RECOMBINANT DNA TECHNOLOGY AND BIOTECHNOLOGY

Biosafety and ethical issues related to genetically modified organism (GMO) and recombinant products

Amulya K. Panda
Scientist
National Institute of Immunology
Jawahar Lal Nehru University Campus
Aruna Asaf Ali Marg
New Delhi – 110067

CONTENTS

Keywords

Biosafety; Genetic engineering; Recombinant products; Toxicity containment; Biosafety levels; Transgenic food; Gene pollution; Bio-diversity

Introduction

Advancements in molecular biology have allowed us to do genetic manipulations on varieties of living organisms particularly microbes, viruses and plant species. All these genetic manipulations result in genetically modified living organism (GMO) with altered /extra nucleic acid sequence than the existing ones. Therefore, it is extremely important that research programs, field trials and commercial activities involving GMOs are monitored right from the time of initiation for due assessment of risks and incorporation of required management measures as per the regulations in the country. The main fear associated with genetically engineered microorganism is that they could escape from the laboratory in to the environment with unpredictable and perhaps catastrophic consequences. Such GMOs once released in to the environment could upset the balance of nature or the foreign DNA in the new organism could affect the metaboilic activity in undesirable manner. Genetically modified plant can promote gene pollution (escape of transgene through pollen to related species) and promote antibiotic resistances through horizontal gene transfer in to microbes. The main difference between classical selection methods and improvement by recombinant DNA technology is that the later trespasses the species barrier, leading to gene transfer between any two species such as microorganisms, plants and animals, related or otherwise. Further, gene transfers are accomplished by manipulations outside of cells allowing rearrangement and modification of genetic material before transfer including introduction of novel genes synthesized in the laboratory. Therefore, this transgenic approach of genetically modifying organisms goes beyond the possibilities of nature leading to safety concerns. Thus, it is of paramount importance to understand the safety and risk associated with GMOs. It is imperative not only to know what are the safety concerns with GMOs but also necessary to frame rules and regulation so that there is minimal risk to environment and humanity. Safety concerns with recombinant pharmaceutical products are low as they are carried out in contained environment in the laboratory. Genetic alterations in plants and animals need special safety requirements as they are directly exposed to the environment. Apart from this, there are public concerns for transgenic crops and their use. It is also important to note that assessment procedures and criteria vary for genetic modification in microbes, plants, animals and viruses. Testing and safety evaluation procedures for a therapeutic product from GMO is vastly different from a food crop made by transgenic route. Thus safety issues for both the above cases are different and need different approaches for containment.

Risks associated with recombinant products

There is no evidence that unique hazards exist either in the use of r-DNA techniques or in the transfer of genes between unrelated organisms. Till date, all the products particularly bio-therapeutics produced by the biotechnology industry have been found to be safe. However, specific GMOs may be harmful by virtue of the novel combinations of traits they possess. The concerns associated with use of GMOs can differ greatly, depending on the particular gene-organism combination and a case-by-case approach is required for assessment of safety concerns. Downstream from the laboratory, the GMOs have found applications in agriculture and healthcare industry, often claiming better value and quality of the products. Healthcare is a highly regulated domain and the products address often life threatening situations in a context where risk and benefits must be balanced. Genetically modified organisms are used in contained environment and only the purified and well characterized products are widely used. In view of the above, there is not much public debate on the risks associated with GMOs and products thereof being used in healthcare. The safety concern from an environmental point of view is very low as compared to genetically modified plant or

transgenic food. However, because of the genetic modification there are few areas of safety concern associated with recombinant products or organism harboring the foreign gene. These are:

1. Contamination, infection or mutation of genetically altered strain
2. Pathogenicity or infectivity of the modified organism
3. Toxicity and allergy associated with microbial growth and product formation
4. Development of antibiotic resistance in microorganism
5. Release of recombinant organism in to the environment during growth by aerosol formation or contamination with the exhaust air in the fermentation system or during purification step
6. Problem associated with disposal of spent microbial mass containing modified genetic element
7. Exposure of the personnel handling the modified organisms particularly at industrial scale
8. Immunogenicity of the recombinant product
9. Toxicity associated with repeated use of recombinant proteins or therapeutics in humans

Risks associated with genetically modified crops

Genetically modified crops as well as transgenic animals deal with the growth of plants and animals with altered characteristics. Transgenic plants and animals are exposed to the open environment and the interaction takes place with other organisms in the field. Many of them contain toxic gene and antibiotic resistance marker with them. Therefore, genetic modification in agriculture has become a more sensitive issue than those experiences with recombinant biotherapeutics. Transgenic plants need special attention because they are exposed to environment and have chances of entering in to animal system through food chain. Till today, there is no major risk concern associated with the marketed transgenic crops such as cotton, tomato, corn and soybean. Unfortunately, the public debate over the hazards of transgenic plant or transgenic food suffers from misinformation and misunderstanding of the basis of genetic manipulation in plant system. These GM foods carry a label and have been to extensive field trials for safety and environmental impact before they are approved for commercialization. With the continuing accumulation of evidence of safety and efficiency and no harm to public or the environment, more and more transgenic plants and food are getting appreciated and used by the people. Nevertheless, thorough assessment of the risk and safety associated with GM crops and food need complete evaluation before they are released to the environment. The major safety concern associated with GM crops and GM food are as follows:

1. The effect of GM crops on environment and biodiversity
2. Gene pollution (escape of gene through pollen)
3. Toxicity of the GM plant due to altered metabolism
4. Safety, toxicity and allergenicity of the GM food
5. Insect and herbicide resistant varieties of plant
6. Undesirable effect of transgenic plant on non-targeted or beneficial insect or plant in the environment

History of biosafety protocol and regulations

Because of the risk associated with genetically modified organisms, there were concerns world wide to control it. It is also important to protect the biological diversity of the nature while releasing or accidental escpae of GMO to the environment. This leads in to the formation of biosafety protocols dealing with genetically modified organisms. The origins of the Biosafety Protocol were found in the UN Convention on Biological Diversity, which was signed by over 150 governments at the Rio "Earth Summit" in 1992, and which came into force in December 1993. In the Convention on Biological Diversity (CBD), it was acknowledged that release of GMOs (referred to in the CBD as 'living modified organisms' or LMOs) may have adverse effects on the conservation and sustainable use of biological diversity. All countries that signed up to the CBD were expected to:

a) "Establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts, taking also into account the risks to human health."

and

b) "Consider the need for and modalities of a protocol setting out appropriate procedures in the field of the safe transfer, handling and use of any living modified organism resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity."

In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of the Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of 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 Cartagena Protocol on Biosafety, the first international regulatory framework for safe transfer, handling and use of living Modified Organisms (LMOs) was negotiated under the aegis of the Convention on Biological Diversity. The Protocol contains reference to a precautionary approach and reaffirms the precaution language in Principle 15 of the Rio Declaration on Environment and Development. The Protocol also establishes a Biosafety Clearing-House to facilitate the exchange of information on living modified organisms and to assist countries in the implementation of the Protocol.

The protocol was adopted on 29th January 2000. The protocol has been signed by 103 countries (except USA). The Cabinet (GOI) approved the proposal and India signed the Biosafety Protocol on 23rd January 2001. Subsequent to the Cabinet approval on 5th September, 2002, India has acceded to the Biosafety Protocol on 17th January 2003. So far, 43 countries have ratified the protocol. The Protocol will come into force on the 90th day after the date of deposit of the fiftieth instrument for ratification by countries that are Parties to the Convention.

Biosafety rules in India for GMO and GM plants

In recent years, activities on development of r-DNA pharmaceutical and genetically modified plant with special characteristics have increased substantially. Even though both activities result in different end products, the basic manipulation and genetic handling remain same.

Thus both the activities are monitored and regulated by the Indian government under different departments with special expert committees.

Ministry of Environment & Forests, Government of India has notified the rules for the manufacture, use/import/export and storage of hazardous microorganisms/genetically engineered organisms or cells, (Rules 1989) under Environmental Protection Act (1986). Rule 1989 also defines the regulatory authorities responsible for according various approvals. Presently, there are three regulatory authorities to regulate recombinant DNA related activities including research, product development and commercialization activity. These are (a) Institutional Biosafety Committee (IBSC); (b) Review Committee on Genetic Manipulation (RCGM) and Genetic Engineering Approval Committee (GEAC).

Institutional Biosafety Committee (IBSC) is engaged in research and production activities related to GMOs in every organization. Functions of IBSCs have been elaborated in the "Recombinant DNA Safety Guidelines, 1990" and "Revised guidelines for research in transgenic plants & guidelines for toxicity and allergenicity evaluation of transgenic seeds, plants and plant parts, 1998" issued by the Department of Biotechnology.

IBSC has to review all recombinant research carried out by an organization. The r-DNA Safety Guidelines of DBT (http://www.dbtindia.nic.in) stipulate three categories of research activities i.e. Category I, II and III with increasing level of containment requirements. Category I experiments involving self cloning, using strains and also inter species cloning belonging to organism in the same exchanger group etc. and are exempted for the purpose of intimation and approval. Category II experiments falling under containment levels II, III and IV, large scale use of recombinants made of self cloning in systems belonging to exempt category etc. require prior intimation to IBSC. Category III experiments involving toxin gene cloning, cloning of genes for vaccine production, use of infectious animals and plant viruses, self fusion experiments, field testing and release etc. require review and approval of IBSC before commencement. Depending upon the category of experiments, IBSC can simply note the information provided by PI, give permission before start of the experiments or forward it to RCGM for approval.

The categories of genetic engineering experiments on plants have been notified specifically under the "Revised Guidelines for Research in Transgenic Plant, 1998" by DBT (http://www.dbtindia.nic.in). In this categorization, routine recombinant DNA experiments fall in Category I and need only intimation to the IBSC in the prescribed performa. Category II include lab and greenhouse/nethouse experiments in contained environment where defined DNA fragments that are non pathogenic to human and animals are used for genetic transformation of plants. Permission for performing Category II experiments is provided by IBSC but the decision of the IBSC needs to be intimated to the RCGM before execution of the experiment and RCGM would put this information on record. Category III 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 cannot be judged precisely. All experiments conducted in greenhouse and open field conditions not belonging to the above 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.

Review committee on genetic manipulation (RCGM) is serviced by the department of Biotechnology, GOI. Its mandate is to monitor all safety related aspects of all ongoing research projects on genetic manipulation. All on going projects involving genetic

manipulation and controlled field trials are reviewed by RCGM to ensure that adequate precautions and containment conditions are followed as per the guidelines. It works on the basic inputs from the respective IBSC before taking decision on a safety aspect of particular genetic engineering activities. RCGM is also responsible to bring out a manual and guide lines specifying the procedures for undertaking genetic engineering activities either for research work or for commercial production. It is empowered to lay down procedures for restrictions or prohibiting production, sale, import and use of genetically engineered organism or cells as per rule 1989.

Genetic Engineering Approval Committee (GEAC) is serviced by Ministry of Environment and Forest. GEAC is responsible for the approval of activities involving large scale use of genetically modified microorganism and products thereof in research and industrial production from environmental angle. GEAC also approves proposals relating to the release of genetically modified /hazardous organisms and products in to the environment.

Biosafety regulations and requirements in GMO and biotherapeutics

Products arising from genetic manipulation are increasing day by day. These includes simple protein molecules to complex glycoprotein (monoclonal antibody), nucleic acid based therapeutics to live modified organism as a final product. In general, there is no risk associated with the final product, however as these are produced with the genetically modified organism, it is very much essential to consider all three steps involved in generation of such organism. Many times processing of these organisms may leave behind traces of genetic materials of the donor organism, gene construct, and host. The final modified organism may have entirely different undesirable characteristics. Processing steps during production and purification of such product from live genetically modified organism results in generation of genetic waste which need proper disposal. Thus it is essential to understand the risks associated with each step and have guidelines to have safe procedures for the generation of biopharmaceuticals using GMOs. It is extremely important to have reliable host system expressing the correct gene and ensuring its proper insertion along with any promoter. A proper extraction and purification process should be developed for extraction of the bioactive products, because presence of protein contaminants can result in unwanted immunological and non-immunological actions. The toxicology protocol should also consist of tests for detecting possible immune alteration, mutagenicity/ oncogenicity assessments and teratogenicity impact.

Characterization of the organisms and steps involved in generation of GMO

Recombinant DNA technology basically involves many different steps i.e. the selection of gene from the donor organisms, the vector used for transfer of the gene, molecular biology techniques used to generate the modified organism and the host organisms, production process involving GMO and finally the purified product. It is essential to asses the risks associates with each step so that regulatory steps are taken to minimize the risk.

The first step of risk assessment is to detail the characterization of the host, donor and gene transfer process including the molecular and phenotypic characterization. The risk identification requires knowledge of genes which are expressed, the characteristics, concentration and localization of expressed products and the consequences of expression. It is also essential to ascertain genetic stability of the introduced gene. The modified organisms are analyzed for the risks of pathogenicity, toxicity, allergenicity, teratogenicity etc. as

relevant in the particular situation. It may be noted that assessment procedures and criteria vary for the genetic modification in microorganisms, plants, animals etc. and products thereof, thus each product should be studied and assessed on a case by case basis.

The properties of the insert are extremely important in risk assessment of GMOs. If the insert encodes a toxic gene product, or one which is known to modify the pathogenicity of the organism into which it is inserted, it is expected that the GMO will have greater risk. However, if the gene product is non-toxic and is not one which may pose a risk to the people working with the organism in containment, the risk management will largely be based on the pathogenicity of the host organism. Individual components used in the preparation of the construct i.e. promoters, enhancers and marker genes also need to be carefully reviewed. The vector has to be characterized both for its own potential for pathogenicity and for its ability to transfer the insert to organisms other than the intended horizontal transfer. The function of the genetic material on the vector should be known as this would ensure that the vector is free from sequences that could be harmful to humans or the environment. The presence of genes coding for antibiotic resistance might be of concern, although, for most of the vectors the antibiotic resistance is already common in the environment.

If the donor organism is merely used as a source of well-characterized DNA for a selectable phenotype or a promoter or other control sequence, the characteristics of the donor are not very important to the risk assessment. If, however, the insert contain genes which are biologically active, producing toxins or virulence factors, then information from the donor organism is extremely important. The construction of cDNA or genomic libraries helps in consideration of all the possible hazards associated with the donor organism. Although, the characteristics of the donor organism are of less relevance to the risk assessment than those of the host, the hazard group selected would be generally higher of the two within which the host and donor fall.

The characterization of the host provides the starting point for the risk assessment. It is assumed that, the level of risk associated with the modified organism is at least as great as that of the host organism (until proved otherwise). The identity of the host must be established and the taxonomy well understood. There should be adequate and documented experience of the safe use of the host organism. In case of microorganisms, the pathogenicity of the organism is extremely important for the risk assessment and subsequent categorization. The host must be evaluated to determine that it is not pathogenic. The microorganisms have been in categorized based on infectivity towards humans into four groups, out of which the first group is that of non-pathogens (Table 1). This categorization is generally applicable only for the assessment of containment requirements, as greater containment is required to control the organism in the higher hazard groups to ensure that the organism do not infect those working with it. The details of microorganisms falling into each category are given in the Recombinant DNA Biosafety Guidelines, 1990, of the Department of Biotechnology, Ministry of Science and Technology, Government of India.

Risks associated with a GMO can be assessed by considering three factors i.e. access, damage and expression. Access is a measure of the probability that a modified organism, or the DNA contained within it, will be able to enter the human body and survive there or escape into the environment as the case may be. It is a function of both host and vector. The properties of the vector, particularly mobilization functions need to be taken into account. Expression and damage are usually associated with the insert and the gene product. Expression is a measure of the anticipated or known level of expression of the inserted DNA. If the 'gene' inserted is intended to be expressed at a high level, for example, by deliberate in-

frame insertion down-stream of a strong promoter, expression is likely to be high. If the insert is simply there to allow probes to detect the DNA, and is non-expressible DNA, i.e. with no foreseeable biological effect or gene containing introns, which the host is incapable of processing, then the expression factor will be low. Examination of the modified organism determines the actual expression, which may be higher or lower than expected.

Table 1: Categorization of microorganisms based on pathogenicity

Hazard Group 1	Organisms that are most unlikely to cause human disease
Hazard Group 2	Organisms capable of causing human disease and which may be a hazard to laboratory workers, but are unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylaxis or effective treatment is usually available
Hazard Group 3	Organisms that may cause severe human disease and present a serious hazard to laboratory workers. They may present a risk of spread to the community, but there is usually effective prophylaxis or treatment available
Hazard Group 4	Organisms that cause severe human disease and are a serious hazard to laboratory workers. They may present a high risk of spread to the community, and there is usually no effective prophylaxis or treatment

Damage is a measure of the likelihood of harm being caused to a person by exposure to the GMO, and is independent of either expression or access. It is associated with the known or suspected biological activity of the DNA or of the gene product. The activity of the organism, which results in any toxic, allergenic or pathogenic effect need to be taken into account within this parameter. Molecular characterization of the GMO is used to provide information about the composition and integrity of inserted DNA, the number of copies of inserted DNA, the number of sites of insertion and the level of expression of novel proteins over time and in different tissues in case of plants and animals. Molecular characterization can provide useful information but cannot by itself answer all questions on risk assessment and safety of GMOs.

Containment facilities

In general, biosafety begins with ensuring the workplace whether it is a laboratory, fermentation plant or open fields, safe for the working staff, the general population and finally, the environment by proper containment. Containment covers both the research stage, when modifications are made, development work in the laboratory, greenhouse or growth room, manufacturing units where GMOs are used for production and open fields where they are released. When a new research project is initiated, it involves the modification of organisms within a laboratory under very controlled conditions. The risks are perceived only to those working in the laboratory and containment conditions are devised to ensure that the organism would not escape into the environment, or if it should, it would have been so designed not to survive in the open. However, when the GMOs are used in an industrial or commercial environment, or in open cultivation, the volume of material is considerably larger and the individuals working with GMOs may be less knowledgeable or competent at handling the situation. This implies that there is possibility of accidental escape in a volume large enough for the GMO to survive and persist in the open environment. There is also a risk of accidental release where the waste from industrial unit/fields is not as carefully

monitored as in the laboratory. Therefore, the containment requirements in these cases would take into account both impact on human health and possible environmental effects.

The containment could be physical, where there are real barriers to prevent escape or biological where the organism is designed not to be able to survive in any environment other than that of the laboratory. The containment facilities and biosafety practices have been defined in detail in "Recombinant DNA Safety Guidelines, 1990" of DBT. Recommended biosafety levels for infectious agents are as follows:

A. Biosafety level 1: To carry out standard microbiological practices. Non primary containment provided by adherence to standard laboratory practices.

B. Biosafety level 2: Level 1 practices plus laboratory coats; decontamination of all infectious wastes limited access; protective gloves and biohazard warning signs as indicated. Partial containment equipment (i.e. Class I or II Biological Safety Cabinets) used to conduct mechanical and manipulative procedures that have aerosol potential that may increase the risk of exposure to personnel.

C. Biosafety level 3: Level 2 practice plus special laboratory clothing and controlled access to the laboratory. Partial contained equipment used for all manipulations of infectious material. The facility need to be well contained.

D. Biosafety level 4: Level 3 practices plus entrance through change room where street clothing is removed and laboratory clothing is put on shower on exit, all wastes are decontaminated on exit from the facility. Maximum containment equipment (i.e. class III biological safety cabinet or partial containment equipment in combination with full body air supplied, positive pressure personnel suit used for all procedures and activities. This level needs maximum containment with strict adherence to safety rules.

It may be noted that effective physical containment of bacteria, viruses and other microbes can be extremely difficult because they cannot be seen and once disbursed cannot be recovered. Biological measures often provide better containment options in these cases. Using biological and physical containment measures in concert offers advantages to achieve a specified level of containment. Apart from this laboratory premises, maintenance and operation of equipments used for growth of GMO need special care. This include: laboratory design according to regulatory requirements, provision for storage and disposal, Standard Operating Procedures (SOP): validation of all equipments; calibration of all instruments; SOPs to minimize contamination; monitoring frequency; methods for viable counts in air, water, surface and non viable particulates in air. The whole idea is to take care each minute steps involved in generation and handling of the modified organism that there is minimal risk to the people who are working, lower risk to the environment.

Safety considerations associated with the biopharmaceutical products

The quality of each biotechnology derived product requires careful control because of the concerns about immunogenic proteins or peptides, endotoxins released by the harvesting of culture systems that secrete the product and other chemical contaminants that may emanate from processing procedures. Regarding the finished byproduct, three essential informations are required, purity, safety and efficacy. A detailed analysis of above three parameters using

proper biological experimentation is needed. Important areas of concern regarding the biotechnology-derived products are:

- Toxicological issues arising from the producing system
- Prokaryotic production system (recombinant DNA): Correct gene and promoter, stable expression,
- Eukaryotic Production System (recombinant DNA): Correct gene and promoter, stable expression, presence of antigens, other bioactive peptide, etc.
- Toxicological issues arising from the production process
- De-and renaturation of protein(s)
- Presence of other bioactive molecules
- Presence of chemical residues
- Microbial contamination (e.g. endotoxins)
- Toxicological issues arising from biopharmaceuticals
- Pharmacological and toxicological actions
- Immunogenicity of the product
- DNA and potential oncogenicity
- Genetic variants and mutants
- Glycosylation patterns
- Host-cell-derived proteins
- Process-related impurities
- Product-related impurities or variants
- Presence of Pyrogens
- Viruses (human, simian, murine, bovine)

Biosafety regulation and safety requirements in GM crops and food

In case of crops being used as the hosts for genetic manipulation, additional factors such as potential invasiveness of the species need to be considered. The safety requirements used for generation of modified organism using molecular biology techniques as described for generation of GMO applies to GM plants and its plant product. However special attention is required for GM plants and GM food because they directly interact with the environment. Apart from this, eating of these plant by different species may allow the gene pollution through food chain which may have severe consequences. Plant species have different geographical ranges and estimates of invasiveness may vary in different regions. Crops can be divided broadly into six categories in accordance with their invasive potential:

1. Crops that have no compatible relatives, carry few weediness traits (less than 40 percent), and do not persist in natural environments.
2. Crops that have no compatible relatives, carry intermediate numbers of weediness traits, rarely escape, and do not persist in natural environments.
3. Crops that have no compatible wild relatives, carry many weediness traits, and can escape and persist in natural environments.
4. Crops that have compatible relatives, carry few weediness traits, and can escape but do not persist in natural environments and dot not spread aggressively.
5. Crops that have compatible relatives, carry intermediate numbers of weediness traits, and can escape but do not persist in natural environments; their compatible relatives also carry few weediness traits and do not spread aggressively.

6. Crops that have compatible wild relatives, carry many weediness traits, and can escape and persist in natural environments; their compatible relatives also carry many weediness traits and spread aggressively.

Risk to the environment

Introduction of GMOs in the environment necessitates a close examination for potential ecosystem disruption. Ecological risks include the impact of introduced traits introgressing into other related species through out crossing, the potential buildup of resistance in insect populations to engineered insecticidal traits, unintended secondary effects on non target organisms, and potential effects on biodiversity.

Persistence of the transgene or of the transgene products

The gene transferred into an organism or the resultant products can actually remain in environment leading to environmental problems. For example, in case of Bt crops it was suspected that insecticidal proteins can persist in the environment but experiments have proved that these are degraded in the soil. There are also concerns in case of microorganisms about their capacity to adapt to new environment conditions and persistance in the environment as spores. The existence of virus resistant plant could encourage viruses to grow stronger or give rise to new or stronger variants that can infect plants. Virus life cycle is very short which speeds up the process of viral mutation. This can occur through recombination and transcapsidation. Recombination can occur between GMO produced viral genes and closely related gene of any incoming virus infecting that GMO. Such recombination may produce viruses that can infect a wider range of hosts or that may be more virulent than the parent viruses. On the other hand transcapsidation involves the encapsulation of the genetic material of infecting virus by the GMO produced viral proteins. Such hybrid virus could transfer viral genetic material to a new host plant that it could not otherwise infect.

Gene flow

Accidental cross breeding between GMO plants and traditional varieties through pollen transfer can contaminate the traditional local varieties with GMO genes resulting in the loss of traditional varieties for the farmers. The wind, rain and insect pollinators can contribute to the spreading of the pollen resulting in the contamination of local varieties through cross pollination/breeding. One such example, which has been reported, is that of Mexican corn. Corn originates from Mexico, which holds the greatest biodiversity of corn species in the world of both wild and cultivated species. Recently it has been reported that wild corn variety located in some remote areas of Mexico has been contaminated by GMO genes.

The consequences associated with such gene transfers may impact intellectual property, increase weediness if transferred to compatible weedy relatives or lead to extinction of endangered varieties of the same genera. However, these risks can be anticipated easily and then evaluated by experiments prior to any commercial release, as many factors influence the potential for gene from crop to crop. Some crops are highly out crossing, with pollen carried to other fields by winds and by insects whereas other species are highly self-pollinating with little potential for pollen transfer to neighboring plants. Because of such differences, every case is evaluated individually for potential to contribute to gene flow from transgenic to conventional crops.

Resistance/tolerance of target organisms

The potential benefits of planting insect-resistant transgenic crops include decreased insecticide use and reduced crop damage. However, the innate ability of insect populations to rapidly adapt to environmental pressures poses a serious threat to the long-term efficacy of insect-resistant biotechnologies. Adaptation by insects and other pest to pest protection mechanisms can have environmental and health impacts. For example, adaptation by insect populations to an environmentally benign pest control technique could result in the use of chemical pesticides with higher toxicity. There are apprehensions about development of stronger i.e. more virulent viruses by either alteration in the process through which plant viruses are transmitted or widening of range of hosts which can be infected by one particular virus. However, there are no detailed scientific studies on the actual existence of such effects and a case-by-case assessment is undertaken.

Loss of biodiversity

There have been concerns about reduction in the genetic diversity in cropping systems (i.e. *in situ*) by the development and global spread of improved crop varieties to the green revolution. This genetic erosion has occurred as the farmers have replaced the use of traditional varieties with monocultures. This is expected to further intensify as more and more transgenic crops are introduced which bring in considerable economic benefits to the farmers. The relative rate of susceptibility to any unforeseen infections or destructive situations increases when single variety is used in cropping system in place of multiple varieties. However, it is argued that there is always a continuous and localized experimentation going on for the development of more effective crops which helps in maintaining genetic diversity. As regards the conservation of traditional land bases, their germplasm should be maintained in seed banks (*ex situ*). In fact, biotechnology applications have a critical role in making seed banks storage more effective by accurate tracking of genetic materials through molecular biology techniques.

Changes in the soil ecology

Many plants leak chemical compounds into the soil through their roots. There are concerns that transgenic plants may leak different compounds than conventional plants, as and unintended sequence of their changed DNA. Speculations are that this may change the ecology of the soil in terms of functional composition and biodiversity. The interaction between plants and soil microorganisms is very complex, with the microorganisms living around plant roots also secreting chemical compounds into the soil. Research till date on the genes which have been released such as *Cry1Ac* shows that any such new proteins released by the plants are degraded and not taken up by microorganisms.

Changes in nutritional level

There have been concerns about the accidental changes in the nutritional components of transgenic crops while incorporating other traits. For example, the comparison of Isoflavone levels in the RoundupReady soybeans (GM variety incorporating herbicide tolerant gene) with the conventional varieties showed minor differences. Because Isoflavones are thought to play a role in preventing heart disease, breast cancer and osteoporosis, this became a matter of concern and was investigated. However, studies in support of applications for permission to sell transgenic soybean indicated similarity in commonly tested nutritional components in transgenic and conventional variety.

Containment facility for GM plant

While working out the molecular details for producing a GMO in the laboratory such as identification of DNA sequences encoding the desired trait, choice of marker gene and nature of regulatory sequences that will direct expression of the transgene, care should be taken to minimize extraneous DNA, target the site of insertion as well as method of transformation. For example, concerns have been expressed about the use of antibiotic resistance gene as marker gene, although, several studies unanimously concluded that the risk was immeasurably low. Therefore, ongoing efforts are undergoing to identify other types of genes useful as markers and to develop methods for removing marker genes before GM products are commercialized. In addition, a number of specific promoter gene sequences have been identified which can turn on gene expression in specific tissues/situations. For example, a leaf specific promoter, directing toxin production in the leaves but not roots, stems or flowers could control a gene encoding a toxin active against a leaf-attacking pest. In another case, use of a promoter responding to a chemical signal can help in activation of the particular transgenic plant or plant part when the chemical is sprayed. These sophisticated gene regulatory sequences can contribute to reducing potential risks. The method of transformation should be such so as to avoid including any extraneous DNA sequence e.g. direct gene transfer by a gene gun in plants avoids the potential for inserting unnecessary vector DNA because no vector is used. On the other hand, cells transformed by *Agrobacterium* mediated DNA transfer methods usually contain extra pieces of DNA coming from the vector in addition to the desired gene or genes. Such sequences have generally been proven as safe but it is better to avoid, if possible. It may be noted that right from the initiation of research, risk assessment and management considerations be kept in mind and integrated appropriately into the research plan for production of GMOs.

Containment is a term used for physical barriers to restrict the spread within a structure or enclosed space. The basic biosafety requirement in the development of a GMO is to limit spread of the GMO and its genetic material. A relatively high level of control can be achieved in the laboratory facilities including pilot scale fermentation and small-scale field trials in greenhouses.

(A) **Laboratory containment**: Physical containment of GMOs is maintained by good laboratory practices. Care should be taken that the laboratory facilities are in line with the risk category of the target organism. It is necessary to ensure that the organisms produced under lab conditions are carefully collected for subsequent use or disposal. Appropriate labeling helps in avoiding accidental mixing of the GMOs with other strains. Materials to be disposed of should be treated in a way that prevents their survival or growth outside the contained facility. This may be achieved by autoclaving, steam sterilization etc. Guidelines have been evolved by various agencies even for different GMOs such as microorganism, which should be strictly followed.

(B) **Greenhouse containment**: Greenhouse facilities should be made as per the specifications required for the category of transgenic plant being handled. Conventional greenhouses designed to keep insects and animals out and plant and plant parts are in can be made suitable for GMOs by structural upgrades. For higher level of containment, facilities have to meet specifications such as controlled and filtered airflow, systems to control and disinfect water leaving the facility, autoclaves for on-site sterilization of plant material and equipment, disinfecting the facility after experiments, strict limits on who is allowed to enter including that of staff and trainee.

Field trials and monitoring

The risk management procedures in the field vary depending on the nature and magnitude of identified risks, which in turn depend on basic characterization of the organism, the nature of genetic modification and most important, the site where the GMO is to be released. The local ecosystem of the site should be carefully examined before planning the release of a GMO. Some of the risk management strategies are explained below:

Confinement

For preventing and minimizing the unintentional spread of GMO or a genetic material, measures should be taken to confine them within a site/zone having designated borders/limits. This can be used by both physical and biological means. Physical means to confine GMOs particularly in case of plants and animals consist of geographical or spatial isolation by the use of structures such as fences, screens, mesh etc. The access to the site should be controlled. In case of plants, appropriate isolation distance should be worked out to control the fertilization of sexually compatible species growing in the vicinity by transgenic pollen. It is essential to collect information about the presence and distribution of cross-fertile wild or weedy relatives of cultivated species near the proposed site.

A common method of biological confinement is reproductive isolation, which can be achieved by adopting some of the following strategies:

- GM plants may be grown in an area where sexually compatible wild or weedy species are not found.
- All plants of sexually compatible wild or weedy species found within the known effective pollinating distance of the GM crop may be removed.
- Flowers may be covered with bags to screen out insect pollinators or prevent wind pollination.
- Production of viable pollen may be prevented by using genetic male sterility, applying a gametocyte, or removing all reproductive structures at an early stage of development.
- Tubers, rhizomes, storage roots, and all tissues capable of developing into mature plants under natural conditions may be recovered.
- Differences in flowering time may be exploited so that GM pollen is not shed at the time when sexually compatible plants nearby are receptive.

In addition, there is the option of incorporating genes into chloroplast DNA instead of chromosomal DNA, since pollen from most species does not contain chloroplasts. This technology is still in its infancy, may not be effective for all genes, and would not be effective in plants in which chloroplasts are transferred by pollen. Another option is to genetically engineer transgenic plants to produce sterile seeds. This technology was developed as a “technology protection” system to secure intellectual property rights for the improved seed (the so-called Terminator gene). It is highly effective for risk-management purposes, but has raised ethical questions regarding seed saving and the role of multinational corporations in controlling seed and therefore food supplies in developing countries. Environmental conditions such as temperature, water supply and humidity can also be manipulated to limit reproduction, survival or dissemination of GMOs outside the experimental area. Chemicals such as herbicides, fungicides, insecticides and disinfectants can also be used to limit survival and reproduction of GMOs outside the trial area. At the end of the experiment, the whole experimental area can be sterilized or treated with appropriate

chemicals. Similar measures are taken in case of transgenic animals such as physical confinement by appropriate means such as fences, islands and ponds, reproductive isolation by using sterile animals and controlling the persistence or dispersal of reproductive structures such as larvae or eggs. It may be noted that these measures will be applicable on case-by-case basis on organism with novel traits. Generally, the risks are acceptably low at the time of field-testing, as a result of testing during research and development.

Monitoring

In view of the speculations about potential harm from GMOs introduced into the environment, there has been considerable focus on the monitoring to follow the fate of these organisms and the transgenes they carry and to be vigilant about the unanticipated consequences. Monitoring programmes have been classified into three categories i.e. experimentation, tracking and surveillance corresponding to progressive scale up in field test and commercialization. In view of different monitoring objectives for each successive stage, there is a need to consider larger geographic sampling areas and longer term observation regimes. The development of the monitoring plan will take into account considerations of the objectives, available knowledge about the organisms, environment, conditions of release and potential risk as determined in a risk assessment and the regulatory requirements. The designing of the plan includes specific sampling regimes and testing procedures. It is important to keep the monitoring plan dynamic so as to incorporate modifications in response to changing conditions or unanticipated problems that might develop during the implementation of the plan.

GM food can be divided into technical and non-technical components. The technical components are generally regarding the scientific hazards evaluated in a risk assessment and the management options arising from the assessment. The nontechnical components include the cultural and ethical issues generally raised by non experts, allegations about secretive regulatory decisions etc. It is a valuable exercise to have effective risk communication strategies and some of the strategies are as follows:

- Accept and involve the public as a legitimate partner and treat adversaries with respect
- Coordinate, collaborate and provide information through credible sources
- Be honest, frank and open, don’t keep secrets and acknowledge mistakes made
- Listen to and acknowledge people’s concerns
- Be proactive and speak clearly with a balanced and realistic information strategy
- Meet the needs of the media and identify and train communicators

Public opinion about biotechnology is based on misperceptions of risks fueled by insufficient or inaccurate information. More fully informed opinions can arise only when people have a better and more realistic understanding of how biotechnology will affect their immediate lives and the environment in which they live. Risk communication is thus an important first step towards public dialogue concerning the development and use of GMOs.

Safety associated with GM food

Eating foreign DNA

There have been apprehensions about danger from eating the foreign DNA in GM foods i.e. the pieces of DNA that did not originally occur in that food plant. DNA being present in all

living things such as plants, animals, microorganisms is eaten by human beings with every meal. Most of it is broken down into more basic molecules during the digestion process whereas a small amount that is not broken down is either absorbed into the blood stream or excreted. In an experiment of feeding mice with a harmless detectable DNA sequence, its progress was tracked through the gastrointestinal tract and the body. About 5% of DNA was detectable in small intestine, large intestine and feces upto eight hours after the meal, 0.05% in the blood stream upto eight hours, very small fragments in liver and spleen upto 18 hours and no foreign DNA after 42 hours. It has been reported that even if foreign DNA finds its way into tissues of an organism, it is destroyed by body's normal defense system. So far, there is no evidence that DNA from GMOs including transgenic crops is more dangerous to human health than DNA from conventional crops, animals or associated microorganisms that are normally eaten. According to a FAO/WHO document on safety of aspects of GMOs of plants origin, published in year 2000, the amount of DNA which is ingested varies widely, but it is estimated to be in the area of 0.1 to 1.0 grams per day. Novel DNA from a GM crop would represent less than 1/250,000 of the total amount consumed. This means that the possibility of the transfer of genes that have been introduced through genetic modification is extremely low. Thus, the DNA of the modified crop will usually be processed and broken down by the digestive system in the same way as that of conventionally bred, or otherwise modified crops and it has been demonstrated that the normal body defense eliminate any stray fragments of foreign DNA that enter into the bloodstream from the digestive tract.

Antibiotic resistance

Production of GMOs generally involves use of genes for antibiotic resistance as selectable markers. Early in the modification process in the laboratory, these markers help in selecting cells that have taken up foreign genes. The use of these antibiotic resistance markers has raised the concerns that eating foods carrying antibiotic resistance marker would reduce the effectiveness of antibiotic to fight disease when these antibiotics are taken with meals. The antibiotic resistance genes produce enzymes that can degrade antibiotics. Therefore, theoretically if a transgenic tomato with an antibiotic resistance gene is eaten at the same time as an antibiotic, it could destroy the antibiotic in the stomach. This issue was raised during the approval processes of Calgene's FlavrSavr tomato and Ciba-Geigy's Bt corn 176. However, in both cases tests showed that the enzyme is produced at such low levels that it is absolutely ineffective on the antibiotic. As a result the effect of orally administered antibiotic is same whether taken along with the food having antibiotic resistance gene or not. Further, in many cases, high processing temperatures would inactivate the enzyme in processed foods.

There are apprehensions about the risk of horizontal gene transfer i.e. transfer of DNA from one organism to another, outside of the parent to offspring channel. Transfer of resistance gene from transgenic food to microorganisms that normally inhabit stomach and intestines, or to bacteria that are ingested along with food could help those microorganisms to survive an oral dose of antibiotic medicine. The resistance gene could also be transferred to human or animal pathogens making them impervious to antibiotics. If such transfers were to occur, it could aggravate the already serious health problem of antibiotic resistant organisms. Although, horizontal transfer of DNA does occur under natural circumstances and laboratory conditions, its probability is extremely rare in the acidic environment of the human stomach or even outside environment. Recently, it has been demonstrated that horizontal transfer of DNA containing nptII gene (a deletion mutation) can occur at a rate of 1×10^{-8} i.e. one transformant per 10 million cells, under strong kanamycin selection pressure. The probability of such transfer would further decrease in natural circumstances. While the risks from antibiotic resistance gene in GMOs appear to be low, steps are being taken to reduce the risk

by phasing out their use. Using genes imparting resistance to those antibiotics which are no longer in therapeutic use could be one of the options for use as the selectable markers and is being seriously pursued.

Suggested Reading

1. Recombinant DNA safety guidelines, 1990. Department of Biotechnology, Ministry of Science and Technology, Government of India, July 2002.
2. Biosafety issues related to genetically modified organisms, 2002, Biotech Consortium India Limited, New Delhi.
3. Biosafety issues related to transgenic crops, 2004, Biotech Consortium India Limited, New Delhi
4. Ghosh, P.K., The rules for handling transgenic organisms and biopharmaceuticals produced there from in India, 2003, Journal of Scientific & Industrial Research, Vol. 62, June 2003, p. 554-572
5. Report on the task force on recombinant pharma, Ministry of Environment and Forest (Government of India) 2005.
6. Hand book for IBSC members and additional documents. BCIL, Delhi and Dept. of Biotechnology, 2005.
7. Laboratory Biosafety Manual, 2nd Edition, 2003, World Health Organization, Geneva
8. Biosafety and the Environment: An introduction to the Cartagena Protocol, 2003,
9. NIH Guidelines for Research Involving Recombinant DNA Molecules, 2002, National Institute of Health, USA
10. http://www.biodiv.org
11. http://www.bio.org
12. http://www.bioint.org/biosafety
13. http://www.fao.org