INSTITUTIONAL BIOSAFETY

Indian regulations require that every organisation intending to conduct research on genetically modified organisms (GMOs) should constitute an Institutional Biosafety Committee (IBSC) to approve and periodically review the biosafety aspects of the research projects. The IBSC of our University is constituted as per DBT, Govt of India guidelines and the constitution is as follows :

1.	**Prof S C Lakhotia**	**Dean, Faculty of Science, BHU**	**Chairperson**
2.	**Dr Mathura Rai**	**Director, Indian Inst Vegetable Research, Varanasi**	**DBT Nominee**
3.	**Dr S K Apte**	**Bhabha Atomic Research Centre, Mumbai**	**External Expert**
4.	**Prof A K Joshi**	**Dept Genetics & Plant Breeding, Inst Agricultural Sci**	**Member**
5.	**Prof S Kundu**	**School of Biochem Engg, Inst of Technology, BHU**	**Member**
6.	**Prof S Pradhan**	**Dept Radiotherapy, Inst Medical Science, BHU**	**Member**
7.	**Prof O P Shrama**	**Dept of Radiodiagnosis, Inst Medical Sci, BHU**	**Member**
8.	**Prof B D Singh**	**School of Biotechnology, Faculty of Science, BHU**	**Member**
9.	**Dr B K Singh**	**Dept Physics, Faculty of Science, BHU**	**Member**
10.	**Prof A K Tripathi**	**School of Biotechnology, Faculty of Science, BHU**	**Member**
11.	**Prof J K Roy**	**Dept Zoology, Faculty of Science, BHU**	**Member Secretary**

ROLE OF IBSC

Each IBSC has to meet at least twice a year to review the status of rDNA projects in the institution. It is important that the Chairman and Member Secretary ensure that regular meetings take place. More than two meetings may be held as per requirement of the projects. The IBSC members are expected to look into the following during the meetings:

i. Action taken on the decisions of earlier IBSC meetings.

ii. Characterization of work and approval as per risk category.

iii. Evaluation of projects and direction to submission for appropriate agencies for approvals

iv. Inspection of containment facilities and greenhouses etc.

v. Review the medical reports of employees

vi. Maintaining procedures and other approval requirements.

IBSC has to furnish half yearly reports on the ongoing projects in the organization to RCGM regarding the observance of the safety guidelines including accidents, risks and deviations, if any.

The role of IBSCs assumes major importance in the regulatory framework since it is a Statutory Committee that operates from the premises of the institution and hence is in a position to conduct onsite evaluation, assessment and monitoring of adherence to the biosafety guidelines. The decisions taken by the next higher committee i.e., Review Committee on Genetic Manipulation (RCGM), which operates from DBT are based on the applications submitted by the investigators with the approval of IBSC. Therefore, it is pertinent that the members of the IBSCs and DBT nominees to the IBSCs have expertise in evaluation, assessment and monitoring of projects as per the rDNA guidelines.

The functions in the IBSC of the head of the organization, members, DBT nominees, and Principal Investigator are explained below:

Head of the institution

The head of the institution, who is also the chairman of IBSC has the responsibility to ensure that

- the biosafety guidelines are followed in his institution.
- regular meetings of IBSC are held to review recombinant research projects in the institution.
- open discussion takes place amongst the members in the meetings and the views of external members as well DBT nominee recorded.
- the facilities at the institution are sufficient to meet the containment levels stipulated for rDNA products and processes.

IBSC members

The main functions of IBSC members as defined in the rDNA Safety Guidelines by DBT are as follows:

i) Review and clearance of project proposals falling under restricted category, which fulfill the requirements under the guidelines.

ii) Tailoring biosafety programme as per the level of risk assessed.

iii) Training of personnel on biosafety.

iv) Instituting a health-monitoring programme for laboratory personnel.

v) Adopting emergency plans.

An indicative list of functions falling under each of the above heads is given below:

i) **Review and clearance of project proposals falling under restricted category and meet the requirements under the guidelines:**

- Review biosafety aspects of all projects submitted to IBSC.
- Review categorization of projects as per appropriate risk category.
- Timely action as per biosafety guidelines i.e. to be noted, approved for initiating work or forwarded to RCGM.
- Focus on scientific details and containment facilities but corresponding review also for the organization set up, status of other approvals required, and socioeconomic aspects of rDNA projects.

ii) **Tailoring biosafety programme to the level of risk assessment:**

- Risk assessment of the projects by examining the intentional and unintentional consequences of the specific modifications being targeted through genetic engineering.
- Confirm assignment of the appropriate containment level for the proposed work based on independent assessment. The proper containment level for the unmodified organism should be considered first followed by whether the proposed modified organism would leave the level higher, lower or unchanged.
- Review compliance with the biosafety guidelines by evaluating facilities, procedures and the expertise of personnel involved in the research projects.

iii) **Training of personnel on biosafety:**

- To oversee that procedures are in place in the organization for training of all staff and students working with GMOs appropriate for the risk category under which the experiments are planned/conducted. Emphasis on the procedures to be preferably documented including specific responsibility and accountability for persons within the organization.
- To ensure that training includes laboratory work practices for dealing with GMOs, awareness about relevant rules and regulations and specific modules for persons dealing with high-risk experiments. Training manual and other information to be provided to new staff/students in regular orientation programs.
- To ensure that the record of all training provided is maintained by the organization for review as well as onward submission to RCGM if required.

iv) **Instituting health-monitoring programme for laboratory personnel:**

- To establish and maintain a health surveillance programme involved in connection with the individual rDNA projects, particularly those requiring higher containment levels.
- To review the complete medical check-up of personnel working in projects involving work with GMOs as required prior to starting such projects.
- To review the follow up medical checkups including pathological tests done periodically, at least annually for scientific workers involved in such projects.
- To ensure that medical records are accessible to the RCGM.

v) **Adopting emergency plans:**

- To review the emergency plan proposed by the Principal Investigator for responding to an accidental release and those adopted to meet any exigencies.
- Copies of site emergency plan to be submitted to RCGM, GEAC, State Biotechnology Coordination Committee (SBCC) or District Level Committee (DLC) as the case may be.

DBT Nominee

Each IBSC has a nominee from DBT who oversees the activities to ensure that safety aspects are being fully adhered by the organization. The DBT nominee serves as the link between the department and the respective IBSC. In addition to the responsibilities as an IBSC member, the duty of the DBT nominee is to ensure that:

- The committee has been constituted as per the norms of the guidelines.
- The Recombinant DNA Safety Guidelines are strictly followed in the institution.
- The IBSC meets regularly, at least twice in a year to review the ongoing activities and provides half yearly reports to RCGM/DBT in the prescribed performa.
- All the activities are within the purview of the guidelines and in the knowledge of RCGM/DBT.
- The DBT nominee is expected to guide the IBSC on biosafety issues.

Principal Investigator

All recombinant research projects carried out by an organization have a Principal Investigator (PI) and it is the duty of the PI to apprise the IBSC about the nature of the experiments being carried out. Depending upon the risk category, the PI has to inform the IBSC, seek permission of IBSC before starting the experiments or seek permission of the RCGM through its IBSC.

The PI is primarily responsible for ensuring compliance with biosafety standards. The PI functions as a project manager as well as a researcher, communicating with the IBSC and bearing responsibility for training and supervising personnel.

Based on the nature of the GMO, the PI determines the proper containment level for the project and, in accordance with the DBT Guidelines, develops the necessary experimental protocols. This information is then submitted to IBSC for review. The responsibilities of PI to IBSC are summarized below:

- to make an initial determination of the required levels of physical and biological containment in accordance with the DBT guidelines.
- to submit the initial research protocol and any subsequent changes (such as changes in the source of DNA or host vector system) to the IBSC for review and approval.
- to ensure that no work is initiated until the research project has been approved by the IBSC and has met all requirements of DBT guidelines.
- remain in communication with the IBSC throughout the conduct of the project.
- To ensure the safe conduct of the rDNA experiments in his laboratory.
- To make available the protocols that describe the potential biohazards and the precautions to be taken to all laboratory staff.
- To instruct laboratory staff about the practices and techniques required to ensure safety, and the procedures for dealing with accidents including the reasons and provisions for any precautionary medical practices advised or requested (e.g. vaccinations or serum collection).
- To supervise the performance of the laboratory staff to ensure that the required safety practices and techniques are employed.
- To undertake corrective measures promptly for any work errors and conditions that may result in the release of recombinant DNA materials.

ROLE OF IBSC IN APPROVAL

The rDNA activities within an organization could be broadly categorized into research, large-scale experiments/production/field release and import and shipment. The role of IBSC in each of these activities is explained below:

Research

IBSC has to review all recombinant research carried out by an organization. The rDNA 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 exempt 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.

Different levels of containment have been prescribed for different categories of rDNA experiments in the guidelines. IBSC should allow genetic engineering activity on classified organisms only at places where such work should be performed as per guidelines. Provision of suitable safe storage facility of donor, vectors, recipients and other materials involved in experimental work should be made and may be subject to inspection on accountability.

Large scale trials and production

Although the approval for small scale field trials fall under the purview of RCGM and approval for large-scale trials and production needs to be taken from GEAC, as per the Rules 1989, in all these cases, IBSC has an extremely important role in terms of verifying the information being forwarded to RCGM and GEAC in terms of physical containment conditions, categorization in terms of risk assessment etc. being the statutory body functioning on the premises of the institution. Both RCGM and GEAC depend on the review of the IBSC on the submissions made.

IBSC has to recommend emergency plan in case of large-scale operations, as and when required, which would be then approved by competent authorities. Emergency plan shall include methods and procedures for handling large losses of cultures and organisms.

Import and shipment

The interstate shipment of indigenous etiological agents, diagnostic specimens and biological products need clearance of IBSC and is subject to appropriate packaging, labeling and shipping requirements.

The import of regulated materials for research (e.g. toxin genes, hybridomas, cell cultures, organelle) and specifying conditions under which the agent or vector is shipped, handled and use are issued by RCGM while large scale imports for industrial use are regulated by GEAC. In case of plants, the import is routed through the Director, National Bureau of Plant Genetic Resources on the basis of the import permit issued by the DBT, based on recommendations of the RCGM. However, all these proposals need to be submitted by the PIs through their IBSCs.

Molecular biology details:

Recombinant DNA technology basically uses three components for manipulation i.e. the selected gene from the donor organisms, the vector used for transfer of the gene and the host organisms. Therefore, the first step in risk assessment is to examine these three entities, followed by the modified organism and the resultant gene products.

i. Characteristics of the donor organisms:

If the donor organism is merely used as a source of well-characterized DNA for a selectable phenotype or a promoter or other control sequence, the characteristics of the donor are not very important 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 and of consequence. 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.

ii. Characteristics of the host/recipient organisms:

A thorough knowledge of the host or recipient organism is extremely important in assessment of the risks of the GMOs particularly keeping in view the concept of substantial equivalence as a starting point. The identity of the host must be established and the taxonomy well understood. There should be adequate and documented experience of the safe use of the host organism. The characterization of the host provides the starting point for the risk assessment. The assumption that is generally taken is that, the level of risk associated with the modified organism is at least as great as that of the host organism (until proved otherwise).

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. Infection by a microorganism followed by disease depends on its ability to multiply in the host and on the host's ability to resist or control the infection. 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 2). 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.

Table : 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

The details of microorganisms falling into each category are given in the Recombinant DNA Biosafety Guidelines, 1990.

Some organisms have been used in rDNA technology experiments frequently and their characteristics have been described in detail e.g. *E. coli* or *Saccharomyces cerevisae* are organisms about which a great deal is known. Further no pathogenic strains of bakers' or brewers' yeast have ever been observed. This type of familiarity allows some confidence in attempting to identify risks associated with their modification. Some strains for example, *E. coli* K12 has been disabled to remove some of the factors that might be associated with pathogenicity (wild type *E. coli* is a Hazard Group 2 pathogen). The factors which have been lost include the cell-surface K antigen, part of the LPS side chain, the adherence factor (fimbriae) that enable adherence to epithelial cells of human gut,

resistance to lysis by complement and some resistance to phagocytosis. This variant of *E. coli* is a common host organism for genetic modifications within the laboratory.

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. 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:

i. Crops that have no compatible relatives, carry few weediness traits (less than 40 percent), and do not persist in natural environments.
ii. Crops that have no compatible relatives, carry intermediate numbers of weediness traits, rarely escape, and do not persist in natural environments.
iii. Crops that have no compatible wild relatives, carry many weediness traits, and can escape and persist in natural environments.
iv. Crops that have compatible relatives, carry few weediness traits, and can escape but do not persist in natural environments; their compatible relatives also carry few weediness traits and dot not aggressively spread
v. 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 aggressively spread.
vi. 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 aggressively spread.

The relative risk of using a transgenic crop will increase with the degree of invasiveness.

The relatedness between the host and the donor organisms is also important in the risk assessment particularly with respect to exchange of genetic material between them as well as with other organisms.

iii. Characteristics of the insert/ gene construct:

The properties of the insert are extremely important in risk assessment of GMOs. For example if the information encodes a toxic gene product, or one which is known to be likely to modify the pathogenicity of the organism into which it is inserted, the greater the 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. In case of plants, the transgenes for herbicides and pest resistance need more careful scrutiny as compared to the ones that are selectively neutral in the natural environment.

Individual components used in the preparation of the construct i.e. promoters, enhancers and marker genes also need to the carefully reviewed.

iv. Characteristics of the vector and method of transformation:

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

vector should be limited in size as much as possible to the genetic sequence required to perform the intended functions. This decreases the probability of introduction and expression of cryptic functions or the acquisition of unwanted traits. 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.

The methods of transformation used for introducing the required gene should be considered for the risk assessment of the modified organism. For example, in case of plants, the two principle methods of transformation that are widely used are the *Agrobacterium* mediated transformation and particle bombardment. Whereas *Agrobacterium* mediated transformations result in a low transgene copy number, minimal rearrangement and higher transformation efficiency, particle bombardment causes extensive rearrangements to transformed sequences.

v. Characteristics of the modified organism:

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 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.

The inheritance and stability of each introduced trait i.e. functional in the modified organism must be determined. For each novel trait the pattern and stability of inheritance must be demonstrated as well as the level of expression of the trait by estimation of protein and its analysis. If the new

trait is one that does not result in the expression of new or modified protein then its inheritance will have to be determined by examining the DNA insert directly or by measuring RNA transcript production.

The first presumption for risk assessment is that the modified organism is at least as hazardous as the host. For example, work with modified haemolytic streptococci will proceed in the laboratory in a similar way as with other streptococci of this type and of known pathogenicity. However, more precautions are normally required for modified organisms as introduced external DNA might increase the hazard usually attached to these haemolytic streptococci. Formally such potential increase of the hazard is expressed by classification of the manipulated strain in higher risk category. The formulation "might increase" is important since it reflects the lack of familiarity with the new strain. In some cases it may be observed that the opposite happens i.e. the new strain will be less invasive, the haemolysis less expressed. In short - the strain will represent less hazard to human health. Nevertheless, the new strain has to be treated as more dangerous until confirmed otherwise.
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.

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 be taken into account within this parameter. It may be that the biological activity of a protein is dependent on the host cell system in which it is expressed. An oncogene expressed in a bacterium will have no discernible effect, but when it is present in a human cell, problems may arise. The full biological function of many gene products requires post-translational modification, which will not occur within a bacterial cell normally. The potential biological activity of the gene product should be considered in the context of where and how it has been expressed and the effect on its structure and activity of the mode of manufacture.

Once an estimate of each of these parameters has been made, they may be combined. The result provides a qualitative measure of the risk, and allows a containment level to be assigned for the use of the organism.

The categorization scheme based on risk assessment has been given in Recombinant DNA Guidelines 1990, which should be referred to for evaluating the containment requirements as well as approvals to be taken.

Human health considerations:

Impact on human health is studied by analyzing the modified organism for the risks of toxigenicity, allergenicity, pathogenicity ,teratogenicity etc. as relevant in the particular situation. Assessment procedures and criteria vary in each case of genetic modification carried out in microorganisms, plants, animals etc. and products thereof, some of which are briefly explained below:

i. Toxicity studies:

The main toxicological assessment of a GMO deal with the protein expression studies of inserted gene(s). Another concern is the expression of novel proteins in host organisms due to genetic modification and resulting changes in the metabolism, if any. *In vitro* and *in vivo* studies are needed to assess the toxicity levels of GMOs and products thereof. The standard toxicology methods are often well documented in the scientific and technical literature and the appropriate protocols can be drawn for each GMO.

In transgenic plant tissues, the concentration of novel protein expressed can be very low, often much less than 0.1% on a dry weight basis. Studies, such as acute toxicity testing, which require relatively large amounts of material are often not feasible using the protein purified from plant tissue. Instead, these studies normally make use of protein purified from bacterial expression systems. In such cases, it is necessary to demonstrate the functional equivalence (i.e., equivalence of physicochemical properties and biological activities) of proteins purified from the two sources. When equivalence is demonstrated based on serological cross-reactivity, it is important to use antisera (either polyclonal or monoclonal) that have been well characterized with respect to their specificity.

ii. Allergenicity:

These risks are more difficult to determine except in simple cases where the transgenes come from a species that is known to involve a risk of allergic reactions or even codes for an already identified allergen. If not, the assessment may be based on the structural similarities between the product of the transgene and known allergens and on the residual levels of the proteins coded by the transgene in the product for use/consumption. Databanks for potentially allergic peptides are available that facilitate these studies. Detailed protocols have been defined in the guidelines which may be referred to while evaluating the proposals.

As an example, in genetically modified plants the common criteria to make decisions regarding allergenicity can include:

a. whether the source of genetic material is known to contain allergens.
b. assessment of amino acid sequence of allergens.
c. immunoreactivity assessment.
d. effect of pH and/or digestion since most allergens are resistant to gastric acidity and to digestive proteases
e. heat or processing stability studies

iii. Nutritional analysis:

Nutritional analysis is necessary for GM food crops being developed. Unintended changes in level of nutrients and expression of other biochemicals can occur in many ways including through insertion of genetic material. Food safety assessments should consider the potential for any charge in nutritional composition. For genetically engineered plants aiming at altered nutritional value, the nutritional evaluation should demonstrate that there has been non intentional charges in the levels of key nutrients, natural toxicants or anti nutrients or the bioavailability of nutrients.

iv. Environmental considerations:

In addition to the effect of inserted gene(s) and their impact on genotype and phenotype of a modified organism, it is important to study the proliferation of the GMO in the environment and the effect on its equilibrium.

Environmental risk assessment of GMOs must be undertaken on a case to case basis and there can be no single method or model to follow. Broader issues include the potential adverse effects, likelihood of these risks becoming a reality, consideration of risk management strategies and assessment of overall potential environmental impact.

Possible adverse effects include outcrossing between GM organisms and pathogens,negative effect on population of non target organisms, including indirect effects on population levels of predators, competitors, herbivores, symbionts, parasites and pathogens.

Identification of any potential adverse effect is followed by a stage in which an estimation is made of the likelihood that the identified potential adverse effect will actually occur. It is important to estimate the chances of each of potential effect for assessment purposes.

The likelihood of certain potential adverse effects occurring can be influenced by characteristics of the size and scale of application in addition to those of inserted transgene and the recipient organism. A precautionary approach is useful in assessment of ecological effects.

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 design not to survive in the open. At this stage, the associated risks are mainly to the human health. 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. In brief, the basic laboratory guidelines have been detailed that are fundamental to all classes of risk groups followed by modifications for work with more dangerous

pathogens. For more details, the most reliable reference is Laboratory Biosafety Manual of the World Health Organization is available at its website. A summary of recommended biosafety levels for infectious agents is given in Table 3:

Table : Summary of recommended Biosafety Levels for Infectious Agents

Biosafety Level	Practice and Techniques	Safety	Facilities
1.	Standard microbiological practices	Non primary containment provided by adherence to standard laboratory practices	Basic
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	Basic.
3.	Level 2 practice plus special laboratory clothing, controlled access	Partial containment equipment used for all manipulations of infectious material	Containment
4.	Level 3 practices plus entrance through change room where street clothing is	Maximum containment equipment (i.e. class III biological safety cabinet or partial containment	Maximum containment

	removed and laboratory clothing is put on shower on exit, all wastes are decontaminated on exit from the facility	equipment in combination with full body air supplied, positive pressure personnel suit used for all procedures and activities

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. It may also reduce the physical requirements to those of the next lower biosafety level. For example, an experiment design to evaluate tomato plants genetically engineered for resistance to tomato spotted wilt virus involves three organisms i.e. tomatoes, the virus and thrips, the insect vector that transmits the virus. Whereas physical containment would be provided by a greenhouse with antivirus screening or by conducting the experiment in insect proof cages within the greenhouse, biological containment could be added by removing alternate host plants for the virus both in and outside of the greenhouse and by applying stringent insect control measures in the surrounding area.

A detailed checklist for according approval to a laboratory for carrying out recombinant DNA technology work has been given in “Recombinant DNA Safety Guidelines, 1990” of DBT. In addition to these guidelines, some of the key points as reviewed from WHO guidelines are detailed below:

i. Premises and lab:

- Appropriate containment: code of practice; lab design and facilities; health and medical surveillance; specification for gene technology lab; specification for large scale operations
- Prevention against entry of pests (air pressure, exhaust air, input air)
- Provisions for emergency
- Provisions for storage and disposal: In process material; starting material; finished product; infected material/rejected
- Cleanliness and hygiene
- Repair facilities

ii. Equipment:

- Adequacy of equipment: appropriate design; set up and maintenance
- Standard Operating Procedures (SOP): validation of all equipment; calibration of all instruments; investigating recording all deviations and expertise
- Automated equipment: computer controlled system; back-up file maintenance and hard copy systems

iii. Animal facilities:

- Receipt of animals, including identification of person responsible and required documentation; maintenance, evaluation of health status; housing, feeding, handling; isolation of sick animals, preventive measures, treatment and quarantine for newly received animals
- Pest control system; facilities for waste, carcass; cleaning, sterilization and maintenance of supplies and equipment (animal cages, racks)

iv. Environment:

- SOPs to minimize contamination; monitoring frequency; methods for viable counts in air, water, surface and non viable particulates in air.

RECOMMENDED BIOSAFETY LEVEL-I LABORATORY PRACTICES FOR RECOMBINANT DNA RESEARCH

1. Biohazard symbol should be placed at the entrance of recombinant DNA work area.
2. Access to the laboratory is limited or restricted only to the workers.
3. Eating, drinking, smoking, applying cosmetics are not permitted in the work area.
4. Laboratory Costs, grown or uniforms are worn while in the laboratory. The protective clothing should be removed before leaving laboratory for non-laboratory areas.
5. Gloves should worn while handling the rDNA materials to avoid skin contamination.
6. All contaminated liquid or wastes are decontaminated by ethanol treatment or autoclaving before disposal.
7. Contaminated materials should be stored in leakproof containers till decontamination.
8. Mechanical pipetting devices should be used; mouth pipetting is prohibited.
9. Work surfaces are decontaminated atleast once a day.
10. Persons should wash their hands after handling recombinant DNA materials.
11. Creation of aerosols is minimized.

12. Biological Safety Cabinets should be used for routine work with rDNA materials.
13. Control measures should be taken to avoid entry of insects and rodents.
14. Spills and accidents which result in rDNA exposures to organisms should be immediately reported to laboratory incharge and IBSC.