implement its obligations under this Protocol. It also declares that nothing in this Protocol shall be interpreted as restricting the right of a Party to take action that is more protective of the conservation and sustainable use of biological diversity than that called for in this Protocol, provided that such action is consistent with the objective and the provisions of this Protocol and is in accordance with that Party's other obligations under international law.

National Measures for Implementation of the Protocol

Each Party is required to make legislations or regulations consistent with the protocol for implementing its obligations. Such national framework is required to designate a unified entity or separate entities as national focal point (NFP) and one or more competent national authorities (CNA). The NFP, on its behalf will be liaising with the Secretariat of the CBD and the Biosafety Clearing-House (BCH) and the CAN shall be responsible for the administrative functions required by this Protocol. On regulation of transboundary movement of LMOs pertaining to its jurisdiction, a Party has right to apply its own domestic regulatory framework, so long as it is in place and consistent with the Protocol (Art. 9(3) and Art. 14(4)).

The Biosafety Clearing-House

The BCH is established at the CBD Secretariat to facilitate the exchange of scientific, technical, environmental and legal information on, and experience with, LMOs and to assist Parties to implement the Protocol. It may also serve as a hub for accessing other international biosafety information exchange mechanisms. Each Party, without prejudice to the protection of confidential information, shall make available all relevant information that is required to the BCH. The information may include existing laws, regulations and guidelines for Protocol implementation at national level and information required by the Parties for the AIAP, bilateral, regional and multilateral agreements and arrangements, generated summaries of its risk assessments or environmental reviews of LMOs, decisions of Parties on the importation or release of LMOs, and national reports on implementation of its obligations under this Protocol,

Differential Treatment for Different Classes of LMOs

While the scope of the Protocol is on transboundary movement, transit, handling and use of all LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, it proposes different kind of treatment to different classes of LMOs. These classes are determined based on the risks posed by the LMOs on their exposure to environment, method of their handling, the nature their transboundary movement, and potentially possible adverse effects from them. For example, those classes of LMOs which are pharmaceuticals for humans are not dealt under the Protocol. Among the LMOs dealt under this Protocol, the class destined for contained use is dealt differently from the other class of LMOs, which are intended for introduction into the environment. The first transboundary movement (import) of LMOs intended for introduction into the environment of the country of import is to be done only after advance informed agreement procedure (AIAP).

Application of the Advance Informed Agreement Procedure

The AIAP is an important step provided in the Protocol to regulate transboundary entry of LMOs, which may adversely impact the conservation and sustainable use of biological diversity, taking also into account risks to human health. This has to be effectively applied prior to the transboundary movement. The AIAP involves few steps including risk assessment, risk management and risk communication. The AIAP applies to the first time a country is allowing transboundary movement of a given LMO of the class requiring such procedure. The steps of the procedure are:

- Notification by the Party of export (PoE);
- Acknowledgement of the receipt of notification by the Party of import (PoI); and
- Decision by the PoI on the notification.

The notification has to be in writing and addressed to the CAN of the PoI well ahead of the intentional transboundary movement of a LMO. Such notification should include the minimum information on the LMO, which is legally accurate, as prescribed in the Protocol (Annex I). With respect to the information being shared, the PoE has a privilege to request the PoI for treating any specified part of the shared information as confidential. The right to maintain confidentiality of information is discussed later.

Under the acknowledgement process, the PoI shall acknowledge in writing the receipt of the notification from the PoE, normally within 90 days of receipt of notification. Such acknowledgement shall state the date of receipt of the notification, whether the notification has all specified information and in required detail, and whether the import shall be in compliance with the domestic regulatory framework of the PoI (in case the PoI has such national framework). However, a failure by the PoI to make such acknowledgement shall not be construed as its consent to an intentional transboundary movement.

One of the important consideration for making a decision by the PoI is risk assessment on the LMO in question, which at minimum shall be done in accordance with the steps laid down in the Protocol (Annex III), deploying recognized risk assessment techniques, at a minimum, based on the information provided by the PoE and available scientific evidence in order to identify and evaluate the possible adverse effects of the LMO. It is important that PoI shall ensure that its decisions on import are based on risk assessments carried out in scientifically sound manner. Risk assessment may be done by the PoE or if so requires, the PoE shall bear the cost of risk assessment. (Please see more details in lecture on “Risk assessment”).

The POI within 90 days of receipt of notification shall, in writing, inform the PoE whether the intentional transboundary movement may proceed only after it has given its written consent or after no less than 90 days without a subsequent written consent. The decision of the PoI on transboundary movement of the LMO in question shall be conveyed in writing to the PoE as well as to the BCH, within 270 days of the date of receipt of notification. Such decision may be affirmative, with or without conditions and also indicating whether the decision shall apply to subsequent imports of the same LMO, or negative or requesting additional relevant information in accordance with its domestic regulatory framework or Annex I, along with extension of time for completing the process. Whenever the decision is other than non-conditional affirmation, the relevant reasons justifying such decision have to be conveyed. The lack of scientific certainty due to insufficient relevant scientific information on the potential adverse effects of a LMO shall not prevent a PoI from taking a decision in order to avoid or minimize such potential adverse effects. Also, failure by the PoI in conveying its decision within stipulated 270 days shall not be interpreted as consent to an intentional transboundary movement.

Confidential Information

The PoE has privilege to classify some of the information on the LMO or associated matters exchanged under AIAP as confidential and request PoI that it shall be treated accordingly. When called up on, the PoE is required to offer justification for such classification of information. The PoI also has privilege to question the propriety of such classification and any disclosure of such 'confidential' information by the PoI has to be done only after prior notice to the PoE, including opportunity for consultation and internal review of such decision. All such information concurred up on as 'confidential' has to be protected most effectively like one's own confidential information. The PoI shall also not use such confidential information for commercial purpose, unless with written consent. In the event of the PoE withdrawing the notification, all information classified as confidential by the PoE have to be totally respected by the PoI. Normally following information are not considered confidential: the name and address of PoE, general description of the LMO, a summary of the risk assessment conducted in accordance with the Protocol, and any methods and plans for emergency response.

Review of Decisions

Having a PoI taken a decision regarding the grant of intentional transboundary movement, it is entitled to review and change the decision, if required, in light of new scientific information on potential adverse effects on the conservation and sustainable use of biological diversity, taking also into account the risks to human health. Such decision, along with the reasons for the same, has to be conveyed to the PoE and the BCH within 30 days. Following this the PoE could request the PoI to review the decision either on the basis of a change in circumstances that has influenced the outcome of the risk assessment upon which the decision was based or on the basis of additional relevant scientific or technical information that has become available. This request from PoE for revised decision shall be responded by the PoI within 90 days along with the reasons for its decision. In this context, the PoI has right to demand a risk assessment for subsequent imports.

Simplified procedure

The Protocol also offers a simplified procedure for cutting time and pre-grant processes. This provides a PoI, on having applied adequate measures to ensure the safe intentional transboundary movement of LMO, to notify the BCH in advance the cases of intentional transboundary movement that may take place in to it and LMOs exempted from the AIAP during their import. Such notification to the BCH should be as specified in the Annex I.

LMOs Excluded from the AIAP

LMOs destined for different uses or moving along different routes are exempted from the AIAP by the Protocol. These are:

- LMOs intended for direct use as food or feed, or for processing (Art. 7 (2))
- LMOs identified in a decision of the CoP serving as the MoP to this Protocol as being not likely to have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health (Art 7 (4)).
- LMOs in transit, but going through a country towards its final destination elsewhere (Art. 6(1)).
- LMOs destined for contained use, like seeds and genetic material brought for experimental use and research (Art. 6(2)).
- Intentional trans-boundary movements in pursuant of bi- or multi-lateral agreements and arrangements among Parties to the Protocol (Art.14).

The exclusion of certain of above categories of LMOs from the purview of AIAP had generated considerable concern among many developing country Parties during negotiation of the Protocol. Hence *the Protocol has offered a way out by providing flexibility to Parties with right to regulate import of these LMOs in their domestic legislation* (Art. 11(6)).

Procedure for LMOs Intended for Direct use as Food or Feed, or for Processing

The LMOs intended for direct use as food, feed or for processing constitute a distinct class of LMOs, which are either traded or transported across national boundaries, or moved under food aid and other kinds of emergency support. More frequently these LMOs are crop or livestock produces or derivates, which may be subject to transportation inside the territory of the PoI, marketed across, or processed as food or feed products, etc like any other crop or livestock produce.

A PoI, either on the request of a PoE or on its own, may take a decision on transboundary movement of a LMO, which is either fit for direct use as food or feed, or for such use after processing, for domestic use and internal marketing. On having taken such decision, within 15 days of making that decision, the PoI shall inform the other Parties through the BCH. The information provided by the PoE shall be at least in accordance with the Annex II of the Protocol and legally accurate. Such minimum information shall include apart from identity of applicant, specific identity of LMO, details of gene modification, technique used and resulting characteristics of the LMO, particulars of taxonomy, source of origin, etc, known centres of origin and genetic diversity of the species, habitat description, approved use of LMO, risk assessment report consistent with Annex III of the Protocol, advisories on safe handling, storage, transport, use, etc. This information shall also be provided, in writing, to the NFP of each of those Parties, which had notified the Secretariat that it does not have access to the BCH. This provision, however, shall not apply to decisions regarding field trials.

A decision on the import of this class LMOs shall also be in compliance with the domestic regulatory framework of the PoI, where ever such framework exists and such framework is consistent with the objective of this Protocol. When Parties have laws, regulations and guidelines applicable to the import of LMOs of this class, these documents shall be deposited with the BCH. For developing countries and countries with economy in transition, the Protocol provides flexibility to declare through the BCH, in exercise of their domestic jurisdiction, that their decision prior to the first import of this class of LMOs shall be taken on the basis of risk assessment undertaken in accordance with the Protocol and the decision made thereof be made available within 270 days. Nonetheless, failure by a Party to communicate its decision within 270 days shall not be construed as its consent or refusal to the import of the LMO of this class.

As precaution is the underlying principle of the Protocol, lack of scientific certainty either due to insufficient relevant scientific information or knowledge regarding the extent of the potential adverse effects of a LMO on the conservation and sustainable use of biological diversity in the PoI, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of that LMO intended for direct use as food or feed, or for processing, in order to avoid or minimize such potential adverse effects.

A PoI may also indicate its needs for financial and technical assistance and capacity-building with respect to this class of LMOs and in such cases Parties shall cooperate to address these needs in accordance with the provisions on capacity building and public awareness.

Bilateral, Regional and Multilateral Agreements and Arrangements

In the current era of globalization and regional, bilateral or plurilateral agreement on trade in commodities including the LMOs, there is need for space to such agreements and arrangements. Hence the Protocol allows Parties to enter into bilateral, regional and plurilateral agreements and arrangements regarding intentional transboundary movements of LMOs. The Protocol insists that such agreements and arrangements have to be consistent with the objective of this Protocol and should not in any way lower the level of protection provided for by the Protocol. These agreements and arrangements that have been entered into before or after the date of entry into force of this Protocol, shall be informed to the Parties through the BCH. When agreements and arrangements are invoked on the intentional transboundary movements between the concerned parties, the provisions of this Protocol on that aspect shall not be operational. However, nothing shall prevent a Party from applying its domestic regulations with respect to specific imports into it and such decision shall be notified to the BCH.

Unintentional Transboundary Movements and Emergency Measures

There are many chances for unintentional transboundary movement of LMOs by natural causes like pollen transfer across geographical boundaries, or by human errors. Such occurrence within the jurisdiction of a Party may either affect adversely or potentially on the conservation and sustainable use of biological diversity, taking also into account risks to human health. Such occurrences shall be reported to the BCH and other relevant international organizations. Such reports should include the estimated quantities and relevant characteristics and/or traits of the LMO; the circumstances of release; estimated date of the event; on the use of the LMO in the originating Party; information about the possible adverse effects on the conservation and sustainable use of biological diversity and risks to human health; about possible risk management measures; any other relevant information; and a point of contact for further information.

Towards minimizing any significant adverse effects by such transboundary movement, each Party, from whose jurisdiction the movement of the LMO had occurred shall immediately consult the affected or potentially affected Parties, enable them to determine appropriate responses and initiate necessary action, including emergency measures.

Transboundary Movements of LMOs Involving Non Parties

As of now only 150 countries are Party to CPB. This offers scope for transboundary movements of LMOs between the jurisdictions of Parties and non-Parties to the Protocol. Under the Vienna Convention on the Law of Treaties, a protocol cannot create rights and obligations for non-Parties without their consent. However, when the involved Parties are members of bilateral, regional and plurilateral agreements or arrangements, the issue may be settled within that framework. If that is not the case with respect to the non-Party, the latter may be encouraged to voluntarily adhere to the CPB and to contribute appropriate information to the BCH on LMOs released in, or moved into or out of, areas within their national jurisdictions.

Illegal Transboundary Movements

Protocol provides measures to be followed to monitor and check illegal transboundary movement of LMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. These measures include regulations at domestic level and penalizing illegal transboundary movements. In the event of an illegal transboundary movement, the affected Party may request the Party of origin to dispose, at its own expense, of the LMO in question either by repatriation or destruction. The information concerned shall be communicated to the BCH by each Party. The preventive or punitive measures are however not easy when natural contamination takes place.

Liability and Redress

This pertains to the rules and procedures in the field of liability and redress for damage resulting from transboundary movements of LMOs. The Protocol text does not contain any provisions on this aspect. This is an area which faced strong conflict during negotiation. The matter was referred as an outstanding issue to the first meeting of the Parties to this Protocol.

Institutional Arrangements

Institutional mechanism provided in the Protocol includes those mechanisms for implementation of the Protocol at the level of each Party and those at the international level. The institutional mechanisms at the level of each Party level include the National laws and regulations on the biosafety consistent with Protocol, Competent National Authority and National Focal Points, and country-level Biosafety Clearing-House. The institutional mechanisms at the International level include CoP of the CBD, Meeting of the Parties (Protocol), BCH, the Protocol Secretariat (which is the CBD Secretariat), and other relevant international institutions. (For details consult Art.: 19, 29, 30 and 31).

Dispute Settlement

The Protocol provides no specific provision in on the settlement of disputes arising from its implementation and interpretation. But it falls back to the relevant provisions of the CBD in this respect (Article 32). Article 27 of the CBD provides for optional recourse to judicial settlement or arbitration, or a conciliation procedure that is mandatory at the request of one of the parties to a dispute. With a view to minimize disputes, the Protocol mandates the CoP-MoP to develop procedures and mechanisms to promote compliance with the provisions of the Protocol (Art. 34). In the case of international trade in LMOs, it is governed by the Protocol as well as the rules of the relevant WTO Agreements. This overlap and existence of a powerful dispute settlement mechanism under WTO, renders the Protocol subordinate to the WTO in the LMO-trade related disputes.

Capacity-Building and Financial Resources

The financial resource for the implementation of this Protocol is generated in accordance with the Art. 20 of the CBD. Similarly, the financial mechanism established in Art. 21 of the CBD (operated by the GEF) shall be serving as the financial mechanism for this Protocol. In this respect, biosafety issues may be “competing” with other biodiversity issues for financial support from the GEF.

Development and strengthening of HR and institutional capacities in biosafety, including biotechnology relevant to the biosafety assume high importance for the purpose of the effective implementation of this Protocol in country Parties, particularly the developing, the least developed, small island developing states, and countries with economies in transition.

The Protocol has no specific finance generation plan or specific guidance on the level of financial resources needed for implementation. A number of capacity-building initiatives in relation to biosafety are already underway since some time.

Public Awareness and Participation

Each Party is required to promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of LMOs in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. And this may be done in cooperation with other States and international bodies. Such public awareness and education should encompass access to information on LMOs imported in accordance with this Protocol. Involvement of public in the decision-making through transparent consultative process, while respecting confidentiality of relevant information is important. The public needs to be informed about the means of public access to the BCH.

Socio-Economic Considerations

Any decision of a Party to import a LMO should carefully weigh, consistent with its international obligations and socio-economic considerations, the risks arising from the impact of LMOs on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities. Cooperation of Parties on research and information exchange on any socio-economic impacts of LMOs on indigenous and local communities is encouraged.

The international framework: The Codex Alimentarius, the World Organization of Animal Health, the International Plant Protection Convention, and the Agreement on Sanitary and Phytosanitary Measures

For many foods, the level of food safety generally accepted by the society reflects the history of safe consumption by these societies. It is recognised that in many cases the knowledge required to manage the risks associated with foods has been acquired in the course of their long history of use. Foods are generally considered safe, provided that care is taken during development, primary production, processing, storage, handling and preparation. Similarly, movements of live plants and animals as well as the commodities therefrom are also regulated both within and across countries to prevent concurrent spread of serious plant or animal pests and diseases as well as possible emergence of alien invasive species. Earliest food safety and quality laws are reported to have introduced in the Austro-Hungarian Empire between 1897 and 1911, where a collection of voluntary standards and product descriptions for a wide variety of foods was developed as the *Codex Alimentarius Austriacus*. With later expansion of trade in food, plants or animals, across countries, they had developed their own

mandatory laws/ regulations to define their standards. However, these standards and enforcement varied across countries causing risks to consumer health or threat to the health and safety of animals and plants. Similarly, food standards higher than what is scientifically justifiable are also foisted as non-tariff barriers to trade in food and farm commodity. These concerns resulted in establishing a collection of internationally harmonized and scientifically validated safety standards, which could serve as a benchmark, to protect the health of people, animals and plants, to promote fair trade and to settle disputes between countries arising from safety standards applied in trade. Towards evolving such internationally harmonized and scientifically validated safety standards three major intergovernmental mechanisms to protect of people, animals and plants, particularly to prevent the undesirable consequences of international movements of people and traded goods are established under the auspices of FAO and WHO. These are:

- **Codex Alimentarius Commission (CAC)**, which sets sanitary and technical standards for food safety, including food standards for commodities, codes of hygienic or technological practice, limits for pesticide residues in foods, and standards for contaminants and food additives;
- **Office International des Épizooties (OIE)**, which deals with animal health and zoonoses, and sets sanitary standards for the international movement of animals or animal products.
- **International Plant Protection Convention (IPPC)**, which provides international phytosanitary standards on how to prevent the spread and introduction of pests of plants and plant products;

The standards developed under these mechanisms have following key features in common:

- They are designed to protect the environment and human health while facilitating international trade and traffic.
- They are designed to be transparent and to harmonize regulations for trade and international traffic so that their application should remove artificial trade barriers and other causes of trade disputes between countries.
- They are developed on the basis of best scientific knowledge at that time (which implies revision in sync with advancing scientific knowledge).

Codex Alimentarius Commission

CAC is an inter-governmental body established in 1963 by joint covenant of the two organizations of United Nations, the Food and Agriculture Organization and the World Health Organization. The main tasks of the CAC as set out in Article 1 are as follows:

(a) protecting the health of consumers and ensuring fair practices in the food trade;

(b) promoting coordination of all food standards work undertaken by international governmental and non-governmental organizations;

(c) determining priorities and initiating and guiding the preparation of draft standards through and with the aid of appropriate organizations;

(d) finalizing draft standards after acceptance by governments, publishing them in a Codex Alimentarius either as regional or global standards, together with international standards already finalized by other bodies, wherever this is practicable;

(e) amending published standards, after appropriate survey in the light of developments.

Codex Alimentarius (Latin, meaning Food Law or Code) is a collection of internationally adopted food standards presented in a uniform manner along with provisions of an advisory nature in the form of codes of practice, guidelines and other recommended measures to assist in achieving the purposes of the Codex Alimentarius (CA). Two landmark years in the foundation of the CA were 1960 and 1961. In October 1960, the first FAO Regional Conference for Europe recognized a widely held view on: "*[t]he desirability of international agreement on minimum food standards and related questions (including labelling requirements, methods of analysis, etc.) ...as an important means of protecting the consumer's health, of ensuring quality and of reducing trade barriers, particularly in the rapidly integrating market of Europe"*. The Conference also felt that: *"... coordination of the growing number of food standards programmes undertaken by many organizations presented a particular problem"*. Subsequent discussions among the FAO, the WHO, the United Nations Economic Commission for Europe (UNECE), the Organisation for Economic Co-operation and Development (OECD) and the Council of the *Codex Alimentarius Europaeus* reached to a proposal, which led to the establishment of an international food standards programme. In November 1961, the Eleventh Session of the FAO Conference passed a resolution to set up the CAC. In May 1963, the Sixteenth World Health Assembly approved the establishment of the Joint FAO/WHO Food Standards Programme and adopted the statutes of the CAC.

The CA includes all the main foods encompassing of processed, semi-processed or raw, materials used in the further processing of food products, hygienic and nutritional quality of food, including microbiological norms, food additives, pesticide and veterinary drug residues, contaminants, labelling and presentation, and methods of sampling and risk analysis. It also includes individual standards, advisory codes of practice, guidelines and other recommended measures. The CA is a single most important international reference point on food biosafety for consumers, food producers and processors, national food control agencies and the international food trade-like a unified global food code. The CA presents a unique opportunity for all countries to join the international community in formulating and harmonizing food standards and ensuring their global implementation. It also allows them a role in the development of codes governing hygienic processing practices and recommendations relating to compliance with those standards. National food safety measures and regulations are evaluated against this benchmark, particularly in the context of international food trade. There is an advantage in having universally uniform food standards for the protection of consumers. The two WTO agreements, namely, the SPS Agreement and the TBT Agreement further

promote the international harmonization of food standards. These Agreements cite international standards, guidelines and recommendations as the preferred measures for facilitating international trade in food. As such, Codex standards have become the benchmarks against which national food measures and regulations are evaluated within the legal parameters of the World Trade Organization (WTO) Agreements.

Food-borne illnesses are at best unpleasant and at worst they can be fatal. The other consequences are outbreaks of food-borne illness, damaging trade and tourism and also leading to loss of earnings, unemployment and litigation. Poor quality food can destroy the commercial credibility of suppliers, both nationally and internationally, while food spoilage is wasteful and costly and can adversely affect trade and consumer confidence. The Codex system also contributes in sensitizing the global community on the importance of food quality and the need for food standards to prevent the danger of food hazards. A formal evaluation of the Codex programme undertaken by FAO and WHO in 2002 found among other things, there is need for increased inclusiveness of developing member countries in the Codex standard development process, including risk assessment and more effective capacity-building for development of national food control systems.

Codex Standards

The two basic aspects for the food safety concerns are the "visible" and "invisible" aspects. The "visibles" include underweight, size variations, misleading labelling and poor quality. The "invisibles" comprise the hidden health hazards, smell or taste, such as microbial contamination, pesticide or chemical residues, adulterants, food additives and environmental contaminants.

Codex standards normally refer to one or more product characteristics appropriate to a commodity depending on how many of these characteristics are regulated by the government. These standards include general as well as commodity specific standards, such as standards for food additives and contaminants and toxins in foods. Because standards relate to product characteristics, they can be applied wherever the products are traded. For example, maximum residue limits (MRLs) for residues of pesticides or veterinary drugs, etc in foods. The Codex General Standard for the Labelling of Prepackaged Foods covers all foods in this category. *Codex methods of analysis and sampling*, including those for contaminants and residues of pesticides and vet drugs in foods, are also considered as Codex standards. *Codex codes of practice* includes codes of hygienic practice, which define the production, processing, manufacturing, transport and storage practices for individual foods or groups of foods that are considered essential to ensure the safety and suitability of food for consumption. A code of practice on the control of the use of veterinary drugs provides general guidance in this area. For food hygiene, the basic text is the Codex General Principles of Food Hygiene, which introduces the use of the Hazard Analysis and Critical Control Point (HACCP) food safety management system.

Codex guidelines fall into two categories: (1) principles that set out policy in certain key areas; and (2) guidelines for the interpretation of these principles or for the interpretation of the provisions of the Codex general standards. In the cases of food additives, contaminants, food hygiene and meat hygiene, the basic principles governing the regulation of these matters are built into the relevant standards and codes of practice. *Interpretative Codex guidelines* include those for food labelling, especially the regulation of claims made on the label. These guidelines pertain to nutrition and health claims; conditions for production, organic foods; and foods claimed to be "halal". There are also free-standing *Codex principles* covering more guidelines that interpret the provisions of the Codex for Food Import and Export Inspection and Certification, guidelines on the conduct of safety assessments of foods from DNA-modified plants and micro-organisms, the Establishment and Application of Microbiological Criteria for Foods, Levels for Radio-nuclides in Foods following Accidental Nuclear Contamination for Use in International Trade and the conduct of microbiological risk assessment.

By far the largest number of (more than 200) specific standards in the CA is the group called "commodity standards". These standards include individual foods within groups of foods and groups of commodities. Commodity standards tend to follow a fixed format defined in the *Procedural Manual of the CAC*. In this category, there are nine groups of foods as follows:

- Cereals, pulses & derived products including vegetable proteins
- Fats and oils and related products
- Fish and fishery products
- Fresh fruits and vegetables
- Processed and quick-frozen fruits and vegetables
- Fruit juices
- Meat and meat products; soups and broths
- Milk and milk products
- Sugars, cocoa products & chocolate & other miscellaneous products

Functional framework of CAC

Eligibility for membership of the Commission, which is open to all Member Nations and Associate Members of FAO and WHO, is defined in Article 2. The Commission is truly an international body. Currently (as on December 2008) the CAC is participated by 177 countries. The Commission normally meets once in two years, alternately at FAO headquarters in Rome and at WHO headquarters in Geneva. On occasions, it may meet more frequently or in special or extraordinary sessions.

The Codex Alimentarius Commission at its Secretariat at FAO headquarters in Rome is constituted by elected Chair person and three Vice Chair persons, who are on tenure, and an

Executive Committee. The Executive Committee assists the Commission on reviewing project proposal and determines its relevance on the basis of established criteria and priorities. Day to day administration is entrusted with the Secretary of the Codex Alimentarius Commission, who is appointed jointly by the Director-Generals of FAO and WHO following an open worldwide search. The Secretary is supported by a small staff of professional and technical officers. The Commission at every member country level has *Codex Contact Points* constituted by the national governments. Many members also have *National Codex Committees* to coordinate activities nationally. National Codex Committees at each the six regions are coordinated by the *Coordinating Committees*. The six regions are Asia, Near East, Africa, Europe, Latin America and Caribbean, and North America and Southwest Pacific. The coordination ensures that the work of the Commission is responsive to regional interests and to the concerns of developing countries. The country that chairs the Coordinating Committee is also the *Regional Coordinator* for the region concerned. The Secretariat and the regional coordinators constitute the core administrative framework.

Under its Rules of Procedure, the CAC is empowered to establish two kinds of subsidiary body. These are (1) *Codex Committees*, which prepare draft standards for submission to the Commission; and (2) *Coordinating Committees*, which coordinate food standards activities in the region, including the development of regional standards. A feature of the committee system is that, with few exceptions, each committee is hosted by a member country, which is chiefly responsible for the cost of the committee's maintenance and administration and for providing its chairperson.

There two kinds of Codex Committees. These are the General Subject Committees (GSCs) and the Commodity Committees. The GSCs are so called because their work has relevance for all Commodity Committees and often applies across the board to all commodity standards. These Committees are also referred to as "horizontal committees". GSCs develop all-embracing concepts and principles applying to foods in general, specific foods or groups of foods; endorse or review relevant provisions in Codex commodity standards; and, based on the advice of expert scientific bodies, develop major recommendations pertaining to consumers' health and safety. The GSCs advise the CAC on such basic matters as definitions, the Rules of Procedure, rules and working procedures for the establishment and operation of Codex Committees and Task Forces, relations with other organizations and the general principles that underlie the preparation of all Codex standards, codes of practice and other texts. The six GSCs are the Committee on Food Additives; on Contaminants in Foods; on Food Hygiene; on Food Labelling; on Methods of Analysis and Sampling; and on Nutrition and Foods for Special Dietary Uses. The Committee on Pesticide Residues and the Committee on Residues of Veterinary Drugs in Foods prepare MRLs for these two categories of chemicals used in agricultural production. The Committee on Food Import and Export Inspection and Certification Systems deals with the application of standards to foods moving in international trade, in relation to the regulatory measures applied by governments.

The responsibility for developing standards for specific foods or classes of food lies with the Commodity Committees (CCs). In recognition of their exclusive responsibilities, they are often referred to as "vertical committees". CCs are constituted and abolished on need basis. The common five CCs are Committees on Fats and Oils; on Fish and Fishery Products; on Fresh Fruits and Vegetables; on Milk and Milk Products; and on Processed Fruits and Vegetables.

A third type of subsidiary body called a Codex ad hoc Intergovernmental Task Force is a Codex Committee with very limited terms of reference established for a fixed period of time. The following ad hoc Intergovernmental Task Forces are constituted till date: Task Force on Animal Feeding, 1999–2004; Task Force on Foods Derived from Biotechnology, 1999–2003 and 2005–2009; Task Force on Fruit and Vegetable Juices, 1999–2005; Task Force on the Handling and Processing of Quick Frozen Foods, 2006-; and Task Force on Antimicrobial Resistance, 2006- .

Guidelines of *Ad hoc* Intergovernmental Task Force on foods derived from Modern Biotechnology

The CAC at its 26th session in 2003 adopted Principles and Guidelines on foods derived from biotechnology. These are overarching principles on the risk analysis of foods derived from modern biotechnology and guidelines for food safety assessment of foods derived from recombinant-DNA plants and microorganisms. Guideline for the conduct of food safety assessment provide two major sections, one on foods derived from recombinant-DNA plants and the other on food produced using recombinant-DNA microorganisms. The conduct of risk analysis is guided by general decisions of the CAC as well as the Codex Working Principles for Risk Analysis. The Risk Analysis of Foods Derived from Modern Biotechnology does not address animal feed or animals fed with the feed or the environmental risks.

Risk assessment includes a safety assessment, which is designed to identify whether a hazard, nutritional or other safety concern is present, and if present, to gather information on its nature and severity. The safety assessment should include a comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences. If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health. Risk analysis methodology and its application should be consistent with new scientific knowledge and other information relevant to this. With the availability of new scientific information, risk assessment may be reviewed to incorporate this information and, if necessary, with suitable adaptation of risk management measures.

Safety assessment on foods derived from recombinant-DNA plants

A safety assessment is characterized by comparative assessment the whole food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences with consideration to:

a) the intended and unintended effects;

b) identification of new or altered hazards;

c) identification of changes, relevant to human health, in key nutrients.

Risk management measures for foods derived from modern biotechnology should be proportional to the risk and these measures may include, as appropriate, food labeling, conditions for marketing approvals and post-market monitoring.

Due to difficulties in using animal models for assessing the risks associated with whole foods, a more focused approach is required for the safety assessment of foods derived from food plants, including recombinant-DNA plants. This has been addressed by the development of a multidisciplinary science-based approach for assessing safety which takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it, on case by case, using the concept of substantial equivalence. The concept of substantial equivalence is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. The safety assessment of foods derived from recombinant-DNA plants involves methods to identify and detect any unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information are necessary to assess unintended effects because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health. These data and information, when considered in total, provide assurance that the food is unlikely to have an adverse effect on human health.

The safety assessment of a food derived from a recombinant-DNA plant follows a stepwise process of addressing relevant factors that include: A) Description of the recombinant-DNA plant; B) Description of the host plant and its use as food; C) Description of the donor organism(s); D) Description of the genetic modification(s); E) Characterization of the genetic modification(s); F) Safety assessment, which may involve: i) expressed substances (non-nucleic acid substances); ii) compositional analyses of key components; iii) evaluation of metabolites ; iv) food processing; v) nutritional modification; and G) Other considerations. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use.

When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. An integrated, stepwise, case-by-case approach used in the assessment of the potential allergenicity of the newly-expressed

protein(s) should rely upon various criteria used in combination (since no single criterion is sufficiently predictive on either allergenicity or non-allergenicity). Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. The assessment of possible compositional changes to key nutrients should be conducted for all recombinant-DNA plants.

Safety assessment on foods produced using recombinant-DNA microorganisms

This addresses safety and nutritional aspects of foods produced through the actions of recombinant-DNA microorganisms. The recombinant-DNA microorganisms that are used to produce these foods are typically derived using the techniques of modern biotechnology either from strains that have a history of safe, purposeful use in food production or the recipient strains do not have a history of safe use. The risk assessment may be different in the case of these two types of recombinant-DNA microorganisms.

Effective risk communication is essential at all phases of risk assessment and risk management. It is an interactive process involving all interested parties, including government, industry, academia, media and consumers. Transparent safety assessment and risk management decision making processes should be part of the risk communication. These processes should be fully documented at all stages and open to public scrutiny, whilst respecting legitimate concerns to safeguard the confidentiality of commercial and industrial information. In particular, reports prepared on the safety assessments and other aspects of the decision-making process should be made available to all interested parties. Effective risk communication should include responsive and interactive consultation processes, where the views of all interested parties should be sought and relevant food safety and nutritional issues that are raised during consultation should be addressed during the risk analysis process.

Applying Codex standards

The two major concerns of the CAC are protecting the health of consumers and ensuring fair practices in the food trade. Harmonization of food laws of countries following internationally agreed standards is expected to largely address the above concerns. In addition to CA, the efforts on international harmonization of food standards are taken forward by the SPS and TBT Agreements. However, despite growing global interest in all Codex activities, in practice it is difficult for many countries to accept Codex standards in the statutory sense. Differing legal formats and administrative systems, varying political systems and sometimes the influence of national attitudes and concepts of sovereign rights impede the progress of harmonization. An increasing number of countries are aligning their national food standards,

or those relating to safety, with those of the CA. Such compliance is more in the case of invisibles such as additives, contaminants and residues.

Codex standards are also gaining recognition as reference point in some regional trade agreements/ trade groupings like NAFTA, MERCOSUR, and APEC for compliance by the member countries on safety of food products traded. The Mutual Recognition Arrangement on Conformity Assessment of Foods and Food Products approved by the APEC has consistency with the standards of SPS, TBT and Codex on food trade and Inspection and Certification Systems. Several other plurilateral and bilateral agreements have accepted Codex for benchmarking safety aspects of food products.

A Trust Fund launched in 2003 seeking US$ 40 million over a 12-year period is being established to help developing countries and countries in transition to increase their participation in the work of the Commission. This fund is leveraged to increase the participation of these countries both by assisting for the involvement of their regulators and food experts in activities on setting in international standards and enhancing their capacity for establishing effective domestic food safety and quality standards and fair practices in the food trade, both in the framework of the CA and in their own countries.

The World Organization of Animal Health

Many animal disease pandemics required fighting at global level. This led to the creation of the Office International des Epizooties (OIE) through the international Agreement signed on January 25th 1924 in Paris. In May 2003 this organization changed its name to the World Organisation for Animal Health, while keeping its historical acronym OIE. The OIE is the intergovernmental organisation with objective to work towards ensuring safe food world wide, by reducing animal food-borne risks to human health due to hazards arising from animals, globally fighting animal diseases and improving the safety of the " food production to consumption continuum". It works in tandem with FAO, WHO, the CAC and relevant Codex Committees and other organisations.

OIE is recognised as a referal organisation by the WTO. As on October 2008, OIE is joined by a total of 172 Member Countries and Territories. The OIE maintains permanent relations with 36 other international and regional organizations and has Regional and sub-regional Offices on every continent.

OIE provides guidance to Members on the role and responsibilities of National Veterinary Services - for educating and training the veterinarians in both animal health (including zoonoses) and food hygiene components- to equip them to play a central role in ensuring safety food of animal origin.

OIE defines a hazard as a biological, chemical or physical agent in food with the potential to cause an adverse health effect in humans, whether or not it causes disease in animals. The Third OIE Strategic Plan for 2001-2005 enlarged OIE activity to the area of public health and

consumer protection, including "zoonoses and diseases transmissible to humans through food, whether or not animals are affected by such diseases“. Zoonose is any infectious disease that is able to be transmitted (by a vector) from other animals, both wild and domestic, to humans or from humans to animals (the latter is sometimes called reverse zoonosis). In 2002, the OIE established a permanent Working Group on Animal Production Food Safety (APFSWG) to coordinate its food safety activities. The Working Group includes in its membership high-level experts from the FAO, the WHO, the CAC and relevant Codex Committees, and reflects a broad geographical basis. The fourth OIE Strategic Plan (2006-2010) continues this mandate, recommending that the APFSWG "continue to work with other relevant organisations, especially the CAC, in reducing food-borne risks to human health due to hazards arising from animals".

More recent pandemics witnessed by the world are mad cow disease or Bovine spongiform encephalopathy (BSE), Avian influenza (AI), and Severe Acute Respiratory Syndrome (SARS).

Bovine spongiform encephalopathy (BSE), or “mad cow disease (MCD)” erupted in UK in the 1980s was a fatal, neurodegenerative disease in cattle causing a spongy degeneration in the brain, spinal cord and retina. The causal agent is believed to be a specific type of misfolded protein called a prion. It is transmittable to humans eating the meat of infected animals to cause Creutzfeldt-Jacob disease (CJD). The first case of CJD was reported in the USA in December 2003. CJD was detected in UK and Europe. By April 2008, it had killed 204 people in these countries. While the origin of disease is unknown, it is believed the epidemic was caused by cattle being fed by meat and bone meal prepared from other cattle. This led Codex to take up feed safety of food-producing animals and evolving the Code of Practice on Good Animal Feeding, production and use of all materials destined for animal feed and feed ingredients at all levels, grazing or free-range feeding, forage crop production and aquaculture.

Severe Acute Respiratory Syndrome (SARS)

SARS is a respiratory disease in humans caused by the SARS coronavirus (SARS-CoV). During November 2002 and July 2003, SARS assumed near pandemic state with worldwide 8,096 known infected cases and 774 deaths (a case-fatality rate of 9.6%). The epidemics of SARS appear to have started in Guangdong Province, China in November 2002. Within a matter of weeks, SARS rapidly spread from the Guangdong province of China to some 37 countries around the world. SARS is believed to have occurred following breaches in laboratory biosafety, or human exposure to an animal reservoir or other environmental source.

Avian influenza (AI) is a highly contagious pandemic viral disease affecting several species of food producing birds (chickens, turkeys, quails, guinea fowl, etc.). Also affects pet birds and wild birds. Based on their pathogenicity, there are two types of AI viruses. Highly pathogenic AI virus (Eg. H5N1) spreads rapidly and causes serious disease and high mortality

rates (up to 100% within 48 hours). Symptoms of mild disease caused by low pathogenic AI (LPAI) are undetectable or no symptoms at all in some species of birds. The virus spreads to human and cause death. Till Sept 2008, 387 cases and 245 deaths due to AI are reported from 16 countries.

The OIE has established the *Terrestrial Animal Health Code* (also referred to as *Terrestrial Code*) to ensure the sanitary safety of international trade in terrestrial animals and their products. This is achieved through the detailing of health measures to be used by the veterinary authorities of importing and exporting countries to avoid the transfer of agents pathogenic for animals or humans, while avoiding unjustified sanitary barriers. The value of the *Terrestrial Code* is twofold: that the measures published in it are the result of consensus among the veterinary authorities of OIE Members, and that it constitutes a reference within the WTO on the Application of SPS Measures as an international standard for animal health and zoonoses. The health measures in the *Terrestrial Code* are in the form of standards and recommendations, and have been formally adopted by the OIE International Committee, the general assembly of all Delegates of OIE Members. The 17th edition of the *Terrestrial Code* revised in May 2008 includes revised chapters on general definitions, notification criteria for listing diseases, obligations and ethics in international trade, import risk analysis, the Veterinary Services, evaluation of Veterinary Services, zoning and compartmentalisation, animal health measures applicable before and at departure, border posts and quarantine stations in the importing country, international transfer and laboratory containment of animal pathogens, rabies, foot and mouth disease, rinderpest, contagious caprine pleuropneumonia, bovine tuberculosis, bovine spongiform encephalopathy, equine influenza, equine rhinopneumonitis, equine viral arteritis, African horse sickness, African swine fever, classical swine fever, avian influenza and Newcastle disease. The revision also includes alternative diagnostic tests for OIE listed diseases, on categorisation of diseases and pathogenic agents by the International Embryo Transfer Society, on inactivation procedures of foot and mouth disease virus and of avian influenza virus, on surveillance for bovine spongiform encephalopathy, for foot and mouth disease, for classical swine fever, for avian influenza and for bluetongue, on animal welfare (including introduction to the recommendations on animal welfare, transport of animals by sea, transport of animals by land, transport by air, slaughter of animals and killing of animals for disease control purposes), on factors to consider in conducting the bovine spongiform encephalopathy risk assessment as well as on model veterinary certificates have also been included.

This OIE draws upon the expertise of internationally renowned specialists to prepare draft texts for new articles of the *Terrestrial Code* or revise existing articles in the light of advances in veterinary science. The *Terrestrial Code* is published annually in paper form in the three official OIE languages (English, French and Spanish), and in Russian.

The document on the "Cooperation between the CAC and the OIE on Food Safety throughout the Food Chain" presents the regulatory perspective on the "food production to

consumption continuum" and establishes a context for the document on “The Role of Veterinary Services in Food Safety”. This document provides guidance to OIE Members on the role and responsibilities of national Veterinary Services. It notes that the education and training of veterinarians, which includes both animal health (including zoonoses) and food hygiene components, makes them uniquely equipped to play a central role in ensuring food safety, especially the safety of food of animal origin. In order for them to make the best possible contribution to food safety, it is important that the education and training of veterinarians meet high standards and that there are national programmes for ongoing professional training. The Veterinary Services should comply with the fundamental principles of quality in the *Terrestrial Code* and guidelines for the evaluation of Veterinary Services are provided in the *Terrestrial Code*. The document also highlights the need for cooperation with other authorities in the food chain continuum to ensure the protection of both animal and public health.

The International Plant Protection Convention

The International Plant Protection Convention (IPPC) is an international treaty to secure action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control by leveraging international cooperation. It is governed by the Commission on Phytosanitary Measures (CPM) which adopts International Standards for Phytosanitary Measures (ISPMs). The CPM has confirmed the IPP as the preferred forum for national IPPC reporting and the exchange of more general information among the phytosanitary community. At national and regional levels, IPPC is assisted by the National Plant Protection Organization (NPPO) and Regional Plant Protection Organization (RPPO), respectively. The IPPC is deposited with and governed by the FAO. The Secretariat of the IPPC located at the FAO, Rome coordinates the activities of the Convention. As of October 2008, the IPPC has 170 contracting parties.

History: The forerunner of the IPPC was a first international agreement for plant protection, the *Phylloxera vasatrix* Convention, concluded in Berne in 1881. The first draft of the IPPC was made in Rome in 1929. However, it was adopted only in 1951 at the Sixth session of the FAO and came into force in 1952 superceding all pre-existed international agreements on plant protection. The Convention was revised in 1979 and 1997. The Uruguay Round of negotiations on GATT recognized the IPPC as one of the standard-setting organizations for the SPS Agreement in 1989. The IPPC Secretariat was established and began standard-setting programme in 1992. The Committee of Experts on Phytosanitary Measures (CEPM) was first constituted in 1993. The first International Standard for Phytosanitary Measures (ISPM) was approved in 1995 by the 27th Session of the FAO. In the mean time, the SPS Agreement negotiated as part of the WTO came into force in 1995. The first meeting of the Interim Commission on Phytosanitary Measures was held in 1998. In 2001, the Standards Committee and dispute resolution procedures were established. The last

revision made in 1997 was wide ranging, based on the recommendations of an Expert Consultation held in 1996 as well as a review and further elaboration undertaken by a technical consultation on the Revision. The revised text of the Convention came into force with respect to all Contracting Parties (CPs) from 2 October 2005.

The existing Convention has 23 articles and model phytosanitary certificate for export and re-export. This document outlines the aims of the IPPC, its organizational structure and the major principles underpinning its implementation. The 1997-revised text of the IPPC provides a framework and a forum for international cooperation and harmonization and technical exchange between contracting parties dedicated to these goals. In addition to describing national plant protection responsibilities, it also addresses important elements of international cooperation for the protection of plant health and the establishment and use of ISPMs.

The Convention extends to the protection of natural flora and plant products. It includes both direct and indirect damage by pests (including weeds). The provisions extend to cover conveyances, containers, storage places, soil and other objects or material capable of harbouring plant pests. The Convention recognizes that countries have sovereign authority to use phytosanitary measures to regulate the entry of plants and plant products and other objects or material capable of harbouring plant pests. Countries can refuse entry, require treatment or specify other requirements for regulated material. In applying phytosanitary measures, CPs have obligations to comply with the Convention's principles of necessity, technical justification and transparency. For example, phytosanitary requirements must be applied only when made necessary by phytosanitary considerations, scientifically justified, consistent with the risk, the least restrictive measure available, and result in the minimum impediment to international trade and traffic. It is important phytosanitary measures are to be followed in consistence with the pest risk and the least restrictive measures available. The restrictive measures must also be modified if conditions change. All these are to be guided by an important principle that the phytosanitary measures are applied non-discriminatory manner between countries of the same phytosanitary status. All relevant information and the rationale for such measures must be promptly made available to any affected CPs, if requested. Under the international cooperation, parties have to exchange with other CPs their information on plant pests, in particular the reporting of any outbreak or spread of pests. Cooperation on such matters is established through the regional plant protection organizations which, in turn, cooperate with the IPPC Secretariat.

The IPPC has always been an important agreement to countries that trade in agricultural, horticultural and forestry products. As governments become more concerned by the adverse impact of weeds and other invasive organisms, not only on commercial crops but also on biodiversity and natural habitats, the Convention is assuming increasingly important role as a framework that can be applied to matters of environmental protection. The principal

organizations administering and implementing the IPPC are: (1) CPM (the ICPM prior to CPM); (2) IPPC Secretariat; (3) the FAO; (4) NPPOs; and (5) RPPOs.

The establishment of the CPM is a major development for the Convention. It provides a global forum for discussion of phytosanitary issues and allows a wide representation of contracting parties in work programmes and strategic planning. The CPM meets annually to implement the objectives of the Convention. It may also meet in more special sessions. CPs try to reach agreement by consensus on matters under discussion, although decisions can be taken by a two-thirds majority of the CPs present and voting, as a last resort. The main tasks of the CPM are:

- reviews global plant protection needs;
- develops and adopts ISPMs;
- establishes procedures for the resolution of disputes;
- promotes the provision of technical assistance to develop the phytosanitary capacity of contracting parties; and
- cooperates with RPPOs and other relevant international organizations on matters relating to the Convention.

Basic funding and resources for the work programme of the commission are currently provided by countries mainly through the FAO budget.

Initially, the IPPC Secretariat was established with the responsibility for coordinating the work programme for the global harmonization of phytosanitary measures. With the subsequent establishment of CPM, the roles of the IPPC Secretariat have shifted so that development of ISPMs has become a joint endeavour between the CPM and Secretariat. Thus the IPPC Secretariat implements the policies and activities of the CPM; publishes information relating to the IPPC; facilitates information exchange between CPs; and coordinates with the technical cooperation programmes of FAO to provide technical support on matters concerned to the IPPC, particularly to least developed nations. The FAO's Plant Protection Service is part of its agriculture department. In support of the IPPC, FAO provides the Convention's Secretariat through the Plant Protection Service; a source of legal advice; technical assistance projects and logistical back up for many of the activities of the international phytosanitary community.

NPPOs are listed on the IPPC Web site (www.ippc.int/IPP/En/default.jsp) together with their contact details. The principal roles, NPPOs are:

- responsibility for issuing phytosanitary certificates;
- managing surveillance for pest outbreaks and control of pests;
- conducting inspection and, if necessary, disinfestation of traded consignments of plants and plant products;
- ensuring phytosanitary security of consignments from certification until export;
- establishing and protecting pest free areas;

- undertaking pest risk analyses for the development of phytosanitary measures.

The last-mentioned three roles were invested with the NPPOs by the 1997 revision of the Convention to make them responsible and important in implementing the concepts of the Convention at a national level. Pest risk analysis (PRA), for example, is a modern phytosanitary practice which provides the technical justification for application of phytosanitary measures. A standardized wording and format are followed for official phytosanitary certificates so that such a certificate is easily recognized and contain the essential information describing the consignment.

RPPO is an intergovernmental organization providing coordination on a regional level for the activities and objectives of the IPPC as laid down in Article IX. The 1997 revised Convention extends the responsibilities of RPPOs to specify their cooperation with the IPPC Secretariat and CPM. The RPPOs are to participate in activities to achieve the objectives of the Convention; to disseminate information relating to the IPPC; and to cooperate with the CPM and the IPPC Secretariat in developing international standards.

Currently there are the following nine RPPOs:

- Asia and Pacific Plant Protection Commission (APPPC) with 24 members representing 24 countries;
- Caribbean Plant Protection Commission (CPPC) with 22 members representing 26 countries;
- Comite Regional de Sanidad Vegetal Para el Cono Sur (COSAVE) with five member countries;
- Comunidad Andina (CA) with five member countries;
- European and Mediterranean Plant Protection Organization (EPPO) with 41 member countries;
- InterAfrican Phytosanitary Council (IAPSC) with 51 member countries;
- North American Plant Protection Organization (NAPPO) with three member countries;
- Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) with eight member countries;
- Pacific Plant Protection Organization (PPPO) with 21 members representing 25 countries.

Not all CPs to the IPPC are members of RPPOs, nor are all members of RPPOs CPs to the IPPC.

Standard setting

The process for developing an ISPM comprises three stages: a draft stage, consultation stage and approval stage. The time taken to go from proposal to approval varies between

standards, and usually not less than 12 months. Suggestions for topics for ISPMs can be made by the NPPOs and RPPOs, the IPPC Secretariat or the WTO-SPS Committee. Other organizations, such as the CBD, industry groups or individuals may submit proposals for standards (or amendments to existing specifications) through the IPPC Secretariat. Priorities for dealing with proposed standards are decided by the CPM in consultation with the Secretariat.

A Standards Committee oversees the standard-setting process and assists in the development of ISPMs. This committee (established by the CPM in 2001), comprises 20 members drawn from the seven FAO regions: two from North America and three from each of the other six regions. Members are senior experts designated by their governments and confirmed by the CPM. The Standards Committee selects from within its members a subgroup of seven experts, the Standards Committee Working Group (SC-7), to undertake detailed work on draft standards. The IPPC Secretariat provides administrative and technical support for the Standards Committee and prepares records and reports of the standard-setting process.

NPPOs, RPPOs or working groups duly established or Standards Committee draft the standard and submit it to the IPPC Secretariat. The draft then passes between the IPPC Secretariat and the Standards Committee. One of the members of the committee takes responsibility for overseeing development of a particular standard from draft to approval. The committee reviews the draft and recommends what further action is to be taken. The Secretariat and Committee may arrange for a technical working group or a consultant to modify a draft standard, if necessary. The Committee continues to review the standard and in due course recommends it for submission to governments for technical comment.

Suggestions by individual member countries and RPPOs for change should be supported by an explanation of their purpose and alternative text should be proposed where appropriate. This is considered by the Standards Committee which, in consultation with the IPPC Secretariat, determines the nature and extent of changes to be made to the draft in response to the comments received. Acceptance of a redrafted standard by the Standards Committee results in submission of the standard to the CPM. The redrafted standard is considered by the CPM, amended if necessary, and adopted. The standard is then published and distributed by the IPPC Secretariat.

Pest risk analysis (PRA) is an important element in preventing the spread and introduction of plant pests. PRA has become increasingly important in modern phytosanitary practice. It provides the technical justification for the application of phytosanitary measures. The CPM has agreed that addressing standards relating to PRA should be a priority for collaborative work with environmental organizations such as the CBD. Information gathering and record keeping are important aspects of PRA. Any PRA should be well documented so that the information sources and the decisions can be evaluated in the event of a review or a dispute over the chosen phytosanitary measures. There are general guidelines for PRA (ISPM

2) and specific PRA for quarantine pests (ISPM 11). A pest is assessed as a quarantine pest in terms of its potential economic importance and possible official control measures in the area endangered by its presence. Complete definitions of quarantine pest and of pest risk assessment and pest risk management as they apply to quarantine pests are published as part of the standard. Over all, the PRA has three stages, comprising pest risk analysis, pest risk assessment and pest risk management. Economic consequences are assessed in terms of direct and indirect pest effects, including the effects on domestic and export markets, particularly on market access. Any PRA must refer to a defined PRA area - an area within a country, the whole country or a region of countries.

Agreement on SPS Measures

This Agreement seeks to protect human, animal or plant life or health, subject to the requirement that these measures are not applied in a manner which would constitute a means of arbitrary or unjustifiable discrimination between Members to restrict international trade. Agreement recognises (Art. 2.2) that governments have the right to take SPS measures but that they should be applied only to the extent necessary to meet the objective, but not as disguised non-tariff barrier in trade between Members where identical or similar conditions prevail. Agreement calls for harmonizing SPS measures on as wide a basis as possible, by basing them on international standards, guidelines or recommendations, where they exist, except as otherwise provided for in this Agreement (Art. 3.1). It allows Members to maintain or introduce measures which result in higher levels of standards, if there is scientific justification or as a consequence of consistent risk decisions based on appropriate risk determinations made by a Member. In case of introduction of new standards, the governments are required to provide advance notice of new or changed SPS regulations, and establish a national enquiry point to provide information. The SPS provision on equivalence requires Members to accept the SPS measures of other Members as equivalent, even if these measures differ from their own or from those used by other Members trading in the same product, if the exporting Member objectively demonstrates to the importing Member that its measures achieve the importing Member's appropriate level of SPS protection. For this purpose, reasonable access shall be given, upon request, to the importing Member for inspection, testing and other relevant procedures. The SPS Agreement spells out procedures and criteria for the assessment of risk and the determination of appropriate levels of sanitary or phytosanitary protection. When international standards are followed, there is no chance for being legally challenged in a WTO dispute. Art 5.7 of the SPS Agreement allows temporary “precautionary” measures to deal with scientific uncertainty.

The international framework: Certification, Traceability, Segregation, Preserved identity and Labelling

The Codex Alimenarius Commission places stress on food labelling and one of its General Subject Committees has mandate on 'Food Labelling'. Food label is a most reliable, effective and ethical practice to communicate to the consumer about the product in terms of its identity, quality and safety. A food label normally declares the name of the food, its ingredients, proximate amount of specific ingredient(s) which are high lighted, prominent indication on durability/shelf life, special storage conditions, if any, name of manufacturer and distributor, the place of origin and the process used in manufacture in certain cases, and instructions for use. A label, backed by suitable consumer protection laws, safeguards consumer rights and immensely helps to check the unfair commercial practice. When certain essential products of consumers are not labelled, they exercise their choice largely on the basis of trust they have built over a period or on the reputation of the seller. Label is more useful to the consumer and seller when the merchandise in question is novel and unfamiliar. For the manufacturer, labelling is a powerful tool in marketing when used effectively and responsibly. Thus, labelling offers a win-win situation for consumer, seller and producer.

"Label" means any tag, brand, mark, pictorial or other descriptive matter, written, printed, stencilled, marked, embossed or impressed on, or attached to, a *container* of *food*. "Labelling" includes any written, printed or graphic matter including a claim that is present on the label, which accompanies the food, or is displayed near the food, including that for the purpose of promoting its sale or disposal. "Food" includes processed, semi-processed or raw, and including drinks, chewing gum and any substance which has been used in the manufacture, preparation or treatment of "food". On the other hand, "Food Additive" is that substance, which is not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The food additives are not "contaminants" or substances added to food for maintaining or improving nutritional qualities. "Claim" means any representation which states, suggests or implies that a food has particular qualities relating to its origin, nutritional properties, nature, processing, composition or any other quality. Food labelling, especially the claims made on the label is guided by the Interpretative Codex guidelines. These guidelines cover the nutrition and health claims, conditions for production, marketing and labeling of organic foods, and foods claimed to be "halal".

Food adulteration is a common undesirable trade practice in many countries threatening food safety. Food safety laws, including labelling, are directed to address this evil. Some of the examples of these regulations are: (1) Using inappropriate name in relation to the

composition of food, (2) Adulteration of high value food with cheaper visually undetectable ingredients, (3) Use of low value fillers for extending a food, (4) Incorrect labelling of the true origin of the food or ingredients, (5) Suppressing or misleading information on geographical or country of origin, (6) Incorrect description of suppression of information on a process or treatment given to the food, and (7) Incorrect quantitative declaration of components of a food. Thus, there are a number of areas that regulate labelling to protect consumer interest and health.

Label is used both in food and nonfood products. Consumer products (toys, detergents, electronic appliances, cosmetics, etc) require safety labelling (symbols and safety phrases, composition and environmental information). Then, the issue arises whether food and non-food products should have similar or different labelling pattern? If food labelling has to be different as a class, then there is need for providing certain commodity-wise general information as well as specific or detailed information on foods in the label. Providing label with more general information on different aspects is called horizontal lebelling or *lex generalis.* The provision of specific and more detailed information on the label on specific aspects such as composition/quality standards, etc is called vertical labeling or *lex specialis.* Consumers may also like to have certain labelling information mandatory and clearly distinguishable from optional and marketing information. Therefore, a legislation on labelling needs to address all these aspects and describe a minimum Standardized framework for presentation of label information.

General Mandatory Labelling Standards of Prepackaged Foods

The information essentially to be provided in the label of prepackaged food, according to Codex standard, includes name of the food, list of ingredients, net content and drained weight, name and address of the manufacturer, packer, distributor, importer, exporter or vendor, country of origin, lot identification, date marking (“Date of Manufacture”, “Date of Packaging” , “Sell-by-Date”, “Date of Minimum Durability”, “best before” , etc) and storage instruction, and instruction for use. Additional mandatory labelling may include quantitative labelling of ingredients to place special emphasis on the presence of one or more valuable and/or characterizing ingredients or on the low content of one or more ingredients, etc and to display prominently whether or not the food has been pre-treated with ionizing radiation or freezing. Irradiation treatment may be optionally indicated with international food irradiation symbol placed in close proximity to the name of the food. Other optional labelling information may include pictorial device written, printed, or graphic matter, provided that these are not in conflict with the specified mandatory requirements and those relating to claims. Commodities exempted from mandatory labeling with information on ingredients, lot number and instruction to use are spices and herbs, small units, where the largest surface area is less than 10 cm^2.

List of ingredients

A list of ingredients shall be declared on the label, except for single ingredient foods, which shall be headed or preceded by a title consisting of /including the term 'ingredient'. All ingredients shall be listed in descending order of ingoing weight (mg) at the time of the manufacture of the food. Where an ingredient is itself the product of two or more ingredients, such a compound ingredient may be declared, as such, in the list of ingredients, provided that it is immediately accompanied by a list, in brackets, of its ingredients in descending order of proportion. A compound ingredient, except food additives, need not be declared when it constitutes less than 5% of the food. All listed foods and ingredients known to cause hypersensitivity shall always be declared. Added water has to be declared as the ingredient except when it forms natural part of an ingredient in a compound food such as syrup. A food additive carried over into a food in a significant quantity or in an amount sufficient to perform a technological function in that food as a result of the use of raw materials or other ingredients in which the additive was used shall be included in the list of ingredients. The presence in any food or food ingredients obtained through biotechnology of an allergen transferred from any of the products shall be declared.

General Principles

Labels shall be applied in such a manner that they are not detachable from the container. All statements mandatory on the label in accordance with Codex standard shall be clear, prominent, indelible and readily legible by the consumer under normal conditions of purchase and use. When the container is covered by a wrapper, the wrapper shall carry the necessary information or the label on the container shall be readily legible through the outer wrapper or not obscured by it. The name and net contents of the food shall appear in a prominent position and in the same field of vision.

If the language on the original label is not sensible to the consumer for whom it is intended, a supplementary label containing the mandatory information in the required language may be used instead of re-labelling. When re-labelling or a supplementary label is used, the mandatory information provided in the original label shall be fully and accurately reflect in.

The Labelling of Food Additives

Food additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods,

but excluding contaminants, or substances added to food for maintaining or improving nutritional qualities, or sodium chloride.

In the context of food additives, it is important to distinguish them from food processing aids and contaminants. Contaminant means any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination.

General Principles on labelling additives

Food additives should not be presented on any label in a manner to give false, misleading or deceptive or in a manner to create an erroneous impression regarding their character in any respect. Food additives should neither be described nor presented in any labelling by words, pictorial nor other devices in a manner that it might be confused, either directly or indirectly, with any other product. The labels of all food additives sold shall bear the prescribed information on details of food additive such as name(s), whether it is "natural", "nature-identical", "artificial", or their combination, instruction on keeping and use, net contents, name and address of the manufacturer, packer, distributor, importer, exporter or vendor, country of origin, and the identity of the producing factory and the lot. Irradiated food additives shall be so designated.

Labelling of and Claims for Prepackaged Foods for Special Dietary Uses

'Foods for special dietary uses' are those foods which are specially processed or formulated to satisfy particular dietary requirements which exist because of a particular physical or physiological condition and/or specific diseases and disorders and which are presented as such. The composition of these foodstuffs must differ significantly from the composition of ordinary foods of comparable nature, if such ordinary foods exist.

The General principle of labeling prepackaged foods for special dietary uses is that it shall not be described or presented in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding their character in any respect. The mandatory labeling of this group of foods shall bear the following information: (1) name of the food with designation such as "special dietary" or "special dietetic" food, together with the characterizing essential feature, but not the condition for which the food is intended, in appropriate descriptive terms in close proximity to the name of the food; (2) the list of ingredients; (3) nutrition labelling including available carbohydrate, fat and energy per unit quantity (100 g), protein content, and total quantity of those specific nutrients or other components per unit quantity which provide the characterizing essential feature for the special dietary use; (4) the net content and drained weight; (5) the name and address of the

manufacturer, packer, distributor, importer, exporter or vendor of the food; (6) the country of origin; (7) the lot identification; and (8) date marking and storage instructions for opened and unopened packages with warning if storage is not desirable for opened package.

Any claims made on this group of foods such as those intended for "special dietary use" shall be in accordance with the General Guidelines on Claims specified by the Codex Alimentarius Commission (CAC/GL 1-1979). A food that is not modified, but is suitable for use in a particular dietary regimen because of its natural composition, shall not be designated "special dietary" or "special dietetic". Claims such as the food is beneficial for the prevention, alleviation, treatment or cure of a disease, disorder or particular physiological condition are prohibited unless they are (a) in accordance with the provisions of Codex standards or guidelines for foods for special dietary uses, and follow the principles set forth in such standards or guidelines; or (b) in the absence of an applicable Codex standard or guideline, permitted under the laws of the country in which the food is distributed. If the foods for special dietary uses are irradiated, it shall be labelled in accordance with guidelines given above. Claims which are misleading or potentially misleading, such as those without meaning or incomplete comparatives and superlatives; and claims as to good hygienic practice, such as "wholesome", "healthful", "sound", are prohibited.

Guidelines on Nutrition Labelling

A separate guideline is developed for precise nutrition labelling to convey information of the nutrient content of a food including those for special dietary use and thereby facilitate the consumer to make informed choice of a food, and to encourage the use of sound nutrition principles in food formulations for the larger public health benefit. Nutrition labelling should not deliberately imply that a food which carries such labelling has necessarily any nutritional advantage over a food which is not so labelled.

The principles of nutrition labeling include nutrient declaration and supplementary nutrition information. The nutrient declaration should have standardized statement or that the nutrients present and considered to be of important are to be listed along with an indication of the quantity of such nutrients. Nutrition claim means a statement that a food has particular nutritional properties including but not limited to the energy value and to the content of protein, fat, carbohydrates, vitamins and minerals. A mere listing of ingredients as a mandatory requirement of nutrition labeling and quantitative or qualitative declaration of certain nutrients as a requirement under national legislation do not constitute nutrition claim.

A nutrient declaration should have the following mandatory information: energy value, amounts of protein, available carbohydrate (i.e., carbohydrate excluding dietary fibre) and fat, amount of any other nutrient as required by national legislation or national dietary guidelines. Where a specific nutrition or health claim is applied, then the declaration of the amount of any other nutrient considered relevant for maintaining a good nutritional status as required by national legislation or national dietary guidelines should be mandatory. Only vitamins and

minerals for which recommended intakes have been established and/or which are of nutritional importance in the country concerned should also be declared. Vitamins and minerals which are present in amounts less than 5% of the Nutrient Reference Value or of the officially recognized guidelines of the national authority having jurisdiction per 100 g or 100 ml or per serving as quantified on the label should not be declared. Where a product is subject to labelling requirements of a Codex standard, the provisions for nutrient declaration set out in that standard should take precedence.

The supplementary nutrition information may vary across countries and target groups within a country according to the needs of the target groups. This is intended to increase the consumer's understanding of the nutritional value of their food and to assist in interpreting the nutrient declaration. This should be optional and should only be given in addition to, and not substituting the nutrient declaration, except for target populations who have a high illiteracy rate and/or comparatively little knowledge of nutrition.

Health claims must be based on current relevant scientific substantiation and the level of proof must be sufficient to substantiate the type of claimed effect and the relationship to health as recognised by generally accepted scientific review of the data and the scientific substantiation should be reviewed as new knowledge becomes available. Health claims should have a clear regulatory framework for qualifying and/or disqualifying conditions for eligibility to use the specific claim, including the ability of competent national authorities to prohibit claims made for foods that contain nutrients or constituents in amounts that increase the risk of disease or an adverse health-related condition. The health claim should not be made if it encourages or condones excessive consumption of any food or disparages good dietary practice. If appropriate, advice to vulnerable groups on how to use the food and to groups, if any, who need to avoid the food.

General Guidelines on "halal" claim in food labelling

Halal food means food permitted under the Islamic Law (lawful food) and fulfilling conditions that it does not consist of or contain anything which is considered to be unlawful according to Islamic Law, that has not been prepared, processed, transported or stored using any appliance or facility that was not free from anything unlawful according to Islamic Law and that has not in the course of preparation, processing, transportation or storage been in direct contact with any food, which is considered to be unlawful according to Islamic Law. However, production, processing and storage as well as transporting of halal and non-halal foods may be acceptable even if same premises or same transport facility are used, provided all necessary measures are taken to prevent any contact or mixing between these two types of foods. Lawful food means those foods of animal origin, which excludes products or derivatives of animals considered as unlawful. In the case of food of plant origin, lawful food shall exclude intoxicating and hazardous plants except where the toxin or hazard can be eliminated during processing. The food shall not include alcoholic drinks, food additives

derived from prohibited animals or plants. Slaughtering of animals shall also follow according to the procedure described under Islamic Law and in compliance with the rules laid down in the Codex Recommended Code of Hygienic Practice for Fresh Meat.

The CAC accepts that there may be minor differences in opinion in the interpretation of lawful and unlawful animals and in the slaughter act, according to the different Islamic Schools of Thought. As such, these general guidelines are subjected to the interpretation of the appropriate authorities of the importing countries. However, the certificates granted by the religious authorities of the exporting country should be accepted in principle by the importing country, except when the latter provides justification for other specific requirements.

Additional labeling requirements are that the word halal or equivalent terms should appear on the label and the claims on halal should not be used in ways which could give rise to doubt about the safety of similar food or claims that halal foods are nutritionally superior to, or healthier than, other foods.

GM Food and Labelling

With the advent of GM-crops, traders, processors and consumers are being posed with the problem of segregation, traceability and labeling GM foods to offer options to consumers. Introduction of GM-technology in crops such as grains, fruits or vegetables might pose situations for their consumption either as raw or cooked or processed products or food derived from animals/birds, which are raised on GM feed or other GM products. Processed products may originate either partially or totally from GM foods. Food processing industry also may have to exercise option for using GMO-derived flavoring agents and enzymes. The issue is whether such food should be compulsorily labeled to provide informed choice to the consumer and processor. One argument is that as most of the biotech foods that are placed on the market have been found to be "substantially (essentially) equivalent" to their conventional counterparts, and does not differ significantly in composition and therefore, labeling of biotech food for GM origin is unnecessary from this technical point of view. A GM food is deemed to be substantively equivalent to the conventional counterpart, if both are found to be *largely* similar. This similarity is determined on molecular and compositional analysis, amount of toxins and allergins, nutritional value and also in terms of its specific use and safety for the environment and for human and animal health. The counter argument is that the GM-food is an unfamiliar product where an informed choice is not feasible without appropriate labeling and hence denial of right to the consumer in exercising a choice against GM-food and therefore such denial raises legal or ethical issues.

Recognising the global free-choice-consumer-opinion on the GM food, there is a justified apprehension among GM food producers and processors that either an affirmative label or advertisement of GM-origin may adversely affect the consumer demand and therefore their business interests. However, producers are willing to label with positive consumption attributes of GM food, like better flavor, nutritional composition, etc. Despite assurances from

GMO producers and some governments about the safety of biotech foods on the market, many consumers insist on their right to distinguish GM-derived food and food products from conventional foods. European Union, which has taken some pro-active measures on GM food production, processing and marketing under label, offers some lessons to those who wish to offer free-choice to consumer and accede their right to distinguish. Two new rules concerning GMOs became legally binding in EU on 18 April 2004. One covered Traceability and Labelling of GMOs (EC No. 1830/2003). The other, the GM Food and Feed Regulation (EC No. 1829/2003), deals with authorisation procedures and labelling issues.

Under the food and feed regulation, labelling is essential for all food and feed products derived from GM sources, regardless of the presence of detectable novel genetic material in the final product and regardless of the quantity of intentionally used GM ingredient present. Such labelling rules apply at the sale point, for consumers exercising their choice on foods containing GM ingredients, such as soya oil, soya flour, corn starch or glucose syrup. Example of a GM fresh produce approved for consumption is sweet Bt-maize (Bt 11). Currently, only ingredients derived from specific varieties of GM soya, maize, oilseed rape and cotton are allowed for food in the EU. The GM Food and Feed Regulation also provides for a threshold for the adventitious, or accidental presence of GM material in non-GM food or feed sources. This threshold for food, additives and flavours is 0.9%, but only applies to GMOs that have an EU authorisation. It is zero tolerance for any GM variety that is not approved. So any GMOs which do not fall within the approved categories cannot be imported into the EU. The food manufacturers are made responsible to ensure that any foods or food ingredients imported are at designated threshold and the GM contamination is only from approved GM crop varieties.

The Food Labelling Regulations, 1966 make falsified description, advertisement or presentation of food an offence. European Marketing Standards and few other laws protect consumers against dishonest labelling and mis-description. The labelling standards for GM foods are different in different countries. In Denmark GM labelling is required not only when it can be proven scientifically but if it is possible that the food will contain GM material. In Japan, presence of GM material above 5 % requires labelling. In the USA GM foods are required to meet equivalent safety standards as the conventional foods, which mean that no labelling for GM foods is required solely because they are of GM origin. Pre-market permit and labelling is required only if it differs substantially from its conventional counterpart. These country to country variations expose the insufficiency of the risk data available on GM foods to arrive at consensus decision on risks and the level of arbitrariness in these decisions.

The Traceability of food from farm to fork

The *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology constituted by the CAC recognised the importance of traceability/product tracing as a risk management tool of GMOs at all stages of food chain, from farm to market, as well as a

useful measure for the control and verification of labelling claims. It can help consumers for making informed choices to protect them against food-borne hazards and deceptive marketing practices and facilitate trade through precise product descriptions. This regulation demands business operators using or handling GM products to transmit and retain information at every stage of placing on the market. For example, where production starts with a GM crop, the company selling the crop for feed production would have to inform any buyer that it is GM. This information has to be retained for 5 years. This will enable products to be withdrawn from the market if any unexpected adverse effects were to arise.

In addition, it is desirable that food and beverage marketing firms augment their existing product traceability protocol by providing continuous visibility on its production and to provide positive assurance that *every* production unit adheres to its business rules. Such Positively Assured Traceability^{TM} (PAT) places the consumers at high confidence for exercising their choice of foods and help to buy according to their particular requirements and cost, be it for diet and health or personal taste and preferences. The essence of PAT is providing continuous visibility regarding: (i) the relevant attributes of each incoming shipment/production lot, (ii) the preserved identity of the material from each incoming shipment/production lot as it moves through an operation across various transformational and commingling steps, such that the attributes of the constituent components of each finished goods are known; and (iii) comparison of data from Steps (i) and (ii) with the firm's operational and quality business rules. This may substantially help a firm in defending any unjustified public or media complaint on the product and recall decisions.

The Food Standards Agency's role is to prevent mislabelling or misdescription of foods. Mislabelling when deliberately done constitutes a crime of fraud. The Codex standards specify that the traceability tool "should not be more trade restrictive than necessary" and should be practical, technically feasible and economically viable. The *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology recognised the importance of traceability/product segregation as a tool to manage risk and a useful measure to control and verify labelling claims.

Segregation and ID preservation of GM- and non-GM crops/products all along the food chain may add substantive overhead cost, which may finally end up in consumer paying higher prices for segregated and ID preserved foods. While deployment of contract farming or production at larger farms may minimise intermixing between GM- and non-GM produces at production level and also cut the cost on segregation and ID preservation, it is challenging and virtually impracticable in countries with small farm holdings and many such farms growing both GM- and non-GM crops. However, price premiums to either the GM- or non-GM foods may largely help in neutralizing the extra cost incurred on segregation and ID preservation. In this context, use of specific ID markers in GM- or non-GM crops as well as in their products may facilitate easy detection from farm to market, within legally allowed tolerance level.

Segregation, ID preservation of GM-produce is difficult not only under small farmer production systems, but also with small and medium sized enterprises (SMEs). On reasons of cost and infrastructure, it is difficult to track the GM-product traceability with database on transactions across the food chain. It becomes more complex in developing and least developing countries, where production is undertaken in many small farms, markets are not organized to preserve identity and traceability, and forward trading and transportation from local markets are equally unorganized. Under such situation, a credible non-GM label may be very seriously compromised. More over, in the case of perishable produces/commodities, such segregation and ID preservation becomes extremely difficult. These limitations and failures may erode credibility of GM-labelling and consumer trust on the system. Notwithstanding these limitations, consistent enforcement of standards, testing, and certification would decrease transaction costs and increase market efficiency in segregation and ID preservation. Tolerance levels for GM-mixing are guided by the level of risk, consumer preference, the carrying capacity of the national system to segregate GM crops from non-GM and ready availability of low cost testing services.

Interestingly when several GM-crops hit and flood the market with poor ID preservation, the non-GM crops may command specific market demand at premium prices, if there are large non-GM preference consumers. This may offer an opportunity to preserve the ID of non-GM crop and market them on a premium akin to the current market for organic products. Such scenario may promote standards, testing, certification, and enforcement for non-biotech foods as well as promoting accreditation of private labs for the testing service. Ultimately, the credibility of GM labelling heavily depends on consistency achievable in standards, IP enforcement, testing services and its quality, certification services and enforcement of all aspects of the law. Many food manufacturers and traders depend on these services and enforcement at every level to ensure credible labelling. It is also important that social/public benefits of credible label shall be outweighing the cost so as to become sustainable.

Packaging, marking and labelling requirements of industrial and agricultural products including food are also regulated by the Agreement on Technical Barriers to Trade under the WTO. The Agreement on TBT seeks to ensure that technical negotiations and standards, as well as testing and certification procedures, do not create unnecessary obstacles to trade. Technical regulations shall not be more trade-restrictive than necessary to fulfil a legitimate objective, taking account of the risks non-fulfilment would create. Such legitimate objectives are, *inter alia*, national security requirements; the prevention of deceptive practices; safety of human/animal/plant health, or the environment. Where relevant international standards are readily or immediately available, Member countries shall use them, or the relevant parts of them, as a basis for their technical regulations. However, the agreement recognizes that countries have the right to establish protection, at levels they consider appropriate, of human, animal or plant life or health or the environment, and shall not be prevented from taking measures at justifiably determined levels of protection.

The international framework: Examples of biosafety legislation and its regional harmonisation

Biosafety laws and regulations concern the food, feed, health of humans, animals and plants and the environment. These laws and regulations are both international and national in nature with regional integration in few groups of countries. Harmonisation of these legislations becomes relevant at international, national and regional levels. Although since long time the CAC, the OIE and the IPPC are concerned with setting standards, guidelines and recommendations on food safety, animal health and zoonoses, and on plant health, respectively, the emergence of WTO demanded substantial harmonization, both horizontal and vertical, among these international biosafety instruments vis-à-vis WTO. Similarly, in the case of the Cartagena Protocol on Biosafety, the processes involving Advance Informed Agreement (AIA), particularly the risk analysis, are being enforced with considerable variation by countries under their national laws and regulations. Conflict potential exists between the Cartagena Protocol on Biosafety and some of the internationally enforceable WTO Agreements, in particular, the SPS Agreement and the TBT Agreement with respect to trade in GMOs.

Developing countries, countries in transition and least developed countries face difficulties in conducting risk-analysis in relation to GMOs. This risk analysis may call for international standard-setting and harmonization. Such harmonization is an integral part of existing pest and phytosanitary risk analysis programmes, and of risk analysis in relation to human health sanitary measures, as signalled in the WTO Agreements on SPS and TBT. Within the WTO, biosafety in relation to GMOs appears to fall chiefly under the SPS Agreement. This Agreement concerns sanitary and phytosanitary measures:

(a) to protect animal or plant life or health from risks arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms or disease-causing organisms;

(b) to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs;

(c) to protect human life or health risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests; or

(d) to prevent or limit other damage from the entry, establishment or spread of pests.

The SPS Agreement recognizes specific international standards, guidelines and recommendations set by the CAC relating to food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling, and codes and guidelines of hygienic practice; by the OIE relating to animal health and zoonoses; by the IPPC relating to plant health; and by the other relevant international organizations open for membership to all Members, as identified by the Committee relating to matters not covered by the above organizations. The TBT Agreement covers a large number of technical measures that seek to protect consumers from economic fraud and deception and measures concerning human, animal and plant life and health not covered by the SPS Agreement, and the environment.

Codex Alimentarius provisions concerning quality and compositional requirements, labelling, nutrition and methods of analysis are relevant to the TBT Agreement.

With respect to a legal framework on biosafety, the harmonization process may work at different levels, within a country, across countries and even groups of countries at regional level. The harmonization is always with reference to an over riding international agreement or convention to which countries have joined party with binding for compliance. At country level, there could state-specific laws enacted by the national legislative body of a country's government, national regulations, which are subordinate legislation of administrative nature authorized by the national law and national guidelines, which are intended to assist by providing ways of complying with national laws and regulations. At the inter-country level there could be bilateral or multilateral agreement, which deals with transactions under the respective national laws, regulations and guidelines. This could accrete into a regional agreement as well. For example, among the eight countries who are members of the South Asia Association for Regional Cooperation (SAARC), biosafety protocols, either as executive order or legislation, are in place in Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan and Sri Lanka. Although all these regulations or laws are in compliance with CPB, they differ in administrative details and public involvement in decision making process. For example, while Bangladesh classifies the risks into four categories, Pakistan does this in three. The huge difference among countries in implementing the biosafety is a matter of concern.

India is currently revamping its regulations on protocol and GMO administration. Recently India initiated an integrated, science-based, competent and transparent system for biosafety regulation under an autonomous agency called National Biosafety Regulatory Authority (NBRA). A draft legislation to establish this system is currently before the Indian Parliament. An initiative is also made by the ten countries under Association of South East Asian Nations (ASEAN) to harmonise their legislation for products derived from modern biotechnology and intellectual property rights, R&D in biotechnology and related environmental protection. Asian Bio Net, an official web site of FAO in this region is assisting Asia in capacity building in the biosafety of GM crops. Regional harmonization and cooperation offers opportunity for sharing of risk-assessment methodologies and results between developing countries with similar ecological environments, as suggested by the Nuffield Council of Bioethics.

The evolution of IPPC over years provides as a good example of harmonization of an international convention. IPPC more recently harmonized with the SPS and TBT Agreements of the WTO. The first text of the IPPC drafted in 1929 underwent revision and was adopted at the FAO Conference in 1951. The Convention came into force in 1952. In 1979, the IPPC was amended and the revised text of the Convention came into force in 1991. Soon after, with the conclusion of the Uruguay Round of trade negotiations and arrival of the SPS Agreement under WTO, the international regulatory landscape on plant protection changed substantially. The leveraging of important technical and legal role to the IPPC by the SPS Agreement

necessitated the IPPC to harmonize its framework in sync with the SPS Agreement for evolving elaborate international standards and to ensure that phytosanitary measures are fairly used in trade. The FAO, in response to this, established a Secretariat for the IPPC in 1992, followed by the formation of the Committee of Experts on Phytosanitary Measures (CEPM) in 1993. The IPPC Secretariat immediately began a major program of standard setting with concurrent amendment of the Convention to more accurately reflect its enhanced role, particularly in harmony with the SPS Agreement. The amended Convention was concluded in November, 1997 and the revised text of the IPPC was approved. It emphasizes cooperation and the exchange of information toward the objective of global harmonization. In addition to describing national plant protection responsibilities, it also addresses important elements of international cooperation for the protection of plant health and the establishment and use of International Standards for Phytosanitary Measures (ISPMs). The revised IPPC entered into force in October 2005.

At national level, the biosafety regulatory frameworks vary according to national priorities and statutory structures. In addition, the different social conditions that prevail in countries make it difficult to typecast the appropriate regulatory systems that should be enforced by developing countries. Notwithstanding such variation, a number of elements are essential and constitute the core of many regulatory frameworks. These are national policy and strategy; regulatory framework consisting of regulations and guidelines; mechanism for handling applications and issuing permits (including risk assessment and management); monitoring and enforcement measures and systems for information dissemination. The regulatory processes involve pre-approval risk assessment evaluation and post approval risk management practices with the requisite monitoring and enforcement measures usually indicated in the conditions of the permit.

Although agreement has been reached on the scientific principles of environmental risk and food safety assessment, consensus has not been achieved on the extent of data required to comply with these principles or on the role of the data for decision-making. A running dispute on the "science" underlying the risk analysis and related restrictions on GMO has been raging between USA and EU for long. This apart, the harmonization process is handicapped, particularly in developing and least developed countries due to lack of resources including local expertise. In order to make informed decisions on the safety of GMOs, governments of these countries need substantial human and institutional resources in the disciplines required for the assessment of risks to the environment and for human food. The role of regional cooperative measures in drawing on a wider resource base in handling risk assessment and management issues has also been a subject of several dialogues. These countries have limited expertise in the required fields of science, as the small number of biotechnologists in these countries is generally engaged in research and therefore mostly unavailable to the regulatory bodies and as policy-makers. In many developing countries, the same scientists who conduct biotechnology research, sit on decision making bodies, and also involved in both risk

assessment and risk management related decision-making. There are three vulnerabilities in this scenario: (a) when developers are also risk assessors, the potential for conflict of interest is magnified; (b) because most members of the decision making bodies are recruited on a voluntary basis, they do not devote much time to this responsibility; (c) because membership of the decision making bodies including the scientific advisory groups generally rotates, there is no continuity in the capacity gained through experience. This, therefore calls for more capacity building for scientists in the regulatory agencies to take a more prominent role in the regulation of modern biotechnology activities.

Building capacity of developing, least developed and transition counties in developing and managing biosafety is hence an important priority area for a few international organization such ad GEF, UNDP, UNEP and FAO. For example in Africa, while South Africa was the first and only country to develop GMO regulations with its own funding (its GMO Act came into force in 1997), national biosafety frameworks (NBFs) of ten African countries (Cameroon, Egypt, Kenya, Malawi, Mauritania, Mauritius, Namibia, Tunisia, Uganda, and Zambia) were developed between 1997 and 2000 only with financial assistance from the Global Environmental Facility (GEF) and project implementation support from the U.N. Environmental Program (UNEP). Since 1998 Zimbabwe was another country which established an independently developed GMO regulation, although this was later made consistent with the provisions of the Cartagena Protocol on Biosafety with GEF assistance. GEF also assisted another 38 African countries (Algeria, Angola, Benin, Botswana, Burkina Faso, Burundi, CAR, Cape Verde, Congo, Comoros, DR Congo, Djibouti, Ethiopia, Eritrea, Gabon, Gambia, Ghana, Guinea Bissau, Ivory Coast, Lesotho, Libya, Liberia, Madagascar, Mali, Morocco, Mozambique, Niger, Nigeria, Rwanda, Senegal, Seychelles, Sierra Leone, South Africa, Sudan, Swaziland, Tanzania and Togo) to develop their NBFs with project implementation support from UNEP. This programme of GEF has so far assisted 139 countries from Asia, Africa, Latin America, and Central & Eastern Europe to prepare their NBFs to facilitate the entry into force of the Cartagena Protocol on Biosafety. As of 27 October 2008, more than 106 countries, including Bangladesh, have completed development of their National Biosafety Projects and their draft NBFs.

On harmonization of Law on Safety in Biotechnology across countries, an African nationalist viewpoint argues for a pan Africa Model, with space for individual African governments to adopt its own national biosafety regulations while adhering to a broader and unified continental biosafety framework that leverages the discretionary components provided in the Cartagena Protocol on Biosafety. This would allow countries to adopt more protective measures than the agreed minimum set out in the Protocol. The Model law is far more comprehensive than that is required by the Biosafety Protocol, it recognizes the importance of Africa as both a centre of origin and a centre of diversity with regard to food and other crops, the precautionary principle and the sovereign right of every country for conducting rigorous risk assessment of any GMO before any decision regarding its use is made. It seeks to

incorporate a liability and redress regime and stricter controls including AIA procedure, notification provision and prior informed consent for the introduction and use of GM food as food aid. The 74th Ordinary Session of the OAU Council of Ministers held in Lusaka in July 2001 had endorsed a Model law, although it has not been made legally binding to Members. The Model Law applies to the import, export, transit, contained use, release and placing on the market of any GMO and a product of a GMO, whether it is intended for release into the environment, for use as a pharmaceutical, for food, feed or processing.

The need for harmonisation of biosafety protocols is not only an issue for developing, least developed and transition countries, but also one for the developed countries. For instance, different strategies for analysing specific risk categories are followed across Europe. Hence, an action to co-ordinate, harmonise and exchange of biosecurity practices, particularly safety assurance (risk containment - risk assessment applicable to BSL3 and 4 laboratories) criteria within a pan European network was initiated recently. This created a pan European network of biosafety experts and a consortium website including an updateable inventory of biosafety relevant elements. It also assesses the cost-effectiveness of measures and methods designed to ensure the safety of the public and private research infrastructures, compile all information into a report and undertakes a program for training and seminars involving national and international organizations in the field of biosafety.

Cardinal area of biosafety regulations is the precautionary approach and risk based analysis and science based approach in risk analysis, identification, management and monitoring. The “science” underlying the risk analysis and related restrictions on GMO trade have been embroiled in two decade long dispute between the US and EU. There are unsettled grey areas between them in standards and burdens of proof, attitudes to uncertainty, and the legitimacy of the “precautionary approach and risk based analysis”. In the on-going discourse, developing and least developed countries are thrown into a quagmire on the merits of the concepts of the “precautionary principle” and the “risk based’ approach to regulation based on a priori scientific proof of likelihood of harm. There is also geographical dispersion to styles of risk management and regulation, as much as there is geography to the scientific practices of risk assessment. Given current divergence in national and international approaches to biosafety, there is wider appreciation for fair harmonization of biosafety laws. It also raises a concern that some countries are coming under pressure to harmonize their laws with one school of regulatory regime. It mentions, for example, that powerful states have been pressuring a number of developing and transition countries, including Bolivia, China, Croatia, Ethiopia, and Sri Lanka, not to implement stringent regulations on GMOs. Caution is also conveyed that ‘harmonisation of biosafety regulation’ is designed to create a one-stop GMO approval system at the sub-regional level, so as to side step a country-by-country, case-by-case risk assessment and decision-making process. In this way, fast-track GM approval systems can be created for the expeditious introduction of GMOs. In this context, it is important that developing, least developed and transition countries are not put to pressure to

deny them the flexibilities available in the protocol on implementing stringent need-based regulations on GMOs which are pivotal from the point of their unique ecological, biodiversity, socio-cultural and farming practices.

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Chapter 6: Status of Relevant Laws and Regulations on Biotechnology in Bangladesh

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Introduction

Currently there are no special laws regulating biotechnology and biosafety in Bangladesh even though the country ratified the Cartagena Protocol on Biosafety on 5 February, 2004. This report attempts to review the status of laws and regulations that are relevant to biotechnology and biosafety issues in Bangladesh. These laws broadly fall under three distinct areas: Sanitary and Phytosanitary (SPS) Measures, Food Safety and Intellectual Property Rights (IPR). In addition to making a separate law on biosafety, this paper recommends that relevant laws and regulations should also be updated in order to develop a comprehensive legal framework for biotechnology and biosafety in Bangladesh bearing in mind its international obligations under the relevant treaties. SPS related laws would ensure that handling, transfer, export and import of genetically modified products do not cause any threat to human beings, plants and the environment. Food safety related laws would ensure that food made of genetically modified organisms do not also pose threat to the consumers. IPR related laws, if amended, would give legal protection to biotechnological research and inventions. Following paragraphs review the current legal status of laws and regulations on these three related areas in Bangladesh.

Biotechnology and relevant laws and regulations on sanitary and phytosanitary (SPS) measures in Bangladesh

Currently we have the following laws on SPS in Bangladesh: On Plant and Plant Products: Destructive Insects and Pests Act, 1914; Destructive Insects and Pests Rules, 1966. On Seeds: Seeds Ordinance, 1977, Seeds Rules, 1988. On Fish and Fish Products: Fish and Fish Products (Inspection and Quality Control) Ordinance, 1983; Fish and Fish Products Rules, 1997. On Animal and Animal Products: Animal and Animal Product Quarantine Act, 2005. On Food: Pure Food Ordinance, 1959, Pure Food Rules, 1968.

The laws on destructive insects and pests regulate the quarantine measures for exported and imported plant and plant products. Rule 3 makes import permits mandatory. Under rule 4 an import permit may be granted with certain conditions. Imposition of conditions on import may help reduce the adverse impacts of releasing biotechnological products, such as the

potential impact of Genetically Modified (GM) plants on the environment, biodiversity and human health.

Under rule 8(1) a phytosanitary certificate from the country of origin is required. Additional information may be required for GM plant or in order to reduce the risks of adverse impacts of biotechnological inventions on environment and human health.

Under Rule 8(6), plants and plant products imported under a valid import permit but without phytosanitary certificate shall either be released after the necessary fumigation or treatment, or returned to the shipper or confiscated and destroyed at the expense of the consignee. These provisions may be used to prohibit unauthorised transboundary movements of GM plants or plant products. The above quarantine measures have been designed to reduce the threats that might arise from the introduction of foreign pests with the imported plant and plant products.

Seed related laws regulate the quality of certain seeds to be made available for sale in Bangladesh. The definition of 'seeds' given under section 2(j), as amended by the 1997 (Amendment) Act, is wide enough to include GM seeds. Section 3 of the Ordinance establishes the National Seed Board. The major functions of the Board, described in Rule 3, are: to advise the government to notify any kind or variety of seeds for regulation; to advise the government to withdraw or denotify outdated varieties of seeds; and to advise the government on a seed security system, among other functions. Section 8 of the Ordinance also establishes a Seed Certification Agency. The major functions of the Agency, described in Rule 6, are: to certify seed of any notified kinds or varieties; to certify seed of other registered varieties; to inspect fields to ensure that the minimum standards for isolation, rouging etc are maintained; and to ensure that seed borne diseases are not present in the field beyond the prescribed limit, among other functions.

Section 7 of the Ordinance prohibits the sale of notified seeds unless (a) such kind or variety of seed and the Seed Dealer is registered with the Board (b) such seed is identifiable as its kind or variety (c) such seed conforms to the standards of seed quality and the container of such seed bears, in the prescribed manner, the mark or label containing the correct particulars thereof.

These laws may be used to set up standards for GM seeds and to regulate their sale through notification in Bangladesh. These laws do not make any distinction between GM seeds and non-GM seeds. Therefore, these laws apply equally to GM seeds of a notified variety in Bangladesh. Furthermore, there is no provision that requires special measures to reduce the threats arising form use, handling or transfer of GM seeds.

The Animal and Animal Product Quarantine Act 2005 regulates the import and export of animal and animal products with a view to controlling the spread of animal diseases and protecting public health. Under section 3 of the Act, animal or animal products that might be the cause of animal or human disease, could be subjected to quarantine or their import or export could be prohibited or restricted, or otherwise regulated by imposing conditions in the

Import or Export Policy Order, passed from time to time by the Government, under the Imports and Exports (Control) Act, 1950.

Section 12 regulates the export of animal and animal products while section 13 regulates the import of animal and animal products. A licence is required for the import of animal and animal products and a health certificate is needed from the country of import. Under section 10, animal and animal products that are found to be infected with disease may be forfeited. This law could be used to prohibit or restrict the import or export of GM animal species or products that have adverse impacts on environment, biodiversity or human health. Necessary rules may be made under section 24 of the Act to regulate the import and export of GM animal species.

Fish and Fish Products (Inspection and Quality Control) Ordinance 1983 and Fish and Fish Products (Inspection and Quality Control) Rules, 1997. These laws deal with inspection and quality control of fish and fish products intended for exports from Bangladesh. Under section 5 of the Ordinance no person is allowed to export, sell for export or have in his possession for export, or deal in any fish or fish products intended for human consumption which is decomposed, unwholesome or contaminated with pathogenic organisms. This provision may be used to prohibit dealings with GM fish or fish products that might pose a threat to environment or human health.

The 1997 Rules regulate the major activities from the production to marketing of fish and fish products with a view to maintaining their export quality. Under Rule 14, a licence is needed for processing, exporting, and servicing factories. Under Rule 5, a licence will not be issued for supply to internal market, for sale, or processing for the purpose of export on the international market unless the quality assurance programme (QAP) stated in Schedule 9 to the Rules is followed. These provisions may be used to reduce the threats that might arise form the use, handling and transfer of GM fish and fish products.

At present there is no quarantine law for fish and fish products imported into Bangladesh. As a result, GM fish and fish products having adverse impacts on environment or other fish species or human health might enter into Bangladesh without any restriction. Furthermore, there is no law to regulate breeding, or crossbreeding activities in local firms. As a result, GM fish with adverse impacts might be developed locally for commercial purposes without any restriction. These laws do not regulate research, production, contained use nor the direct release of GM fish or fish products that might pose threat to environment, biodiversity and human health.

The challenges associated with implementation of the SPS laws in Bangladesh are numerous, including primarily inadequate resources and a lack of scientific equipment and modern laboratories.

Weak enforcement or no enforcement at all is another reason for poor sanitary and phytosanitary regulation in Bangladesh. The required administrative power to enforce the SPS laws is inadequate and ineffective. Thus, it is important to take steps to strengthen

administrative enforcement mechanisms. In addition, lodging complaints and litigation in this area is complex and subject to cumbersome procedures. The relevant Department such as,

Plant Protection Wing, has to go through a number of Ministries or Departments to file a claim against the violators of SPS laws.

Biotechnology and relevant laws and regulations on food safety in Bangladesh

Currently, the following laws are in place in Bangladesh:

- on Food Safety: Pure Food Ordinance, 1959; Pure Food Rules, 1967; and
- on Food Standards: Bangladesh Standards and Testing Institution (BSTI) Ordinance, 1985 and Bangladesh Standards and Testing Institution (BSTI) Rules, 1989.

These laws provide for better control and regulation of the manufacture and sale of food for human consumption. According to Section 3(5) of the Food Ordinance, 'food' means 'any kind of edible oil, fish, fruit, meat, or vegetable or any other article used as food…. and those articles which will be notified by the Government from time to time,…'. Thus, the definition is wide enough to include GM foods.

Section 4A establishes a National Food Safety Advisory Council to advise the Government on matters related to the safety of food, food standards and quality control as well as policies and strategies, all with a view to ensuring the purity, safety and proper nutritional value of food. This power can be used to ensure the safety of GM foods and to set up standards and quality control measures for GM foods.

Section 18 prohibits the use of false labels. It says, 'no person shall…give to the purchaser a label, whether attached to or printed on the container…which falsely describes that article or is otherwise calculated to mislead as to its nature, substance or quality'. Section 19 prohibits the false advertisements of food articles. It says, 'no person shall publish...an advertisement which falsely describes any article of food or is otherwise calculated to mislead the public as to its nature, substance or quality'. This provision may be used to require special labelling for GM food or food products.

These laws do not make any distinction between GM food and non-GM food. They do not require special measures for GM food and food products in order to protect public health.

The BSTI Ordinance provides for the establishment of an institution for standardisation, testing, metrology, quality control, grading and marking of goods. Section 3 of the Ordinance empowers the Government to establish the Bangladesh Standards and Testing Institution (BSTI). The major functions of the Institute, described in section 5, are, among others, to set up Bangladesh Standards of quality and dimensions relating to materials, commodities, structures, practices and operations; to secure compliance with the Bangladesh Standards; to implement Bangladesh Standards through the administration of a national certification mark

scheme or inspection of goods, or both; to grant, renew, reject, suspend, or cancel a licence for the use of Standard Mark etc.

BSTI adopts international standards such as standards developed by ISO, IEC, Codex. BSTI is the Codex focal point for Bangladesh. BSTI also develops its own standard where there is no Codex standard. If, for a given product Codex standard is not available and BSTI does not have its own standard, it then looks for countries where such product is available with the corresponding standard. In developing new standards BSTI follows the procedure of standard developments as laid down by ISO.

BSTI is mostly concerned with finished products and setting standards for these products. Food safety for raw products is the concern of the Department of Heath and the Local Government. They have their own inspectors to enforce laws. Obviously, there is no coordination between the activities of these departments and the BSTI.

In addition to standard setting BSTI is also responsible for certifying products manufactured either for domestic consumption or for export. According to current practice, for all food products certification is not needed, certification is compulsory for only 52 items, the list of which is available on their website. For other goods certification by BSTI is merely optional.

BSTI plays important role in the enforcement of BSTI laws. It is also empowered to take action under Pure Food Ordinance. It has inspectors, but the number is very limited as are its resources. It has its own laboratory. It makes arrangement for a mobile court to inspect market places to try summarily the offenders of BSTI laws and Pure Food Ordinance.

The Directorate General of Food under the Ministry of Food is only responsible to ensure the quality of food grain exported by the Directorate itself. It has its own testing laboratory. In case of food exported by it, it follows the standard mentioned in the contract. Where food is received as aid from other countries the Directorate follows Codex or BSTI standards. It has its own officials at Chittagong and Khulna sea ports who make necessary onsite inspections and investigations regarding the quality food.

Under section 23 of the Ordinance, the Government may, subject to certain conditions, prohibit, restrict, or control the taking out of Bangladesh, articles of any specified description, which do not bear the Standard Mark or regulate generally all practices including trade practices and procedures connected with the export of such articles. Under section 24 the Government may, by notification in the official Gazette, prohibit the sale and distribution of any article specified therein which does not conform to the relevant Bangladesh Standard. These powers may be used to set up Bangladesh standards for GM goods and to control their export, sale and distribution.

The challenges relating to implementation of food safety laws include the multitude of Ministries and Departments that enjoy overlapping jurisdictions, and a lack of coordination among them. The Ministry of Health, Ministry of Industries, Ministry of Local Government

etc are all involved in supervising and administering food safety related activities in Bangladesh. No single organization exists to oversee or coordinate food safety activities.

Poor enforcement of food safety related laws and regulations is another significant problem in Bangladesh. Foods are sold in open markets and on streets. As a result it is difficult to monitor or even enforce food safety laws given the fact that the Government gives priority to other important issues such as, security, crime prevention etc. Recently, mobile courts were organized by the Government to bring provide greater regulation and control of food safety and food quality in Bangladesh.

Biotechnology and relevant laws and regulations on intellectual property rights in Bangladesh

The following laws are in place in Bangladesh on intellectual property rights (IPR):

- Laws on Patents/Designs: Patents and Designs Act, 1911; and
- Patents and Designs Rules, 1933 (a new draft Patents and Designs Act is under formulation by the Department of Patents and Designs);
- Trade Marks Act, 2009 replacing the Trade Marks Act, 1940 and the Trade Marks Rules, 1963;
- Copyright Act, 2000 as amended in 2005.

IPR laws were enacted during the British rule in this sub-continent and thus, Bangladesh inherited its IPR laws from this period which served the purposes of trade and business of that time. Although certain amendments were made subsequently, they do not fulfil the needs of the present day. These laws are inadequate at least in two ways: firstly, they do not fulfil the requirements of the Trade Related Aspects of Intellectual Property Rights (TRIPs); and secondly, they do not provide an adequate legal framework for the promotion of biotechnological research and investment in the country.

The importance of IPR laws is gaining ground in Bangladesh as a result of its increasing participation in WTO and the need to protect its trade interests. Furthermore, the potential role of biotechnology in increasing the country's agricultural production and fulfil the ever-growing food needs of the people, is another reason why IPR laws need renewed attention.

Although IPR laws can take different forms (Patent, Design, Trademark, Copyright), in order to strengthen the legal framework for biotechnology in Bangladesh, a revision of the law of patents seems to be important. Currently, two separate Departments administer the IPR laws in Bangladesh. The Department of Patents, Designs and Trademarks is responsible for regulating aspects relating to patents, designs and trademarks and is headed by a Registrar. On the other hand, the Office of Copyrights, also headed by a Registrar, administers matters relating to copyrights in Bangladesh.

The Patents and Designs Act, 1911 and the Patents and Designs Rules, 1933 are the main laws on patents and designs in Bangladesh. These laws lay down the rules and procedures for

patents and designs in Bangladesh. Section 2(11) of the Act defines 'patent' as 'a patent granted under the provisions of this Act'. In view of the scope of TRIPS, this definition may be reformulated. Under section 3 an application in the prescribed form is required by the applicant expressing the desire to get a patent with declaration claiming to be the true and first inventor. The application must be accompanied by specification information and a fee. The conditions that need to be fulfilled to get a patent for an invention, are: novelty, non-obviousness (inventive step) and utility (industrial application). Under section 6, the Registrar is required to advertise the acceptance of the application. However, the applicant shall have the privileges and rights as if the patent had been sealed on the date of the acceptance of the application (section 7). Any person (within four months from the date of the advertisement of the acceptance of an application) may give notice of opposition to patent on certain specified grounds for example, that the invention is publicly used (section 9).

A patent confers on the patentee the exclusive privilege of making, selling, and using the invention throughout Bangladesh and of authorizing others to do so (section 12).

Under section 14, the duration of protection is 16 years but under section 15 it may be extended for another 5 to 10 years. This provision contradicts the provision of TRIPS as TRIPS provides for a minimum 20 years protection.

According to section 21, a patent has the like effect as against the Government as it has against any person. Under section 21A an inventor of munitions of war may assign to the Government all the benefits of the invention.

Section 22 provides for a compulsory licensing system. Any person interested in a patent may thus make a petition alleging that the demand for the patented article in Bangladesh is not being met to an adequate extent and therefore request the grant of a compulsory license. The government may either dispose such petition or refer it to the High Court Division.

Under section 26 revocation of a patent is possible in a suit before High Court Division on grounds such as fraud. Under section 29, a suit for infringement of patents may be filed in the Court of District Judge that has jurisdiction. The court is allowed to grant relief including temporary injunctions and damages. However, a person may be exempted for innocent infringement (section 30). The court may take the help of an expert in infringement cases (section 35).

Under section 65 the Registrar is given quasi-judicial power. In any proceedings under this Act he or she will have the powers of a civil court for the purpose of receiving evidence, administering oaths, enforcing the attendance of witnesses, compelling the discovery and production of documents, issuing commission for examining witnesses. The Registrar can also award costs and such awards shall be executable in any court having jurisdiction as if it were a decree of that court. The Registrar may however refuse to grant a patent for an invention if the use is contrary to law or morality (section 69).

Appeals against the decisions of the Registrar shall be made within 3 months of the date of the order (section 70). In addition to filing a civil suit under the Patents and Designs Act,

1911, a criminal case may be filed for the infringement of a patent on certain grounds described in the Penal Code, 1860, for example, counterfeiting products and trademarks.

The IPR laws do not comply with the requirements of TRIPS. For example the emphasis in Bangladesh law is on the patenting of processes but TRIPS requires patents for both processes and products. While Bangladesh patent legislation provides protection for 16 years, TRIPS requires protection for 20 years.

Furthermore, TRIPS allows exemption from patent on many grounds such as, plants, animals on grounds of protection to the health of animal, human beings, on the need for environmental protection etc. But under Bangladesh law these grounds of exemptions are not available.

The issue of parallel importation needs to be addressed. Current laws do not address this issue.

A significant problem with the IPR laws is that enforcement is very weak: litigation is very costly, time consuming and the damages and punishment provided is not adequate. The Department of Patents, Designs and Trademarks does not have adequate resources nor the technological support to administer the IPR related laws effectively.

A lack of awareness of the importance of IPR laws and the need to protect the rights granted by such laws is another reason why people infringe IPR laws. Therefore, relevant Departments must have adequate resources and training facilities for public awareness and capacity building in Bangladesh.

A draft law on Patents and Designs has been prepared. It aims to comply with the requirements of TRIPS and is under formulation by the Department of Patents and Designs. However, one of the outcomes of this project could be to make necessary recommendations so as to ensure that biotechnological inventions are properly protected by IPR laws.

As TRIPS allows for the legal protection of plant variety a law has been drafted on Plant Variety Protection. It contains provisions on farmer's rights in Bangladesh. But the enforcement aspect of the draft law is very weak and needs reconsideration. It does not disclose what will constitute an infringement of the Act, what remedies are available, which court has the competence to hear these cases, nor the powers of such court or the procedures to be followed.

The preceding discussion reveals the followings facts. Firstly, the laws and regulations reviewed in this paper are generally quite old. Secondly, the laws under review were not adopted specifically to address the possible threats of GMOs/LMOs. Thirdly, while some of the provisions of the laws under review might be relevant, the respective scopes of the laws are nevertheless limited. They do not provide a comprehensive regulatory regime for biosafety in Bangladesh. For example, the Destructive Insects and Pests Act, 1914 and the Destructive Insects and Pests Rules, 1966 regulate only import, export and transit of plant and plant products. They do not regulate use, transfer, handling, contained use, direct release etc

of plant and plant products which could be GM plant or plant products. Lastly, many institutions have overlapping jurisdictions which creates confusion and delay in the regulation of GMOs/LMOs. For example, the Seeds Ordinance, 1977 and the Seeds Rules, 1988 regulate the quality, sale and distribution of seeds in Bangladesh and is implemented by the Seeds Wing of the _____. But in order to import seeds, further permission is needed from the Plant Protection Wing to ensure that such seeds are pest free. If such seeds are used as foods than other institutions, for example, Department of Food and BSTI will have a role. This lack of coordination and lack of delineated competences may cause problems in the regulations of for example, GM seeds and any potentially adverse impacts on environment, biodiversity and human health.

The only relevant document currently in place to address biotechnology and biosafety aspects is the Biosafety Guidelines which is not a legally-binding instrument. It contains guidelines on conducting biosafety related research and provides an administrative procedure for handling relevant applications. This paper concludes that the laws under review do not provide a comprehensive legal framework to address biotechnology and biosafety issues. It recommends that a comprehensive law should be developed for this purpose backed by a national policy on biosafety for Bangladesh.

ISBN 978-92-5-106447-4

I1252E/1/11.09/300