ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ

UNIVERSITY OF CRETE
DEPARTMENT OF BIOLOGY

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UNIVERSITY OF CRETE

GENERAL RULES FOR SAFE LABORATORY PRACTICE

July 2013

Introduction

This booklet points out some of the hazards which are faced by people working in the Department of Biology, it gives the rules which have been established to minimize these hazards and it explains the procedures to be followed if accidents or emergencies occur.

A copy of this booklet must be kept inside every laboratory at all times and is also accessible from the Department's web site. All students, visitor scientists and members of staff must read this document on their first day at work in a laboratory of the Department of Biology.

Much of this manual deals with the obvious dangers of harmful chemicals, radioisotopes, biological materials and fire but most accidents involve more ordinary dangers (*e.g*. broken glass, splashes of corrosive liquids, slipping on wet floors) which should be avoided by using care and common sense rather than a rule book.

Please, therefore, always:
In situations where specific rules apply, R*EAD THE RULES, REMEMBER THEM AND FOLLOW THEM.*

In all situations, *WORK CAREFULLY AND AVOID ENDANGERING YOURSELF OR OTHERS*.

In situations where accidents could occur, *BE PREPARED IN ADVANCE TO DEAL WITH THEM.*

If an accident does occur, *REPORT IT IMMEDIATELY TO THE APPROPRIATE PRINCIPAL INVESTIGATOR (PI) OR SAFETY OFFICER*.

Management Responsibilities

PIs are responsible for the health & safety of staff in their lab by ensuring

- safe working practices i.e.
  - hazards are identified
  - risk control measures are properly maintained
  - procedures are monitored and reviewed
- competence of staff (see also Staff Training)

with advice from the Departments' Safety Officers.

It is not an offence to have an accident but it is an offence to ignore it or not to know. All accidents or potentially hazardous incidents must be reported to the PI and Safety Officers.

Penalties
A serious offence may invoke the disciplinary procedure.
Otherwise a less serious offence may result in a penalty, agreed by the Head of the Department, the PI and the Safety Officer after interviewing the offender.
First offence - verbal warning from the PI.
Second offence - ban from relevant experimental work for proscribed period and/or invoking the disciplinary procedure.

Staff Training

PIs or designated supervisory staff should arrange for all new staff, students and visitor scientists, to receive basic Induction Training in general laboratory practice.

Additional training by experienced personnel is required for the correct use of equipment and, in particular, the use of centrifuges. Further training by experienced personnel is also required for the correct use of specialised areas (e.g. dark rooms, tissue culture suite, microscopy suite, animal house), radioactivity or high risk chemicals.

This Safety Manual is issued to all new staff. All personnel must then sign an Induction Training Form (copy attached) which must also be signed by the appropriate PI and kept in a safe place.

Security of premises

Protection against theft
There were numerous incidents of theft in offices and laboratories in the past, both during and outside working hours. The risk of intrusion and theft can be reduced by **keeping office areas locked at all times**. In the laboratories, make sure **you place personal high risk items** (e.g. handbags, wallets, mobile phones, cameras) **in drawers or cupboards and keep them out of sight at all times.**

Never leave an open door or window unattended outside normal working hours (typically from 9 am to 6 pm). Close them.

If for any reason you are suspicious of any persons or group loitering inside the building or in the car park you should inform the Departments' security guard (ext. 4150). Never put your personal safety at risk by approaching such groups.

Stray Dogs and other Animals

Stray dogs and their excrements pose a significant health hazard to cleaners, University students and staff. **Keep outside doors closed at all times** to prevent stray animals from entering the building. **Keep also the internal door** in the corridor linking the security guards office to the Departments' undergraduate labs **in the first floor of the building closed after 5 pm and at weekends** as this is also a common entry point for stray animals.

Do not feed animals inside the building!

Do not allow stray or domestic animals inside the laboratories at any time.

Do not allow stray or domestic animals inside the restaurant or cafeteria at any time! This is a serious offence and can cause health inspectors to revoke the restaurant's or cafeteria's licence.

Fire Safety

1. Fire Prevention

- Store flammable solvents in metal cabinets
- Portable gas burners should only ever be used in cleared areas of lab bench. Before lighting a gas burner **check that the immediate area is clear of flammable material or solvents**
- **Never leave a naked flame unattended**

2. Electrical equipment

DO NOT fit plugs or fuses to equipment yourself: bring to Building Services.

DO NOT overload electrical points. If you need to run more than one piece of equipment from a single point, again check with Building Services.

DO NOT leave electrical equipment running overnight, unless unavoidable. This is particularly important for high voltage sequencing apparatus, hot-plates or heated stirrers which must never be left unattended outside normal laboratory hours.

3. Smoking

- Smoking is only permitted outdoors. **Indoors smoking is prohibited by law in all laboratories, corridors, lecture theatres, offices and other University areas**. Take care to completely extinguish your cigarettes before discarding in bins outdoors.

Actions on discovering a Fire

1. Fire Alarm

If you cause or discover a fire, you **must decide** your course of action.
If the fire is very small and easily extinguished then put it out.
If the fire is too large to be quickly extinguished, or you are unsure **it is imperative** you warn your colleagues by activating the fire alarm - find the nearest **Fire Alarm Call Point** (red break-glass) and press firmly on the glass. The alarm (electronic klaxon) then sounds continuously.

All members of staff must take the time to learn for themselves the location of the fire alarm call points. These are clearly labelled in red.
If you hear the alarm, stop what you are doing and make it safe and evacuate the building by the nearest exit (see below). Tell anyone you see still working to evacuate the building too. **Finally remember to phone the Security Guard (39 4150) or the fire brigade (199).**

2. Fire Exits

DO NOT leave clutter or obstruct access to fire exits. When you hear the fire alarm, you **should immediately make safe whatever you are doing, close the door of your office or laboratory (if applicable), and evacuate the building, quickly and calmly, by the NEAREST fire exit** (unless blocked by fire). Walk around the outside of the building to the assembly point at the car park between the Departments of Biology and Chemistry. Assist anyone having difficulties evacuating the building. **DO NOT delay your departure by collecting personal belongings. DO NOT use lifts. DO NOT return to the building until the alarm stops or the Fire Brigade Officer has stated that it is safe to do so**.

3. Fire Extinguishers

Fire extinguishers are serviced once per year by the Building Services (Tehniki Ipiresia). Water fire extinguishers are suitable for paper, cardboard and wood fires. Foam fire extinguishers are suitable for liquid fires. CO2 and dry powder fire extinguishers are suitable for all fires including electrical. Most fire extinguishers in the Department of Biology are CO2 but please check the instructions before using.

Personal Safety and Good Laboratory Practice

1. Laboratory Areas

- Eating, drinking or storing food is forbidden in laboratories or adjacent desk areas unless separated from the lab area by a wall or partition.
- **Smoking is strictly prohibited in all laboratories**.
- Minors should not enter laboratory areas. Untrained adults should only enter laboratory areas under supervision of a trained individual.
- Keep your workplace clean and free of clutter.
- Always wash your hands thoroughly before leaving the laboratory area.

2. Personal Protective Equipment

- **Wear a lab coat** and take it off before leaving the lab. Remember to have your lab coat washed from time to time!
- Wear gloves when handling chemicals. Remove them to avoid spreading contamination (for example before handling telephones, light switches, computer keyboards, elevator buttons, etc).
- Wear safety glasses when handling concentrated corrosive or volatile acids, organic solvents, concentrated bases, liquid nitrogen or radioactivity.
- Always wear a lab coat with sleeves rolled down, protective mask for your face AND a pair of UV-grade safety goggles for your eyes when exposed to UV radiation.
- Do not wear open-toed shoes when handling corrosive acids or concentrated bases.

3. Instruments

- Read the instruction manuals carefully. Do not use any instrument before given instructions from a trained user.
- Strictly follow the safety requirements given by the manufacturer (for example always balance rotors during centrifugation and never interrupt centrifugations by immobilizing rotors manually).
- **Mouth pipetting is strictly forbidden.**

4. Various Hazards

- Be careful when using liquid Nitrogen or dry ice as contact may cause burns.
- Be careful when inserting glass pipettes into a mechanical pipetboy. There is danger of glass breaking and injury.
- Glass, broken or not, syringe needles and blades should be disposed of in special sharp bins.
- Familiarise yourself with the position of first-aid kits, fire extinguishers and fire exits closest to your working area.

Handling Chemicals

1. Classification of Chemicals
Chemicals are rated according to their hazardous properties to human health and assigned a COSHH (Control of Substances Hazardous to Health) risk number.

- Chemicals with risk number **0 (zero)** are not considered hazardous.
- Chemicals with risk number **1 (one)** are corrosive, harmful by indigestion, skin contact or inhalation that can cause organ damage or burn. However, exposure to those chemicals doesn't carry a risk of inducing chronic effects.
- Chemicals with risk number **2 (two)** are poisonous and have *a possible risk* of irreversible chronic effects (for example infertility, mutagenesis or organ failure) when inhaled, ingested or absorbed through the skin.
- Chemicals with risk number **3 (three)** are poisonous/ carcinogens/ teratogens and have *a known risk* of causing systemic effects, reproductive disorders, mutagenesis or human carcinogenesis when inhaled, ingested or absorbed through the skin.

The hazardous properties of every chemical are described in its material data sheet (MSDS). All laboratory chemicals have to be considered dangerous but **extra care must be taken when handling COSHH3 chemicals**. If you have no knowledge on the exact hazardousness, please consider the chemical COSHH3-dangerous!

A list of commonly used chemicals and their risk number can be found in **Appendix I** and is accessible through the Department of Biology web site. It is important this list is kept updated.

2. Storage
- Harmful chemicals should be stored inside closets and not on bench shelves.
- Flammable liquids should be stored in metal safety cabinets and volatile chemicals in hood cabinets.
- Concentrated acids should be kept separately from bases.

3. Use of Chemicals
- Wear lab coat, gloves and never mouth pipette.
- Work in the hood and wear protective glasses when using **concentrated corrosive or volatile acids** (e.g. hydrochloric, sulphuric, nitric, phosphoric, glacial acetic acid), **organic solvents** (e.g. chloroform, ether, isoamyl alcohol, phenol, formaldehyde, dimethylformamide), **reducing agents** (e.g. 2-mercaptoethanol) and **concentrated bases** (e.g. NaOH, KOH).
- Wear protective respiratory mask when weighing fine powdered chemicals such as SDS or nutrients.

4. Chemical Waste
4.1 COSHH 0 and 1 Chemical Waste
- Liquid waste can be safely poured down the laboratory sink with plenty of water. This includes the majority of day-to-day used buffers.

- Solid waste such as plastic pipettes, tips, gloves, paper towels, etc is discarded in regular bin bags.

4.2 COSHH 2 and 3 Chemical Waste

- Concentrated COSHH 2 and 3 organic solvents (such as phenol, chloroform, formaline, formamide, glutaraldehyde, isoamyl alcohol and solutions of more than 16% formaldehyde) should be collected in special recipients and removed from the Department of Biology by qualified personnel.
- Working solutions of COSHH 2 and 3 chemicals should be aspirated in 10% v/v chlorinated water for at least 30 min before being poured down the laboratory or fume hood sink with plenty of water.
- Plasticware containing more than 100 µl of concentrated COSHH 2 or 3 organic solvents (for example eppendorf tubes with small amounts of phenol) or paper towels used to mop small spillages of concentrated/ stock COSHH 2 or 3 chemicals should be disposed of in designated bins for incineration. Tips, gloves and other plasticware containing only traces of COSHH 2 or 3 chemicals can be disposed of with regular waste.
- Ethidium bromide is a COSHH 2 carcinogen. International regulations allow disposal in the laboratory sink of liquid waste containing less than 10 µg/ml ethidium bromide and in regular waste of gels containing less than 0.1%. Those concentrations are within the range routinely used in the laboratory. Higher concentrations of ethidium bromide should be disposed of as previously described for COSHH2 and 3 chemicals. **Use of SYBR green (Invitrogen) as a DNA stain is recommended as it cuts down the need to use ethidium bromide.**

5. Spillages

Before attempting to clean-up a spill, the lab responder must put on splash goggles, lab coat with sleeves rolled down and nitrile or neoprene gloves in good condition.

Clean-Up Procedures

- Control the source of the spill
- Absorb any free liquid
- Remove broken glass
- Neutralise spill residues with spray neutralizer and decontaminate the area with soapy water
- Package all spill residues in heavy duty plastic bag and seal.

Handling Biological Material

Biological agents are placed into 4 Biological Safety Levels.

Level 1: unlikely to cause disease
Level 2: can cause disease and may be a hazard to users but are unlikely to spread to the community and there is usually effective prophylaxis or treatment available
Level 3: can cause serious human disease, may be serious hazard to users, may spread in community but there is effective prophylaxis.
Level 4: causes severe human disease, serious hazard to users and no effective prophylaxis available.

Only agents in Levels 1 and 2 are handled in the Department of Biology.

Bacteria and yeast

Laboratories in the Department of Biology are suitable for working with Biological Safety Level 1 agents. These are well-characterized biological agents not known to consistently cause disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment, such as yeast, non-infectious bacteria and *Escherichia coli*.

Safety considerations

- Work is generally conducted on open bench tops using standard microbiological practices.
- Precautions against the biohazardous materials in question involve wearing protective lab coat and gloves and washing hands before leaving the laboratory.

Cell lines

Primary and continuous cell lines are classified as Biosafety Level 2 organisms, even if they pose no direct threat to humans. This designation is used to limit the release of genetically modified organisms into the environment. **Personnel must receive training in handling cells by qualified scientists.**

Safety Considerations

- Assume all cultures are hazardous since they may harbour latent viruses or other uncharacterized organisms.
- Wear protective lab coat and gloves.

- A Category II biological safety cabinet (laminar flow hood) should be used for culturing, treating and manipulating cell lines. The cabinet protects the worker by preventing airborne cells and viruses released during experimental activity from escaping the cabinet; there is an air barrier at the front opening and exhaust air is filtered through a HEPA filter before returned to the room.
- Decontaminate work surfaces with 70% ethanol before and after use.
- Use aseptic technique.
- Do not use open flames inside Tissue Culture hoods! This is unnecessary since the laminar flow already provides a sterile environment. Furthermore, open flames might damage the hood's HEPA filter and are hazardous when handling flammable liquids such as 70% ethanol!
- Immediately mop any spillage of medium (even if cells were not growing in it) and disinfect with 70% ethanol.
- Flasks and dishes contaminated with bacteria should be discarded immediately and NOT be opened in tissue culture areas or hoods.
- Wash hands after handling cultures and before leaving the laboratory.

Other Level 2 organisms

These are biological agents of moderate potential hazard to personnel and the environment. This Biosafety Level includes amphotropic viruses and *all* eukaryotic viruses (wild-type, mutant, recombinant) that are not Level 3 or higher (see also below) and work must be conducted in a **Category II biological safety cabinet.**

Safety Considerations

- **Laboratory personnel must receive specific training in handling pathogenic agents by qualified scientists.**
- Procedures in which infectious aerosols or splashes may be created should be conducted in a **Category II biological safety cabinet**.
- **Extreme caution should be taken with contaminated sharp items**.
- Wash hands after handling agents and before leaving the laboratory.

Biological waste (Levels 1 and 2)

- Solid waste (e.g. tips, eppendorfs, plastic tubes, Petri dishes, flasks, paper towels, gloves, etc) should be discarded in available autoclave (blue) bags and sterilised by autoclaving before final disposal.
- Liquid waste including remaining bacteria or cell cultures should be aspirated in chlorinated water for at least 30 min before being poured down the laboratory or fume hood sink with plenty of water.
- Contaminated glassware should be soaked in chlorinated water for at least 30 min and rinsed thoroughly with water prior to being washed and reused.

Biological agents of Levels 3 and 4

These are organisms (bacteria or viruses) of high hazard to laboratory personnel and the environment. **Work with such agents is NOT permitted in the Department of Biology of the University of Crete**, subject to establishing specialised facilities and operation procedures in conformity with international safety regulations. An indicative list of such biological agents is shown below.

Biosafety Level 3 agents
These are biological agents which may cause serious or potentially lethal disease after inhalation. This Biosafety Level includes various bacteria and viruses that can cause severe to fatal disease in humans, but for which vaccines or other treatment exist, such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, West Nile virus, Venezuelan equine encephalitis virus, Hendra virus, SARS coronavirus, *Salmonella typhi*, *Coxiella burnetii*, Rift Valley fever virus, *Rickettsia rickettsii* and yellow fever virus.

Biosafety Level 4 agents
These are exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and cause severe to fatal disease in humans for which vaccines or other treatments are *not* available, such as Bolivian and Argentine hemorrhagic fevers, Marburg virus, Ebola virus, Lassa fever, Crimean-Congo hemorrhagic fever, and other various hemorrhagic diseases.

Working with Radioactive Isotopes

It is imperative the user is suitably trained and their practice approved by the PI or the Department of Biology Radiation Officer. Radioactive work is only permitted in specific areas designated by representatives of the Greek Atomic Energy Commission. Some general guidelines are also provided below:

1. General Guidelines

- It is imperative to use protective glasses (or face shield), labcoat and resistant gloves. For radioactive material emitting radiation to a distance (like P32), use a protective Plexiglas shield. It is also recommended to strictly follow standard laboratory safety rules when using radioactive isotopes and avoid leaving the laboratory area during the experiment.
- Transferring isotopes and radiolabelled material should be done only with a mechanical pipette and NEVER practice mouth pipetting.
- All laboratory areas (hoods, benches, refrigerators, etc) where radioactive material is used or stored should be checked regularly and thoroughly. Any contamination should be removed promptly. Glassware, plastics or other objects that come in contact with radioactive material should be cleaned thoroughly or marked with a radioactivity label to exclude any other use.
- The use of radioactive material should be confined to the smallest possible area to simplify protection and cleaning. The area should be kept tidy and free from unnecessary objects. It is imperative to cover all working surfaces with special absorbing, waterproof material.
- In case of extensive contamination, a cleaning procedure with an appropriate detergent (DECON) is followed and care is taken to prevent transfer of the contamination to other parts of the area and covering-warning of possible spots not yet sufficiently decontaminated. NEVER use DECON for contaminated parts of your body (follow the first aid instructions)
- It is necessary to label every laboratory area designated for use and storage of radioactive material or waste.
- It is imperative to monitor your area BOTH BEFORE AND AFTER working with radioactivity for spillages using a scintillation counter and clean up any contaminations.
- In labs working with radioactivity, lab areas and working surfaces should periodically be examined for possible contamination.

2. Radioactive waste

The P32 and S35 solid waste are disposed of in special barrels in the basement area of the Department of Biology. Liquid waste is disposed of ONLY in designated sinks. For the disposal of other isotopes (such as C14, H3 that are rarely used) specially labelled recipients exist in the basement area of the Department. Small amounts of Iodine 131 like the ones used to measure thyroid hormones by commercially available kits are disposed of in the sink with plenty of tap water. For further information please consult with the Department of Biology Radiation Officer.

Accident Procedure

Following an accident that results in:

- **superficial contamination of the skin**.
  The affected area should be washed with soap and running water, with gentle scrubbing.
- **contamination of the eye.**

The eye should be washed rigorously - keeping the eyelid open - for at least 5 minutes.

- **contamination of nose or mouth**.
  They should be washed out with copious amounts of tap water.
- **breakage of the skin.**
  The wound should be encouraged to bleed, and the area washed with soap and water but without scrubbing. The wound should be covered with a waterproof dressing.
- **thermal burns.**

Do not remove burned and stuck clothes. Wash the burn with abundant cool water. If the skin remains intact, apply special ointment or spray and a loose bandage.

- **chemical burns.**

Remove the clothes from the region of burn. Wash with abundant cool water under moderate pressure for at least 10 minutes. Do not rub the region.

- **contact with high doses of a chemical substance.**

If **swallowed,** you should induce vomiting and seek hospital treatment.
If you have a **skin** spill wash rigorously with clean water.
If the substance has been **inhaled** move the individual to an open air space.

- **haemorrhagic trauma.**

If the trauma has been caused by glass, remove the splinters that have not been inserted into the wound, disinfect and bandage the region.
If there is severe bleeding, pressure should be applied to interrupt the blood flow. You should then seek medical attention.

- **contamination with radioactivity.**

Wash the contaminated region with abundant water and seek for medical assistance.

In any case, after first aids, you should visit your nearest hospital.

Safety Forms

Department of Biology, University of Crete
Induction Training Form

Name of New Staff/ Student/ visiting scientist: _____
_____ **Group Leader (PI) :** _____
I have received brief verbal induction training. **Signature:** _____
I have received a Safety Manual. **Signature:** _____
I have read, understood and agree to abide by the local rules detailed in the Safety Manual and Appendices. **Signature:** _____
New Staff/Graduate Student/ Visiting Scientist **I confirm that the above named new staff/graduate student/ visiting scientist will have training needs identified, and appropriate training provided, ensuring competence to work safely in the Department of Biology within the local rules.** **Group Leader's Signature:** _____

Date: _____

Useful Contact Numbers

Security Guard
Department of Biology (39 4150)
IMBB (39 1168)

Building Services ("Tehniki Ipiresia")
39 4008, 39 4007

Department of Biology Health and Safety Secretary
Ms Georgia Papadaki (39 4400)

Department of Biology Safety Officers
Dr George Zachos (39 4365, 39 4380)
Dr Dimitrios Tzamarias (39 4057, 39 4050)

Department of Biology Radiation Officer
Dr Ioannis Vontas (39 4077, 39 4438)

Police
100

Fire Department
199

Ambulance
166