REVISED GUIDELINES FOR SAFETY IN BIOTECHNOLOGY

MAY 1994

DEPARTMENT OF BIOTECHNOLOGY
(PID Division)

MINISTRY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

It gives me a great pleasure to introduce the revised guidelines for biosafety in biotechnology being brought out by the Department. The first guidelines were brought out in January, 1990. During the last one decade, the country has made noteworthy progress in handling and manipulation of plasmids, construction of vectors and through them transformation of various hosts ranging from bacteria to yeast, animal and plant cell lines as well as transformed plants.

After the signing of the Biodiversity Convention by the Government of India, the Ministry of Environment & Forests requested the Department to revise the earlier guidelines, specially to take note of Genetically Modified Organisms (GMOs) from safety angle in research, application and also for transfer of technology.

In consonance with the above, the Department has revised the guidelines with the advice of an Expert Group as well as the Ministry of Environment and Forests, Indian Council of Medical Research and the Indian Council of Agriculture Research. The revised guidelines incorporate the consolidatd views of all the user Ministries and organisations as well as the R&D and academic institutes.

I wish to thank all the experts, the institutions and the Ministries which provided valuable inputs for the revised guidelines. I am confident that the revised guidelines would be of immense help in our future programmes in the field of biotechnology.

Sd-

New Delhi
May, 1994

(C.R. Bhatia)
Secretary

GUIDELINES FOR SAFETY IN BIOTECHNOLOGY

1. Introduction

Biotechnology, in its modern version, is the application of techniques like gene splicing, oligonucleotide synthesis, construction of expression vectors and expression hosts, indentification of proteins and nucleic acids by blotting, protein engineering, affinity purification of proteins and their down stream processing which are aimed at developing specific applications in different areas. A good deal of effort is being made in the areas of diagnostics, vaccines, bioactive proteins, transform microbes, biocides, tissue culture raised high value plants, including vegetables and crops to develop technologies and useful products. The success in indigenous research efforts would eventually be translated into commercially viable technologies. These developments have generated a sense of concern among scientists working in biological areas and others to find ways as to how safely the research in the field should be carried out and also the possible means to regulate the work involving pathogenic microorganisms and virulent genes. With the safety considerations in view, the Department of Biotechnology had published "Recombinant DNA Safety Guidelines" in January 1990. The "Recombinant DNA Advisory Committee (RDAC)" functioning under the Department had framed the guidelines taking into account the local factors such as resistance to infection (immunity), host and parasite burden in the community, laboratory enviroment and chances of survival and growth of altered organisms under the tropical condition. The Committee will update/modify the guidelines from time to time, on the basis of scientific information and from the experience gained locally and outside the country on the use of the new techniques in the area of research, manufacture and applications. Safety measures to be practiced, in general, have been formulated which are given in Appendix-I.

2. Coverage of Guidelines including Research:

The current guidelines cover areas of research involving genetically engineered organisms, genetic transformation of plants, rDNA technology in vaccine and diagnostics development and on large scale production and deliberate/accidental release of organisms, plants, animals and products derived by rDNA technology into the environment and also import and shipment of GMOs/transgenics for laboratory research and large scale use.

3. Classification of pathogenic microorganisms:

The classification of infective microorganisms are drawn up under **4 risk groups in** in increasing order of risk based on the following parameters:

— pathogenecity of the agent

— modes of transmission and host range of the agent

— availability of effective preventive treatments or curative medicines

— capability to cause diseases to human/animal/plants

— epidemic causing microbial strains in India

The above mentioned parameters may be influenced by levels of immunity, density and movement of host population , presence of vectors for transmission and standards of environmental hygiene.

The summary of [illegible]

categories mandating different levels of [illegible]

out research activities involving genetic manipulation of microorganisms, plants or animals should constitute the IBSC.

Presently, IBSC has been constituted in 52 institutions including industries in the country. Any research projects which are likely to have biohazard potential during the execution stage or which involve the production of eithter microorganisms or biologically active molecules that might cause bio-hazard should be notified to IBSC. The on-site emergency plan should be prepared by the institution or occupier for each of the above activities with the help of IBSC. IBSC will recommend genetic engineering activity on classified organisms only at places where such work could be performed as per guidelines. IBSC has to meet atleast twice in a year and provide atleast two half yearly reports on the on-going projects to RCGM.

In addition to the above, the activity of IBSC also include:

(i) Training of personnel on biosafety.

(ii) Instituting health monitoring programme for laboratory personnel. Medical check-ups including pathological tests are to be done periodically.

(b) *Review Committee on Genetic Manipulation (RCGM):*

The RCGM is functioning under the Department of Biotechnology and has the following functions:

(i) To review the reports in all approved ongoing research projects involving high risk category and controlled field experiments.

(ii) To visit site of experimental facilities periodically where projects with biohazard potential are being pursued and also at a time prior to the commencement of the activity to ensure that adequate safety measures are taken as per the guidelines.

(iii) To issue clearance for import/export of etiologic agents and vectors, germ plasms, organelle, etc. needed for experimental work/training and research.

The RCGM has to meet atleast twice in a year.

(c) *Genetic Engineering Approval Committee (GEAC):*

The GEAC is functioning under the Ministry of Environment and Forests to examine and issue the clearance from the view point of environmental safety on the case by case basis for:

(i) activities involving large scale use of hazardous microorganisms and recombinants in research and industrial production from enviornmental angle.

(ii) proposals relating to the release of genetically engineered organisms and products into the environment including experimental field trials.

(iii) production, sale, import or use of substances and products including food stuffs and additives including processing aids containing or consisting genetically engineered organisms or cells or microorganisms.

(iv) import, export, transport, manufacture, process, use or sale of any hazardous microorganisms or genetically engineered organisms/substances or cells.

(v) scale up or pilot operations for facilities using genetically engineered organisms/microorganisms mentioned in the schedule.

The GEAC shall give the directions to the occupier to determine or take measures concerning the discharge of microorganisms/genetically engineered organisms or cells mentioned in the schedule from the laboratories, hospitals and other areas including prohibition of such discharges and laying down measures to be taken to prevent such discharges. The GEAC shall supervise the implementation of the terms and conditions laid down in connection with the approvals accorded through the State Biotechnology Coordination Committee or the State Pollution Control Boards/District Level Committee or through any person authorised in this behalf.

(d) *State Biotechnology Co-ordination Committee (SBCC):*

There shall be a State Biotechnology Coordination Committee in the states wherever necessary to:

(i) inspect, investigate and take punitive action in case of violations of statutory provisions through the Nodal Department and the State pollution Control Board/Directorate of Health/Medical Services.

(ii) review periodically the safety and control measures in the various industries/institutions handling genetically engineered organisms/hazardous microorganisms.

(e) *District Level Committee (DLC):*

There shall be a District Level Biotechnology Committee (DLC) in the districts wherever necessary under the District Collectors.

(i) The Committee monitor the safety regulations in installations engaged in the use of genetically modified organisms/ hazardous microorganisms and its applications in the environment.

(ii) The District Level Committee/or any other person/s authorised in this behalf shall visit the installation engaged in activity involving genetically engineered organisms, hazardous microorganisms, formulate information chart, find out hazards and risks associated with each of these installations and coordinate activities with a view to meeting any emergency.

(iii) They shall also prepare an off-site emergency plan.

The District Level Committee shall regularly submit its report to the State Biotechnology Coordination Committee/Genetic Engineering Approval Committee.

(f) *Funding agency for research proposals:*

The funding agency is responsible for approval and clearing of research proposals for grants in aid in respect of rDNA research activities. Support on approved projects can be withdrawn in case of deliberate violation or avoidable negligence of rDNA guidelines.

The funding agencies have to submit the list of approved projects that come under this area annually to RCGM.

The guidelines suggest compliance of the safeguards through voluntary as well as regulatory approach and are notified in the Gazette of India under the Environment Protection Act, 1986. Any violation and non-compliance including non-reporting of the activity in this area would attract the punitive actions provided under the EPA.

7. Large Scale Industrial Processes and Operations:

All operations requiring the handling of **20 — litre or more** capacities are deemed large-scale. While the exigencies of laboratory-level safety also apply here, additional ones particularly suited to industrial processes have to be stringently observed.

(a) Approval of Competent Authority (GEAC) should be sought in all cases. The fundamental design of strategy of the process together with the operational details, as well as the methods for the proper neutralization/disposal of the potential harmful byproducts should be submitted for prior review.

(b) Regular Monitoring, both by in-house and by outside agencies should be conducted of the control measures and safety equipment pertaining to the operation. Proper training should be imparted to personnel for the safe execution of the process as well as the disposal/dissemination of the primary and by-products. A stringent code of conduct for containment, process control and execution and final dissemination should be laid down and implemented.

(c) Regular Monitoring of viable process organisms in the outside environment is necessary.

(d) Ideally the host organism should not be a pathogen, and although optimized for survival under bioreactor conditions, should not be able to survive in the harsh environment for extended periods of time. Similarly, the vector-inserts should be completely defined structurally and should be devoid of sequences that are harmful either directly (eg. those coding for toxins) or confer advantages for survival (antibiotic resistance, bacteriocin production, etc.) unless there is a demonstrably direct requirement of that particular function in the process. Additionally, the host-vector system should not function as a pathogen.

(e) Health status monitoring of personnel involved in process should be carried out from time to time.

(f) Pathogenic organisms should be destroyed or neutralised in some other demonstrable way. Even seemingly innocous organisms should be disposed off only after loss of viability. Experimental animals should be handled in special facilities. All carcasses and animal waste should be incenerated.

The containment approaches for large scale industrial applications are given in Appendix-4.

8. Field Trials of Transgenic Plants and their Release to the Environment:

Planned release of recombinant organisms should be carried out only after a stepwise evaluation of upscaling of the GMOs from lab scale to a growth chamber and then to greenhouse conditions. All relevant published information should be provided to the **Regulating** Agency on the environmental aspects, as also the predicted effects of the release on the ecosystem. Model experiments to ascertain data on this aspect should be carried out with prior permission of RCGM, and provided to GEAC alongwith request for approval, for release into the environment. Depending on the type of organism handled and the assessment of potential risks involved appropriate containment facilities must be provided to ensure safety and to prevent unwanted release in the environment.

Biowastes resulting from laboratory experiments, industrial operations should be properly treated, so organisms are either destroyed or rendered harmless before disposal into the environment.

Criteria to be followed for field testing of the transgenic plants only on the experimental farms:

(a) Maintain atleast the minimum isolation distance recommended for raising "Foundation Seed" all around the transgenic plants. In the isolation distance a noncompatible corp can be grown. For isolation distance consult the book on "Indian Minimum Seed Certification Standard" by N.S. Tunwar and S.V. Singh published by the Central Seed

Certification Board, Deptt. of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi, July 1988 or any updates that will come in future.

(b) Beyond the isolation distance, grow a few rows of the nontransgenic plants of the same crop to serve as a pollen trap.

(c) Analyse the seed progeny from the plants used as pollen trap for accessing pollen escapes and verifying the effectiveness of the isolation distance. The experiments conducted to monitor pollen escape into compatible border row plants should be maintained that will be useful for risk assessment.

(d) Pre-release tests of genetically engineered organisms in Agricultural applications should include elucidation of genetic markers, host range, requirements for vegetative growth, persistence and stability in small plots and experimental field trials for 2 years.

9. Post harvest handling of the transgenic plants:

(a) destroy by burning all the vegetative parts and left over seeds.

(b) leave the land fellow next year and destroy the plants, if any emerging from the seeds of last year in the soil.

(c) Soil samples in experiments under controlled containment conditions should be tested for the absence of viable cells before disposal into the environment.

GEAC initially may clear a project for limited trials which may be enough to have data for assessing the environmental impact in case it is necessary. Where such environmental impact studies are not warranted, GEAC may give permission for large scale trials.

GEAC has to take a decision within 90 days after receiving the complete information in the prescribed format from the applicant.

10. Risk assessment:

Risk assessment should take into account the following factors wherever relevant:

1. Geographical location, size and nature of the site of release and, physical and biological proximity to man and other significant biota; in the case of plants, proximity to plants which might be cross pollinated.
2. Details of the target ecosystem, and the predicted effects of release on that ecosystem.
3. Method and amount of release, rate frequency and duration of application.
4. Monitoring capabilities and intentions, how many novel organism be traced, e.g. to measure effectiveness of application.
5. On-site worker safety procedures and facilities.
6. Contingency plans in the event of unanticipated effects of novel organism.
7. The nature of the organism or the agent to be released, in the species (or culture), its host range and pathogenecity (if any) to man, animals, plants or micro-organisms.
8. The procedure used to introduce the genetic modification.
9. The nature of any altered nucleic acid and its source its intended function/purpose and the extent to which it has been characterised.
10. Verification. of the genetic structure of the novel organism.
11. Genetic stability of the novel organism.
12. Effects that the manipulation may be predicted to have on the behaviour of the organism in its natural habitat.
13. The ability of the organism to form long-term survival forms, e.g. spores, seeds etc. and the effect that altered nucleic acid may have on this ability.
14. Details of any target biota (e.g. pest in the case of a pest

control agent); known effects of non-manipulated organism and predicted effects of manipulated organism.

15. Growth and survival characteristics of the host organism and the effect that manipulation may have.

16. Susceptibility to temperature, humidity, dessication, UV etc. and ecological stresses.

17. Details of any modification to the organism designed to effect its ability to survive and to transfer genetic material.

18. Potential for transfer of inserted DNA to other organisms including methods for monitoring survival and transfer.

19. Methods to control or eliminate any superfluous organism or nucleic acid surviving in the environment or possibly in a product.

Exact protocol/s and quantitative aspects of the application, together with methods to quantitate the efficacy of the release with respect to intended application/end effect should be ascertained and provided to GEAC.

11. Import and Shipment:

The import or receipt of etiologic agents and vectors of human and animal disease or their carriers is subject to the quarantine regulations. Permits authorising the import or receipt 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 used are issued by the Review Committee on Genetic Manipulation while large scale imports for industrial use are regulated by Genetic Engineering Approval Committee. The Inter-State Shipment of indigenous etiologic agents, diagnostic specimens and biological products is subject to applicable packaging, labelling and shipping requirements specified for etiologic agents. All such shipments would need the clearance of IBSC and should be in the knowledge of RCGM.

12. Quality Control of Biologicals produced by DNA Technology:

The general regulations normally applicable for biologicals are applicable to the recombinant DNA products. The specific relevant aspects to a particular product should be discussed with the appropriate Government Agency on a case by case basis.

A new licence for the product or drug application would be required on products made of recombinant DNA technology even if the product is considered to be chemically and physically similar to the naturally occurring substance or previously approved product produced in conventional system.

A recombinant DNA product produced in the same host and demonstrated to be identical to normally occurring substance would not require toxicological and pharmacological data if the information is already available at dose levels of intended use but fresh clinical trials on a relatively limited basis will be necessary on all such products. A much more stringent regimen of testing evaluation that presupposes the product as a new drug would be followed for totally new products e.g. protein engineered derivatives with novel functionalities.

The Chemical and Biological characterization of DNA products, especially those intended for application to living organisms parenterally should be based on the following criteria.

— Chemical and physical identity of the products.

— Biological Potency

— Purity (percent)

— Nature of contaminants

— Toxicity studies in animals

— Immunogenecity (in case of vaccines)

— Consistency of production

Details of methodology used for cloning, construction of vector, host-type, origin of cloned DNA, nucleotide sequence/fine — detail RE map of cloned segment and vector should be provided to GEAC.

Limited trials for all products derived from DNA technology are necessary to evaluate their safety and efficacy.

13. Contact addresses:

Research, field trials in controlled conditions

The Secretary,
Department of Biotechnology
Block-2, 6th Floor
CGO Complex, Lodi Road
New Delhi-110 003.

Large scale production, environmental applications and use

The Secretary,
M/o Environment & Forests
Paryavaran Bhawan,
CGO Complex, Lodi Road
New Delhi-110 003.

APPENDIX 1

Good Laboratory Practice

— Never do direct mouth pipetting of infectous or toxic fluids; use a pipettor.

— Plug pipettes with cotton.

— Do not blow infectious material out of pipettes.

— Do not prepare mixtures of infectious material by bubbling expiratory air through the liquid with a pipette.

— Use an alcohol-moistened pledget around the stoper and needle when removing a syringe and needle from a rubber stoppered vaccine bottle.

— use only needle-locking hypodermic syringes. Avoid using syringes whenever possible.

— Expel excess fluid and bubbles from a syringe vertically into cotton pledget moistened with disinfectant, or into a small bottle of cotton.

— Before and after infecting an animal, swab the site of injection with a disinfectant.

— Sterilize discarded pipettes and syringes in pan where they were first placed after use.

— Before centrifuging, inspect tubes for cracks. Inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter. Carefully remove all bits of glass from the rubber cushion. A germicidal solution added between the tube and the trunnion cup not only disinfects the surfaces of both these, but also provides and excellent cushion against shocks that otherwise might break the tube.

— Use centrifuge trunnion cups with screw caps or equivalent.

— Avoid decanting centrifuge tubes; if you must do so, afterwards wipe off the outer rim with a disinfectant. Avoid filling the tube to the point that the rim ever becomes wet with culture.

— Wrap a lyophilized culture vial with disinfectant-wetted cotton before breaking. Wear gloves.

— Never leave a discarded tray of infected material unattended.

— Sterilize all contaminated discarded material.

— Periodically, clean out deep-freeze and dry-ice chests in which cultures are stored to remove broken ampoules or tubes. Use rubber gloves and respiratory protection durign the cleaning.

— Handle diagnostic serum specimens carrying a risk of infectious hepatitis with rubber gloves.

— Develop the habit of keeping your hands away from your mouth, nose, eyes and face. This may prevent self-inoculation.

— Avoid smoking, eating, and drinking in the laboratory.

— Make special precautionary arrangements for respiratory, oral, intranasal, and intratracheal inoculation of infectious material.

— Give preference to operating room gowns that fasten at the back.

— Evaluate the extent to which the hands may become contaminated with some agents and operations, forceps or rubber gloves are available.

— Wear only clean laboratory clothing in the dining room, library and other nonlaboratory areas.

— Shake broth cultures in a manner that avoids wetting the plug or cap.

AEROSOL MINIMIZATION

Because of their insidious nature, aerosole pose special problems in that the laboratory worker may be unwillingly exposed. procedures which can produce aerosols include:

— grinding

— blending

— sonicating
— resuspending packed cells or viruses
— inserting a hot loop into a culture
— centrifugation
— flaming an inoculation loop so that it splatters
— forceful ejection of fluid from a pipette or syringe
— opening a tube containing a lyophlized agent
— releasing the vaccum on a freeze dryer
— opening a tube within which the air pressure may differ from that of the room such as may occur when the tube is opened at a temperature different from that which it was sealed.

APPENDIX-2

Summary of recommended biosafety levels for infectious agents

Bio-safety Level	Practice and Techniques	Safety	Facilities
1.	Standard microbiological practices.	None : 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 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.	Maximum Containment

APPENDIX-3

Glasshouse Containment Conditions for Plant Experiments

1. **Glasshouse Containment A** are:

   (i) Plants should be grown in a designated glasshouse or compartment, clearly marked with a bio-hazard sign indicating "glasshouse containment A".

   (ii) Any other plants grown in the designated glasshouse or compartment must be handled under conditions appropriate for the experimental plants.

   (iii) Plants should be managed by suitably trained personnel with the principles of good glasshouse hygiene.

   (iv) The IBSC should consider whether any additional factors such as pest control, screening to prevent ingress by vermin, birds and insects and destruction of surplus plants and seed are relevant to the particular experiment.

2. **Glasshouse Containment B** conditions will be specified by the committee (RCGM) and will vary with the pathogen, being particularly dependant on its mode of dispersal, host range and pathogenicity and they are to be worked out on case by case basis.

Special conditions may be needed in addition to those given under "A" to prevent dissemination of the genetically manipulated plant pathogen especially during transfer between glasshouse and laboratory, during disposal of plants and equipment and through survival of pollen, seeds or other biological vectors.

   (a) Need for negative pressure and air filtration double doors etc. in cases where airborne dispersal is a potential hazard.

   (b) Need for effluent treatment plant where water borne dispersal is a hazard.

   (c) Need for suitable construction of glasshouse (floors, dwarfwalls, threshold at door, etc.) in cases wehre waterborne or soilborne dispersal are potential hazard.

(d) Need to prevent pollination and seeding, or to contain pollen and seed in cases where pollen and seed-borne dispersal is a potential hazard.

(e) Need for measures either to prevent contamination of, or to decontaminate the clothing of personnel or tools, pots, equipment etc., where mechanical transmissions is an above average hazard.

(f) Need to limit the growing of host plants in the vicinity of the containment facility and to provide monitoring for escape.

Inspection of a "Glasshouse Containment B" facility by IBSC will be required before approval.

APPENDIX-4

Examples of Containment Approaches for large scale industrial applications other than GLSP (Good Industrial Large Scale Practice)

	Specifications	Containment Categories 1	2	3
1.	Viable organisms should be handled in a system which physically separates the process from the environment (closed system)	Yes	Yes	Yes
2.	Exhaust gases from the closed system should be treated so as to	Minimise release	Prevent release	Prevent release
3.	Sample collection, addition of materials to a closed system and transfer of viable organisms to another closed system, should be performed so as to	Minimise release	Prevent release	Prevent release
4.	Bulk culture fluids should not be removed from the closed system unless the viable organism have been	Inactivated by validated means	Inactivated by validated means	Inactivated by validated means
5.	Seals should be designed so as to	Minimise release	Prevent release	Prevent release
6.	Closed system should be located within a controlled area	Optional	Optional	Yes, and purpose built
	(a) Biohazard signs should be posted	Optional	Optional	Yes
	(b) Access should be restricted to nominated personnel only	Optional	Yes	Yes, via an airlock

(c)	Personnel should wear protective clothing	Yes	Yes	A complete change
(d)	Decontamination and washing facilities should be provided for personnel	Yes	Yes	Yes
(e)	Personnel should shower before leaving the controlled area	No	Optional	Yes
(f)	Effluent from sinks and showers should be collected and inactivated	No	Optional	Yes
(g)	The controlled area should be adequately ventilated to minimise air contamination	Optional	Optional	Yes
(h)	The controlled area should be maintained at an air pressure negative to atmosphere	No	Optional	Yes
(i)	Input air and extract air to the controlled area should be HEPA filtered	No	Optional	Yes
(j)	The controlled area should be designed to contain spillage of the entire contents of the closed system	No	Optional	Yes
(k)	The controlled area should be sealable to permit fumigation	No	Optional	Yes
(l)	Effluent treatment before final discharge	Inactivated by validated means	Inactivated by validated chemical or physical means	Inactivated by validated chemical or physical means

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