



**CNPEM biosafety Manual**  
**For GMO risk I and risk II**

**v.1 – 26/02/2016**

## 1. Introduction

**Biosafety:** The biosafety guidelines are legally applied to processes involving genetically modified organisms (GMOs) and issues related to scientific research with embryonic stem cells, regarding to the Brazilian Law on Biosafety - n ° 11105 of March 24, 2005, regulated by decree No. 5591 of November 22, 2005.

The National Biosafety Technical Committee - CTNBio is a multidisciplinary collegiate committee, established by Law No. 11,105, of March 24, 2005, whose purpose is to provide technical advisory support and advice to the Federal Government in the formulation, updating and implementation of the National Biosafety rules, the establishment of technical safety standards and technical advice concerning to the protection of human health, living organisms and the environment, for activities involving the construction, testing, cultivation, manipulation, transportation, marketing , consumption, storage, release and disposal of GMOs and derivatives.

Internal committee of biosecurity – CIBio. As regulated by the 5591 decree of November 22, 2005, Art 61. The institution dedicated to education, scientific research, technological development and industrial production, that uses techniques for genetic engineering or perform research using GMOs and their derivatives should create an Internal Biosafety Committee - CIBio whose operating mechanisms is established by CTNBio. CIBio shall deliberate and provide technical advice on projects involving work with GMO organisms, may authorize the execution risk I projects. Projects involving risk II should be addressed to the CTNBio.

Law No. 11,105, of March 24, 2005: This law provides for safety standards and oversight mechanisms on the construction, cultivation, production, handling, transport, transfer, import, export, storage, research, marketing, consumption, release into the environment and disposal of genetically modified organisms - GMOs and derived products, with the guidelines stimulating scientific advances in biosafety and biotechnology, protection of life and human health, animal and plant, and observance of the precautionary principle for the protection of the environment.

Decree No. 5591 of November 22, 2005: This Decree regulates provisions of Law 11.105, of 24 March 2005 laying down safety standards and monitoring mechanisms on the construction, cultivation, production, handling, transportation, transfer, import, export, storage, research, marketing, consumption, release into the environment and disposal of genetically modified-GMOs and their bodies, with the guidelines stimulating scientific breakthrough in area of biosafety and biotechnology, protection of life and human health, animal and plant, and observance of the precautionary principle for the protection of the environment, and standards

for use by authorization of embryonic stem cells obtained from human embryos produced by in vitro fertilization and not used in their procedure for research and therapeutic purposes.

Principal Investigator (PI): The whole project involving the handling of GMOs or embryonic stem cells held in CNPEM should be planned and supervised by a responsible researcher appointed by the CTNBio as head coach. The principal investigator must submit the project to a previous analysis of CIBio, highlighting the risk class of the GMO involved, planning experimental methodologies, handling, transport and disposal where applicable. The projects involving GMOs may be initiated after approval of CIBio (risk I) or CTNBio (risk II). The head coach is responsible for compliance with biosafety standards in accordance with the recommendations of CIBio and Regulatory CTNBio Resolutions and shall ensure that the technical and support staff involved in activities with GMOs and derived products receive appropriate training in biosafety and are aware of the situations of potential risks of these activities and personal protection procedures and collective in the workplace.

specific manuals and procedures: In addition to this manual, each operating unit (national laboratories) should have its own biosafety manual, whenever necessary, as well as subunits (individual laboratories or facilities) should also have specific manuals, approved by CIBio. This manual should provide list of GMOs and classification of biosafety risks, emergency telephones, emergency plan for situations involving contamination with GMOs, specific procedures for handling, disposal, storage and transfer of GMOs model for incident report as Annex I.

## 2. Acronyms and technical definitions

The Normative No. 2 of 27 November 2006 provides for the classification of risks of GMOs and biosafety levels required to work in contention. We transcribe below some risk class definitions and biosafety levels.

### Risk rating

GMOs will be classified into four risk classes, adopting as criteria the pathogenic potential of the donor, recipient, transgene, its potential to cause infection, harms and disease. The RISK rate of the resulting GMO is evaluated considering its adverse effects on human and animal health, plants and the environment. For genes encoding products that are harmful to human and animal health, plants and the environment, the vector used should have limited ability to survive outside the containment environment. All genetically modified organism must have a marker to allow identification among a population of the same species.

The types of risks of GMOs will be defined as follows:

I - Risk Class 1 (low individual risk and low risk to the community): The GMO containing sequences of DNA / donor organism RNA and receiver that do not cause harm to human and animal health and adverse effects to plants and the environment ;

II - Risk class 2 (moderate individual risk and low risk to the community): The GMO containing sequences of DNA / donor organism RNA or receiver with a moderate injury risk to human and animal health, which has low risk of dissemination and to cause adverse effects to plants and the environment;

III - Risk Class 3 (high individual risk and moderate risk to the community): The GMO containing sequences of DNA / RNA donor or recipient organism with a high risk of harm to human and animal health, which has low or moderate risk dissemination and cause adverse effects to plants and the environment;

IV - Risk Class 4 (high individual risk and high risk to the community): The GMO containing sequences of DNA / RNA donor or recipient organism at high risk of injury to human and animal health, which has high risk of dissemination and to cause adverse effects to plants and the environment.

The resulting GMO risk class can not be less than the risk class of the recipient organism, except in cases where there is reduced virulence and pathogenicity of the GMO. The GMO containing sequences of DNA / RNA organisms or infectious agents devoid of potential expression in the proposed activities and projects will be classified in the same risk class of the recipient organism. The GMO containing sequences of DNA / RNA derived from higher risk class of organisms and the potential of expression may, at the discretion of CTNBio, be classified in risk class of the recipient organism, since admittedly not associated with toxicity or pathogenicity in activities and proposed projects.

#### Biosafety Level Rating

The biosafety level of activities and projects will be determined by the higher risk involved class GMOs. The activities and projects involving GMOs and derived products should be preceded by a detailed and careful analysis of all experimental conditions and should be used the biosafety level appropriate to the risk class of the GMO manipulated.

There are four Biosafety Levels, increasing in the higher degree of restraint and complexity of the level of protection, according to the risk class of the GMO.

I - Biosafety Level 1 (BSL-1): adequate to activities and projects that involve risk class OGM 1.

II - Biosafety Level 2 (BSL-2): adequate to activities and projects that involve risk class of GMOs 2.

III - Biosafety Level 3 (BSL-3): adequate to activities and projects that involve risk class of GMOs 3.

IV - Biosafety Level 4 (BSL-4): adequate to activities and projects that involve risk class of GMOs 4.

These biosafety levels are suitable for the manipulation of GMOs on a small scale, less than 10 liters. Activities and projects involving the cultivation of GMOs on a large scale must have supervision and additional containment measures.

The CNPEM has areas for experimental manipulation of GMO risk I and II risk, located at LNBio and CTBE. Each operating unit is responsible to prepare an appropriate biosafety manual for the work, GMOs and scales used, planning operational and emergency procedures, according to their characteristics. The biosecurity manuals for the development work in these units should be presented to the staff and students of these units in the course of their training, in addition, the operative units should keep copies of these manuals available in the lab.

Greenhouses and containment facilities for the development of projects with GM animals such as vivariums, aquaculture tank, aviary, barnyard and infection areas, among others, should follow guidelines recommended by CTNBio, and the principal investigator which is responsible for training and to keep biosafety standards. These areas must also have appropriate biosafety manual for conducting work with GMO, procedures and emergency measures.

Cibio suggest immunocompromised people must not access OGMs areas. An special care should be observed about using medicines like immunosuppressive drugs in laboratorial dependencies CNPEM, due to contact risks with microorganisms handled in the experiments.

### 3. Requirements and General biosafety rules

#### 3.1 Rules for NB-1

a) NB1 facilities don't need to be physically isolated from other facilities of the institution, and the activities and projects conducted usually bench, vivarium or greenhouse;

b) the technical and support team should have specific training in procedures performed on the premises and should be supervised by head coach;

c) the NB-1 facilities should be designed to allow easy cleaning and decontamination;

d) the surface of the tables and benches must be impermeable to water and resistant to acids, alkalis, organic solvents and moderate heat;

e) the spaces between the benches, cabinets and equipment should be sufficient to allow easy cleaning;

f) GMOs will be handled in areas labelled with the universal biohazard symbol, with access restricted to technical staff and support or authorized persons;

g) work surfaces must be decontaminated once a day or whenever contamination (see procedure in Annex II)

h) all liquid or solid waste contaminated must be decontaminated before disposal, as well as any material or equipment that has been in contact with the GMO;

i) It's mandatory using mechanical device for pipetting; Mouth pipetting isn't aloud.

j) foods should be stored in specific areas, outside the biosafety labeled premises. It's forbidden to eat, drink, smoking, wearing contact lens, takink medicines and applying cosmetics in biosafety labeled working areas;

k) hands should be washed whenever there has been manipulation of organisms containing DNA / recombinant RNA and before leaving the premises;

l) sinks for handwashing and individual and collective protective equipment should be used to minimize the risk of exposure to GMOs;

m) the admission of animals (unrelated to work )is prohibited;

n) extreme caution should be taken when handling needles, syringes and broken glass so as to avoid self-inoculating and production of aerosol during use and disposal. Needles should not be bent, broken, recapped or removed from the syringe after use. Needles, syringes and broken glass should be immediately placed in puncture-resistant container and autoclaved before disposal;

o) contaminated materials can only be removed from premises in leak-proof rigid containers;

p) An adequate routine program of insect and rodent control should be conducted. All areas that allow ventilation should have physical barriers to prevent the passage of insects and other animals;

q) A Biosafety Manual must be prepared according to the specificities of activities. All personnel should be instructed about the possible risks and the need to follow the specifications of each routine work, biosafety procedures and practices outlined in the Handbook;

r) It should be kept records of each activity or project developed with GMO and its derivatives;

s) All activities and projects with non-genetically modified organisms occurring simultaneously and in the same premises that are handling GMO handling must comply with the risk classification of the GMO;

t) GMOs and derived products should be discarded in order to avoid their use as food for animals or man, except in the case where this is the purpose of the experiment, or specifically authorized by CIBio or CTNBio;

### 3.2 Rules for NB-2

Apply the same NB-1 rules, complementing the rules listed below.

a) facilities and procedures required for NB-2 must meet the specifications set for NB-1 plus the need for an available autoclave inside, to allow decontamination of all the material before disposal, without the transit GMOs through corridors and other spaces not controlled;

b) For experimentation: always use biological safety cabinets (Class I or II);

c) Everyone who want enter a NB-2 facility should have authorization of the principal investigator or responsible for the area.

d) The NB-2 should have a notice indicating the level of risk, identifying the GMO and the name of the principal investigator, full address and different possibilities of its location or other person responsible and the CIBio contact, for emergencies;

e) The principal investigator must establish policies and procedures, providing comprehensive information to all persons working on the premises of the potential risk related to the activities and there conducted projects as well as on the specific requirements for sites in entrance where there is the presence of animal inoculation;

f) Everyone which enter NB-2 premises should use personal protective proper equipment such as lab coats, gloves, hats, masks, goggles, pro-foot protectors, among others, which must be removed before the person leaves the accredited facilities; The principal investigator can allow a different procedure for a special situation that personal protective equipments won't be used. In this situation all experiments and or procedures will be stopped and the facility will be closed.

g) A non-disposable personal protective equipment should be cleaned and stored out of the contaminated area and people should be trained for handling and proper guard;

h) All the requirements for entry an NB-2 facility, as personal protective equipments, must be indicated at the entrance door;

i) Work surfaces of safety cabinets and other containment equipment should always be decontaminated at the end of activities with GMOs;

j) If a risk 1 experiment is conducted concurrently in the NB-2 site, it should adopt NB-2 level of biosafety;

k) Technical and support staff must be vaccinated against infectious agents related to experiments conducted in BSL-2 facilities, if available; The principal investigator is responsible for this vaccine procedure.

l) periodic medical examinations for workers in facilities where GMOs with activities and projects are conducted can be requested by CTNBio, including laboratory clinical evaluation in accordance with the GMO involved, taking into account the security measures and appropriate prevention.

### 3. Emergencies

Given the particularities of GMOs and procedures, each operative subunit is responsible for the preparation of a biosafety manual must submit the list of organisms and biosafety risk classification, emergency telephones, emergency plan for situations involving contamination with GMOs, specific procedures for handling, disposal, storage and transfer of GMOs. A model for incident report is attached (annex I ). The principal investigator should submit the specific biosafety manual to be approved in advance by the CIBio CNPEM.

As a general rule in emergency procedures, the first rule is containment (isolation area to prevent spread), second is to look for help to do the decontamination of equipment and personnel and to communicate CNPEM of Emergency Personnel. CIBio should always be notified. Never do a procedure that you are not familiar. Call the emergency ( phone extension 686 – CNPEM emergency)

**Useful phone Numbers**

Biosecurity Coordinator: Marcio Chaim Bajgelman, (19) 3512-1104 (Extension 1104)

Email: [marcio.bajgelman@lnbio.cnpem.br](mailto:marcio.bajgelman@lnbio.cnpem.br)

Security / Emergency CNPEM: Extension 686

job security: Celso Rodrigo Lorenzo (Extension 3502)

Health service CNPEM: Extension 1201

Nearest hospital: Medical center, there is 1km away from CNPEM at Rua Edilberto Luiz Pereira da Silva, 150, Cidade Universitaria, Campinas-SP, phone (19) 3789-5300.

fire brigade military police SP: 193 or 190

Annex I

**Incident report involving contamination with GMOs**

1. Name:
2. Phone:
3. Email:
4. Principal Investigator:
5. Incident location:
6. Description of GMO:
7. GMO risk class: ( ) Class I ( ) Class II ( ) other
8. contamination type ( ) solid ( ) liquid ( ) spray / quantity:
9. There was contamination outside the biosafety area? Yes ( ) No ( )
10. If the contamination was outside the cabin, specify the location / equipment:
11. There was contamination of the operator or third parties? Yes ( ) No ( )
12. Brief description of the incident:
13. Measures taken (cite all the measures taken to decontaminate equipment, flow, clothes, floor, centrifuges or others who may have been contaminated)
14. In the case of personal contamination, what steps were taken?
15. The contamination was outside the containment area certified for trial? What action has been taken?
16. Other observations he considers important:

Campinas, \_\_\_\_\_ of \_\_\_\_\_ of \_\_\_\_\_,

\_\_\_\_\_

Name:

position:

Annex II

**Routine procedures for decontamination work surfaces NB-1 and NB-2**

The procedure for routine decontamination is explained in biosafety training. CIBio the CNPEM and each principal investigator is responsible for verifying the correct compliance with biosafety standards including procedures for routine and emergency decontamination of staff, students and trainees at CNPEM.

**BSL-1 labs**

In the BSL-1 laboratory working surfaces should be routinely decontaminated every day at the end of their use, or whenever there is contamination. We suggest decontaminate the work surface routinely before and after its use as protocols below.

Laboratory bench, stands - It should be routinely decontaminated with 70% ethanol, applying the ethanol on the bench and making drag movements from left to right or right to left, from top to bottom using a disposable paper towel . The paper towel should be discarded in the wastebasket with biohazard warning.

PCR cabin - When equipped with germicidal lamps, these lamps can be switched on 15 minutes before and 15 minutes after using. The working stand must be decontaminated as described above for lab benches, using 70% ethanol and towel paper, applying the ethanol on the stand and making drag movements from left to right or right to left, from top to the lower part. The paper towel should be discarded in the wastebasket with biohazard warning.

Laminar Flow - When equipped with germicidal lamps, these lamps can be switched on 15 minutes before and 15 minutes after using. The working stand must be decontaminated as described above, using 70% ethanol and towel paper, applying the ethanol on the stand and making drag movements from left to right or right to left, from top to the lower part. The paper towel should be discarded in the wastebasket with biohazard warning.

**BSL-2 laboratories**

In the BSL-2 laboratories, work surfaces should be routinely decontaminated before and after use. Routine procedures are the same as described above for BSL-1. In the case of spilling should follow specific procedures described in BSL-2 manual, including emergency procedures and incident reports.