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RECOMBINANT DNA SAFETY GUIDELINES AND REGULATIONS

DEPARTMENT OF BIOTECHNOLOGY
MINISTRY OF SCIENCE AND TECHNOLOGY
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1. INTRODUCTION

The new capabilities to manipulate the genetic material present tremendous potential and find use in many novel experiments and applications. 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 possible means to regulate the work involving pathogenic microorganisms and virulent genes. With the safety considerations in view, the Department of Biotechnology is mandated to evolve the Recombinant DNA Safety Guidelines. The Department has set up the Recombinant DNA Advisory Committee (RDAC) for this purpose. On the basis of current scientific information a document on Recombinant DNA Safety Guidelines has been brought out on the use of this technique in the area of research, manufacture and applications. The booklet brings forth the salient features in the document as a guide towards observance of the guidelines in research and also to meet the regulatory requirements by those in production, testing and use of genetically modified organisms and products.

2. RESEARCH

The guidelines cover areas of research involving

i) Genetically engineered organisms,
ii) Genetic transformation of green plants, animals,
iii) rDNA technology in vaccine development and
iv) Large scale production and deliberate/accidental release of organisms, plants, animals & products derived by rDNA technology.

The guidelines prescribe 4 levels of risk, while carrying out experiments with micro-organisms. Classification of organisms within these levels is based on pathogenicity, local prevalence of disease and epidemic causing strains in India.

3. NOTIFICATION

Related to the incremental risk involved in the use of microorganisms, plants, animals in experiments, the corresponding biosafety practices are required. Accordingly the notification procedures are defined under 3 categories.

i) Exempt category—(self cloning experiments)
ii) Intimation to competent authority—(e.g. experiments involving nonpathogenic DNA vector systems)
iii) Review and approval of competent authority—(e.g. Toxin gene cloning, antibiotic resistant genes, etc.)

4. CONTAINMENT

Containment facilities are recommended for necessary safeguards while carrying out experiments involving organisms belonging to 4 different biosafety levels.

a) PHYSICAL CONTAINMENT

Physical containment is to limit the spread of dangerous microorganisms by way of

i) Technique (Good Laboratory Practice)
ii) Safety equipments
iii) Laboratory design and facilities

b) BIOLOGICAL CONTAINMENT

Biological containment involves the use of the combination of vector and host in such a way so that it

i) can limit the infectivity of vector to specific hosts and
ii) control host vector survival in the environment

The growth of whole plants will however require special environmental conditions which may be achieved by using glasshouse containment.

Glasshouse containment A is appropriate to plant experiments involving no plant pathogen and would be suitable for experiments involving non-pathogen DNA vector systems and regeneration from single cells. (Notification to competent authority is needed).

Glasshouse containment B is recommended for experiments involving i) genetically manipulated plant pathogens including plant viruses such as the propagation of genetically manipulated organisms in plant and ii) the growth of plants regenerated from cells transformed by genetically manipulated pathogen vector systems which still contain the pathogen. (Approval of competent authority is required before commencement of activity).

5. RECOGNITION OF FACILITY

Application for recognition of research facility to carry out genetic manipulation should be made to the Department of Environment before the commencement of work in the prescribed proforma as per 7(2) of the rules on hazardous microorganisms/genetically engineered organisms notified under the EPA 1986.

6. COMPETENT AUTHORITY

a) INSTITUTIONAL BIOSAFETY COMMITTEE (IBSC) (IN R&D CENTRES, UNIVERSITY ETC.)

i) The IBSC shall be the nodal point for interaction within the institution for implementation of the guidelines. As such, in the first instance it is necessary that the institutions intending to carry out research activities involving genetic manipulation of

microorganisms, plants or animals should constitute the IBSC. Any research project which is likely to have biohazard potential (as envisaged in the guidelines) during the execution stage or which involve the production of either 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 allow genetic engineering activity on classified organisms only at places where such work should be performed as per guidelines. The prescribed form-Notification of intention to carry on genetic manipulation—Part B should be submitted to funding agency with comments of IBSC for financial support on experiments falling under categories II, III & IV. Support on approved projects will be withdrawn in case of deliberate violation or avoidable negligence of rDNA guidelines. In addition, it may attract action under EPA.

ii) IBSC will provide half yearly reports on the on-going projects to RCGM.

iii) Authorisation for inter-state exchange of etiologic agents, diagnostic specimens and biological products will be done by IBSC on standard proforma evolved for this purpose.

iv) Manipulation of plants under containment would be performed under the regulatory clearance of IBSC. Development of organisms for agricultural and environmental applications should be conducted in a step-wise fashion, moving where appropriate from the laboratory to the growth chamber and green-house under containment conditions and good laboratory practice.

b) REVIEW COMMITTEE ON GENETIC MANIPULATION (RCGM)

The RCGM will have the function :

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 authorisation for import and receipt of etiologic agents and vectors, germ plasms, organelle, etc needed for experimental work/training and research. (Applications should be made in prescribed proforma).

7. LARGE SCALE EXPERIMENTS AND MANUFACTURE

Regulations of large scale production and field testing of engineered organisms and products including environmental release has been laid down under statutory provisions of Environmental Protection Act 1986.

Experiments beyond 20 litres capacity for research as well as industrial purposes are included in the category of large scale experimentation/operations. For such activities it is recommended that one should seek approval of the competent authority

(GEAC) on furnishing the relevant details in a prescribed format (To be issued by GEAC with Department of Environment).

The following safety criteria are to be complied :

i) The host organism should not be a pathogen, should have extended history of safe use and built in environmental limitations.

ii) The vector/insert should be well characterised and free from known harmful sequences, limited in size as much as possible (with insert less than 10 base pair or below 30,000 M.W.) should be poorly mobilisable, should not transfer any resistance markers. In cases, where the insert sequence exceeds the above limit, toxicity screening should be made. The constructs should have 3 markers for bioprocess monitoring.

iii) The genetically manipulated organism should not be a pathogen.

For large scale operations, measures such as proper engineering for containment, quality control, personnel protection, medical surveillance are recommended.

Offsite contingency plans in event of unanticipated effects of novel organisms/products on accidental release are to be worked out and appropriate control measures are to be developed in consultation with the competent authority (State and District level coordination committees of GEAC) to meet any exigency.

8. BIOLOGICALS PRODUCED BY rDNA TECHNOLOGY

The general regulations normally applicable for biologicals are relevant to the recombinant DNA products. The specific relevant aspects to a particular product should be discussed with the appropriate Govt. agency (Drugs controller) on a case by case basis.

i) A new licence for a product drug application whould 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.

ii) A recombinant DNA product demonstrated to be identical to normally occurring substance would not require toxicological and pharmacological data if the information is already available at the dose levels intended for specific use. Otherwise the data on the above would be necessary on such products.

9. RELEASE TO THE ENVIRONMENT & FIELD EXPERIMENTS

i) Depending on the type of organism handled & the assessment of potential risks involved appropriate containment facilities must be provided to ensure safety and to prevent unwanted release in the environment.

ii) It is important to evaluate rDNA modified organism for potential risk, prior to application in agriculture and environment. Prior to introduction of micro-organisms,

properties of the organisms, the possible interaction with other disease causing agents and the infected wild plant species should be evaluated. An independent review of potential risks should be conducted on a case by case basis to application.

iii) Pre-release tests of genetically engineered organisms in Agricultural applications should include elucidation of genetic markers, host range, requirements for vegatative growth, persistence and stability in small plots and experimental field trials for 2 year. Soil samples in experiments under controlled containment conditions should be tested for the absence of viable cells before disposal into the environment.

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

10. GENETIC ENGINEERING APPROVAL COMMITTEE (GEAC)

i) Testing of genetically altered organisms, transgenic animals, plant material tested against pathogens & products in the environment should follow regulatory guidelines seeking field use permit from GEAC in the prescribed proforma (to be evolved by GEAC).

ii) The large scale planned release of organisms into the environment both for environmental and agricultural applications should be done under licence. Applications to this effect should be made to GEAC on the prescribed proforma which will be examined on case by case basis (to be evolved by GEAC). The validity of approval is for a period of 4 years.

iii) Import for large scale use, export, manufacture, process, sell, use of any genetically engineered substances or cells including food stuffs and additives that contains rDNA products are subject to regulatory control by GEAC. Prior approval to this effect should be obtained on applications in the prescribed proforma (to be evolved by GEAC).

11. A copy of the Recombinant DNA Safety Guidelines and the rules under EPA 1986 for the manufacture, use, import and storage of hazardous micro-organisms/genetically engineered organisms or cells may be obtained on request.

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Good Laboratory Practice

- Never do direct mouth pipetting of infectious 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 stopper 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 an 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 during 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.

2. AEROSOL MINIMIZATION

Because of their insidious nature, aerosols 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 lyophilized 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.

TABLE 1* Summary of recommended biosafety levels for infectious agents

Bio-safety Level	Practice and Techniques	Safety	Facilities
1.	Standard micro-biological 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.

* Biosafety in Microbiological and Biomedical Labs. (US Deptt. of Health & Human Services) Ist. Ed., 1984

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	Inactived by validated means	Inactived by validated means	Inactived 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	Yes	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

Specifications		Containment Categories		
		1	2	3
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	Opional	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	Inacti-vated by val-idated means.	Inacti-vated by val-idated chemical or physical means.	Inacti-vated by val-idated chemical or physical means.

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 particulary 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 where waterborne or soilborne dispersal are potential hazards.

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.

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