Safety Manual

Department of Biological Sciences

Table of Contents

Introduction to safety manual

This manual constitutes the main guidelines for laboratory work and safety at Department of Biological Sciences – Indian Institute of Science Education and Research, Kolkata. All new employees and students, as well as guest researchers and others that will work in labs at the department, are requested to read and understand this manual.

Personal safety of all the members is of utmost importance at IISERK and to this end a safety committee was constituted. The safety committee comprises of a group of individuals who strive hard to make the lab a safe place to work. The objectives of the safety committee can be summarized as "Instruct, Equip and Emergency response (IEE)".

Instruct

The potential hazards and safety practices to be followed by everyone. So, our safety committee undertakes the following actions:

- Safety Committee instructs students and new members of IISERK, on formal overview on various safety hazards and lab safety measures.
- We conduct mandatory refreshing seminars to safe guard the users and make them clearly aware of hazards
- Inclusive policy on introducing new resources will be updated.

Equip

Responsibility emergencies would need adequate safety equipment and apparels in place. To that end safety committee takes stock of the various safety requirements and plugs in the various breaches. So that the major duty has also been on gas, fire and chemical safety.

- Smoke detector system with alarms and auto shut off valves in case of a gas leak has been put in place.
- Fire extinguishers and fire alarms have been installed and will be monitored at periodic intervals.
- Chemical spill kits, chemical resistant gowns, shoes, goggles, first aid kits and eye wash and body shower along with a policy for usage are at designated place.

Emergency Response

It is important to know, how to respond to emergencies at the times. Safety committee manuals will help one to understand the precautions required.

Remember, **you are responsible for your own safety**, **and that of others around you**. IISERK provides you with information, recommendations and necessary resources for you to be able to do your work safely. It is up to you to ensure that you take appropriate precautions for your safety and your fellow lab members.

After reading this manual student will have to sign the declaration to confirm this. The manual will be updated regularly to include new recommendations and apply to the current rules for laboratory work. The updated sections will be highlighted in order to be recognized easily.

Emergency Contacts

S.No	Faculty in charge / Safety committee member	Faculty/ Officer/ Member	Email	Phone number
1	Ambulance	Mr.Jainal Mondal Mr. Debu Halder		9836249346 8145549528
2	Fire department local, Nadia	Dr. Rupak Datta		033-25828101 (Kalyani F.S.) 8584027304 8584027305
3	Nursing assistant	Mr.Deepak K. Panigrahi Ms.Purabi Mondol		9002232022 9836249346
4	Institute Medical Officer	Dr. Mayukh Pal		9433863905
5	DBS safety committee member (Radiological Safety Officer - RSO)	Mr. Ritabrata Ghosh	ritabrata.ghosh@iiserkol.ac.in	9231179708
6	DBS safety committee member	Dr. G.Lekha	lekhag@iiserkol.ac.in	9620507671
7	Radiation- facility (FIC)	Dr.Partho Sarothi Ray	psray@iiserkol.ac.in	9874703899
8	DBS safety committee member	Dr. Tapas Kumar Sengupta	senguptk@iiserkol.ac.in	9831417327
9	DBS safety committee member	Prof. Jayasri Das Sarma	dassarmaj@iiserkol.ac.in	9748642423
10	DBS safety committee member	Dr. Rupak Datta	rupakdatta@iiserkol.ac.in	9874477790
11	DBS safety committee convener	Dr. Bidisha Sinha	bidisha.sinha@iiserkol.ac.in	9163608998

A. General guidelines

The following is a short description of general laboratory work rules that has to be implemented and followed. For most part these rules are self-evident and are usually already followed by people. They are here written to avoid misunderstandings, to clarify under which rules everybody in the lab should.

1. Generally, you should always work in a way that is safe for all in the lab. Know your surroundings, exits, location of nearest fire extinguishers and First Aid Kit; phone numbers of Institute medical officer, nursing assistant and ambulance.
2. Follow prescribed instructions while working with Biological Material **(B: B1-B3),** hazardous substances **(C: C1 –C8)** or Radioactive compounds **(D1 – D7**).
3. Work and move around carefully in the lab to avoid accidents. Do not use headphones while
4. working. Food and drinks are not allowed in labs. Mouth pipetting is prohibited.

Fig: First aid kit

5. It is recommended to use lab-coats when working in the lab, for protection. Be properly clothed (covered footwear recommended in areas where footwear is allowed).
6. Protect yourself from hazardous chemicals by using the appropriate precautions. Use gloves where required and discard them properly to avoid exposing others to the chemicals on the gloves.
7. Do not leave equipment, buffers, chemicals or other material anywhere that is not for storage. Ensure while preparing reagents that the tubes/bottles are labelled properly with the material, concentration (if applicable), and your name and date. This will avoid accidental use of unlabeled harmful material.
8. Be particularly careful when working with **radioactive biomolecules (D: D1–D7), acryl amide, SDS, phenol and ethidium bromide (C: C5-C8)**. Always take the recommended precautions.
9. Used glassware, placed for further cleaning, must be already free of buffers and chemicals.
10. All technical equipment must be used according to instructions after consulting the person in charge. Following proper procedure of handling is essential to prevent accidents and to prevent destruction of instruments.

11. Log book entries are a mandatory. You must report if you observe any abnormal functioning of the machine – both in the log book and to the person in charge.
12. Used materials (slides, gels, media, plates, culture dishes, tissues, buffer) should be properly discarded and not left in the vicinity of the instruments **(F: F1 to F3).**

You must also be careful when working with very hot or very cold substance or items. Make sure that everybody knows if you use open fire, heat or boil any liquids, use dry ice or liquid nitrogen. Never handle dry ice or liquid nitrogen with your bare hands since this can cause severe burns. Burning of ethanol can only take place in a hood, but if you knock over the beaker and spread the fire then turn of the hood immediately to avoid spreading.

Heating is often required when working with agar, agarose and for fast sterilization. If you burn yourself apply plenty of cold water to the injured place immediately. Remove any clothing with hot liquid.

Use mercury-thermometers in the labs with outmost care. In case you break an Hg-thermometer be extremely careful not to get in contact with Hg. Do not flush out Hg in the sink.

A1. Routes of exposure associated with laboratory work

Route	**Microbiological practice**
Ingestion	• Mouth pipetting • Splashes of infectious material into mouth • Contaminated articles or fingers placed in mouth • Consumption of food in workplace
Inoculation	• Needlestick accidents • Cuts from sharp objects • Animal and insect bites and scratches
Contamination of skin and mucous membranes	• Spills or splashes into eyes, mouth, nose • Spills or splashes on intact or nonintact skin • Contaminated surfaces, equipment, articles
Inhalation	• Numerous procedures that produce aerosols

Routes of Exposure

The most common routes of exposure associated with laboratory work are listed in Table 1. Specimen collection, specimen processing and manipulation of cultures during routine laboratory operations frequently contaminate containers, bench tops, equipment, laboratory requisitions and fingers from spillage of infectious material and generation of aerosols. Eating, drinking, and applying cosmetics in the laboratory pose such a hazard that these activities are universally prohibited. Food

should not be brought into a laboratory or stored in refrigerators designated for the storage of clinical specimens or cultures.

A2. Laboratory activities that generate aerosols

Laboratory activity	Microbiological practice
Inoculating-loop Manipulation	Sub culturing and streaking culture, 'Cooling" a loop in culture media, Flaming a loop
Pipette	Mixing microbial suspensions, Pipette spills on hard surfaces
Needle and syringe Manipulation	Expelling air, Withdrawing needle from stopper, Injecting animals Spray created when needle separates from Syringe.
Others	Centrifugation, using blenders, shakers, sonicators and mixing instruments Pouring or decanting fluids, Opening culture containers, Spillage of infectious materialLyophilization and filtration under vacuum

PPE (Personal Protective Equipment)

Safety Googles Always wear safety googles to protect your eyes in any activity involving chemicals, flames, or heating or the possibility of broken glass wear.

Plastic Gloves Wear disposable plastic gloves to protect yourself form chemicals or organisms that could be harmful. Keep your hands away from your face. Dispose the glove after use as per the instruction.

Laboratory Apron Wear a laboratory apron to protect your skin and clothing.

Use **ear protectors** while using Sonicators.

Use **Proper Shoes** while working in biology laboratory.

Safety rules to be followed

Be careful when handling hot glassware

Turn off all heating appliances when not in use. Keep flammable objects away from your workspace.

Handle glassware carefully

Properly dispose of anything that breaks. Report cuts, spills, and broken glass to your instructor immediately.

ID hazards

Identify hazardous materials before beginning labs.

No food

Don't eat or drink in the lab—and never taste chemicals.

Clean up

After completing the lab, carefully clean your workspace and the equipment, and wash your hands.

Be attentive

Be attentive while in the lab. Don't leave lit Bunsen burners unattended or leave an experiment in progress.

B. Working with Biological material

All biological material is a potential health hazard. The different kinds of biological material are all under regulations as shown below. Since all biological material can contain pathogens, all waste containing such material must be discarded in designated risk wastebaskets.

B1. Animals

All work with experimental animals should be performed in the animal facilities after consultation with the person in charge.

B2. Human material

All work with human material should be performed after consulting with the person in charge.

B3. Microorganisms and cell culture

All cell cultures (bacterial, mammalian or pathogenic microorganisms) should be performed following biosafety norms. The following general rules should therefore be applied:

1) Spills should be treated with 70% ethanol or detergent solution and removed. Be particularly careful with bacteriophages to avoid contamination of cultures. Always wash your hands before leaving the lab in order to avoid unnecessary spreading of bacteria.
2) Infected pipettes, contaminated glassware and left-over cultures must be collected in designated bottles and decontaminated.
3) All infected disposable material should be discarded in risk waste boxes (having an inner plastic bag).

Fig: Biological spill clean-up

C. Working with hazardous substance

It is likely that there will be two levels of risk assessments that we have to apply to. One will be on **"non-hazardous or moderately hazardous"** which will not demand any further action, whereas work with the **"hazardous or very hazardous"** chemicals will require risk assessment documentation.

Work with all hazardous chemical substances must be performed according to the recommendations of the manufacturer. Some chemicals are regulated in this safety manual to avoid misunderstandings.

Please consult the Appendix for lists of carcinogenic chemicals that are prohibited to use (**Group A and B**) and those that are allowed in limited amounts (**Group C**).

C1. Corrosive Compounds

Always use gloves and if possible safety goggles. If you in spite of this get corrosive substance on your skin: rinse with plenty of water.

Do not store acids, bases or other corrosive chemicals high up on a shelf. Remember never to pour water into strong acids or bases always pour those into water.

If you get something corrosive under your contact lens it has to be removed otherwise washing with water will not help.

<u>If you get some chemical agent into the eyes...</u>

a) Go immediately to the nearest eye bottle-shower and rinse thoroughly with water for at least 5 minutes. If you get <u>NaOH</u> or <u>phenol</u> in your eyes, rinse for at least 30 minutes.
b) Visit as soon as possible the (eye) emergency intake at the hospital.

<u>If somebody else gets some chemical agent in the eyes...</u>

a) Help him/her to the nearest eye-shower and see to it that his/her eyes are kept open during rinsing.
b) Contact the hospital and follow the injured person there.

C2. Inflammable and explosives compounds

All (organic) solvents are more or less inflammable (and besides, more or less toxic). A maximum of 10 L of solvents (flashpoint below +21°C) per lab unit can be kept on benches etc.

Up to 50 L of inflammable liquids can be kept in ventilated cabinets. Larger volumes are not allowed. Labs must have absorption material available to be used in case of a larger spill.

All work with solvents should be carried out in ventilation hoods. A list in the Appendix shows the common organic solvent that you can only handle in a ventilated hood.

Be especially careful with organic peroxides in ether, dioxane and tetrahydrofuran. Never evaporate these kinds of solvents if they have been standing for a longer time without testing for peroxides.

C3. Carcinogenic and mutagenic compounds

It is not allowed to store or work with carcinogenic substances belonging to group A and B (see Appendix). Work with group C carcinogens must be conducted with outmost caution.

When handling carcinogens/mutagens you should mark and cover the area where you are going to work with bench cover like aluminum foil.

Always use gloves and preferably also protective goggles when you handle these substances. Consider the gloves as contaminated, i.e. when you work with carcinogens do not open doors, refrigerators etc. with a glove-covered hand.

Transferring from storage-container to weighing-container should normally be performed in a hood. In certain cases, it could, however, be safer to do the transfer outside the hood.

You should always use a container that can be sealed if you need to transport the substance within the lab. Residual solutions and waste with carcinogenic substances must be treated as hazardous garbage.

C4. Poisons

Substances classified as particularly toxic should be kept in the designated locked compartments. The same directions, as for carcinogens and mutagens, apply for work and waste handling of these substances.

A common risk assessment has been worked out for working with acrylamide, SDS, phenol and ethidium bromide as presented below.

C5. Risk assessment and instructions for working with Acrylamide

Be particularly careful when working with these compounds. Never get in direct contact with them. Always use gloves and preferably also goggles and mouth protection when weighing powder.

Always weigh in the hood. Be careful when dissolving in water or buffer and avoid dispersion of powder in the air.

Fig: Weighing hood

Risk characterization

Upon single exposure, acrylamide is toxic or harmful by all routes of administration. The principal effect observed as a result of repeated exposure, by all routes, is peripheral neuropathy.

The key toxicological endpoints for acrylamide are neurotoxicity, genotoxicity, carcinogenicity.

Acrylamide is a skin irritant, with skin peeling being a particular problem. There is clear evidence for skin sensitization potential. It should be considered as an eye irritant too.

Acrylamide is a direct-acting mutagen *in vitro* and there is also an evidence clearly demonstrating that acrylamide is genotoxic *in vivo* to both somatic cells and germ cells.

It is noted that acrylamide should be stored, transported and handled under the correct conditions. The recommended conditions for acrylamide dry crystals are to avoid direct sunlight; crystal temperatures above 50°C; and initiators such as bisulphites, peroxides, reducing agents, oxidising agents and redox systems.

For aqueous solutions of acrylamide, the recommended conditions are to store below 32°C and above the crystallisation point. Avoid contamination with iron or rust, initiators such as bisulphites, peroxides, reducing agents, oxidising agents and redox systems and prevent the loss of dissolved oxygen.

There should be a requirement to reduce exposure to acrylamide as far as is reasonably practicable.

C6: Risk assessment and instructions for working with Sodium dodecyl Sulphate (SDS)

An anionic surfactant (detergent) used for denaturing proteins. It works by disrupting non-covalent bonds in proteins

Potential Hazards

Inhalation Causes respiratory tract irritation. Moderately harmful if inhaled. Toxic if absorbed through skin. Causes skin and eye irritation on exposure. Harmful if swallowed.

Waste disposal procedures

Prevent further leakage or spillage and do not let product enter drains.

Decontamination of Equipment

Wear appropriate PPE and wet any spills of SDS powder and very carefully mop up spill with paper towels and place into appropriate waste container.

Handling and Storage Requirements

- Avoid contact with skin and eyes.
- Avoid formation of dust and aerosols.
- Provide appropriate exhaust ventilation at places where dust is formed. Keep away from sources of ignition – No smoking.
- Take measures to prevent the build-up of electrostatic charge.
- Keep container tightly closed in a dry and well-ventilated place, store in cool place.
- Stable under recommended storage conditions.

Conditions to avoid: Heat, flames and sparks.

Materials to avoid: Oxidizing agents

Accident response

a) General advice consults a physician. Move out of dangerous area.
b) If inhaled or if breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

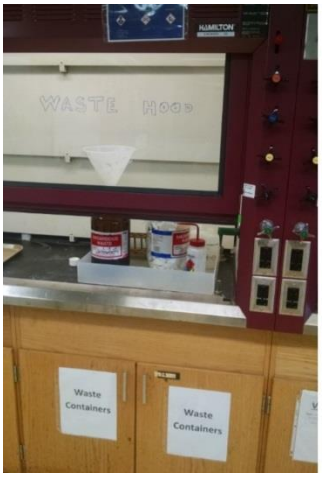
c) In case of skin contact wash off with soap and plenty of water. Consult a physician.

d) In case of eye contact Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

e) If Swallowed Do not induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water.

Spill Clean Up

Contains Spillage and then collect with an electrically protect vacuum cleaner or by wet brushing and place in container for disposal according to local regulations. Keep it suitable, closed containers for disposal.



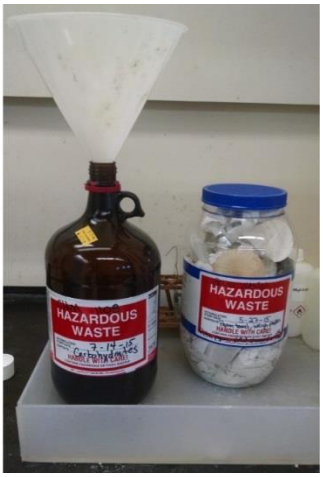


Fig: Disposal containers of hazardous waste

C7. Risk assessment and instructions for work with phenol

Main use: Extraction of nucleic acids from biological samples. If solid phenol is used one normally melts it in a water bath and makes a water (or buffer) solution that is stored in a refrigerator or freezer.

Hazard identification: Corrosive and can cause severe burns. If ingested or inhaled in larger amounts phenol can cause severe tissue damage and may even be fatal.

Phenol on the skin causes initially an itchy feeling that can pass over to numbness and if not removed cause severe deep topical damage as well as systemic effects. Phenol in the eyes can rapidly cause irreversible damage and blindness.

Risk assessment: Phenol is a very toxic chemical and must be handled with care. The risk of getting phenol on the skin is rather large, but through the itching feeling it is rapidly recognized and could be washed off. The largest risks for severe accidents are connected to melting solid phenol.

Safety measures:

The most important safety measures are therefore to:

- Use only the liquidities phenol
- Work in the hood
- Use lab coat, gloves and preferably also safety goggles
- Discard immediately contaminated gloves and change gloves each time leaving the hood
- Use the right type of centrifugation tubes (size and material, not polycarbonate (clear type) which will be destroyed by phenol and contaminate e.g. the centrifuge)

- Use only tubes with a good cap and make sure that tubes are closed and that they are not contaminated on the outside
- Balance the centrifuge well
- Be aware of possible contamination and remove it ASAP

If you do not want to use phenol, there are alternative methods for extracting nucleic acids and there are also nucleic acid extraction kits available on the market.

First aid measures: If you get phenol on the skin wash immediately with plenty of water and a soap solution (special phenol removing detergents are available on the market). After longer time of exposure the skin area of contact will turn white, be swollen and in the worsted case develop into a deep wound that does not heal easily. If you start experiencing these symptoms you should immediately go to the hospital emergency unit. If you get phenol in the eye you have to wash immediately with plenty of water and go to see the hospital emergency unit.

Waste handling: All solutions containing phenol should be collected in plastic bottles. If you prepare phenol solution make sure you rinse all used glass before taking it out from the hood.

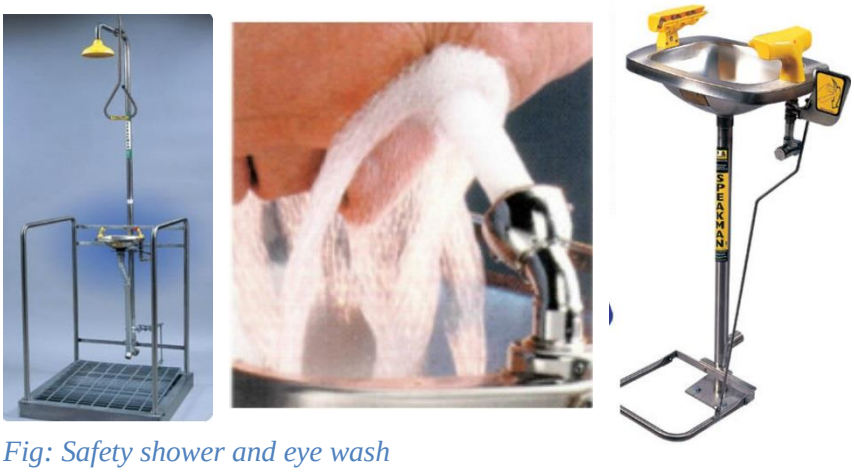


Fig: Safety shower and eye wash

C8: Risk assessment and instructions for working with <u>ethidium bromide</u>

Main use: Fluorescence labeling of nucleic acids on agarose or acryl amide gels. A stock solution of ethidium bromide (typically 10 mg/ml) is prepared by dissolving tablets or powder in water. It is <u>strongly</u> recommended to use the tablets that can be handled with much greater safety. This stock solution is diluted approximately 10 times and the final concentration when treating gels is 1 µg/ml or less.

Hazard identification: Irritating, mutagenic, probably inducer of reproductive toxicity and possibly carcinogenic, although no such data are available.

Risk assessment: Primarily because of its mutagenic and probable reproduction toxic properties, ethidium bromide should be handled with care. Very low concentrations of ethidium bromide are used when labeling nucleic acids and if gloves are used the exposure to ethidium bromide is very small and the risk of adverse health effects is probably small compared to other risks.

Safety measures: Risks connected with the use of ethidium bromide can be significantly reduced by following some simple safety measures.

- Use gloves (ethidium bromide will pass latex gloves, but not nitrile gloves.)
- Use only tablets since that will reduce exposures
- Label all solutions and collect all waste
- If possible, do the staining of the gel afterwards (not when casting) since that will reduce exposures and contamination.
- Use some kind of designated tray or box to carry the ethidium bromide stained gel in, e.g. when bringing it for documentation.
- It is advised to use a slice when moving gels from and to trays and boxes and not touch it at all with hands. Discard contaminated gloves.

First aid measures: If you get ethidium bromide solution on the skin wash with plenty of water (at least 15 min). If you get ethidium bromide in the eye wash immediately with water for at least 15 min and if irritation persists go to the hospital emergency unit.

Waste handling: Stock and other solutions can be destroyed by adding activated charcoal or alternative methods and thereby reducing the amount of waste. Gels with ethidium bromide must be collected in plastic containers that can be sealed and handled as risk waste.

D. Working with Radioactivity

D1. Types of Ionizing Radiation

1. Alpha particles,
2. Beta particles,
3. Gamma radiations.

On the basis of forms of Sealed sources and Unsealed sources

D2. Adverse effects of radiation

Ionizing radiation and injury the hazards that are usually encountered in using radioisotopes arise from: External exposure and Internal exposure

External exposure - When the person gets exposed to radiation emitted from external sources of radiation. The resulting damage will depend upon the type of the radiation emitted, energy of the source and duration to which the person is exposed. In this situation the person can be seriously injured but is not radioactive.

Internal exposure - When there is deposition of radioactive material in a target organ it leads to internal contamination and exposure. Ionizing radiation transfers energy to any material that comes in contact with it. This results in disruption of chemical bonds in cells resulting in permanent damage; the extent of which on chronic or acute exposure is decided by the energy received and its effectiveness is either somatic or genetic. Once the radionuclides are inside the body, they get metabolized and distributed in the organs according to their chemical properties.

Some instances of detrimental effects of radiations are:

Radiation Units

Becquerel (Bq) - one disintegration per second.

Curie (Ci) - one curie corresponds to 3.7 x 1010 disintegration per second. 1 Ci = 37000 MBq.

Radiation symbol. The warning sign must be conspicuously and prominently displayed at all times.

-on external surfaces of radiation equipment.

-containers for storage of radioactive materials.

-packages for radioactive materials

-at the entrance to the room housing the radiation generating source.

Radiation Dose and Dose Limits

The different kinds of radiation dose fall under:

a) Absorbed Dose (DT, R)

b) Equivalent Dose (HT)

c) Effective Dose (E).

Dose Limits- Protective measures should be optimized that the radiation exposures are kept as Low as Reasonably Achievable (ALARA). Social-economic consideration should be factored in optimizing the level of protection.

1.Occupational – 20 mSv per year.

2. Non-occupational – 1 mSv per year for public.

D3. Radiation protection

1. Time – Lesser time of exposure to radiation, better is the protection.

2. Distance – More the distance, lesser is the exposure, therefore more is the protection.

3. Shielding – Use of specific material (lead boxes for sealed sources(SS) and lead vials for storage and transport of unsealed sources(US) and Perspex shielding in work area is mandatory. The thickness of shields varies with respect to different types of radiations, ensuring better protection. Light metals like aluminum are preferred for shielding beta particles as they produce less bremsstrahlung radiation. Use of gloves, eye shield and lab coat are mandatory for handling sources.

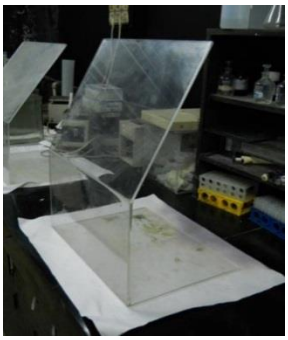


Fig: Shield in the work area

D4. Determination of radiation exposure

1.Personnel monitoring – This is done by wearing TLD badges and comparing the effective luminescence against a control badge.

2.Area monitoring – This is done with radiation monitor, and by taking periodic swipe tests, with respect to the background.

Fig: TLD badges

D5. Radioactive waste disposal

The method of disposal should be such, that it does not increase any type of radioactive contamination into the environment. For Sealed sources, the isotopes of interest need to be taken back by the manufacturer.

For Unsealed sources, periodic storage is followed with monitoring; before proceeding to conventional disposal methods (for solid waste). For unsealed liquid waste, similar protocol is followed before draining the same under running normal tap water. For detailed methods please refer to the specific Lab safety manual for Radioactivity.

D6. Radiation Detection

This is done by specific radiation monitors/GM counters, effective body contamination monitors, collecting swipes from the effective area for body fluids (blood, sputum, etc.) or cell and tissue samples and then measuring with standard methods.

D7. Emergency preparedness

- Charts mentioning the various steps of the radiation worker should take in case of an emergency should be conspicuously displayed in the laboratory.

-All radiation monitoring and measuring instruments should be checked routinely and kept in working conditions.

-A kit comprising of accessories for the decontamination operation should be available, ready to handle accidental spillage.

-Lab coat must be worn and eye shielding with glasses should be used while working.

-A proper inventory of isotopes received, used and disposed should be maintained.

E. Emergency response

Fires in beakers or other containers can be extinguished by using a lid or other cover to prevent the oxygen supply. Do not pour water on to burning liquid. Fires in clothing, paper, equipment and on the floor, can be extinguished by water from the hoses or sink.

E1. Fire

Steps should be taken such that radioactive contamination does not escape into the environment like opening of the windows, etc. The workers/public in and around should be made aware. The area should be restricted to access. Inform adjoining laboratories. Break glass of manual call point and actuate the fire alarm. Evacuation should be done ensuring all doors and windows are closed. The firefighting personnel should be made aware there is radiation.

Fig: Steps to use fire extinguisher

Fig:Fire extinguisher

It is important that everybody knows where to find fire extinction equipment, the first aid kit, the liquid-absorbing material (each lab must have a box available), showers for decontamination and how to use these, and also know the escapes routes out of the building. Always take responsibility and stop the accident (or at least inhibits its expansion), help injured people, warn all others. When the fire alarm goes of, leave the building calmly and make sure you do not leave anything behind which needs attention (people in distress, running equipment, open chemical bottles or gas tubes for example).

E2. Theft

All the sources should be kept under lock and key in a monitored area. The area should be accessed by authorized personnel only. Efficient monitoring by installation of CCD cameras at the entrance is also practiced. In case theft happens, the same should be brought to notice of the RSO,facility-in-charge,& the CSO. Practical steps should be taken to retrieve the missing source.

E3. Health

For **external** contamination

The immediate washing of contaminated areas with water and soap must be done for removing loose contamination. Washing must be done gently without causing any damage to the skin. After washing for a few minutes, the skin must be dried and monitored. Washing may be repeated if necessary without causing skin damage. While washing care must be taken to prevent contamination of uncontaminated parts or internal contamination. In case of contaminated small open wounds, cuts or other injuries, the wound must be immediately washed and medical attention must be sought.

For **internal** contamination

The purpose of treatment in case of an internal contamination will be to eliminate much of the contaminant still remaining in the mouth, gastro-intestinal or respiratory tract as fast as possible. Internal contamination requires special medical advice and supervision. All should be brought to notice of the Radiological Safety Officer (RSO).

F. Waste Handling

We are currently working to have as safe and simple a handling of laboratory waste as possible. In the labs there will be two types of waste baskets, one type for biological waste and waste which can be harmful (risk waste) and one type for all other waste (ordinary garbage). It is here defined which waste that has to be collected in the risk waste and the rest can then be discarded through the ordinary waste, excluding some solvents, radioactivity, electronic equipment and items for recycling. Organic liquids have to be collected in plastic containers and radioactive material in leaded baskets for storage or destruction. However, a collection of chemicals can be poured into the drain with extensive flushing with water. These chemicals are listed in the Appendix.

F1. Risk waste

All biological material (except decontaminated media and buffers) has to be considered as a health hazard and be collected in the risk waste (cardboard box with thick inner plastic bag). Into this waste also goes all gels with acryl-amide or other two-component polymers, gels with ethidium bromide, all material with chemicals from the risk groups described above. Pipette tips, and other such material used for handling of biological material as well as hazardous chemicals, also has to go to the risk waste. Used plastic scintillation vials can generally also be thrown into risk waste. Broken glass, needles, scalpels and other sharp material that can hurt personnel must be collected in plastic containers, which can be closed (can be old rinsed salt containers or similar) and then put in the risk wastebaskets.

F2. Solvents

Those solvents that cannot be poured into the sink and flushed out with water (see Appendix), has to be collected in labeled 5 L plastic bottles with screw caps. Several common organic solvents belong to this group like ether and formaldehyde. This also applies to develop and fixation solutions, used oil from equipment and solutions containing heavy metals (like silver, cobalt and copper) and other hazardous chemicals. Always mark on the container what is inside and avoid collecting more than one chemical in each container. Never fill these containers to more than the 5 L, since some expansion may occur.

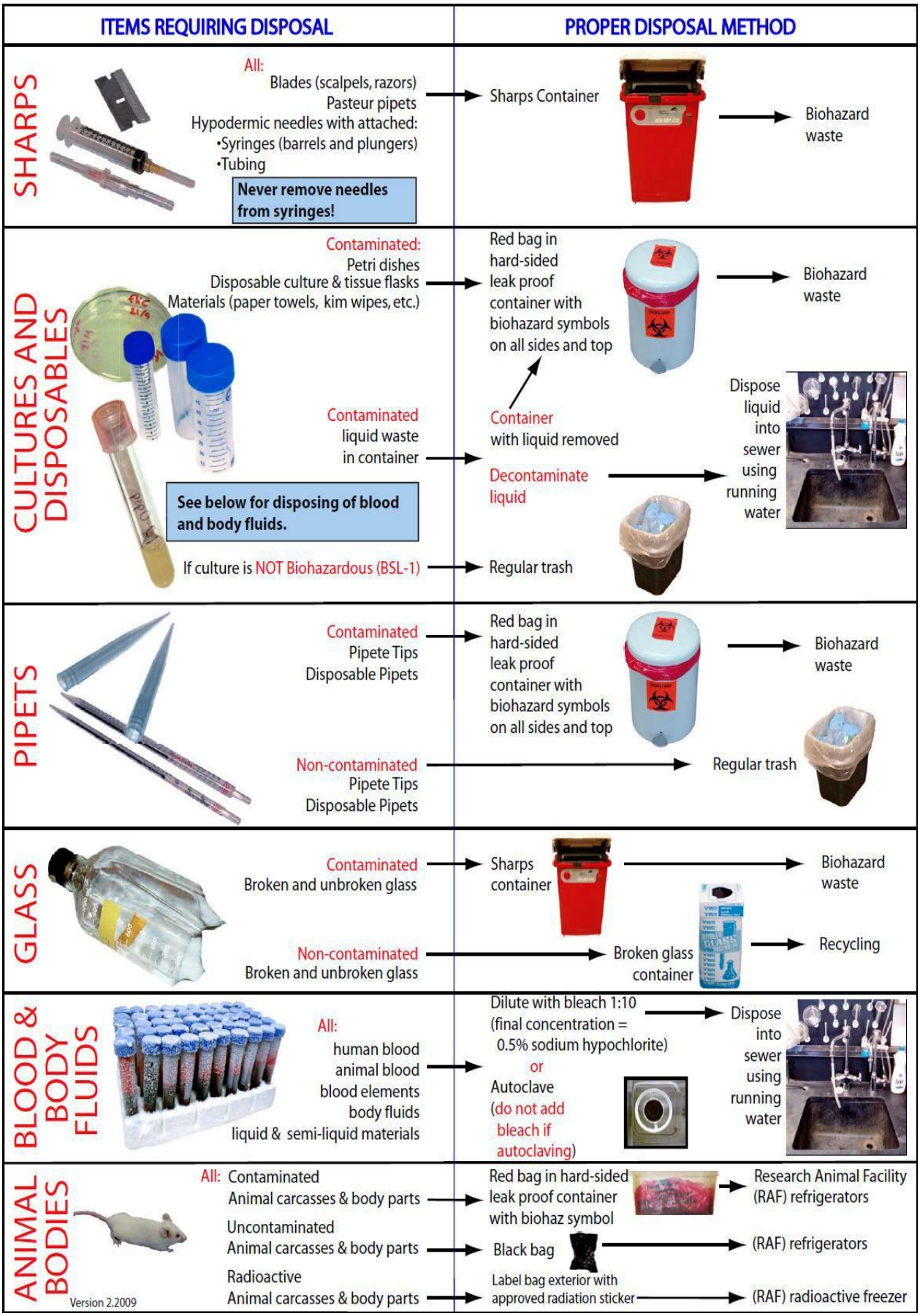
F3. Radioactive waste disposal

The method of disposal should be such, that it does not increase any type of radioactive contamination into the environment.

For **Sealed sources**, the isotopes of interest need to be taken back by the manufacturer.

For **Unsealed sources**, periodic storage w.r.t. the half-life of the isotope of interest needs to be done, with continuous monitoring and keeping records of the same before following conventional disposal methods (for **solid waste**). For unsealed **liquid waste**, a similar protocol should be followed as mentioned, before draining it out under running normal tap water.

Biohazard waste management



G. Instruments and equipment

All laboratory machines and equipment must be used according to the recommendations of the manufacturer. If you do not know how to operate a particular instrument you must contact the responsible person for an introduction. If an instrument or set of equipment does not work properly or breaks down immediately contact the responsible person.

In the lab make sure that refrigerators and freezers have the correct temperature. Use microwave ovens with care, never have metals or foil inside and open the lid of bottles and other containers when heating. Make sure that bottles are not wet on the outside when heating in microwave oven. Never use older types of glass that are not designed for use in microwaves.

The Sonicator can only be used wearing ear protection. Other people in the same room are required to also wear ear protection when the Sonicator is operated. Never forget running equipment, use a timer to remind you. Remember that ventilation hoods use tremendous amounts of energy. Only open the hoods when you are working there and always take care to close them when you have finished the work, if you are leaving to do something else or have things running there on their own.

When using the house vacuum make sure you have attached tubes and flasks correctly and avoid any leakage. Also close the vacuum valve before dismantling your equipment. CO_2 flasks for the CO_2 incubators should be replaced with outmost care. Always close all valves before replacing the flask and always attach the flask to the wall using the chain. Used glass, paper and cardboard boxes must be taken to the ramp and placed in the appropriate containers.

Material Preparation

1. Ensure material is safe for autoclaving.
2. NEVER AUTOCLAVE FLAMMABLE, REACTIVE, CORROSIVE, TOXIC, or RADIOACTIVE MATERIALS.
3. Glassware must be inspected for cracks prior to autoclaving.
4. Prepare and pack material suitably.
5. Place items in heat-resistant secondary containers to secure and contain spills.
6. Biohazardous waste must be processed according to IISERK guidelines.

Fig: Autoclave

Loading Autoclave

Fig: Autoclave

1. Wear Personnel Protective Equipment (PPE) including laboratory coat, eye protection, heat-insulating gloves and closed-toe shoes. Wear an apron and face shield when handling liquids.
2. Inspect drain strainer daily. Clean when blocked.
3. Place materials in autoclave. Do not mix incompatible materials.
4. Do not overload; leave sufficient room for steam circulation. If necessary, place container on its side to maximize steam penetration and avoid entrapment of air.
5. Close and latch door firmly.

Operating Autoclave

1. Close and lock door.
2. Choose appropriate cycle (e.g., gravity, liquid, or dry cycle) for the material. Consult autoclave manual for assistance in choosing the appropriate cycle.
3. Only designated individuals are allowed to set and/or change parameters for the autoclaves. Use indicators to determine the best treatment time.
4. Start your cycle and fill out the autoclave user log with your contact information. A completed cycle usually takes between 1 to 1.5 hours.
5. Check chamber/jacket pressure gauge for minimum pressure of 15 pounds per square inch (psi). Check temperature for 250^0F (121^0C) every load.
6. Do not attempt to open the door while autoclave is operating.
7. If problems with the autoclave are perceived, abort cycle and report it to the supervisor and Facilities Maintenance immediately.

Unloading Autoclave

1. Wear PPE including heat-insulating gloves, eye protection, laboratory coat, and closedtoe shoes.
2. Ensure cycle has completed and both temperature and pressure have returned to a safe range.
3. Wearing PPE, stand back from the door as a precaution and carefully open door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.
4. Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.

5. Do not agitate containers of super-heated liquids or remove caps before unloading.
6. Wear PPE, plus an apron and face shield for liquids, to remove items from the autoclave and place them in an area which clearly indicates the items are "hot" until the items cool to room temp.
7. Shut autoclave door.
8. Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.

Autoclave Use Log

1. Entries must be made in the logbook each time the autoclave is used. These records are used for maintenance/service schedules and reporting of incidents, accidents and/or faults.
2. Entries should include: operator's names, phone number, date, time and duration.

Maintenance and Repair

1. No person shall operate the autoclave unless the autoclave is in good repair.
2. Only qualified professionals are permitted to make repairs.

Equipment Malfunction

1. If the autoclave does not operate exactly as expected, do not attempt to fix the problem.
2. Record the problem in the autoclave log book.
3. Report the problem to the supervisor and Facilities Maintenance immediately.

Spill Cleanup

1. Spills may occur from a boil-over, breakage of containers, or a blocked drain.
2. No operation of the autoclave is allowed until the spill is cleaned up.
3. The operator is responsible for the cleanup of spills. Contain spilled material with paper towels or other absorbent material. Use your laboratory spill kit, if necessary. Wait until the autoclave and materials have cooled to room temperature before attempting cleanup.
4. Review the manufacturer's instructions to determine appropriate PPE for spill cleanup and disposal protocols.
5. Dispose of waste in accordance with regulatory requirements. If materials have been mixed, follow the cleanup and disposal protocol for the most hazardous component.
6. Cracked glassware must be disposed of properly.
7. Record the spill and cleanup procedure in the autoclave logbook.

* NEVER ALLOW WASTE TO ACCUMULATE IN THE LAB. NEVER LEAVE WASTE UNATTENDED.

Incident Response

1. All incidents, including a spill or release of biohazardous materials (including recombinant / synthetic nucleic acids) must be reported to the supervisor.
2. If clothing is soaked, remove it and place the injury in cool water.
3. Place a sign on the unit indicating that it is not to be used until it is safe for operation.

H. Laser Safety

Below is a brief description of each of the current laser classes.

The standards apply equally to lasers and LEDs. Generally, LEDs fall under lower Classes (1, 1M, 2, 2M, 3R) of lasers, except Class 4 LEDs*(laser Class 3B).

Generally speaking lasers are point sources while LEDs are extended sources. Extended sources have higher power limits than point sources for a given laser Class. Therefore, a visible LED emitting 10 mW may be Class 2, while a visible laser pointer of the same power would be Class 3B. NB Laser pointers above Class 2 are banned for sale to the public by trading standards.

Class 1 This class is eye-safe under all operating conditions.
Class 1M This class is safe for viewing directly with the naked eye, but may be hazardous to view with the aid of optical instruments. In general, the use of magnifying glasses increases the hazard from a widely-diverging beam (eg LEDs and bare laser diodes), and binoculars or telescopes increase the hazard from a wide, collimated beam (such as those used in open-beam telecommunications systems).
Class 2 These are visible lasers which are safe for accidental viewing under all operating conditions. However, it may not be safe for a person who deliberately stares into the laser beam for longer than 0.25 s, by overcoming their natural aversion response to the very bright light.
Class 2M These are also visible lasers. This class is safe for accidental viewing with the naked eye, as long as the natural aversion response is not overcome as with Class 2 but may be hazardous (even for

accidental viewing) when viewed with the aid of optical instruments, as with class 1M.
Class 3R The class limit for 3R is 5x the applicable class limit for Class 1 (for invisible radiation) or class 2 (for visible radiation). Hence CW visible lasers emitting between 1 and 5 mW are normally Class 3R. Radiation in this class is considered low risk, but potentially hazardous.
Class 3B For a continuous wave laser the maximum output into the eye must not exceed 500mW. The radiation can be a hazard to the eye or skin. However, viewing of the diffuse reflection is safe. Radiation in this class is very likely to be dangerous.
Class 4 This is the highest class of laser radiation. Radiation in this class is very dangerous, and viewing of the diffuse reflection may be dangerous. Class 4 laser beams are capable of setting fire to materials onto which they are projected.

Any laser product of a given Class may contain 'embedded' lasers which are greater than the Class assigned to the product, but in these cases engineering controls (protective housings and interlocks) ensure that human access to radiation in excess of product Class is not possible. Notable examples of this are CD and DVD players which are Class 1 laser products while containing Class 3R or Class 3B lasers and laser printers which are Class 1 laser products but contain Class 4 embedded lasers.

Terminology used in case of lasers

CW (Continuous Wave)- i.e. not pulsed

Diffuse reflection - The reflection of radiation from a matt surface such as a wall

Optical instruments - Binoculars, telescopes, microscopes, magnifying glasses (but not prescription glasses)

Table of laser classes and their degree of safety according to standards** (detailed of which mentioned below).

CLASS 1	Safe
CLASS 1M	Safe provided optical instruments are not used
CLASS 2	Visible lasers. Safe for accidental exposure (<0.25s)
CLASS 2M	Visible lasers. Safe for accidental exposure (<0.25s) provided optical instruments are

	not used
CLASS 3R	Not safe, but low risk.
CLASS 3B	Hazardous. Viewing of diffuse reflection* is safe.
CLASS 4	Hazardous. Viewing of diffuse reflection* is also hazardous. Fire risk.

Optical instruments here mean – binocular, telescopes, microscopes, magnifying glasses

*Diffuse reflection means – The reflection of radiation from a matt surface such as a wall.

This classification is according to the current version of EN-60825-1 and IEC 60825-1.

standards**-Standard governing the safety of laser products in Europe (EN) and International(IEC)

The phrase "eye-safe" is applicable to the whole optical spectrum from 180nm to 1mm wavelength, not just in the retinal hazard range of 400nm to 1400nm. Outside the retinal hazard range there is potentially a hazard to the cornea. A wavelength outside the retinal hazard range is therefore not automatically eye-safe!

Safety precautions

Use of proper protective eye glasses in case one is using an instrument, working with lasers.

Instruments should be properly monitored, to check repetitively to stop any leak of lasers when system is under operation.

Mostly all lasers come with the effective control and safety measures. Users should strictly abide by it to avoid any serious accidents.

Users should never look directly when the lasers are under operation; as discussed above, even partial exposures can have a harmful and temporary/permanent effect.

Proper aeration and cooling effect should always be functional when lasers are functioning, to avoid overheating.

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APPENDIX

Here are lists of hazardous chemicals and chemicals which can be discarded through the sewage system. The hazardous chemical lists cover those which are not allowed in the labs (Group A and B) and those that can be used with outmost caution and in limited amounts (Group C). **Group A1 - Forbidden Chemicals** Carcinogenic chemical

	CAS-nr
2-Acetylaminofluorene (AAF)	53-96-3
3-Methylcholanthrene; 20-methylcholanthrene	56-49-5
4-Dimethylaminoazobenzene; Solvent Yellow 2; Butter Yellow	60-11-7
β-Naphthylamine; 2-Aminonaphthalene	91-59-8
4-Aminodiphenyl; p-phenylaniline	92-67-1
Benzidine; 1,1'-Biphenyl-4,4'-Diamine	92-87-5
4-nitrobiphenyl; 1-Nitro-4-Phenylbenzene	92-93-3
1,2-Dibromo-3-Chloropropane (DBCP)	96-12-8
Chloromethyl Methyl Ether (CMME)	107-30-2
Bis(chloromethyl)ether; 1,1'-Dichlorodimethyl Ether	542-88-1
Hexamethylphosphoramide (HMPA)	680-31-9
N-Nitroso-N-Methylurea; methylnitrosourea (MNU)	684-93-5
Crocidolite Asbestos; Crocidolite	12001-28-4
Erionite	66733-21-9

Group B1 – Forbidden chemicals (which can be used with permission from authorities)

Carcinogenic chemical	CAS-nr
Urethane; Ethyl carbamate	51-79-6
1,1-Dimethylhydrazine; Dimethylhydrazine (asymmetrical)	57-14-7
β-Propiolactone; 1,3-Propiolactone	57-57-8
4-Aminoazobenzene; *p*-Aminoazobenzene	60-09-3
Monomethyl Hydrazine; hydrazomethane	60-34-4
Ethyl methanesulfonate (EMS)	62-50-0
Thioacetamide	62-55-5
Thiourea, Thiocarbamide	62-56-6
N-Nitrosodimethylamine; Dimethylnitrosoamine	62-75-9
Diethyl Sulphate	64-67-5
Methyl methanesulfonate (MMS)	66-27-3
Propylenimine; 1,2-Propylene Imine	75-55-8
Dimethyl Sulfate (DMS)	77-78-1

3,3'-Dichlorobenzidine	91-94-1
2,4-Diaminotoluene; Toluene-2,4-Diamine	95-80-7
Ethylenethiourea; 1,3-ethylene-2-thiourea	96-45-7
(Dichloromethyl)benzene; Benzal Chloride	98-87-3
Benzotrichloride; 1-(trichloromethyl)benzene	98-07-7
4,4'-Methylenebis-(2-Chlorobenzenamine); 4,4'-methylenebis(2-chloroaniline)	101-14-4
4,4'-Methylenebisbenzeneamine; 4-(4-aminobenzyl)aniline	101-77-9
Ethylene Dibromide; 1,2-dibromoethane	106-93-4
2,2'-dichlorodiethylether	111-44-4
o-dianisidine; 3,3'-dimethoxybenzidine	119-90-4
o-Tolidine; 3,3'-Dimethyl-1,1'-Biphenyl-4,4'-Diamine; Dimethylbenzidine	119-93-7
Tris(2,3-dibromopropyl) phosphate	126-72-7
α-Naphthylamine; 1-Aminonaphthalene	134-32-7
N-Phenyl-2-naphthylamine	135-88-6
Ethyleneimine; Aziridine	151-56-4
Hydrazine	302-01-2
Diazomethane	334-88-3
Auramine; 4,4'-(Imidocarbonyl)bis(N,N-Dimethylaniline)	492-80-8
Mustard Gas; 1-chloro-2-(beta-chloroethylthio)ethane	505-60-2
1,2-dimethylhydrazine (symmetrical)	540-73-8
2,4-Diaminoanisole; 4-methoxy-m-phenylendiamine	615-05-4
1,2-Oxathiolane 2,2-dioxide; 1,3-Propane Sultone	1120-71-4
Diepoxybutane; 1,2:3,4-Diepoxibutane	1464-53-5
β-Butyrolactone; 3-Hydroxybutanoic Acid beta-Lactone	3068-88-0
Sensitizing chemicals	
Hexahydrophthalic Anhydride; 1,2-Cyclohexanedicarboxylic anhydride	85-42-7
Tetrahydrophthalic anhydride; 1,3-Isobenzofurandione	85-43-8
3,3'-Dichlorobenzidine	91-94-1
Toluene-2,4-Diamine; 1,3-diamino-4-methylbenzene	95-80-7
N-Phenyl-p-phenylenediamine; 4-Aminodiphenylamine	101-54-2
p-Phenylenediamine; 1,4-Benzenediamine	106-50-3
Carbamimidothioic acid; 2-(dimethylamino)ethyl ester, dihydrochloride	16111-27-6
Methylhexahydrophthalic anhydride	25550-51-0
Methyltetrahydrophthalic anhydride	26590-20-5

Group C – Carcinogenic chemicals that are allowed but must be use with caution

Carcinogenic compound	CAS-nr
Chlorodiphenyl; PCB (Polychlorinated diphenyl)	
Chromates	
Nickel compounds	
Wood dust	
Formaldehyde	50-00-0
Benzo[a]pyrene	50-32-8
Carbon tetrachloride	56-23-5
β-mercaptoethanol	60-24-2
Acetamide; Acetic acid amide	60-35-5
Aniline; Aminobenzene	62-53-3
Chloroform; Trichloromethane	67-66-3
Benzene	71-43-2
Iodomethane; Methyl iodine	74-88-4
Vinyl chloride; Chloroethene	75-01-4
Acetaldehyde; Acetic aldehyde	75-07-0
Dichloromethane; Methylene chloride	75-09-2
Ethylene Oxide; 1,2-epoxyethane	75-21-8
Propylene oxide; 1,2-Epoxypropane	75-56-9
Trichloroethylene	79-01-6
Acrylamide	79-06-1
2-nitro-Propane; Isonitro propane	79-46-9
Benzyl chloride;	100-44-7
p-Dichlorobenzene	106-46-7
Epichlorohydrin; 1,2-epoxy-3-chloropropane	106-89-8
Butadiene; 1,3-Butadiene	106-99-0
1,2-Dichloroethane; 1,2-Ethylene dichloride	107-06-2
Acrylonitrile	107-13-1
Tetramethylethylenediamine (TEMED)	110-18-9
Tetrachloroethylene	127-18-4
p-Dioxane; 1,4-diethylene dioxide	123-91-1
2,4-toluene diisocyanate; 2,4-diisocyanato-1-methylbenzene	584-84-9
Arsenic and its organic molecules	7440-38-2
Beryllium and compounds	7440-41-7
Cadmium and inorganic compounds	7440-43-9

Chromic acid	7738-94-5
Cobalt sulphate	10124-43-3
Radon	10043-92-2
Cobalt chloride	23670-59-9
Trichlorophenol and salts	25167-82-2
Dinitrotoluene (mixed isomers)	25321-14-6
Asbestos (except Crocidolite - see Group A)	132207-33-1

Some organic solvents that in small amounts could be poured out into the drain

Small amounts of the following solvents can be poured into the drain - if it can be done without risk for fire ignition. These substances are decomposed very quickly or have such a low toxicity that small amounts in the common drain do not involve any environmental risk.

Acetone (2-propanone)
Acetonitrile
Dimethylsulfoxide (DMSO)
Ethanol
Ethyleneglycol (glycol)
Formaldehyde (formalin)
Methanol
N-Methylpyrrolidone
1,2-Propandiol (propyleneglycol)
1-Propanol (propylalcohol)
2-Propanol (isopropanol)

Do not flush down more than one (1) L per 24 hours. Concentrated solutions should only be poured out in a hood. Flush thoroughly with water afterwards.

Common organic solvents that have to be used in a ventilated hood

Acetaldehyde
Acetone (2-propanone)
Acetonitrile
Benzene
Chloroform
Dimethylsulfoxide (DMSO)
Ethanol (in larger amounts, < 10 ml)
Ethyleneglycol (glycol)
Formaldehyde (formalin)
β-mercaptoethanol

Methanol

N-Methylpyrrolidone

Phenol

1,2-Propandiol (propyleneglycol)

1-Propanol (propylalcohol)

2-Propanol (isopropanol)

Tetramethylethylenediamine (TEMED)

Note that this is not a complete list. All organic solvent must be regarded hazardous and be handled in a ventilated hood to avoid inhalation and minimise the risk of spills and ignition.

IISER Kolkata - safety manual declaration

This declaration is to certify that I ……...
(insert name above) have read and understood the Safety manual of IISER Kolkata.

I hereby agree to follow the rules stated in the Safety manual when I am at the IISER K premises and perform my work according to the rules and recommendations.

Location:……
Date:……
Signature:……

Safety manual

for

Working with Radioactive Materials

Department of Biological Sciences

IISER – Kolkata

Introduction

This laboratory safety guidelines for radioactive users in research purpose will serve as a manual to be used as a binding document for all personnel working in laboratories to ensure safe work conduct and practices regarding radioisotope usage (unsealed) in the Department of Biological Sciences, IISER KOLKATA campus.

Procedures and Rules within this Manual are formulated for three reasons:
1.) To avoid health risks and accidents for our personnel.
2.) To be in a position to act appropriately in case of emergencies.
3.) To minimize the environmental burden and risks caused by our work.

This manual cannot cover all circumstances where safety procedures must be applied; it is intended to set up a framework of how Department of Biological Sciences (DBS) of Institute of Science Education and Research (IISER) Kolkata researchers should work in radiation facilities. The avoidance of safety risks for the personnel at the institutional level requires knowledge of possible hazards in our environment, especially radioactivity. In this regard the students/researchers of DBS, IISER Kolkata are required to familiarize themselves with safe practices for applied radioactive laboratory operations.

This manual will be amended as various situations might arise and come to the attention of the concerned personnel.

EMERGENCY CONTACTS

Sl.no.	Faculty-in-charge (FIC)/safety committee member	FACULTY/officer/ member	Email: id @iiserkol.ac.in	Phone number
1	Radiation-facility (FIC)	Dr. Partho Sarothi Ray	psray	9874703899
2	DBS safety committee member (Radiological Safety Officer-RSO)	Mr. Ritabrata Ghosh	ritabrata.ghosh	9231179708
3	DBS safety committee Convener	Dr.Bidisha Sinha	bidisha.sinha	9163608998
4	DBS safety committee Member	Dr. Rupak Datta	rupakdatta	9874477790
5	DBS safety committee Member	Prof.Jayasri Das sarma	dassarmaj	9748642423
6	DBS safety committee member (DBS chairperson)	Dr. Tapas Kumar Sengupta	senguptk	9831417327
7	DBS safety committee member (Scientific officer)	Dr. G Lekha	lekhag	9620507671
8	Institute Medical Officer	Dr. Mayukh Pal		9433863905
9	Nursing Assistant	Mr. Deepak K. Panigrahi Ms. Purabi Mondal		9002232022 9836249346
10	Fire department local, Nadia			033 -2582 8101 (KALYANI F.S.) 8584027304, 8584027305
11	Ambulance/(Ambulance Driver)	Mr. Jainal Mondal Mr. Debu Halder		9038469584 8145549528

IONISING RADIATIONS

Alpha particles

This has two protons and two neutrons and combine with electrons in medium to form helium atoms. They can travel short distance in air. They can be stopped by a thin sheet of paper or clothing worn. They do not pose an external health hazard due to their low penetration power. They can cause serious damage if the source enters the body. Their disintegration causes tissue damage in the immediate vicinity where they are deposited.

Beta particles

Beta particles are high energy electrons produced by disintegration of the nucleus. Their energies will vary depending upon the radioactive isotope concerned. Higher the energy the deeper will be the penetration. Longer exposure to beta particles can result in skin burn. They cause internal hazard, if they enter the body by means of ingestion or inhalation. Secondary X radiations known as bremsstrahlung may be produced from them; so light metals like aluminum are preferred for shielding.

Gamma radiation

These are very high energy electromagnetic radiation of short wavelength having high penetration power; emitted when a nucleus undergoes a transition from a higher to a lower energy state. They cause external exposure hazard. Thick shielding of lead or concrete is required for attenuating gamma radiation.

Activity - The average number of spontaneous nuclear transformation (or disintegration) taking place per unit time.

i. **Becquerel** (Bq) ,**1 Bq = 1 disintegration per second = 1 dps**
ii.**Curie** (Ci) ,**1 Ci** = 3.7 x 1010 disintegration per second = **3.7 x 1010 Bq** = 37 Gbq

Types of sources

I. Atomic Energy Regulatory Board (**AERB)** -on the basis of **relative radiotoxicity per unit activity**)

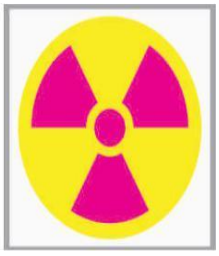
Group I (e.g. Po210, Th230, Ra226.)	**Group II** (e.g. I125, Co60, Na22),
Group III (e.g. P32, S35, C14)	**Group IV** (H3, O15, U235).

ii. Forms -

a. **Sealed** sources. b. **Unsealed** sources.

Radiation symbol

The symbol should be accompanied with appropriate legend in English, Hindi, Local language)- indicating radiation hazard and restricted entry, **e.g. CAUTION-RADIOACTIVITY**



RADIATION DOSE and DOSE LIMITS

Absorbed Dose (DT, R) -

The amount of energy absorbed per unit mass of the medium at the point of interest. The SI unit of dose is **Gray(Gy). 1 Gy = 1 J Kg-1**

Tissue weighing factor (wT)

Based on the extent to which the risk from stochastic effects in a tissue/organ may contribute to the total risk from stochastic effects, a weighting factor, called the tissue-weighting factor, wT is assigned to each tissue/organ.

Equivalent Dose (HT)

The sum equivalent of the product of the absorbed dose and tissue weighing factor (wR). The unit for equivalent dose is **Sievert(Sv).**

Equivalent dose in Sv = equivalent dose i.e. **HT = ΣR DT, R wR** = **(Dose in Gy) x (wR) J Kg-1**

Effective Dose (E)

This is defined as **E = ΣT HT. wT Sieverts; where wT**
represents the contribution of tissue T to the total risk due to stochastic effects resulting from uniform irradiation of whole body.

Application	Annual Dose Limit	
	Occupational	Public
Effective Dose	20mSv per year (average over a defined period of 5 years with no more than 50mSv in any single year)	1mSv per year
Annual equivalent Dose		
Individual organ		
• eye lens	150mSv	15 mSv
• skin	500 mSv	50 mSv
• hands and feet	500 mSv	-
Equivalent Dose		
• Pregnant woman	2mSv for the surface of the abdomen and 0.05 ALI for intake of radionuclides after declaration of pregnancy upto termination of pregnancy.	

Protective measures are optimized following the ***A**s Low **A**s **R**easonably **A**chievable **(ALARA) principle.*** This appropriate social-economic consideration should be factored in optimizing the level of protection.

PREVENTIVE MEASURES- working with radiation

Unsealed radioactive sources must not be manipulated with unprotected hands. Laboratory coat, hand gloves and safety glasses must be used while working with unsealed radioactive substances.

Equipment, tools or glassware used in radioactive areas must be marked/identified and must not be taken out of the radioactive laboratory.
The source must be handled in secondary containers to contain spills.

Work surfaces must be covered with absorbent material to soak up minor spills. Same must be considered as radioactive waste after use.
Shielding must be provided as near to the container of radioactive substances as possible.

Wet operations must be used instead of dry ones. Frequent transfers must be avoided.

Care must be taken not to contaminate objects needlessly, in particular, light switches, taps, door knobs, etc. The gloves should be either taken off or a piece of non-contaminated material (tissue paper) should be used for the same.

The gloves must be worn and removed in a manner without contaminating the inner side and the hands.
Mouth pipetting must not be done in the laboratories.

Precautions must be taken to avoid punctures or cuts while handling/manipulating radioactive isotopes.
Any person who has a wound or abrasion below the wrist must not handle radioactive isotopes.

The following items must not be introduced into the radiation facility.
Food or drinks, Books/note pads, Handkerchiefs.
Disposable paper towels and paper handkerchiefs must be used in the laboratory.

Hands must be washed thoroughly before leaving the laboratory with special attention to nails, in between fingers and outer edges of the hands.
Hands, shoes and clothing worn must be monitored for contamination before leaving the lab.

Radiation Protection

Time – Lesser time of exposure to radiation, better is the protection**.**

Distance – More the distance, lesser is the exposure, therefore more is the protection.

Shielding – Use of specific material (**lead boxes** for **sealed sources(SS)** and **lead vials** for storage and transport of **unsealed sources(US)** & **Perspex** shielding in **work area** is mandatory**.** The **thickness** of shields varies with respect to (w.r.t) to different types of radiations, ensuring better protection. Light metals like aluminum are preferred for shielding beta particles as they produce less **bremsstrahlung** radiation. Use of gloves, eye shield and lab coat is **mandatory** for handling sources.

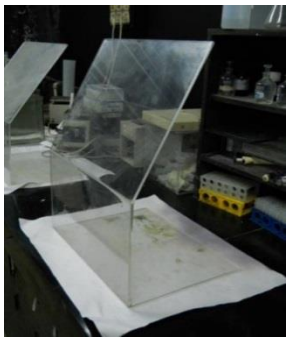


Fig: Shield in work area

Working with Unsealed sources- User Responsibilities

1) The user needs to make a count of the work area and background before and after the experiment and make a log book entry each time when he/she will be doing their experiments. This will be counter signed and maintained by the RSO.

2) The instrumentation of the facility (scintillation counter, radiation monitor etc.) should be used carefully , so as not to contaminate them.

3) All users need to be very careful and avoid any kind of spillage as we are dealing with unsealed sources where the chance of radioactive contamination (both external and internal) is quite high and alarming. Wearing aprons, gloves and using eye glass for shielding body parts and minimum exposures should be strictly practiced.

4) The user needs to make a count of the work area and background before and after the experiment and make a log book entry each time when he/she will be doing their experiments. This will be counter signed and maintained by the RSO.

5) The RSO will be performing periodic swipe tests and keep a record in accordance with the guidelines of AERB. The RSO will maintain the storage record, usage record, waste disposal record with the help and cooperation of the users

6) All radioactive users are to inform the RSO immediately if there is any mishap, without panicking. The RSO will guide the user and help the user through the necessary steps for cleaning and decontamination.

DETERMINATION OF RADIATION EXPOSURE

Determination of radiation exposure

1.Personnel monitoring – This is done by wearing TLD badges & comparing the effective luminescence against a control badge.

2.Area monitoring – This is done with radiation monitor, and by taking periodic swipe tests, with respect to the background.

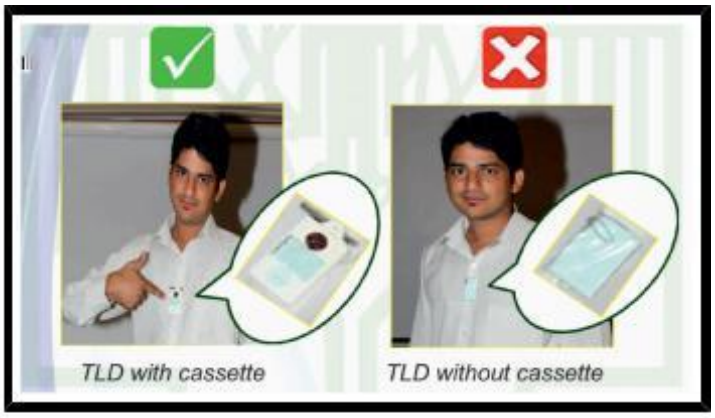


Fig: Thermo luminescent badges (TLD) and Radiation workers showing the right way to wear a TLD badge

RADIATION MONITORING INSTRUMENTS - based on their applications

1. Area monitoring instruments,
2. Portable survey instruments and
3. Personnel monitoring instruments.

Four basic types of radiation measuring *(monitoring)* instruments used in research labs

- Dose rate meters
- Dosimeter
- Surface contamination meters:
- Airborne contamination meters

Radiation monitoring instruments has the key components:

- Detector
- Amplifier
- Processor
- Display

RADIOACTIVE WASTE DISPOSAL AND RECORD MAINTENANCE

The method of disposal should be such, that it does not increase any type of radioactive contamination into the environment.
For the waste generated (solid and liquid) should be mentioned in the log book, and a protocol of sequential storage must be followed.
This requires designated three compartmental enclosed shielded waste disposal areas, within the facility, namely

a) Working Discard,

Fig: Waste disposal box

b) Decaying,
c) Decayed.

Protocol of periodic storage and shifting of waste from a. to b. and then to c. to be followed. Finally, the waste from c. after the time period of 2-3 months (or when it has insignificant activity as background whichever is quicker), will now be treated and discarded as normal waste (solid).
For unsealed liquid waste, a similar protocol should be followed as mentioned, before draining it out under running normal tap water.

Before final disposal of the solid waste, by conventional methods, a record must be maintained by the radioactive users.

Waste disposal in an emergency

Wastes generated can be of the following types

Solid waste

- Solid waste includes filter papers, contaminated glass, plastic tips, hand gloves, etc.
- The waste must be stored in a Perspex box. Based on the half-life, after the level of radiation is reduced below permissible limits, same can be disposed of as normal waste
- Wastes with a longer half-life must be stored until they are handed over to statutory body. **Liquid waste**
- If a solution contains low activity below the permissible level, it may be disposed of in drain followed by a large flushing of water. Statutory norms must be followed for the same.
- Active sources, active spills, etc., must be collected in polyethylene carboys for disposal.
- All containers used for storing radioactive waste must be properly labeled.
- Any swabs collected from users in health emergency will be treated as radioactive waste.

ADVERSE EFFECTS OF RADIATION

1. In research applications, radio nuclides in unsealed forms are used, which may enter the body through -injection, -inhalation, -untreated wounds, -absorption by skin

2. Accumulation of **internal contamination** depends on
    - deposition of the absorbed radio nuclides presents in air and water,
    - rate of intake and metabolism.

3. Effect of radiation on cells
    (a) inhibition of cell division
    (b) chromosome aberrations
    (c) genes mutation
    (d) cell death

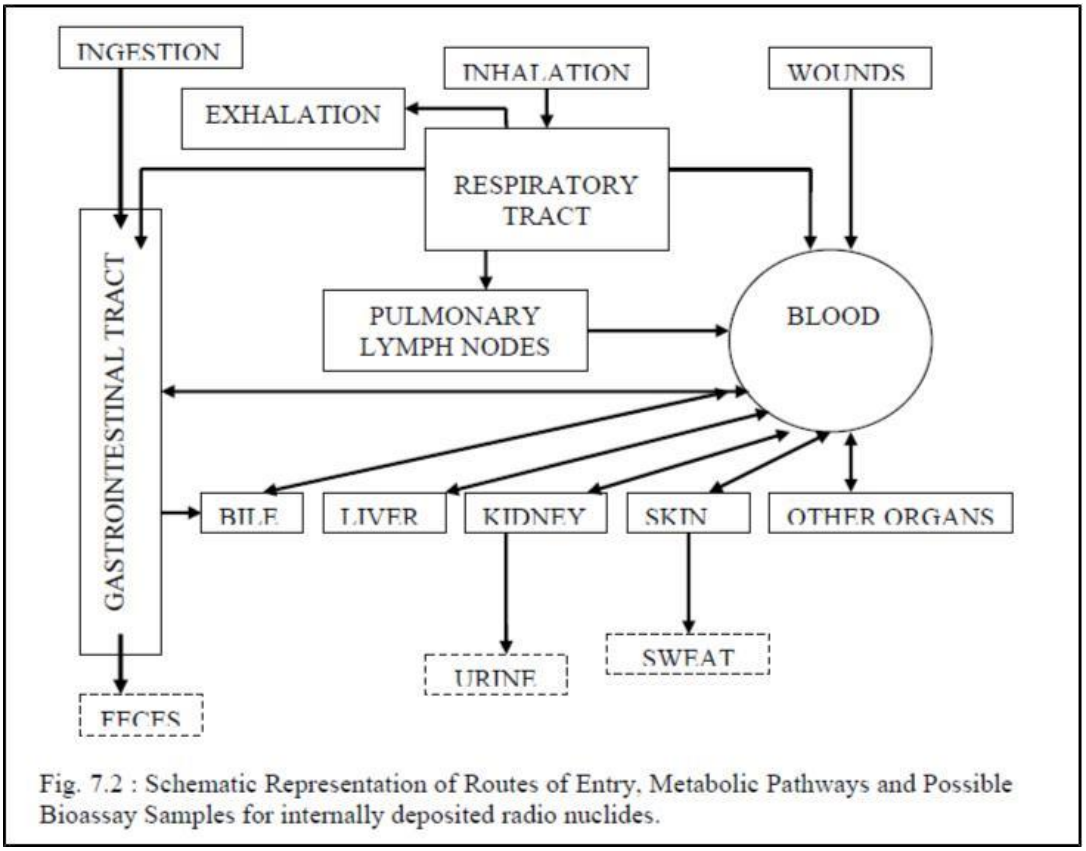


Fig. 7.2 : Schematic Representation of Routes of Entry, Metabolic Pathways and Possible Bioassay Samples for internally deposited radio nuclides.

EMERGENCY PREPAREDNESS

a) Charts, which detail various steps to be taken by a radiation worker, in case of a radiation emergency, should be conspicuously displayed in the laboratory.

b) All the radiation monitoring and measuring instruments should be checked routinely and kept always in working condition.

c) The ventilation system of the radioisotope laboratory should be checked periodically and maintained properly.

d) A kit comprising of accessories like tongs, forceps, waste receptacles etc. which are required for the decontamination operation should be available readily to handle an accidental spillage.

e) A proper inventory of radioisotopes received, used and disposed should be maintained.

EMERGENCY RESPONSE

FIRE	Steps to be taken to **restrict radioactive contamination** in the **accidental** area like not opening of the windows, etc. - The **workers/public** should be made aware immediately. - The area should be **restricted to access**. - **Inform** adjoining laboratories. - Break glass of manual call point and **activate** the **fire alarm**. - **Evacuation** should be done following standard **protocols** of the **fire dept**. - The **fire fighting** personnel should be made aware there is **radiation**
THEFT	• All **sources** should be kept **under lock and key** in a **monitored area**. • Location of radioactive substances or sources is often more quickly done using a suitable detection instrument rather than by visual inspection. • **Area** should be **restricted** to authorized personnel only. • Efficient monitoring by **installation of cameras** at the **entrance** should be practiced. • In case **theft** happens, it should be brought to **notice** of the **RSO**, **facility-in-charge FIC)**, & the Chief security officer (**CSO)**. When the RSO and the committee confirms that there are reasonable grounds for believing that a source or radioactive substances have been lost or stolen then the • a. The RSO will inform Head, Radiological Safety Division, Atomic Energy Regulatory Board (AERB) and the police as soon as possible of the suspected theft or loss; and • b. All reasonably practicable steps will be taken forthwith to recover the source. An investigation must be carried out into how the source was lost or stolen. • If the source is not recovered, its loss should be recorded on the appropriate radiation databases. • The RSO, the Faculty-in-charge, the Licensee, the convener of institute radiological safety committee, (Institute laboratory safety committee) should be informed, who will decide to inform the AERB and take necessary steps.
HEALTH External-	The **immediate washing** with water and soap gently, must be done for removing loose contamination. Sequential washes to be followed with effective drying and **monitoring** without causing any skin damage and internal contamination. In case of contaminated small open wounds or cuts, the wound must be immediately washed and bleeding encouraged and medical attention must be sought.
Internal	The purpose of treatment in case of an internal contamination will be to eliminate much of the contaminant still remaining in the mouth, gastro-intestinal or respiratory tract as fast as possible. Internal contamination requires special medical advice and supervision. All should be brought to notice of the RSO.

Radiation incidents involving contamination, with no immediate personal injury

In case of spills of radioactive substances

The spill should be contained and prevented from spreading by taking appropriate measures such as placing absorbent pads available in the laboratory on and around the spill. The surrounding areas should be immediately monitored using a survey meter to determine the extent of contamination.

The workers are instructed to evacuate the laboratory / area. Then go for monitoring for personal contamination (checking clothes, hands and feet). Decontaminate contaminated personnel in a safe area. (See decontamination notes below).

Contaminated clothing or materials used to contain spills or clean contaminated areas must be placed in a polythene bag, which must be marked "radioactive," tagged and placed in the decay store for disposal.

The workers in the immediate vicinity of the lab must be informed and made aware of the incident.

The spreading of contamination into the environment should be prevented (via inappropriate drains, under doors and via river courses etc.).

Turn off appropriate lab services, close windows and doors.
Lock the lab or erect a barrier to prevent further access.
The incident / accident must be reported, in writing, to the Head, Radiological Safety Division, Atomic Energy Regulatory Board (AERB), government of India by the RSO in charge.

Further notes on Decontamination

Damp, but not dripping, paper swabs should be used first with soap and water, followed by detergents such as 1% Decon 90 (contrad: 2%-5% KOH) in water, if time permits.

- Ensure the skin is not broken by excessive rubbing during these procedures or contamination may be pushed deeper into the skin or the body.
- Soap and water, or if necessary detergent or EDTA soap, should be used to remove any contamination of the hair.
- Great care is needed when decontaminating the face to ensure that active liquid does not touch the lips or enter the eyes.
- Any contamination of the skin surrounding the eye should be removed before irrigation using water or eyewash.
- If the skin is actually broken in the area of contamination, the wound should be allowed to bleed, within reason and irrigated immediately with tap water or a saline solution, taking care to limit any spread of contamination on the skin.

- Both the area of the wound and the object which caused it should be monitored for radioactive contamination.
- It may be necessary to lay a mat of paper towels on the floor to absorb any spillages and prevent the floor from becoming slippery.

Recovery plan for the decontamination of the laboratory area, fixtures or fittings

- Permission and direction from the RSO will direct the recovery phase of the contingency plan.
- Persons must wear appropriate Personal Protective Equipment (PPE) according to the Local Rules. A full contamination survey must be mounted using the appropriate monitors. In the case of H-3 smears must be taken and read by Liquid Scintillation Counting.
- Starting from the outer edge, decontaminate the area removing heavy contamination by blotting paper or absorbent tissues, then by wiping and scrubbing with detergent and water. If it is suspected that contaminated chemicals are hydrophobic, caustic, toxic, flammable or emit a heavy vapor then a new risk assessment and further written instructions must be drawn up.
- Monitor all persons and equipment involved in cleaning. Any contaminated equipment should be placed in polythene bags which must be labeled as radioactive.
- Contaminated equipment and materials that cannot be decontaminated must be tagged and placed in the decay store for disposal as radiation waste.

Incidents and radiation accidents involving personal injury with contamination

The treatment of serious injuries must take precedence over decontamination and containment of contamination. Give first aid. Call for first aid/ ambulance (Ph no.: 9836267955/8145549528, medical officer :03364510543, Nursing Assistant :9002232022 /9836249346).

- Warn everyone in the area and control movement and the spread of contamination.
- Contact the RSO, and Faculty in charge.
- Remove contaminated clothing carefully so as not to spread contamination.
- Washing must be done gently without causing any damage to the skin.
- After washing for a few minutes, the skin must be dried and monitored.
- Washing may be repeated if necessary without causing skin damage.
- Use of organic solvents or alkaline solutions must be avoided.
- While washing hands special care must be taken to ensure proper cleaning of finger nails, interfinger space, folds and the outer edges of the hands.
- While washing care must be taken to prevent contamination of uncontaminated parts or internal contamination.
- If there is a chance of spreading to other areas, the contamination can be removed locally by
- using an absorbent and also by covering the uncontaminated area.
- Open wounds on the skin must be protected.
- After each decontamination, the affected part must be dried with a fresh non-contaminated tissue and monitored. Same shall be treated as contaminated waste.
- While decontaminating face, care must be taken not to contaminate the eyes and lips.
- In case of contamination of eyes, the eyes must be irrigated with saline or clean water.
- If contamination has entered the mouth collect sputum in a suitable container.

- Wash the skin with mild soap and water, use only mild abrasion wash. Repeat only three times. Monitor after each wash.
- Secure the entrance to the laboratory and attempt to identify a 'clean pathway' for first aider / Ambulance personnel. Make overshoes, gloves available to anyone entering the area.
- Monitor patient for personal contamination, if contamination persists, cover the contaminated area prior to the arrival of the ambulance.
- Retain all swabs, sputum samples and items of clothing place in a suitable bag or container and label.
- The RSO will inform and make a report to the Head, Radiological Safety Division, AERB.

Emergency Equipment

An emergency box must be available in rooms where work on radioisotopes is carried out (3.02). The minimum contents of this box should be:
Soft Nail brush
Washing up liquid Tissues
Eye wash bottle (check date) Cotton wool
Disposable Paper Overall Overshoes
Absorbent paper Refuse bag
Scouring pads (Scotchbrite) Radiation Tape
2 x Radioactive waste bags 2 x general purpose rubber gloves2

Definition of a major incident
A major emergency is defined as an incident involving activities in excess of those listed below.

Sl.no	Unsealed radioisotopes used at DBS, IISER-K	Activity used	Biologically active radioisotopes that might cause a major emergency
1.	32P,35S	250µCi , 500µCirespectively	3.7 x 108 Bq (10mCi)

INCIDENT REPORTING FORM

Employees/researchers/users should understand that the purpose of reporting and documenting accidents is not to affix blame, but instead to determine the cause of the accident so that similar incidents may be prevented in the future.

Use this form to report all accidents

PART I

When did it happen?	Date:	Time:
Where? Place:		

PART II

What happened and how it was happened:

PART III

About the injured person (if no one injured go to IV):

Injured person's full name:

Address/ Contact information:

Age:

Gender: Male/Female

If the injured person was employed by someone else at the time of the accident what is the name and address of his / her employer:

PART IV

Any witnesses to the accident:

PART V

Recommendations to prevent similar accident.

Describe any action, which you recommend to prevent similar accident in the future:

Details about the person who complete this form:

Name:

Contact Information:

(Department, Telephone No and Email ID):

A separate form to intimate the AERB within 24 hours is available presently in eLORA of AERB as the incident reporting from.

Procurement procedures for labeled bio molecules

ORDERING

- The Faculty/PI will send/mail the requisition to the RSO with a general overview of the exp. mentioning the radioisotope and activity that is required

- The compliance of the radioisotope and it's activity will be checked with the present license for the institute.

- The filled up B1 form (picture given) is submitted by the RSO (the consignee) to the Faculty/PI.

- The Faculty/PI needs to sign the form and forward to the DORD for his signature. The RSO will forward a copy (soft copy) of the finally signed form to the Faculty/PI.

- The Faculty/PI concerned needs to attach the current license copy for the institute and place the order via mail to Jonaki, BRIT keeping minimum of 4-5 days before the dispatch date.

Collection and Storage

- After email affirmations of the despatch by BRIT, an **Authorization** for **collection** is given by the consignee.

- The source needs to be brought straight to the designated storage area in radiation facility and kept there following necessary protection**.**

  NO STORAGE IN ANY OTHER PLACE IS ALLOWED.

- The documents in the packing needs to be handed to the RSO, for inventory maintenance.

- JONAKI, REGIONAL CENTRE BOARD OF RADIATION & ISOTOPE TECHNOLOGY **(BRIT)** HYDERABAD**,** Types of bio molecules for detailed information refer to ***Appendix 1.2.***

REFERENCES

- Indian Standard (IS) 4906 -1968 Code of Safety for Radiochemical Laboratory.
- Atomic Energy (Radiation Protection) Rules 2004.
- Guidelines/Codes of Atomic Energy Regulatory Board. www.aerb.gov.in
- Encyclopedia of Occupational Health and Safety (Part–II) – International Labour Office.
- Handbook of Laboratory Safety- Norman V. Steere
- Radiation Protection in the Health Sciences- Marilyn E. Noz, Gerald Q. Maguire Jr.
- Fundamentals of Industrial Hygiene – Julian B. Olishifski
- Handbook of nuclear, biological and chemical agent exposures - Han Leikin, Jerrold McFee, Robin B.
- NCBS lab safety manual

- IIT Bombay lab safety manual
- University of Essex, laboratory safety manual
- University of Cambridge, laboratory safety manual

APPENDIX 1.1

Classification of laboratories in accordance of AERB

Table 8.3 : Classification of Tracer Laboratories using Unsealed Sources

Group of Radionuclide*	Prescribed Limits for Handling Radionuclides		
	Type I	Type II	Type III
I	< 185 kBq	185 kBq-185 MBq	> 185 MBq
II	< 1.85 MBq	1.850 MBq-1.85 GBq	1.850 GBq
III & IV	< 18.5 MBq	18.5 MBq-18.5 GBq	> 18.5 GBq

* group classification according to radio-toxicity.

APPENDIX 1.2

The list of labelled bio molecules that can be obtained from **JONAKI, REGIONAL CENTRE BOARD OF RADIATION & ISOTOPE TECHNOLOGY** (BRIT) are tabulated here.

Sl. No.	CODE	Product Description	Activity
1	LCP-101 PLC-101	**γ^{32}P-ATP**	0.25 **mCi** / 250 **µCi**
2	LCP-102 PLC-102	**α**32**P-dCTP**	0.25 **mCi** / 250 **µCi**
3	LCP-103 PLC-103	**α**32**P-dATP**	0.25 **mCi** / 250 **µCi**
4	LCP-104/1010	**A**32**P-dGTP/pCp**	1.0**mCi (**cannot be ordered for **Type 1** labs**)**
5	LCP-106	**α**32**P-ATP**	0.25 **mCi** / 250 **µCi**
6	LCP-107	**α**32**P-GTP**	0.25 **mCi** / 250 **µCi**
7	LCP-108 PLC-108	**α**32**P-UTP**	0.25 **mCi** / 250 **µCi**

8	LCP-109	**α**32**P-CTP**	0.25 **mCi** / 250 **µCi**
9	LCP-1011	γ32**P-GTP**	0.25 **mCi** / 250 **µCi**
10	LCP-32	Orthophosphoric Acid (aq) research grade	1 **mCi (**cannot be ordered for **Type 1** labs**)**

1. **LCP-101, 102,etc.** are supplied in aqueous solution containing **2-mercaptoethanol** (5mM) with concentration of 370 Mbq/ml(**10 mCi/ml**).
2. **PLC-101**, **102**,etc. Permaluci range of **32P** labeled nucleotides are colour – coded and temperature stabilized (can be transported at room temperature and stored in refrigerators).
3. **P32** labelled nucleotides are normally supplied on fortnightly basis.
4. **LCP-104 (dGTP)** and **LCP-1010(pCp)** are custom made.

5. In general 32P products are dispensed once a fortnight.
6. Request for occasionally required products LCP106 a ATP, LCP 107 a GTP, LCP 1011gGTP may please be sent 10 days in advance from the schedule date.

This list may vary with respect to current availability. It is better to kindly contact the regional center before placing the order in consultation with the RSO.