INTRODUCTION

This guide is a welcome document for new personnel who undertake activity in the laboratories of the Centro Nacional de Biotecnología (CNB-CSIC). It provides a simple summary of the basic health and safety standards to be followed in the laboratory.

Complete detailed information on chemical and biological safety and radiological protection is provided in the CNB Laboratory Health and Safety Manual.

All CNB personnel are required to know and comply with the regulations indicated in this guide and in the more comprehensive manual. All personnel receive an updated copy of the guide. Both this guide and the manual can also be consulted on the CNB website, in the Biosafety Service section:


Both this guide and the manual are updated periodically based on the types of hazardous agents found in the CNB, and on regulatory changes as well as technical developments in radiological protection and biological and chemical safety.
Basic Guide to Health & Safety

2 GENERAL INFORMATION

It is very important that all CNB personnel know:

- **The location and use of safety devices**: emergency showers and eyebaths, alarm buttons, emergency telephones, fire extinguishers, etc.
- **General safety rules** for the handling of materials and equipment
- **The availability** of the online **CNB Laboratory Health & Safety Manual**
- **This guide**, the comprehensive manual, and other technical documents regarding health and safety are available in the **Biosafety Service section** of the **CNB website**


- **The functions of the Biosafety Service** are as follows:
  - Conducting biological, chemical and radiological **risk assessment**
  - Editing **radiological protection and biological and chemical safety standards**
  - Providing **training and information** on health and safety for **personnel exposed** to hazards
  - **Direct management and control** of the Radioactive Facility (IR; Lab 350) and the Biosafety Level 3 Laboratory (NCB3; Lab 150)
  - **Assuring compliance** with health and safety standards in the laboratories
  - Collaboration in **medical surveillance and dosimetry** of exposed personnel
  - **Response to incidents, accidents and emergency situations**
  - **Internal management of hazardous waste**: toxic, biological and radioactive

- **The Biosafety Committee** is comprised of a representative from each department, technical personnel responsible for those services that incorporate a biological containment area, and the head of the Biosafety Service. Its functions are:
  - **To conduct risk assessments** of biological agents and genetically modified organisms
  - **To evaluate** the suitability of the **biocontainment infrastructure** in CNB laboratories
  - **To report** the results of risk assessments and of all relevant biosafety considerations to the **CNB Director**

- **The functions of group leaders and heads of services** are:
  - Group leaders and heads of service are **directly responsible for their personnel** in all aspects of occupational risk prevention
  - **To collaborate with the Biosafety Service** in all aspects of health and safety in their laboratories, including accidents and emergency situations

HOW TO CONTACT THE BIOSAFETY SERVICE (Office 340):
- **CALL EXTENSION 4305 OR 4541**
- **CALL MOBILE PHONE EXTENSION** (internal calls) 63042
- **ADVISE RECEPTION TO NOTIFY THE BIOSAFETY STAFF**
LABORATORY SAFETY SIGNS

SUPERVISED AREA

- Radiation hazard
- Contamination hazard
- Radiation and contamination hazard

CONTROLLED AREA

- Radiation hazard
- Contamination hazard
- Radiation and contamination hazard

- Radioactive
- Low temperature
- High voltage
- General danger
- Laser radiation
- Strong magnetic field
- Non-ionizing radiation
- Biohazard
- Flammable materials
- Explosion risk
- Toxic
- Corrosive
- Oxidizing
- Irritant

- First aid
- Safety shower
- Eyebath
- Evacuation routes/exits

- Fire extinction materials
- Fire extinguisher
- Fire hose
- Fire alarm button
3 PERSONAL INCIDENTS AND ACCIDENTS

SERIOUS OR POTENTIALLY SERIOUS ACCIDENTS:

CALL 112 AND REQUEST IMMEDIATE MEDICAL ATTENTION

MINOR ACCIDENTS:

1. Provide FIRST AID (see procedures below)
2. NOTIFY the Biosafety Service IMMEDIATELY for advice. On holidays and outside working hours (9:00-17:00 h), notify by phone in the following order:
   1. Fernando Usera Mena (mobile phone): 687 542 369
   2. Emergencies, Biosafety Service (mobile phone): 628 415 776
3. MEDICAL ASSISTANCE:
   Students: student medical service / insurance contracted by the CNB / family physician
   CSIC employees: mutual accident insurance contracted by the CSIC
   Non-CSIC staff: mutual accident insurance provider contracted by the employer

IMPORTANT:
   CSIC mutual accident insurance: request the medical assistance form from the Human Resources Service
   CNB accident insurance: call 902 448844 and indicate policy number 0551480497991

---

EMERGENCY TELEPHONE NUMBERS

<table>
<thead>
<tr>
<th>SERIOUS OR POTENTIALLY SERIOUS ACCIDENTS</th>
<th>112</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosafety: Mon-Thu: 9-17 h; Fri: 9-14:30 h</td>
<td>Extensions:</td>
</tr>
<tr>
<td>Biosafety: outside working hours / holidays</td>
<td>1. Fernando Usera</td>
</tr>
<tr>
<td></td>
<td>2. Biosafety Service</td>
</tr>
<tr>
<td></td>
<td>4541 / 4305 / 63042 (internal)</td>
</tr>
<tr>
<td></td>
<td>687 542 369</td>
</tr>
<tr>
<td></td>
<td>628 415 776 / 63043 (internal)</td>
</tr>
<tr>
<td>Physical Security Service</td>
<td>Sócrates Gutiérrez</td>
</tr>
<tr>
<td></td>
<td>Reception</td>
</tr>
<tr>
<td></td>
<td>Ext. 4512</td>
</tr>
<tr>
<td></td>
<td>Ext. 4500</td>
</tr>
<tr>
<td>CSIC employees</td>
<td>FREMAP mutual accident insurance</td>
</tr>
<tr>
<td></td>
<td>900 61 00 61</td>
</tr>
<tr>
<td>External employees, students and visitors</td>
<td>Appropriate insurance provider (see above)</td>
</tr>
<tr>
<td>CSIC Occupational Risk Prevention Service</td>
<td>Health Surveillance Service</td>
</tr>
<tr>
<td></td>
<td>General Information</td>
</tr>
<tr>
<td></td>
<td>915 681 933/32</td>
</tr>
<tr>
<td></td>
<td>915 680 004</td>
</tr>
<tr>
<td>NATIONAL INSTITUTE FOR TOXICOLOGY 24-HOUR EMERGENCY TELEPHONE</td>
<td>915 620 420</td>
</tr>
</tbody>
</table>

---

FIRST AID PROTOCOL

⇒ Basic procedure: wash the affected area with running water
 - Cuts and punctures: use pressure to encourage the wound to bleed under running water for 5 minutes
 - Minor burns: cool the area under running water for 10 min; do not use creams or salves
 - Biological contaminants: wash and apply a cutaneous disinfectant (betadine, alcohol) and cover
 - Radioactive contaminants: use “Radiacwash” towelettes or the cutaneous decontaminant supplied by the Biosafety Service. If there is a wound, use disinfectants; do not use decontaminants for radioactivity.
⇒ Showers and eyebaths: for skin or eye contamination, remove clothing IMMEDIATELY and use the emergency showers and eyebaths in the corridors or in the laboratory sinks (extension building)
⇒ First aid kits: on each floor next to the lifts (main building), next to the service lifts (extension building), in biosecurity labs, the radioactivity installation, and Reception
⇒ Decontaminants, germicide, and change of clothing: these are found in the emergency cupboards in the corridors on all floors in the main building and the extension building
4 DECONTAMINATION OF SURFACES AND MATERIALS

Use the personal protective equipment and decontamination material in the emergency cupboards located in the corridors.

**Radiation decontaminants:** Every radiological unit must stock Radiacwash towelettes and Decon90 (corrosive to metals). The Biosafety Service will provide cutaneous decontaminants and Desox18 and Desup33 decontaminants, appropriate for metals and very effective.

**Biosafety Service contact numbers**

<table>
<thead>
<tr>
<th>Time</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00-17:00 h</td>
<td>Extensions: 4541 / 4305 / 63042 (internal)</td>
</tr>
<tr>
<td>Outside work hours</td>
<td>1. Fernando Usera 687 542 369</td>
</tr>
<tr>
<td></td>
<td>2. Biosafety Service 628 415 776 / 63043 (int.)</td>
</tr>
</tbody>
</table>

✓ **General decontamination procedure:**
- Small spills can be cleaned up with paper towels. If the spill is a very dangerous substance, notify the Biosafety Service to organize decontamination.
- If a large volume is spilled, isolate the area, restrict access, and notify the Biosafety Service.
- If a large volume of radioactive or chemical material is spilled, absorb it with vermiculite. Wash the contaminated surface with standard detergents or with Decon90 (for radioactivity) to eliminate the contamination completely.
- The spilled substance and all clean-up materials should be treated as hazardous waste.

✓ **Biological decontamination:**
- For small spills inside biosafety cabinets, spray with germicide and clean up with paper towels.
- If the spill occurs outside a containment system, spray it with Virkon (in the emergency cupboards) and allow it to act for 20 minutes. Clean up the spill using vermiculite or paper towels, and wash or mop the area again with Virkon.

✓ **Radioactive decontamination**
- Decontaminate by scrubbing from the edges to the centre of the contaminated area and dry with paper towels.
- **Monitor.** If contamination persists, repeat the process as often as necessary.
- The material can also be submerged in a container filled with decontaminating solution.
- **If the contamination cannot be completely eliminated, notify the Biosafety Service.**
- Any material that cannot be decontaminated should be stored as radioactive waste until it has decayed or is transferred to ENRESA (³H or ¹⁴C contamination).
5 MEDICAL AND DOSIMETRIC SURVEILLANCE

The Biosafety Service monitors all personnel working in the laboratories. This control facilitates risk assessment for each work place and is essential for correct management of specific medical surveillance and dosimetry, as well as for training in prevention and personal protection.

Current legislation on the prevention of occupational risks requires specific medical surveillance of all workers. To this end, the Occupational Risk Prevention Service will initially and periodically evaluate the specific risks associated with each work place. The Biosafety Service collaborates with the Occupational Risk Prevention Service in this assessment. Specific medical surveillance of personnel is carried out by the Health Surveillance Service of the Occupational Risk Prevention Service. For CSIC employees, the Biosafety Service informs the Health Surveillance Service of the specific risks to which each employee is subject. The Biosafety Service will collaborate similarly with the prevention services of companies or external entities within a framework to coordinate business activities.

VACCINATION AND PROPHYLAXIS

Where there is risk of exposure to biological agents for which vaccines or other prophylactic methods are available, the health surveillance service must make them available to exposed personnel, as well as written information on the advantages and disadvantages of treatment.

- **Tetanus vaccine**: laboratory personnel who regularly use cutting materials or sharps.
- **Hepatitis A and B vaccine**: personnel who work with human or animal biological material in vivo or in vitro
- **Other vaccines**: personnel who work with samples that contain pathogens for which there are appropriate prophylactic methods.

6 TRAINING AND INFORMATION

In accordance with Procedure 1000 of the Spanish General Government Administration, the CNB operates a programme that provides welcome and initial information as well as work place-specific theoretical training and information for all laboratory personnel. The Biosafety Service offers initial training in health and safety for new personnel, with periodic follow-ups. The Biosafety Service also trains all other internal or external personnel who might be affected directly or indirectly by hazardous agents in CNB laboratories. Each research line or support service is
responsible for initial and periodic practical training of its staff, in collaboration with the Biosafety Department.

In addition, the Occupational Risk Prevention Service conducts periodic general information and training programmes for all laboratory personnel and specific courses and seminars for certain work places. The Biosafety Service specifically labels and delimits all risk areas, posting safety standards and procedures where necessary in the laboratories.

Through the Biosafety Service, the CNB management informs exposed personnel, prevention delegates, and the Occupational Risk Prevention Service of any work-related incidents or accidents. At the request of the exposed personnel or their representatives, it will also report on the list of risk-exposed personnel and the result of risk assessments carried out by the Biosafety Committee or by the Biosafety Service. Through the Biosafety Service and the Risks Prevention Unit, the CNB management will likewise coordinate management of business activities with respect to external personnel affected by risk-associated activities carried out in the laboratories.

7 RISK-SENSITIVE PERSONNEL

✓ Especially risk-sensitive personnel are those more vulnerable to risks that arise from their work in the laboratory due to personal characteristics or physiological condition.

✓ Pregnant or lactating personnel who work in the laboratory are advised to communicate their status to the Biosafety Service and the Occupational Risk Prevention Service, to allow a specific risk assessment of their work places as well as health surveillance that considers their physiological condition.

✓ The procedure is similar for any especially risk-sensitive personnel, with specific evaluation of his/her condition.

✓ If risk assessment and/or health surveillance detect incompatibilities with certain risk agents, adaptation to or a change of work place will be carried out if deemed necessary.

✓ Pregnancy and lactation are incompatible with exposure to carcinogenic, mutagenic and reprotoxic agents, or to pathogenic biological agents of risk groups 2, 3 and 4.
8 BASIC RULES FOR PREVENTION AND PROTECTION IN THE LABORATORY

✓ Order and general cleanliness are to be maintained in the work place. Accumulation of materials should be avoided, especially in overhead storage and above all, those that are heavy and that accumulate dust (paper and cardboard).
✓ Bench areas and specific materials will be allocated for certain high-risk operations such as work with cytostatics, radioisotopes, high-voltage sources, etc.
✓ Smoking is prohibited in the CNB. Eating and drinking are prohibited in laboratories. No food or drinks are to be stored in the research area. Use the refrigerators and freezers located in the cafeteria or in seminar rooms. There is a designated shared-use refrigerator for storage of food and drink for celebrations. The key can be obtained in Reception.
✓ Lockers are available for street clothes and personal effects. To request a locker, contact General Services (extencion 4538).
✓ Do not wear short (skin-exposing) clothing or sandals. Contact lenses cannot be worn as they make decontamination impossible; use prescription eyeglasses.
✓ Labcoat, gown or scrubs are compulsory and are used exclusively in the research area. Do not wear labcoat, gown, scrubs or protective gloves in other areas; remove the labcoat or cover scrubs with a blue labcoat.
✓ Mouth-pipetting is prohibited.
✓ Use protective gloves to handle hazardous agents (basic gloves, or specific for cold, heat, sharp materials).
✓ Use safety glasses if there is risk of splashing hazardous liquids.
✓ Use an anti-particle mask if there is risk of inhaling solid particles or dangerous aerosols (weighing chemical compounds, biological aerosols).
✓ After any laboratory activity, wash your hands. Avoid handling hazardous materials if you have lesions or sores on your hands.
✓ Gloves are for manipulation of hazardous agents; do not touch other elements such as telephones, keyboards, lift/elevator buttons, etc.
✓ Avoid the use of sharp and cutting materials as much as possible.
✓ Contaminated glassware that is recyclable should be decontaminated and rinsed before delivery to the Washing and Sterilization Service. Broken or unusable laboratory glassware should be decontaminated and deposited in specific containers for glass.
✓ Bottles and flasks are transported with both hands, holding the container by its neck and the base. Hazardous materials are to be stored in hermetically sealed, resistant containers for transport outside the laboratory. Carts should be used for transport.
When transporting hazardous materials and waste, use the freight or the service lefts/elevators. **The use of public lifts is prohibited.**

### INOCULATION OR PUNCTURE ACCIDENTS
Needles and syringes should be disposable, and should be used only if necessary. After use, the needle should not be separated from the syringe. Do not re-cap the needle.

### PERSONAL PROTECTIVE EQUIPMENT (PPE)
Personal protective equipment and materials for waste management, containment, shielding and decontamination are available from the warehouse. Direct questions regarding use or acquisition of new PPE or equipment to the Biosafety Service.

### SPLASHES TO THE EYES, EMERGENCY SHOWERS AND EYEBATHS
Use protective eyewear (safety glasses) when handling liquids that can splash into the eyes. Each person should have their own glasses.

**Ocular contamination:** immediately decontaminate the eyes for 10 min using the eyebaths located in the corridors (main building) or in the laboratory sinks (extension building).

**Extensive body contamination:** Immediately remove clothing and use emergency showers (located in all corridors).

### PPE AND DECONTAMINATION MATERIALS FOR ACCIDENTS AND EMERGENCIES
The labelled emergency cabinets located in all corridors contain the PPE and materials for labelling, decontamination and waste collection, for exclusive use in accidents and emergencies. The protocols of action are inside the cabinet.

---

Labelled emergency cabinet with personal protective equipment and decontamination materials and media

Some PPE available at the CNB
9 SPECIFIC CHEMICAL SAFETY STANDARDS

PRELIMINARY INFORMATION ON CHEMICAL PRODUCT HAZARDS

- **Product label**: the hazard pictogram indicates the type of danger, the signal word summarizes the level of danger: "Danger" for more severe hazards and "Warning" for less severe hazards; the H (hazard) and P (precautionary) statements indicate the specific risks and the prevention and protection norms to be adopted (R and S statements in the old labelling system).

- **Safety Data Sheets (SDS)**: the most complete source of information on the preventive and protective measures to be adopted for each chemical product. These sheets are available in the Biosafety Service, or you can consult various databases on the internet. Links to some of these databases are indicated on the CNB website in the Biosafety Service section.

- **Biosafety Service**: Consult the Biosafety Service when in doubt about rules for handling a specific compound or management of its waste.

Chemical product labelling (Globally Harmonized System)

- **Product identifier**: chemical name of the substance
- **Signal word**: "Danger" or "Warning"
- **H statement**: hazard indications. Numeric code or complete text
- **P statement**: precautionary advice. Numeric code or complete text.

- **Physicochemical properties**: Purity, composition, etc.
- **Supplier identification**: Company name, address, and telephone
- **Hazard pictograms**

- ✓ Always use **protective gloves** when handling hazardous chemicals. If these products are liquid, also use **safety goggles**.

- ✓ Always handle **flammable, explosive and volatile chemicals that pose inhalation hazards** in the **fume hoods** (gas extraction cabinets). If these volatile products are **carcinogenic**, also use a **filter mask** (double barrier). Handle flammables and explosives away from any heat source.

- ✓ Use **gloves, anti-particle masks, and safety goggles** when weighing hazardous solids.
✓ Use trays to prevent the dispersion of liquid compounds.
✓ When using precision balances or the Gel Doc, follow the indications on the instructions card and always fill in the usage register.
✓ Gas cylinders must have taps in good condition, be anchored (by the Maintenance Service) and located far from heat sources.
✓ Non-original containers used to store chemical products must be airtight and breakage- and product-resistant. The label must indicate the name of the product and the type of associated hazard (flammable, explosive, corrosive, toxic, etc.).
✓ Safety cabinets for flammable materials: separate flammable and explosive products from products toxic by inhalation, and store them on different shelves.
✓ In the extension building, store acids and bases in the specific module located under the fume hoods. In the main building, store them in a plastic tray in a labelled module under the laboratory bench.
✓ Keep the inventory of compounds and chemicals in a low, closed cabinet. Do not store incompatible chemicals together.
✓ Minimize stock of any hazardous chemicals, especially highly flammable substances such as alcohols.
✓ Liquid nitrogen: use special gloves and face shield and work with caution with cold cryotubes (possible explosion/shattering). Do not store the containers in closed spaces (cold rooms). Follow the instructions in the cryopreservation room.
Based on the risk associated with biological agents and genetically modified organisms (GMO) and thus, the containment infrastructure needs for each type of laboratory, the following classification has been established in the CNB:

- **Biosafety Level 1 laboratories (NCB1):** basic research laboratories and most support service laboratories
- **Biosafety Level 2 laboratories (NCB2):** *In vitro* culture laboratories 180, 480, 10, 13.3 and 36; laboratories for work with bacteria and fungi: part of 211, 223, 280.2 and 20; part of the Confocal Microscopy Service; Animal Facility quarantine, barrier (SPF) and inoculated animals areas; greenhouse NCB2
- **Biosafety Level 3 laboratory (NCB3):** laboratory 150

**Specific regulations for NCB1 and NCB2 laboratories:**

- In NCB1 laboratories, no work is to be done with cell lines, tissues, fluids or blood of human origin or from other primates (human pathogen risk group 2). There may be exceptions for certain techniques (cytometry, microscopy, etc.), which must be processed by the Biosafety Committee.
- Access to NCB1 laboratories will be controlled by heads of laboratories or services, in collaboration with the Biosafety Service.
- Good microbiological practices will be followed in NCB1 labs, with monitoring for possible culture contamination, avoidance of aerosol formation as much as possible, and disinfection of work surfaces at least once a day.
- Access control in NCB2 laboratories: in shared-use laboratories, the access door will have a fingerprint and security card reader, and access will be controlled by the Biosafety Service. In laboratories exclusive to a single research line or service, the access door will at least have a security lock and access will be controlled by the head of the research line or service, in collaboration with the Biosafety Service.
- To access NCB2 areas, staff must be trained and proficient and have undergone specific health surveillance. There may be access restrictions for pregnant or lactating women, as well as for other workers especially sensitive to biological risk. These cases will be evaluated by the Occupational Risk Prevention Service.
- In NCB2 laboratories, the use of specific work clothes (green labcoats) is mandatory. Change labcoats in the access lock or, if there is none, inside the laboratory next to the access door. Washing and sterilization of labcoats (external laundry) is supervised by the Washing and Sterilization Service for shared-use laboratories, and by personnel of the research line or service in exclusive-use laboratories.
- In the Animal Facility, personnel must wear area-specific scrubs and shoe covers in the conventional and quarantine zones, and high protection overall, shoe protectors, gloves and mask in the barrier and inoculation zones.
- All potentially hazardous biological material must be manipulated in biosafety cabinets. Avoid using Bunsen burners and accumulation of materials in the interior of the cabinet so that laminar flow is not interrupted. For microbiology, microincinerators can be used for inoculating loops. Avoid covering the perforated strip on which the arms are supported so that frontal protection flow is not interrupted.
✓ Use **protective gloves** for infectious samples. When there is no biosafety cabinet for protection, use **safety glasses** when working with infectious liquids and/or an **anti-particle mask** if there is risk of infectious aerosol production.

✓ In NCB2 laboratories, all **work surfaces must be decontaminated** with a broad-spectrum germicide after each manipulation or an accidental spill.

AVOID THE USE OF SHARP OR CUTTING OBJECTS AS MUCH AS POSSIBLE.

RULES FOR USE OF NEEDLES AND SYRINGES:

- Use needles only when necessary
- Use the special safety needles available in the warehouse
- Never separate the needle from the syringe; dispose of the set in the sharps container
- Never recap or reuse the needle

Specific rules for the Animal Facility are indicated in the Health and Safety Manual in the laboratories and in the Animal Facility Standard Operating Procedures (SOP). All **new personnel** who will work in the Animal Facility will receive a **specific training course** on the handling of experimental animals.

The specific rules for the NCB3 laboratory are indicated in the Operating Regulations and Emergency Plan of the laboratory and in the NCB3 laboratory-specific SOP of the Biosafety Service.
11 SPECIFIC RADIATION PROTECTION STANDARDS

General standards:
✓ Use the radioactive materials logs found in Radioisotope Laboratory 350 and all radiological zones of the Radioactivity Installation (RI).
✓ Shielding: methacrylate or PVC for \( \beta \)-emitters except \( ^3 \)H, and leaded methacrylate or leaded glass for \( \gamma \)-emitters. Double containment: trays and plasticized bench-liner.
✓ Detection: Geiger-Müller monitors for \( \beta \)-emitters and solid scintillation monitors for \( \gamma \)-emitters. Monitor before, during, and after each operation. Any contamination must be eliminated immediately. For \( ^3 \)H, detection is by smear; contact the Biosafety Service for more information.
✓ Personal dosimeter: obligatory use except if only \( ^3 \)H is used
✓ Use screw-cap vials and tubes for radioactive samples
✓ Radioactive samples and waste must be stored with shielding and labelled.

Standards for the radiological areas of research laboratories:

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Activity</th>
<th>Isotope</th>
<th>Activity</th>
<th>Isotope</th>
<th>Activity</th>
<th>Isotope</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^3)H</td>
<td>5 mCi</td>
<td>(^35)S</td>
<td>2 mCi</td>
<td>(^32)P</td>
<td>1 mCi</td>
<td>(^131)I*</td>
<td>200 µCi</td>
</tr>
<tr>
<td>(^14)C</td>
<td>5 mCi</td>
<td>(^33)P</td>
<td>2 mCi</td>
<td>(^125)I*</td>
<td>200 µCi</td>
<td>(^51)Cr</td>
<td>200 µCi</td>
</tr>
</tbody>
</table>

Activity levels per assay

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Activity</th>
<th>Isotope</th>
<th>Activity</th>
<th>Isotope</th>
<th>Activity</th>
<th>Isotope</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^3)H</td>
<td>2 mCi</td>
<td>(^35)S</td>
<td>1 mCi</td>
<td>(^32)P</td>
<td>500 µCi</td>
<td>(^131)I*</td>
<td>100 µCi</td>
</tr>
<tr>
<td>(^14)C</td>
<td>2 mCi</td>
<td>(^33)P</td>
<td>1 mCi</td>
<td>(^125)I*</td>
<td>100 µCi</td>
<td>(^51)Cr</td>
<td>100 µCi</td>
</tr>
</tbody>
</table>

(*) \( ^{125} \)I and \( ^{131} \)I cannot be used unless they have been incorporated into non-volatile, chemically stable molecules

✓ Each area must have a supervisor who holds a Supervisor or Operator licence and collaborates with the Biosafety Service.
✓ Authorized areas will be marked, covered with plasticized bench-liner, and delimited. The material to be used for radiolabelling must be marked. Sufficient materials must be available for shielding, containment, detection and decontamination.
✓ Somewhat volatile radiolabeled compounds such as \( ^{35} \)S-methionine must be handled exclusively in fume hoods.

Standards for NCB2 laboratories nº 180 and 480
✓ The Biosafety Service is responsible for access to and use of these facilities. Activity limits per experiment are identical to those for basic laboratories.
✓ No vials, samples or radioactive waste can be stored in these laboratories. After labelling, transport these materials to the appropriate radiological area. Shielding, containment and detection material will be supplied by each radiological area.
✓ Record all data for labeling performed in tissue culture laboratories, including incubator and cabinet numbers, in the radioactive materials register of the laboratory of origin.

✓ **Laboratory 480** has an area for radioactive work with the harvester, with a maximum of 5 mCi for \(^3\)H. Users must fill out the specific radioactive materials register. The radioactive waste generated can be stored in the area.

### Standards for the central laboratory of the Radioactivity Installation

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^3)H</td>
<td>25 mCi</td>
</tr>
<tr>
<td>(^3)P</td>
<td>25 mCi</td>
</tr>
<tr>
<td>(^3)I*</td>
<td>10 mCi</td>
</tr>
<tr>
<td>(^3)Fe</td>
<td>20 mCi</td>
</tr>
<tr>
<td>(^3)S</td>
<td>25 mCi</td>
</tr>
</tbody>
</table>

✓ The **Biosafety Service controls access** by shared-use magnetic cards. Wear a labcoat and a dosimeter for access, even if no radioactive material will be used. Fill in the reservation form located at the entrance as well as the laboratory use register.

✓ The laboratory has the necessary materials for protection, containment, shielding, detection and decontamination. Follow the Biosafety Service instructions.

✓ Radioisotope fume hoods will be used for activity \((A) \geq 1\) mCi per assay, or when using volatile radioactive compounds such as \(^{125}\)I-Na. There are additional workstations for low-activity, non-volatile radioisotopes. Use the sink to wash contaminated material, never to eliminate radioactive waste.

✓ There is an emergency shower and eyewash, washbasin, and first aid kit at the laboratory entrance. To use, follow the Biosafety Service instructions.

### Irradiation of cultures and experimental animals

✓ The **Biosafety Service controls access** to the \(\gamma\)-irradiator at all times. Irradiation is carried out by the Biosafety Service. Users should contact the service one day in advance to schedule irradiation, and are responsible for transporting the samples to be irradiated.

### ACQUISITION AND INTERNAL MOVEMENT OF RADIOACTIVE MATERIAL

Request by external purchase order and present in the Biosafety Service before 14:00 h (2 p.m.).

❖ Orders are checked to ensure they do not exceed RI activity limits and are ordered from an authorized supplier. The RI Supervisor approves by signing, and the Purchasing Department processes the order.

❖ On receipt of the radioactive material in the warehouse, the Biosafety Service transports it to the central radioisotope laboratory, records the material in the central laboratory’s radioactive material entry/exit register, and signs for reception.

❖ When the material is collected, the user will be recorded in the register. They can then use the material in the central laboratory or in the laboratory radiological areas. In the latter case, they must record the entry of the material, its handling, and waste transfers in the radioactive material register in their area.

❖ Radioactive material can be moved internally between authorized radiological areas provided entries and exits are recorded in the appropriate registers.
12 TOXIC, BIOLOGICAL AND RADIOACTIVE WASTE HANDLING

✓ Hazardous waste cannot be disposed of in the same way as urban waste. These materials must be separated and packaged as indicated in the attached tables.

✓ Toxic and radioactive liquid waste cannot be poured down the drains. Biological liquid waste can be disposed of in the sink in NCB1 and NCB2 laboratories, provided it has been previously inactivated with a broad-spectrum germicide for at least 20 minutes (e.g., a 1/10 solution of commercial bleach).

✓ Before use, waste containers and bags must be identified by a label that indicates the specific type of waste.

✓ In the main building, full toxic liquid waste containers or those in use are stored in the module beneath the fume hoods, labelled with a skull. In the extension building, laboratories have a specific labelled module for this type of waste.

✓ Waste-producing laboratories must provide specific, approved 30-L or 60-L containers for biological and toxic solid waste, as well as covered bins lined with a bag for low-activity radioactive solid waste. Bins and containers should be kept out of high-traffic areas.

✓ Litter bins for low-activity radioactive waste must be marked on the lid with the specific label for the contaminating radioisotope. As an exception, waste contaminated with $^{35}$S and $^{32}$P can be mixed in the same bin; in this case, labels for both radioisotopes are used.

✓ Containers for biological and toxic material will be marked on a single side, which is visible. Containers for biological solid waste will have three identifications: the dangerous goods class, the waste category, and the BIOHAZARD label with the biosafety rosette.
✓ Intermediate containers for temporary storage of solid biological or toxic material (micropipette tips, vials, etc.) must be identified with a label that indicates the waste category, as on the final containers.

✓ Containers for high-activity radioactive waste generated in the laboratories will be stored labelled in the shielded modules bearing the radioactivity symbol, beneath the fume hoods. Maximum storage time is 1 month, provided the maximum permitted activity level for the laboratory is not exceeded.

✓ Cutting objects and sharps waste. These can be classified as biological: biological risk, yellow sharps container with red cap; as cytotoxic: chemical risk, blue sharps container; or as innocuous: no risk, use a marked plastic container and eliminate the closed container as conventional waste.


✓ NCB2 laboratories. If viruses or other pathogens are used, in addition to the above, the biological waste must be inactivated when it is generated, inside the biosafety cabinets, by mixing with or immersion in a germicide solution.

✓ Waste produced in the Animal Facility and the NCB2 and NCB3 laboratories: follow the procedures indicated in the CNB Laboratory Health and Safety Manual and in the NCB3 Laboratory Internal Regulations. The tables below summarize the separation and handling of this waste.

✓ Waste with several hazards (for example, radioactive and toxic waste): contact the Biosafety Service to determine the most appropriate handling method.

✓ The necessary material for waste handling is available in the central warehouse: containers, bags, labels, closure ties, etc.

WASTE TRANSFER

✓ Before delivery to the Biosafety Service, users must seal approved biological and toxic waste containers, close radioactive waste bags with zipper seals, and completely close bottles and drums.

✓ Radioactive waste: the transfer takes place from 09:00-14:00 h (9 a.m. to 2 p.m.) in the central radioisotope laboratory (Lab 350). Users must notify the Biosafety Service in advance to request the necessary means of transport (shielded carts).

✓ Biological and toxic waste: sealed containers will be left next to the laboratory door on the day established for removal by the waste management company.

✓ The Biosafety Service will inspect the waste delivered or deposited and will reject that which does not comply with separation, packaging, labelling and closure guidelines.
Basic Guide to Health & Safety

SPECIAL WASTE HANDLING IN THE CNB

SOLID TOXIC WASTE

UN3077

Contaminated solid material
- Mixtures of contaminated solid materials and empty plastic containers

SOLID: NON-CUTTING, NON-SHARP

UN3249

SOLID: CUTTING MATERIALS, SHARPS

Although toxic, this waste is treated as cytotoxic

EMPTY GLASS BOTTLES CONTAMINATED WITH TOXIC MATERIAL

UN1950

LABORATORY REAGENTS

UN3287

Laboratory reagents
- Expired, unused, or unknown chemical products, both solid and liquid
- Dispose of in the original container

LIQUID TOXIC WASTE

UN2929

Non-halogenated organic solvents, halogenated solvents, acids, bases, heavy metals, oil (motor oil), photographic developer and fixer

UN1993

For gels ONLY
- Ethidium bromide: Halogenated solvent label
- Other DNA intercalators: Non-halogenated solvent label

SOLID BIOLOGICAL WASTE

SOLID BIOLOGICAL WASTE: NON-CUTTING, NON-SHARP

SOLID BIOLOGICAL WASTE: CUTTING MATERIALS, SHARPS

UN3291

Special biological waste class III

BIOSAFETY SERVICE

NOVEMBER 2017
<table>
<thead>
<tr>
<th>LABEL</th>
<th>DESCRIPTION</th>
<th>EXAMPLES</th>
<th>CONTAINERS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NON-HALOGENATED SOLVENTS</strong>&lt;br/&gt;<strong>DISOLVENTES NO HALOGENADOS</strong></td>
<td>Carbon compounds with no halogen elements</td>
<td>Ethernet, butanol, phenol, formaldehyde, toluene, xylol, xylene, acetone, propanol, methanol, dyes, acetic acid-methanol mixtures, acetonitrile-water mixtures, methanol-water mixtures, etc.</td>
<td>Preferably, 2-L bottles 5-L jerrycans 10-L jerrycans</td>
</tr>
<tr>
<td><strong>HALOGENATED SOLVENTS</strong>&lt;br/&gt;<strong>DISOLVENTES HALOGENADOS</strong></td>
<td>Carbon compounds or mixtures that contain halogen elements (more than 1%)</td>
<td>Chloroform, phenol-chloroform, dichloromethane (methylene chloride), tetrachloroethylene, dichloroethane, carbon tetrachloride, ethidium bromide, propidium iodide</td>
<td></td>
</tr>
<tr>
<td><strong>ACIDS</strong>&lt;br/&gt;<strong>ÁCIDOS</strong></td>
<td>Aqueous acid solutions</td>
<td>Hydrochloric, nitric, sulphuric acids, etc.</td>
<td></td>
</tr>
<tr>
<td><strong>BASES</strong>&lt;br/&gt;<strong>BASES</strong></td>
<td>Aqueous base solutions</td>
<td>Sodium hydroxide, potassium hydroxide, etc.</td>
<td></td>
</tr>
<tr>
<td><strong>HEAVY METALS</strong>&lt;br/&gt;<strong>METALES PESADOS</strong></td>
<td>Solutions that contain heavy metals</td>
<td>Solutions with silver, mercury, chrome, cadmium, arsenic, copper, zinc, etc.</td>
<td></td>
</tr>
<tr>
<td><strong>INDUSTRIAL OILS</strong>&lt;br/&gt;<strong>ACEITES INDUSTRIALES</strong></td>
<td>Mineral oil waste</td>
<td>Pump and motor oils</td>
<td></td>
</tr>
<tr>
<td><strong>HALOGENATED SOLVENTS</strong>&lt;br/&gt;<strong>DISOLVENTES HALOGENADOS</strong></td>
<td>Gel with ethidium bromide</td>
<td>--</td>
<td>5-L canisters</td>
</tr>
<tr>
<td><strong>NON-HALOGENATED SOLVENTS</strong>&lt;br/&gt;<strong>DISOLVENTES NO HALOGENADOS</strong></td>
<td>Gel with other DNA intercalators</td>
<td>Gelred, Gelgreen, Sybrid Safe, Total Red, etc.</td>
<td></td>
</tr>
<tr>
<td><strong>LABORATORY REAGENTS</strong>&lt;br/&gt;<strong>REACTIVOS DE LABORATORIO</strong></td>
<td>Containers or bottles with unknown liquids Liquid chemical products past expiry date or unused, in their original containers</td>
<td>--</td>
<td>Dispose of in original containers</td>
</tr>
<tr>
<td><strong>DEVELOPER</strong>&lt;br/&gt;<strong>REVELADOR</strong></td>
<td>Photographic developer</td>
<td>--</td>
<td>25-L jerrycans</td>
</tr>
<tr>
<td><strong>FIXER</strong>&lt;br/&gt;<strong>FIJADOR</strong></td>
<td>Photographic fixer</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

Mixtures of halogenated and non-halogenated: label as **halogenated solvent**
Mixtures of halogenated, non-halogenated and heavy metals: label as **heavy metals**
<table>
<thead>
<tr>
<th>LABEL</th>
<th>DESCRIPTION</th>
<th>EXAMPLES</th>
<th>CONTAINERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTAMINATED SOLID MATERIAL</td>
<td>Mixture of solid materials contaminated with chemical products in all previously described groups Empty plastic chemical product containers</td>
<td>Paper, gloves, disposable plastic material (tips, flasks), absorbents, etc.</td>
<td>60-L container</td>
</tr>
<tr>
<td>CYTOTOXIC</td>
<td>Blades and sharps contaminated with chemical products</td>
<td>Syringes with needle, scalpel blades, glass Pasteur pipettes, slides, etc., contaminated with chemical products (cytotoxic or not)</td>
<td>10-L blue sharps container</td>
</tr>
<tr>
<td>LABORATORY REAGENTS</td>
<td>Containers or bottles with unknown solid contents Expired or unused chemical products in their original containers</td>
<td>--</td>
<td>Dispose of in original containers</td>
</tr>
<tr>
<td>AUTHORADIOGRAPHY FILM</td>
<td>Photographic film</td>
<td>--</td>
<td>30-L blue bin with clamp closure. Located in darkrooms</td>
</tr>
<tr>
<td>CONTAMINATED SOLID MATERIAL (GLASS)</td>
<td>Empty glass bottles contaminated with chemical products</td>
<td>--</td>
<td>60-L containers. Located in common corridors</td>
</tr>
</tbody>
</table>

Sharps contaminated with biological material or toxic chemicals: **container and label for cytotoxics**
## SOLID BIOLOGICAL WASTE

<table>
<thead>
<tr>
<th>LABEL</th>
<th>DESCRIPTION</th>
<th>PACKAGING/INACTIVATION</th>
<th>DISPOSAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS III SPECIAL BIOLOGICAL WASTE</td>
<td>Not cutting or sharp: Gloves, tips, pipettes, vials, flasks, etc.</td>
<td>NCB1 LABORATORIES Approved 30-L or 60-L container</td>
<td>When full, seal the container and place it outside the laboratory door on pick-up day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCB2 LABORATORIES Approved 60-L container</td>
<td>Remove and autoclave the container without the lid (lids should not be autoclaved, as it damages the seal). In common-use NCB2 laboratories, the Washing and Sterilization Service is responsible for sterilizing and delivering these containers.</td>
</tr>
<tr>
<td></td>
<td>Cutting and sharps: Syringes with needles, scalpel blades, Pasteur pipettes, glass slides, etc.</td>
<td>NCB1 LABORATORIES Approved 3-L, 5-L and 10-L yellow containers</td>
<td>When full, seal the container and place it outside the laboratory door on pick-up day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCB2 LABORATORIES Approved 3-L, 5-L and 10-L yellow containers</td>
<td>Seal and autoclave. In common-use NCB2 laboratories, the Washing and Sterilization Service is responsible for handling this waste.</td>
</tr>
</tbody>
</table>

## LIQUID BIOLOGICAL WASTE

<table>
<thead>
<tr>
<th>LABEL</th>
<th>DESCRIPTION</th>
<th>PACKAGING/INACTIVATION</th>
<th>DISPOSAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO STANDARD LABEL</td>
<td>Liquid culture media Small volumes of blood and other body fluids, human or animal</td>
<td>NCB1 LABORATORIES 2-L brown plastic bottles containing germicide Culture flasks and bottles with added germicide</td>
<td>After 30 min with germicide, pour down the drain while diluting with abundant running water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LEVEL 2 CONTAINMENT TISSUE CULTURE LABORATORIES Collect in vacuum trap that contains germicide</td>
<td>Add more germicide, wait 30 min, and pour down the drain while diluting with abundant running water. In common-use NCB2 laboratories, the Washing and Sterilization Service is responsible for maintaining vacuum traps and eliminating waste</td>
</tr>
<tr>
<td>DESCRIPTION</td>
<td>LABEL</td>
<td>PACKAGING/INACTIVATION</td>
<td>DISPOSAL</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Bedding and excrements</td>
<td>CLASS II BIOLOGICAL WASTE INCORPORABLE INTO URBAN WASTE NO STANDARD LABEL</td>
<td>Load into 300-gauge green bags</td>
<td>Transfer to an authorized handler for biological waste to be incorporated into urban waste</td>
</tr>
<tr>
<td>CLASS III BIOLOGICAL WASTE</td>
<td>ANIMAL FACILITY: NCB2 INOCULATED AND QUARANTINE AREAS</td>
<td>Approved 60-L container</td>
<td>Removed, autoclaved and packaged in 60-L black containers by the Animal Facility</td>
</tr>
<tr>
<td>Animal cadavers</td>
<td>NO STANDARD LABEL</td>
<td>Freezer bags</td>
<td>Freeze at -20°C and transfer to an authorized handler for animal cadavers</td>
</tr>
<tr>
<td>CLASS III BIOLOGICAL WASTE</td>
<td>ANIMAL FACILITY: NCB2 INOCULATED AND QUARANTINE AREAS</td>
<td>Special bins with autoclave bags</td>
<td>Removed, autoclaved and packaged in 60-L black containers by the Animal Facility</td>
</tr>
<tr>
<td>Syringes with needles, scalpel blades, Pasteur pipettes, glass slides, etc.</td>
<td>CLASS III BIOLOGICAL WASTE SHARPS</td>
<td>Approved 3-L, 5-L, and 10-L yellow containers</td>
<td>When full, seal containers and place them near the Animal Facility door on pick-up day</td>
</tr>
<tr>
<td></td>
<td>ANIMAL FACILITY: NCB2 INOCULATED AND QUARANTINE AREAS</td>
<td>Approved 3-L, 5-L, and 10-L yellow containers</td>
<td>Autoclaved and sealed by the Animal Facility. Place near the Animal Facility door on pick-up day.</td>
</tr>
</tbody>
</table>
# SEPARATION OF RADIOACTIVE WASTE: $T_{1/2} <100$ DAYS

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>DESCRIPTION</th>
<th>CONTAINER</th>
<th>SHIELDING</th>
<th>STORAGE/TRANSFER</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOLIDS</td>
<td>Empty commercial vials ($^{32}$P, $^{125}$I)</td>
<td>Leaded commercial container</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>High-activity rigid solids (tips, plates, tubes, etc.). Cutting material and sharps (syringes + needles, scalpel blades, slides, Pasteur pipettes, etc.).</td>
<td>2-L white polypropylene bottle</td>
<td>• Methacrylate box  • $^{32}$P: Methacrylate box inside a 1.5-mm lead box</td>
<td>• Lead box  • Leaded methacrylate container</td>
</tr>
<tr>
<td></td>
<td>Low-activity soft solids (paper, gloves, plastic, etc.)</td>
<td>25-L transparent polypropylene bag and closure ties</td>
<td>Foot-pedal waste bin</td>
<td>Leaded foot-pedal waste bin</td>
</tr>
<tr>
<td>LIQUIDS</td>
<td>High- and low-activity</td>
<td>2-L white polypropylene bottle</td>
<td>• Methacrylate box  • $^{32}$P: Methacrylate box inside a 1.5-mm lead box</td>
<td>• Lead box  • Leaded methacrylate container</td>
</tr>
</tbody>
</table>
## Separation of Radioactive Waste: $T_{1/2} > 100$ días

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Container</th>
<th>Shielding Low-Energy Beta and Gamma</th>
<th>Gamma</th>
<th>Storage/Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solids</strong></td>
<td>Cutting materials and sharps (syringes + needle, scalpel blades, slides, Pasteur pipettes, etc.)</td>
<td>NEEDLES, BLADES, AND METAL MATERIALS Specific sharps container from ENRESA</td>
<td>$^3$H, $^{56}$Fe: No shielding $^{14}$C, $^{45}$Ca: Methacrylate container</td>
<td>$^{22}$Na: Lead box</td>
<td>Deliver separately to the Biosafety Service</td>
</tr>
<tr>
<td></td>
<td>Rigid solids (tips, pipettes, plates, tubes, etc.)</td>
<td>2-L polypropylene bottles 25-L transparent polypropylene bags in cardboard boxes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soft solids (paper, gloves, plastic, etc.)</td>
<td>Directly in 25-L transparent polypropylene bags</td>
<td>Covered foot-pedal waste bin</td>
<td>Leded foot-pedal waste bin</td>
<td>Temporary storage in radioactive waste modules located beneath fume hoods, labelled with the radioactivity symbol</td>
</tr>
<tr>
<td><strong>Liquids</strong></td>
<td>High-activity</td>
<td>2-L polypropylene bottle</td>
<td>$^3$H, $^{56}$Fe: No shielding $^{14}$C, $^{45}$Ca: Methacrylate container</td>
<td>$^{22}$Na: Lead box</td>
<td>Transfer to the Biosafety Service</td>
</tr>
<tr>
<td></td>
<td>Low-activity, large volume</td>
<td>5-L polypropylene container</td>
<td>Radioactive waste modules beneath fume hoods, labelled with the radioactivity symbol</td>
<td>Radioactive waste modules</td>
<td></td>
</tr>
<tr>
<td><strong>Mixed</strong></td>
<td>Closed vials that contain sample and scintillation cocktail</td>
<td>2-L polypropylene bottle 5-L wide-mouth container</td>
<td>Not normally</td>
<td>Not normally</td>
<td></td>
</tr>
</tbody>
</table>

Never mix organic solvents with aqueous solutions. If a technique requires it, inform the Biosafety Service.