

# **Safety Regulations**

**for work in the laboratories  
at the Department of Molecular Biology and Genetics**

**Department of Molecular Biology and Genetics  
Aarhus University**



**2014/2015**

**A copy of this booklet must be available in each laboratory**

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# 1) Introduction

Everybody working in the laboratories at the department must familiarise themselves with the contents of this booklet with safety regulations. They are intended to help staff, students and guests by giving some general or special guidelines for safe work in the laboratory.

It is impossible for this booklet to cover everything, since many different techniques are being used. It is therefore the duty of every group leader (lecturer/professor) and supervisor to provide instructions for the safe use of special techniques. Furthermore, it is the duty of everybody working in the laboratory to seek the information required to work in a safe manner.

The safety instructions are useless unless they are respected by everybody. In this context, it must be stressed as a general principle that

- ***a lab coat must be worn for all laboratory work - this is an absolute demand from the Danish Working Environment Authority (“Arbejdstilsynet”).***
- ***everybody finding a room or a piece of equipment that does not meet the safety requirements must take action immediately so that the situation can be remedied.***

In some situations, it may be something simple so that you can do it yourself, e.g. wiping up a spill. In more complicated situations, help should be sought from one of the persons responsible for the room or the apparatus or one of the Work Environment Representatives.

Please note that if you call from a landline phone at Aarhus University, you need to press 0 before the number to get a line out of the house.

*All information in this booklet can also be found on the department's website <http://mbg.medarbejdere.au.dk/en/work-environment/>.*

This edition of the safety regulations is a thoroughly revised version of that from 2008. The regulations are updated to new practices and rules and regulations at the department, and a number of new sections on for example laboratory practices and equipment have been added. Finally, the order of chapters and sections are adjusted to achieve more consistency. The revision work has been carried out by Karen Marx, Dorthe Bødker Jensen, Niels Sandal, Mogens Duch, Magdalena Pyrz, Ulla Birk Henriksen, Tinna Stevnsner, Michael Bjørn and Dorthe C. Riishøj, who are also available for comments and amendments.

Modification and translation of the Danish version of October 2014 by Lisbeth Heilesen.

## 2) First aid

### The four main steps of first aid:

1. stop the source of the accident
2. give life-saving first aid
3. call for any help needed
4. give general first aid

### Call for help:

Dial 112

When you are connected, state clearly:

- where the accident has happened
- what has happened
- how many persons are injured
- who is calling
- from where the call is being made

Make sure that the Emergency Service is met at the entrance to the building and is informed about all the details.

Inform an Occupational Health and Safety (OHS) representative/supervisor

### Burns:

- Immediately wash the burned area with cold water
- Remove loose clothing from the burned area
- Continue washing while someone else fetches a bowl of tepid water (22- 23oC)
- Dip the burned part into the tepid water and keep it there until the pain disappears for at least half an hour
- Cover the affected area with a cold wet compress and take the victim to the hospital's casualty ward.

### Frostbite:

Frostbite falls into three categories:

*First degree burns:* Produces white numb areas on the skin

*Second degree burns:* Produces white and harder tissue in a large area, often blistering while thawing.

*Third degree burns:* Produces white and hard tissue that when tapped sounds like tapping a piece of wood, in the worst case scenario. The tissue is dead and becomes spongy while thawing.

In all categories, first aid involves immersion in 38 degrees warm water. Be aware that this can be very painful. When the skin becomes red, a sterile bandage should be applied, carefully avoiding any pressure or cooling. For second and third degree burns, seek a doctor.

### Corrosive substances

#### Internal:

- Do not try to induce vomiting
- Give plenty to drink (milk or water)
- Call an ambulance or take the victim to the hospital's casualty ward. Bring information about the corrosive substance (name, chemical formula, and container).

**External:**

- rinse immediately with plenty of water
- remove clothing - continue washing for 10 min.
- if pain continues, continue washing for a further 10 min.
- if there is no improvement, take the victim to the hospital's casualty ward, by ambulance if necessary. Bring information about the corrosive substance, (name, chemical formula, and container).

**Corrosive substances in the eye:**

- rinse immediately with plenty of gently running water
- rinse from the base of the nose outwards
- continue for 5 min.
- always consult a doctor afterwards.
- bring information about the corrosive substance (name, chemical formula, and container).

See also the section on the use of the eye wash bottle.

**Poisoning****When the victim is conscious:**

- if the substance involved is neither an organic solvent nor a corrosive then vomiting can be induced by sticking a finger down the victim's throat, possibly after giving a drink of water.
- place the victim in a recovery position.
- take the victim and the vomit to a casualty ward, by ambulance if necessary

**When the victim is unconscious:**

- place the victim in a recovery position.
- call an ambulance. Provide information about the poison (name, chemical formula, and container).

**First Aid Supplies, gas mask and heart defibrillator**

A First Aid box is to be found on each floor of buildings 1130 and 1131, in each of the teaching laboratories in building 1120, and on each floor of the buildings in the Science Park. Make a note where they are to be found *before* you need. Inform your Occupational Health and Safety representative/supervisor if something is missing from the box.

**Remember:** *all accidents, both big and small, must be reported to an Occupational Health and Safety representative/supervisor.*

In the "Biokæden" (campus) buildings a health and safety station with a heart defibrillator and a gas mask against toxic organic fumes are located on the 3<sup>rd</sup> floor of building 1130.

In the Science Park, health and safety station is found in building 3130, just outside the luncheon room.

### **Use of eye rinsing bottles**

These are found in all laboratories.

- First make sure that the solution is clear and the bottle is sealed.
- Unseal by turning the eye cup.

*Alone:*

- bend yourself over the bottle
- open the eye wide with the thumb and index fingers
- press the cup carefully against the eye while the eye is still open
- rinse with plenty of eye rinse by pressing the bottle repeatedly

*With a helper:*

Some serious injuries result in a reflex eye closure so that the victim is unable to rinse the eye effectively. Assistance will be necessary.

*With a standing or sitting patient:*

- the helper opens the injured eye with the thumb and index fingers
- the eye cup is held a hand's width from the eye
- rinse liberally by pressing on the bottle

*With patient lying down:*

- the eye cup is held a hand's width from the eye
- rinse with plenty of eye rinse by pressing the bottle repeatedly

**The solution in the eye bottle must always be sterile (see instruction on the bottle).**

Efficient eye washing can also be carried out by attaching a piece of tubing directly to a tap. There must always be a piece of tubing attached to at least one tap in a laboratory.

*Please note: Make sure that the eyelid is washed thoroughly, too!!!*

### 3) Extinguishing fires and evacuation

- 1) At "Biokæden": Activate the internal fire alarm located in the corridors (tilt safety lid and press the button).  
In the *Science Park*: Activate evacuation
- 2) Call the fire department by dialling 112 and give them one of the following addresses:

**Fire at Aarhus University**

C.F. Møllers Allé 3, building 1130, 1131, 1134 or 1135.

**Fire at Aarhus University**

C. F. Møllers Allé, building 1120

**Fire in the Science Park**

Gustav Wieds Vej 10C, building 3130, 3131, 3132, 3133, 3134 or 3135

**Fire in the Science Park Pavilion 3142 or Pavilion 3120**

Gustav Wieds Vej 10B

Be ready to give information about **any victims, what is burning, and the telephone number** from which you are ringing.

Extinguishing fires is the job of the Fire Dept., but it is important that certain precautions are taken before the Fire Dept. arrives, in order to minimise the risk to personnel and to limit the burning area.

- 3) Start an evacuation if relevant:  
<http://medarbejdere.au.dk/en/administration/hr/workingenvironment/emergency/evacuation-at-au/>

Take the evacuation package hanging by the elevators ("Biokæden")/in each building (the Science Park) and distribute the safety vests and follow the instructions in the material.

**Please note.** It is extremely dangerous to enter into rooms filled with smoke. Leave this job to the Fire Department's specially trained persons.

- 4) Call AU's alarm number 87151617

An emergency shower is found above the door in most laboratories  
A fire blanket is found just outside all laboratories  
CO<sub>2</sub>- extinguishers and hand pump/fire hoses are found on all floors.

***Make a note of where these things are located and how to use them, before it is too late!***



## 4) Good laboratory practice

### General rules for work and tidiness

- It is compulsory to wear a lab coat in all experimental laboratories.
- Do not begin an experiment until you have collected everything you need (substances and apparatus).
- All smelly and dusty work and cleaning of the used equipment must be carried out in the fume cupboard.
- Follow all instructions closely. Any deviation should only be made after consulting the supervisor or instructor.
- Keep continuously updated logbooks on all lab work.
- Water pumps must NOT be used for suction filtration. Instead use membrane pumps or vacuum pumps with a trap.
- Instructions for use and function should be found on each fume cupboard.
- It is forbidden to eat, drink or smoke in the laboratories.
- Keep the laboratory clean and tidy.
- Put small apparatus back in its place when not in use. Keep the floor free of apparatus, boxes, waste, etc.
- Put containers of chemicals and bottles of reagents back in place after use.
- Keep the fume cupboard clean and tidy.
- Cans and bottles with inflammable liquids (solvents) must not be placed on the bench or on the floor. They must be stored in a special cupboard or cupboards with ventilation.
- Water spills must be wiped up immediately to avoid the risk of slipping.
- Spilled chemicals must be cleared up immediately and disposed of according to the instructions given.
- Glassware must be cleaned as quickly as possible after use before being sent for dishwashing.
- The laboratories must be left clean and tidy after work.

### Preparation for laboratory work

It is important that lab work is carefully prepared, with regard to both safety and the end result. A continuously updated lab logbook is an invaluable and indispensable tool in this respect.

An evaluation of the risk and safety precautions is an important part of the preparation for your work. For example, it can be necessary seek information on the properties of a substance: State, reaction with water, combustibility (kindling temperature, explosion limit), caustic and corrosive properties, odour, toxic properties and especially long-term effects, penetration of rubber and plastic (gloves) its possible hygienic threshold.

For many known chemical reactions and products, much of this information is unavailable. Only typical physical and chemical properties are registered. However, various types of literature have articles describing substances that are hazardous to health and the environment.

When preparing for lab work (whether experimental or routine) each person must consider the risk involved.

- As a result of the physical, chemical and toxic properties of the chemicals, substances and compounds that are to be used or that can be formed.
- As a result of special characteristics (e.g. heat production) for the reaction or procedure that is being followed.
- As a result of the apparatus construction to be used.
- Search the literature for any missing information.
- Note down any relevant information in the laboratory logbook with regard to risks and precautions.
- Whenever alternative procedures are possible, then the least risky must be used.
- When a huge risk cannot be eliminated, consider dropping the experiment. If this is not possible, the project leader should carry it out or should watch over the procedure.
- The scientific staff member who starts up a project (project leader) must be acquainted with the health and environmental risks involved. Furthermore, the project leader is responsible for informing all persons (staff and students) involved of these risks so that they can take the necessary precautions before the project is started.
- Be prepared to give first aid to yourself and to others in the event of an accident or incident.

### **Fume cupboards and how to use them**

All work with substances and reactions that give rise to hazardous or malodorous gases or vapours must be carried out in a fume cupboard. As a general rule, the fume cupboard should be used for all forms of chemical work whenever possible.

The degree of safety provided by the fume cupboard depends partly on its technical and construction conditions and partly on personal and actual conditions, namely:

- The type and amount of the substance being used.
- How the user handles the substance and how the fume cupboard is being used.
- The set-up of apparatus or other kinds of hindrances to the flow of air inside the fume cupboard.
- The temperature inside the fume cupboard.

### **The following rules apply when using a fume cupboard:**

- After opening the fume cupboard check that air is being extracted and that the alarm works. Be wary of a possible failure.
- Always have the least possible opening. It is not always possible to keep the fume cupboard completely closed when working in it.
- Respect the alarm. When it sounds, make sure to find out what is wrong.
- Keep your face (respiratory zone) over the lower edge of the fume cupboard window.
- Apparatus should be placed at the back of the fume cupboard and as far as possible from the side walls. Large apparatus that can interfere with the flow of air should be raised ca. 5 cm.
- Avoid rapid movements when working and when opening the fume cupboard. Make sure to button your lab coat. Do not have windows open and avoid moving quickly past these as this increases the risk of contamination.
- Keep to the general safety rules for working with inflammable material when working in the fume cupboard. An open flame must not be used.

- Keep the fume cupboard clean and tidy. Clear up and wipe the floor of the fume cupboard. It must not be used for storage of for example chemicals.
- In the event of any failure that is likely to be a safety risk, all work must be stopped immediately. Inform the management, the Occupational Health and Safety representative/supervisor or the departmental safety organisation about the incident.
- Equipment with heating must always be placed on a lift table so that heating can be turned off safely.
- Every three months, an own-check must be conducted of each fume cupboard and entered into the logbook or on form kept by the fume cupboard. The own-check includes control of the suction of the fume cupboard by putting a piece of paper on the edge of the fume cupboard, control of alarm with light and sound and clean-up.

### **Fume cupboard alarm:**

The Danish Working Environment Authority ("Arbejdstilsynet") demands that fume cupboards be fitted alarms that are activated when the airflow is inadequate.

Each fume cupboard has its own alarm, which sounds and shows a red light when the airflow falls below a certain level.

When a fume cupboard is closed there is still a slight suction. When the window is opened, the airflow is increased, but when the height of ca. 40 cm is reached, the alarm is activated. Even before this height is reached, air movement around the opening can reduce the effectiveness of the air flow, especially if more than one fume cupboard in a laboratory is open at the same time, as there is a limit to the total capacity for suction.

### **Working outside normal working hours**

- No one who is alone in the building is allowed to carry out experimental or work that carry a risk factor. When working alone with non-risk procedures, you should ensure that at least one other person knows where you are.
- When laboratories or other rooms are left for the night, weekend etc., all windows must be closed and lights turned off.
- Any alarm system must be activated. Electrical apparatus that are not in use should be unplugged and all gas and water taps turned off (also permanent cooling devices).
- If it is necessary to have an apparatus running overnight, then cooling connections and all tubing must be fastened securely. Electrical systems must be secured against any unforeseen temperature rise that could cause a fire.
- The responsibility for ensuring that all the safety rules are followed lies with the person who set up the experiment.

## 5) Personal safety equipment

This section mostly deals with the protection of eyes, skin and respiratory organs.

### Safety goggles and face mask

It is mandatory to wear safety goggles or a face mask when working with liquid nitrogen. It is also required to wear safety goggles or a face mask when working with anything that can splash when boiling, or can splinter, when working with strong acids, bases or radioactive materials.

*When wearing contact lenses:* be extremely careful when working with strong acids, bases or poisonous solutions. If any of these substances come into contact with the eye they may come underneath the contact lens and damage the eye. Therefore, always work in the fume cupboard and wear safety goggles. In the event of an accident, it is extremely important to remove the contact lens so the eye can be thoroughly washed.

Safety goggles are found in various qualities and sizes. They must have side protection so that there is less likelihood of particles coming into the eye from the side. Some have adjustable side lengths, some are adjustable up or down so that they fit the individual. Usually they are made from strong plastic material (e.g. polycarbonate) - their weakness being that some organic solvents can dissolve the surface so they become opaque.

### Gloves

Safety gloves are used when needed to protect the hands from substances that can damage the skin, either directly or by penetrating the skin and cause damage elsewhere.

### When should gloves be used?

- Wearing gloves is absolutely necessary whenever there is a danger for skin contact with hazardous substances (e.g. when cleaning up a spill, when the hands are dipped into a substance, when hands are in contact with skin-penetrating vapours, when there is a risk of spillage etc.).
- Wearing gloves is also necessary when skin contact with experimental solutions can be harmful for the experiment itself (transfer of microorganisms, proteases, nucleases or other skin-borne enzymes).
- In other situations one should consider whether it is at all necessary to wear gloves, since they retain moisture so that the skin becomes overheated and its pores open up. Cotton gloves can be used as under gloves to absorb the moisture. Some glove material can give rise to eczema or allergy (especially latex).
- When working with solids, the cheapest disposable gloves can be used as solids do not penetrate. However, this is assuming that the gloves are not wet and that one is not working with solvents at the same time.

### Penetration times

- Not all gloves are equally resistant to all substances and materials.
- Penetration times provide data showing how much time can elapse from the first contact until the first traces of the substance getting through the glove.
- Penetration times are provided by the glove producer. Notice that penetration times often refer to pure substances and not blends.
- It is always necessary to know penetration times when working with liquids. When working regularly with a special blend, it is possible to test the penetration time by using a special glove tester. Several authorised advisory bodies offer this service.
- See Aarhus University's (SvF AU) "Glove database" ("Handskedatabase")

- See also “Quick selection guide to chemical protective clothing”, Krister Forsberg, S. Z. Mansdorf, Fourth edition.

### **Provisions and precautions**

- Use only gloves approved by DS/EN 374-3. Approval means that the gloves have been tested for one or more chemical with regard to penetration and that it is possible to obtain test data from the producer.
- Preferably use disposable gloves that can be thrown away if they come into contact with a chemical or when the penetration limit has been reached.
- Use gloves that are not powdered (allergy risk). The powder (cornstarch) does not itself cause allergies, but can be an irritant and carry possible allergy-causing molecules from the glove material.

N.B. There may be a risk of allergy even from powder-free gloves.

### **General advice on using gloves**

Before putting on gloves:

- hands should be clean and dry.
- avoid wearing rings inside gloves.
- the gloves should be intact
- when working with liquid chemicals make sure you know the penetration time for the gloves you are using. This is calculated from the first contact with the substance.
- if the gloves are to be worn for more than 15 min, inner cotton gloves will help to absorb moisture from the hands.

### **Gloves must be changed:**

- if they break, are torn, etc.
- before the penetration time has been reached, even if the gloves are intact
- if gloves become dirty on the inside (often with short cuffs)
- when inner gloves become wet
- after work and before breaks, etc.

Always wash hands when changing gloves.

### **Good hand hygiene**

- Good hand hygiene is extremely important as dry and cracked hands increase the risk of picking up substances and materials that can cause eczema and allergic reactions.
- Wash hands thoroughly and often.
- Dry hands carefully and rub in a nourishing hand cream.
- If one type of glove gives problems, change to another type or to another size.

See local policies on the website:

<http://mbg.medarbejdere.au.dk/en/work-environment/leaflets-rules-databases-etc/policy-for-gloves-and-lab-coats/>

**Breathing**

Only under very special circumstances, e.g. in the event of accident, will it be necessary to use respiratory aids (gas or dust masks). Respiratory organs are primarily protected by avoiding situations likely to give rise to hazardous gases, vapours or dust, such as working in a fume cupboard.

In “Biokæden” (campus) a gas mask against toxic organic vapours is located on the 3rd floor, bldg. 1130 and at the 4th floor in bldg. 1130.

In the Science Park it is found in bldg. 3130, 1st floor.

## 6) Gas cylinders, liquid nitrogen, dry ice

Gas cylinders with pressurised gas are frequently used in all types of laboratory work and carry many risk factors. Damage to a gas cylinder can cause it to explode because the gas is under high pressure. A broken valve can result in such a violent rush of gas that the cylinder becomes a projectile. The escaping gas from a damaged cylinder or a badly conducted experiment can cause an explosion and fire, poisoning, corrosion or choking, depending on the type of gas. A list over the most commonly used pressurised gases is found below:

The pressure at 20°C in the cylinders we use:

<b>150 – 200 atm</b>	<b>10 – 60 atm</b>
Hydrogen	Carbon Dioxide (56 atm)
Oxygen	
Nitrogen	
Helium	

Working with and storing liquid nitrogen and dry ice presents the risk of frostbite (N<sub>2</sub>, -196 °C; CO<sub>2</sub>, -78°C) and choking (1 L liquid nitrogen at 20°C and 1 atm will fill ca. ¾ m<sup>3</sup>). Carbon Dioxide has also a physiological effect and in concentrations of 10–20% lead to instant death. Liquid nitrogen is often used in cooling traps and can result in condensation of atmospheric oxygen inside the trap as well in the liquid nitrogen tank. This oxygen can cause violent explosion in contact with oxidising substances, e.g. organic compounds.

- Gas cylinders must be transported on a trolley and must be locked with a chain.
- Gas cylinders must not be moved when the reduction valve is in place. The protective cover must be in place when moved.
- Both empty and full gas cylinders must be secured against falling whenever they are used or stored.
- Gas cylinders must not be subjected to knocks or strong, especially exposed heating (sun, radiators, etc.)
- Gas cylinders must not be opened with heavier tools than those recommended.
- Gas cylinders must be protected against backward suction from wash bottles and reaction containers by inserting a safety trap.
- A triangular warning sign with the text “Gas cylinders to be moved in the event of fire”, must be found wherever gas cylinders are used or stored.
- Safety goggles or a face mask must be worn when drawing off or pouring liquid nitrogen.
- Liquid nitrogen and dry ice must not be transported in a manned elevator. Danger of choking! Neither should these substances be transported in a closed car.
- First Aid for frostbite and choking: See section on First Aid.

## 7) Inflammable liquids

*Liquid:* Substance that is fluid at normal temperatures and pressure.

*Kindling temperature:* The lowest temperature at which a liquid gives off flammable vapours.

*Inflammable liquid:* Liquid with a kindling temperature below 100°C.

*Class I:* Inflammable liquid with a kindling temperature below 21°C.

*Class II:* Inflammable liquid with a kindling temperature of 21-55°C

*Class 3:* Inflammable liquid with a kindling temperature above 55-100°C

All three classes are divided up into *sub-Class I* for liquids that are *not* water miscible under all conditions, and a *sub-Class II* for liquids that are water miscible under all conditions.

Class	Storage amounts	Max. storage in	
		glass	approved plastic or metal container
I	1 L	2,5 L	no limits up to 25 L
II	5 L	5 L	no limits up to 125 L
III	50 L	10 L	no limits up to 1250 L

Plastic containers over 125 ml **must always** be approved by the Danish Emergency Management Agency ("Beredskabsstyrelsen").

The given amounts refer to the **total amount of stock, usage and waste**.

Altogether, there should be no more than 50 storage units per laboratory

Containers with inflammable liquids of Class I-1, I-2, II-1 and III-1 **must not** be placed in any of the escape routes (corridors, stairways, etc.).

Further information: Guidelines#14 July 2010 from the Danish Emergency Management Agency ("Beredskabsstyrelsen") ([www.brs.dk](http://www.brs.dk)).



## 8) Inflammable and explosive chemicals

Do not work close to an open flame or where there is a risk of sparks.

*Please note:* Explosive substances, e.g. diethyl ether and petroleum ether **must not** be stored in a normal refrigerator.

Name	Kindling point ( °C)	Group	Source
Acetylaldehyde	-38	I-2	c
Acetone	-19	I-2	a
Acetonitril (= methylcyanide)	2	I-2	c
iso-Amylalcohol (= iso-entylalcohol)	18	I-1	b
Benzene	-11	I-1	a
n-Butanol	29	II-1C	c
2-Butanol (= sec.butanol)	24	II-1	c
tert-Butanol	11	I-2	c
iso-Butanol	27	II-1	c
Butylacetate	22	II-1	a
n-Butylchloride	-7	I-1	b
Carbon disulphide	<-20	I-1	c
Cellosolve (=2-ethoxyethanol - ethyleneglycol mono-ethylether)	40	II-2	c
Cyanbrinte (= Prussic acid)	-18	I-2	a
Cyclohexane	-18	I-1	b
Cyclohexanone	43	II-2	b
Diethylamine	<-20	I-2	c
Diethylether	-45	I-1	a
N,N-Dimethylformamide	58	III-2	b
Dimethylsulfoxide (DMSO)	95	III-2	b
Dioxane	12	I-1	c
Acetic acid, conc.	40	II-2	c
Acetic anhydride	49	II-2	c
Ethanol	13	I-2	*
Ethylacetate	-4	I-1	c
Ethylalcohol	12	I-2	a
Ethylenechlorohydrine	55	III-2	c
Ethylenediamine	34	II-2	c

Continued

Name	Kindling point ( °C)	Group	Source
iso-Hexane	<-20	I-1	c
n-Hexane	-22	I-1	c
n-Heptyl alcohol (= 1-heptanol)	< 21	I-1	a
Methanol	11	I-2	a
2-Methoxyethanol (= ethylenglycol-monomethylester = methylcellosolve)	37	II-2	c
Methylisobutylketon (= MBIK)	14	I-1	c
Mineral turpentine	< 60	III-1	a
Nitrobenzene	88	III-1	c
iso-Octane	-12	I-1	a
Pentane	<-20	II-1	a
1-Pentanol	38	II-1	a
Propanol (= 1-propanol)	22	II-2	b
iso-Propanol	12	I-2	a
iso-Propylether (= di-iso-propylether)	22	I-1	b
Pyridine	17	I-2	c
Styrene (= vinylbenzene)	32	II-1	a
Tetrahydrofuran	-17	I-2	a
Toluene	6	I-1	a
Trichlorethen (= trichlorethylen = "Tri")	32	II-1	a
Triethylamine	-7	I-2	c
Trimethylamine 30% in water)	<-30	I-2	a
Xylene ( <i>o</i> -, <i>m</i> - and <i>p</i> -)	25-30	II-1	a

The information given above comes from the following sources:

- a: Kemikalier og Sikkerhed, Teknisk Forlag, 1976.
- b: Merck Index, 11<sup>th</sup> edition, 1989
- c: Hommel's Handbuch der gefährlichen Güter, 1973/74.

See also the KIROs database (<http://www.kiros.dk/W/>)

## 9) Chemicals

Before starting to work with chemicals, you must seek information about their hazardous characteristics, e.g. whether they are inflammable, poisonous, caustic or have long-term effects. For labels and instructions for use and guidelines from work place and distributors, see the KIRO database.

### Working with chemicals

Avoid contact with chemicals in all situations where chemicals are handled: Weighing out, pouring, routine lab work, transporting, cleaning up and disposal of waste.

- Avoid any contact of chemicals and solvents with the skin or eyes.
- Wear lab coat, gloves and safety goggles.
- Avoid breathing in chemicals and vapours. Always work in the fume cupboard.
- Weighing out of chemicals must always be carried out in a fume cupboard or point suction.
- Wipe up any spills immediately.

### Storing chemicals

- Chemicals must be stored in closed, clearly labelled containers: Name, formula, melting point/boiling point.
- Possible hazardous characteristics: Explosive, inflammable, self-igniting, water or air sensitive, caustic, poisonous, allergenic or carcinogenic.
- Solutions in ether and other volatile solvents can only be stored in the refrigerator when explosion-safe. Beakers must not be used. Use only closed flasks and bottles.
- Only small amounts of inflammable chemicals/solvents can be stored in the laboratory.

### Transporting chemicals

- Chemicals transported out of the laboratory must be in closed containers. Glass containers must be carried in a carrying basket or on a trolley.
- Specially volatile, fuming, caustic, inflammable and explosive chemicals must not be transported in a manned elevator. This applies for example to volatile solvents, liquid nitrogen, dry ice and fuming acids.

### Chemical spillage

- Spilled chemicals must be wiped up immediately. Liquids are absorbed by porous material (cat litter, sand, vermiculite, etc.) after neutralising and /or diluting with water. Spilled powder can be dusty to wipe up, therefore use suitable personal protection must be used. For disposal, see under the section Waste Disposal.
- Cat litter and chemistry sorb sponges can be found in the common storage room in the Science Park, building 3131. At “Biokæden”, 3rd floor, building 1130 and at the laboratory technician office at the 5th floor in building 1131. In the teaching labs, they are located in building 1120
- Contaminated clothing should be changed as quickly as possible. Shoes, watch straps, etc. that have absorbed liquids should be removed immediately.

Contact your local Occupational Health and Safety representative for guidance.

## 10) Peroxides and other unstable substances

For substances or containers with possible explosive qualities, age, storage temperature, light and air can be crucial for stability, so it is of utmost importance that these substances are not bought and stored in large quantities.

The Danish Emergency Management Agency ("Beredskabsstyrelsen") has complete information on peroxides and formic acid.

If in doubt about high peroxide contents then be very careful about carrying and opening the container. A simple method of checking whether there are peroxides in e.g. ether is to mix a couple of mls with a potassium-iodide solution, add a couple of drops of diluted HCl and shake. The brown colour of iodine is a sure sign of peroxide.

The peroxide content can be checked with Peroxide Strips (Merck 1.10081.0001, level 1-100 mg/L H<sub>2</sub>O<sub>2</sub>). Most peroxide-forming chemicals carry a stabiliser when delivered and chemical companies usually guarantee the shelf-life in unopened containers for three to five years from the production date. For chemicals not containing added stabilisers, there is a shorter shelf life.

Some peroxide-forming substances can reach explosive peroxide levels without a concentration of the solvent, and the general rule for substitution means that there should be a special reason for using diisopropylether.

According to the Danish "ADR" rules for transporting dangerous material, many of the ethers used routinely are classified as class 3 inflammable liquids. These rules (article 2.2.3.2.1) state that Class 3 liquids that easily form peroxides can only be transported by road when the peroxide content is no more than 0.3%, i.e. 3000mg/L. Such a high peroxide content will seldom be found in a laboratory, and unused peroxide-forming substances will normally be disposed of via the waste disposal system.

If in doubt – or it is known – that a substance has a high peroxide content (limit 100ppm), contact an Occupational Health and Safety representative/supervisor for further information, e.g. it could mean destroying the peroxides with an acid solution of ferrous-sulfate.

Containers with unstable chemicals should be labelled with date of purchase, date of opening, stability control, location, etc.

## 11) List of incompatible substances

Unless the mixing process is under control **do not** mix:

- oxidising agents and reducing agents
- acids and bases
- water-reacting substances and water.

### Oxidising agents and reducing agents

<i>Examples of the most commonly used <b>oxidising agents</b> (easily reduced substances)</i>	
Free halogens (Chlorine, Bromine and Iodine)	Chromic sulphuric acid
Perchloric acid and perchlorates	Fuming & concentrated nitric acid
Periodic acid and periodates	Chromium trioxide
Chlorates	Fuming & concentrated sulphuric acid
Hypochlorites – bromites	Chlor sulphonic acid
Peroxides – organic: Di-benzoylperoxide m-chlorperbenzoic acid	Peroxides – inorganic: Hydrogen Peroxide Sodium- & barium oxide Sodium sulphate
Bleach	Sulphurylchloride
Permanganates	Ozone
Manganese oxide	Nitrates
Di-chromates	Nitrites

### These must not be mixed with:

<i>Examples of the most commonly used <b>reducing agents</b> (easily oxidized substances)</i>	
Hydrogen chloride, chlorides	Hydrazine (-hydrate, sulphite)
Hydrogen iodide, iodides	Metallic salts (ferro-, chromo-, stamno-)
Sulphur dioxide	Metals (lithium, natrium, kalium, zinc)
Sulphites	Phenols (hydroquinine, pyrogallol)
Sodium dithionite (sodium dydrisulphite)	Aromatic amines
Organic compounds in general, especially: Methanol Ethanol Formaldehyde Acetaldehyde Formic acid	Metallic hydrides: Sodium- Lithium- Sodiumboron- Lithium aluminium

*Please note:* Concentrated or fuming nitric acid + ethanol must **not** be used for cleaning glassware because of the danger of explosion.

## Acids and bases

The warning is specially meant for concentrated acids and bases

<i>The most common concentrated acids are:</i>	
Fluoric acid and liquid hydrogenfluoride	Chlorsulphoric acid
Hydrochloric acid conc.	Nitric acid, conc. and fuming
Perchloric acid	Phosphoricacid, conc. Polyphosphoric acid
Hydrogenbromic acid, conc.	Phosphoruspentoxide
Sulphuric acid, conc	Acetic acid, conc.
Sulphuric acid, fuming	Acetic acid anhydride
Sulphur trioxide	Formic acid, conc.

### **These must not be mixed with:**

<i>The most common concentrated bases are:</i>	
Sodium hydroxide, solid form (caustic soda)	Barium hydroxide, solid form
Sodium hydroxide, 33% aqueous (lye)	Amines (e.g. triethylamine, 40% aniline)
Potassium hydroxide, solid	Ammonia (water free)
Calcium oxide	Conc. Aqueous ammonia
Calcium hydroxide	Hydrazine, hydrazinehydrate
	Salts of weak volatile acids such as: fluorides, sulphides, sulphites, nitrites, cyanides and carbonates

## Water-reacting substances and water

Water-reacting substances react radically with water, often producing a lot of heat and in many cases producing gases. When mixing, pour the substance carefully into the water – **never the other way round.**

Examples of such substances are:

<i>Produce hydrogen or hydrogen carbons (e.g. methane and butane with water) Risk of explosion and fire!</i>
Alkaline metals (Li, Na, K, Rb, Cs)
Alkaline earth metals (Ca)
Metalhydrides (LiH, NaH, CaH <sub>2</sub> , LiAlH <sub>4</sub> , NaBH <sub>4</sub> , NaAl(OR) <sub>2</sub> H <sub>2</sub> )
Metalalkyles (CH <sub>3</sub> , Li, C <sub>4</sub> , H <sub>9</sub> , Li, CH <sub>3</sub> , MgX)

<i>Produce self-igniting, inflammable or poisonous gases with water or diluted acids Risk of explosion, fire and poisoning</i>
Carbides
Silicides
Phosphides
Sulphides
Tellurides
Selenides
Arsenides
Nitrides
Acid chlorides

<i>Mix or react with water, producing a lot of heat</i>
Concentrated acids (sulphuric acid)
Acid anhydrides (sulphur trioxide, phosphorous pentoxide, acetic acid anhydride)
Acid chlorides (thioyl chloride, sulphuryl chloride, phosphor-oxy-chloride, phosphor trichloride, phosphor-pentachloride, Stannichloride, acetyl chloride, benozyl chloride)
Water-free salts (aluminium chloride, ferric chloride, calcium chloride).
Concentrated bases (solid alkaline hydroxides, lime)

When mixing, pour the chemical carefully into the water.

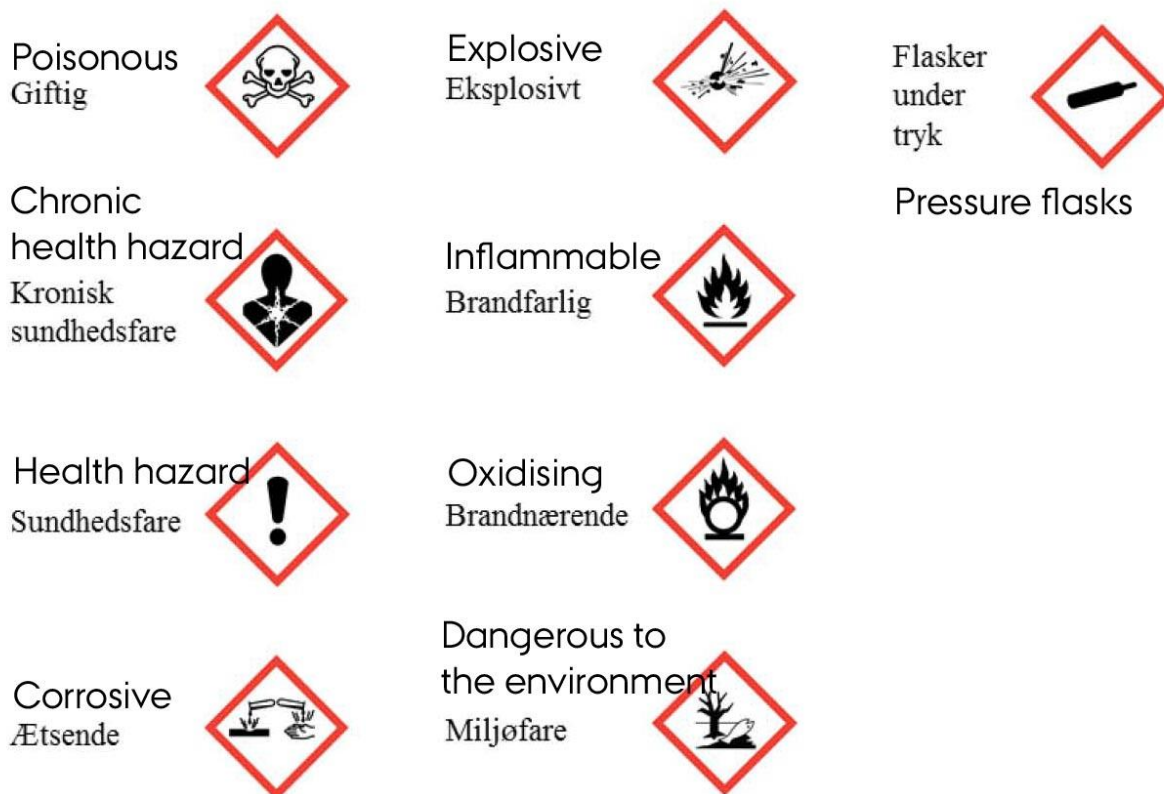
## 12) Instructions for working with hazardous substances

Hazardous substances are defined as those substances that are dangerous for health and the environment.

Information on classification and limits can be found in Danish on website of the Danish Environmental Protection Agency ("Miljøstyrelsen") under the Chemicals (<http://mst.dk/virksomhed-myndighed/kemikalier/>).

Details can be found in Kiros and in the "EU list of harmonised classifications" (see Literature).

Danger is indicated by signal words and hazard pictograms. The signal word is either "Danger" or "Warning" and may use one of the following pictograms:



It is your duty to seek all the necessary information about the chemicals to be used before starting an experiment. These can be found in Kiros and the guidelines from the distributors.

Chemicals marked with a danger symbol, such as media in powder form, can cause allergies and should always be prepared in the fume cupboard.

Moreover, those following substances marked with an H are covered by the Danish Environmental Protection Agency's rules for storage of dangerous agents and must be placed in a locked place after use: H300, H301, H310, H311, H3, H331, H340, H350, H360 and H370.



The seriousness of an injury caused by a chemical - acid, base, or poisonous - is naturally dependent upon the type of chemical, but also on how its concentration in the tissue and the length of time it has been allowed to remain there. Therefore:

- avoid contact with the skin, i.e., wear *protective gloves and a lab coat*.
- avoid inhalation of vapours, i.e., work in a *fume cupboard*.

Remember that gloves provide only limited protection. Some substances penetrate some types of gloves very quickly. There are many different types of gloves. See section on personal safety equipment or ask an Occupational Health and Safety representative/supervisor or a lab technician.

Spills on the working surface or floor must *immediately be wiped up*. Used glassware must be rinsed with plenty of water before being sent for washing.

*All scientific staff members who order or in any other way bring chemicals into the lab must check and take the responsibility for the correct safety procedures. This includes labelling, and instruction of all laboratory personnel regarding storage and use.*

### **13) Safety procedures when working with phenol**

***Injury to the skin:*** If phenol comes into contact with the skin, wash immediately with plenty of water, after which the skin must be wiped for at least 15 min with gauze or a cloth soaked in a mixture of polyethylene glycol (PEG 400) and ethanol in the ratio 7:3. This should continue until every trace of solidified phenol is removed. Wash again with plenty of water. Clothes spotted with phenol must be removed immediately. The person giving aid must wear gloves.

***Wherever phenol is used there must always be found a clearly labelled bottle of polyethylene glycol 400/ethanol in the ratio 7:3.***

If this mixture is not to be found, wash with plenty of water for at least 15 min. If the injury is of a serious nature, take the victim to a hospital and give a detailed description of the type of accident and the procedures taken.

***Injury to the eyes:*** If phenol splashes into the eyes, the phenol must be thoroughly washed away with a mixture of polyethylene glycol 400 and water in the ratio 1:1. Thereafter, wash with water for 5-10 min.

***Wherever phenol is used there must always be found a clearly labelled bottle of polyethylene glycol 400/water in the ratio 1:1.***

If this mixture is not to be found, wash with plenty of water for at least 15 min. Take the victim to a hospital, possibly to the eye department. Washing must be continued during transportation with the aid of an eye bottle, until a doctor has taken over.

***Injury to the mouth, throat, etc.:*** Phenol in the mouth should be washed out with water, and followed by a couple of spoonfuls of edible oil. Do **not** try to induce vomiting.

***A clearly labelled bottle of edible oil should always be found wherever phenol is used.***

If the victim is unconscious and is not breathing, artificial respiration should be administered. If the victim is unconscious but breathing normally, then treat according to general first aid for shock: Turn the person on one side with head lower than the rest of the body and keep warm with a blanket or coat. Unconscious persons must never be given anything to drink.

After necessary first aid, the victim should be taken to a hospital and the staff be informed about the type of accident and the procedures taken.

See also workplace guidelines for Phenol.

## 14) Safety procedures when working with acrylamide

Acrylamide is a white crystalline powder that is easily taken up through the skin, the lungs, and the wall of the intestine. Acrylamide is carcinogenic and can affect the nervous system even when only small amounts are ingested.

Acrylamide must therefore be handled with extreme caution, both in crystalline form and when in solution so that there is minimal risk for skin contact or ingestion by nose or mouth.

Therefore:

- all procedures involving acrylamide must be carried out in the fume cupboard, also when making gels.

**We suggest that all groups buy acrylamide-bisacrylamide as ready-made solutions.**

Thorough cleaning is essential so that others are not subjected to contact with acrylamide.

All glassware must be carefully rinsed.

Always use nitrile-disposable gloves (see section on personal safety equipment), also after polymerisation.

See also workplace guidelines for Acrylamide.

## 15) Safety procedures when working with ethidium bromide

From 1 January 2009, SYBR Safe is to be used instead of Ethidium Bromide – see website for further details. In special cases, if it is not possible to replace ethidium bromide with SYBR Safe, you need to get the permission from the Head of Department to use this.

Ethidium bromide is a powerful mutagen, possibly also carcinogenic, and must be handled with extreme caution. The following guidelines are suggested in order to minimise the risk involved in working with this substance.

- As a general rule, all work with this substance should be carried out in a fume cupboard or in another well-ventilated place. When transporting gels and solutions containing ethidium bromide, always use a closed container.
- Wear gloves whenever handling gels. Dispose of the gloves immediately afterwards so that door handles and suchlike are not contaminated with ethidium bromide.
- Solutions for disposal must be kept in a closed container or destroyed according to the procedure below. The methods suggested do not eliminate other methods of destruction.

See also workplace guidelines for ethidium bromide.

### **Destruction of ethidium bromide in solution:**

#### 1) Destaining Bags from CLP (VWR-Bie & Berntsen, CLP 5459.25)

- Buffer with ethidium bromide should be collected in a suitable container with the “tea bag”. Incubate overnight with stirring or on a rocking table.
- The effectivity is controlled by measuring the absorbance at 343 nm.
- The liquid can then be disposed of into the sink.
- The “tea bag” can decontaminate 1 l buffer with an ethidium bromide concentration of 0.5 mg/ml.

#### 2) Active carbon filter, Carbon Cap 75 (Frisenette APS, 67047500)

- Buffer solutions with less than  $\leq 2$  mg ethidium bromide/l can be cleansed by passing through the filter after which it can be thrown directly into the sink.
- It is a good idea to filter the buffer solution through a normal paper filter before passing it through the carbon filter, because gel pieces can clog the filter.
- Carbon Cap has a capacity of about 200 mg ethidium bromide. Measure the absorbance at 343 nm regularly to make sure the capacity is not overloaded.
- Gels are disposed of in a closed plastic bag as solid hazardous waste.

Disposal of both Destaining Bags and Carbon Cap 75 is treated as B-waste, i.e. in closed plastic bags.

In the event of large spills of ethidium bromide solutions, use absorbing material as described in this leaflet.

## 16) Instructions for working with radioactivity

Reference: "Vejledning om strålebeskyttelse ved arbejde med åbne radioaktive kilder", Statens Institut for Strålehygiejne, 2005 (Guidelines for protection from radiation while working with open radioactive sources), and "Bekendtgørelse om anvendelse af åbne radioaktive kilder på sygehuse, laboratorier m.v.", Bekendtgørelse nr. 954 af 23. oktober 2000 fra Sundhedsstyrelsen. (Act on the use of open radioactive sources in hospitals, laboratories, etc.)

Below, some general and practical advice is given concerning the handling of isotopes which are used in our laboratories at present. Whenever a new isotope is introduced, it will be included in the collection.

It is taken for granted that when working with isotopes, the safety rules which apply for working with hazardous chemicals also apply here, i.e., *wear lab coat, gloves, safety goggles* (when required), etc. Furthermore a **thermoluminescent dosimeter** (TLD/TL dosimeter) must be worn, either a personally registered dosimeter with quarterly/monthly checking, or an extra dosimeter that can be used by other persons as required and then sent for checking after use. The Government Bill 823 of 31 October 1997 form the basis for these rules. In contrast to the earlier Bill, the maximum allowed dose per person has been lowered to 20 mSv, and there are special rules for working with radioactive substances while pregnant (see page 33).

Units of Activity: 1 mCi = 37 MBq    1  $\mu$ Ci =  $2.2 \times 10^6$  dpm (decay per minute)  
1 MBq = 27  $\mu$ Ci    1 Bq = 1 dps (decay per second)

### List of isotopes being used at the department

#### Isotopes emitting $\beta$ -particles

Isotope	Maximum energy	Half-life time	Radionuclide group
$^3\text{H}$	0,018 MeV	12,3 år	4
$^{14}\text{C}$	0,159 MeV	5760 år	3
$^{35}\text{S}$	0,167 MeV	87,2 dage	4
$^{32}\text{P}$	1,71 MeV	14,3 dage	3
$^{33}\text{P}$	0,249 MeV	25,4 dage	3

#### Isotopes emitting $\gamma$ -irradiation

Isotope	Maximum energy	Half-life time	Radionuclide group
$^{125}\text{I}$	0,035 MeV	60,1 days	2

**$^{125}\text{I}$  must not be used in "Biokæden"**

After storage for 10 half-lives, the radioactivity is decreased to about 1/1000. This will often be an appropriate time for contaminated glassware and other highly radioactive waste.

$^{32}\text{P}$  waste must be stored for five months

$^{35}\text{S}$  and  $^{33}\text{P}$  waste must be stored for 30 months

$^{125}\text{I}$  waste must be stored for 20 months

After this time lapse, this waste is considered ordinary, non-radioactive waste.

In the Science Park, waste after iodination (solid in for exemple gel matrix or absorbed in cat litter or similar absorbant) must be stored in closed 1" steel pipes, which are stored for at least 20 months in the isotope waste room (3131- 0.1). It is important to note that <sup>32</sup>P-waste must not be stored in these steel pipes due to the conversion of beta radiation to brake radiation.

### Protection against radiation

For β-particles, the maximum range is dependent on the particle's energy. The particle is slowed down, and the heavier the braking substance, the quicker the particles are slowed down. Please be aware that the absorption of particle radiation in a heavy absorber gives a more powerful braking radiation than absorption in a light absorber. Plexiglas therefore offers better protection against <sup>32</sup>P than ordinary glass.

Range and necessary shielding for selected isotopes:

<u>Isotope</u>	<u>Water</u>	<u>Air</u>	<u>Shielding thickness</u>
<sup>3</sup> H	0.006 mm	6 mm	Not necessary
<sup>35</sup> S	0.3 mm	30 cm	1 cm perspex
<sup>14</sup> C	0.28 mm	24 cm	1 cm perspex
<sup>32</sup> P	0.8 cm	720 cm	1 cm perspex
<sup>33</sup> P			0-1 cm perspex
<sup>125</sup> I			3 mm lead or lead glass

The gamma radiation from <sup>125</sup>I will be halved after penetrating for example 0.2 mm lead, 5 mm aluminum or 3 mm H<sub>2</sub>O.

### Storage of radioactive substances

Radioactive material must be stored in the refrigerator or freezer in one of isotope laboratories, room 320 in building 1131, room 016B in building 1120 and room 3131-0.10 in the Science Park, and must be registered in the isotope inventory database.

The cabinets are fitted with locks where the following keys can be used:

- “BIF” key in building 1131
- A5-1 key in building 1120
- Special key supplied in the Science Park

When planning to purchase radioactive material, the purchaser is responsible for making sure that the total allowable inventory for each isotope is respected. This information can be found in the database on the department's intranet. The purchaser is also responsible for registering all new purchases in this database, and all users must make sure that they register in the database every time they use any of the stored isotope.

In ”Biokæden” the following rules apply:

Only specially authorised employees have access to room 320, building 1131. This authorisation is issued together with a special access card is given by Tinna V. Stevnsner.

In the Science Park, the following rules apply:

Larger quantities of radioactive material can be stored in the freezer in the common class B isotope laboratory (3131-0.10), which can only be accessed with a Science Park keycard where access is authorised by Niels Sandal. Smaller quantities can be stored in approved

storage freezers, etc. by the other isotope laboratories, for which a responsible superuser has been appointed.

List of people with proper instruction can be found on the department's website:  
[http://mbg.medarbejdere.au.dk/fileadmin/site\\_files/mb/internt/arbejdsmiljoe/instrueret\\_personale.pdf](http://mbg.medarbejdere.au.dk/fileadmin/site_files/mb/internt/arbejdsmiljoe/instrueret_personale.pdf)

[http://mbg.medarbejdere.au.dk/fileadmin/site\\_files/mb/internt/arbejdsmiljoe/instrueret\\_personale-biokaeden.pdf](http://mbg.medarbejdere.au.dk/fileadmin/site_files/mb/internt/arbejdsmiljoe/instrueret_personale-biokaeden.pdf)

### **Maximum limits for working with radioactive materials**

*Recognised areas (building numbers 3130 to 3134 are located in the Science Park):*

Type B isotope laboratory: 3131-0.10  
(S2-classification)

Type C isotope laboratory: 1131-320  
(S1-classification)

Conditional Type C isotope laboratory, where one must handle open radioactive sources corresponding to one 1/100 S2 (= S1 /10) authorisation:

1130-210, 1130-214, 1130-215, 1130-222, 1130-317, 1130-415, 1130-417, 1130-418, 1130-420 og 1130-428

1131-316, 1131-317, 1131-319, 1131-326, 1131-415, 1131-419, 1131-423 og 1131-531  
3131-2.15/17 og 3131-3.09

3132-0.15, 3132-0.18, 3132-1.04, 3132-1.14, 3132-2.06 og 3132-2.14

3133-2.16 og 3133-2.18

3134-0.15 og 3134-2.14

Storage rooms:

3131-2.10, 3131-3.10, 3130-0.11 og 1131-320

Waste disposal rooms:

3131-0.01 and 1130 5th floor (locked room on South side)

The isotope laboratory, room 320 in building 1131 is classified as a type C-isotope laboratory (S1). The laboratory is special in that the sink has a direct flow into the sewer. Liquid radioactive waste from all laboratories in "Biokæden" (the Biology buildings) must be disposed of into this sink.

All approved rooms must have a freely available logbook for recording control measurement results, as control measurements must be made at least once a month if isotope work is being carried out. Furthermore, each isotope laboratory must have calibrated beta and/or gamma monitors.

For the **isotope laboratory**, class B, room 313-0.10 in the Science Park (person responsible: Niels Sandal), the following *maximum limits* are applicable:

S2-isotope laboratory (class B)	$^{125}\text{I}$	$^{32}\text{P}$ , $^{14}\text{C}$ , $^{33}\text{P}$	$^3\text{H}$ , $^{35}\text{S}$
Stock (MBq / mCi)	5.000 / 135	50.000 / 1350	500.000 / 13500
<b>MBq / Ci</b> in use at any one time			
simple wet procedures	500 / 13,5	5.000 / 135	50.000 / 1350
wet procedures	50 / 1,35	500 / 13,5	5.000 / 135
procedures with dry material	5 / 0,135	50 / 1,35	500 / 13,5

<sup>125</sup>**I must not be used in “Biokæden”**

For **isotope laboratories**, class C, room 320 in building 1131, the teaching labs in buildings 1120 and 1122 and the laboratories in the Science Park, the following *maximum limits* are applicable:

S1-isotope laboratory (Class C)	$^{125}\text{I}$	$^{32}\text{P}$ , $^{14}\text{C}$ , $^{33}\text{P}$	$^3\text{H}$ , $^{35}\text{S}$
Stock (MBq / mCi)	500 / 13,5	5.000 / 135	50.000 / 1350
<b>MBq / mCi</b> in use at any one time			
simple wet procedures	50 / 1,35	500 / 13,5	5.000 / 135
wet procedures	5 / 0,135	50 / 1,35	500 / 13,5
procedures with dry material	0,5 / 0,0135	5 / 0,135	50 / 1,35

<sup>125</sup>**I must not be used in the Biology buildings (“Biokæden”)**

Tinna V. Stevnsner is responsible for room 320 in building 1131 and the teaching labs in buildings 1120 and 1122. In the Science Park, Niels Sandal is responsible.

If a higher maximum limit is required for a special experiment in “Biokæden”, it is possible to apply for dispensation from “Statens Institut for Strålehygiejne” (the Danish National Institute of Radiation Protection) to carry out this work in room 320, building 1131.

If there is a Class B or C laboratory in the building, work with S 1/100 amounts must be carried out in all other laboratories. If work involves amounts over S 1/100, then individual laboratories can apply for S 1/10 classification. An application is required for each isotope. The classified laboratories in “Biokæden” and in the Science Park have S 1/10 classification for isotopes  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ .

S1/10 = 1/100S2 laboratory	$^{125}\text{I}$	$^{32}\text{P}$ , $^{14}\text{C}$ , $^{33}\text{P}$	$^3\text{H}$ , $^{35}\text{S}$
<b>MBq / <math>\mu\text{Ci}</math></b> at any one time			
simple wet procedures	5 / 135	50 / 1350	500 / 13500
wet procedures	0.5 / 1,35	5 / 135	50 / 1350
procedures with dry material	0.05 / 1.35	0.5 / 13.5	5 / 135

<sup>125</sup>**I must not be used in “Biokæden”**



Below are examples of what can be defined as:

- "*simple wet procedures*": Extraction from stock solutions, dilutions.
- "*wet procedures*": Normal experiments.
- "*procedures with dry material*": Work involving a certain risk: chromatogrammes, evaporation, dry gels.

The word "in use at any one time" means the maximum amount of activity that can go on in any one laboratory at any given time. If several experiments are going on at the same time in the same laboratory, then the maximum amount per experiment must be reduced to compensate for an increased risk of contamination and accident.

The maximum amount of radionucleotide that can be stored in a laboratory is the same as that stated for a *wet procedure*.

## 17) Guidelines for working with isotopes

- Upon extraction from vials with rubber cap: Always insert a needle with a cotton wool in the ampoule before use to equalise pressure differences (many compounds have been on dry ice and therefore assume considerable pressure by warming to room temperature).
- General care must of course be shown. Always use plastic trays, gloves, etc. when working with isotopes.

### Disposal and cleaning-up after working with $^3\text{H}$ , $^{14}\text{C}$ and $^{35}\text{S}$

- All liquid waste must be diluted to  $<2.7 \mu\text{Ci}$  (0.1 MBq) per litre, and poured down the sink. Rinse thoroughly afterwards by letting the water run for about 5 minutes. The maximum monthly limit that can be disposed of in the sink is 13.5 mCi (500 MBq) for each per permit for  $^3\text{H}$  and  $^{35}\text{S}$  (radionuclide group 4) and max 1.35 mCi (50 MBq) for  $^{14}\text{C}$  (radionuclide group 3).
- All solid waste must be collected in the laboratory in a plastic bag inside the special container of 2 cm Plexiglas. In the Science Park, when the work is finished, the waste must be transported to the waste disposal room for radioactive materials beside the ice machine in the basement of building 3131. The plastic bag must be put in a special hazardous waste drum labelled with the name of the group, room number, and type of isotope. When the total radiation level is less than 13.5 mCi (500 MBq) the drum is disposed of as ordinary H-solid waste. Less than 0.27  $\mu\text{Ci/kg}$  (0.01MBq/kg) is considered to be inactive and can be disposed of as ordinary H-solid waste.
- Glassware and similar things used for work with radioactive isotopes must be soaked overnight in soapy water (e.g. Decon 90) and rinsed thoroughly before sending to be washed.
- Check working area at least once a month. Take a piece of wet filter paper (e.g. Whatman 3 MM, diameter 2.4 cm) and wipe this across the work top, sink, etc.
- Dry the paper and count it in a scintillation counter.
- The laboratory should be frequently checked with a monitor. A special monitor is available, which has a large sensitivity to handle large areas.
- Each laboratory must have a protocol where the results of the frequent checks are noted. Accidents should also be noted in this protocol.

### Guidelines for working with isotopes $^{32}\text{P}$ and $^{33}\text{P}$

- $^{32}\text{P}$ -ampoules must always be stored in a lead container.
- All work with isotope amounts larger than 1 mCi (40 MBq) must be carried out in an isotope laboratory, room 320, building 1131 or room 3131-0.10 in the Science Park. Handling of the isotope must take place behind a plexiglass or a similar screen. Work with small amounts of isotopes can be carried out in S1 or S1/10 laboratories.
- Always use a plexiglass tray and use nitrile gloves.
- Avoid using glass when working with  $^{32}\text{P}$ , because “Bremsstrahlung” can be formed.
- Always keep a monitor beside you.
- Check yourself and the work top with the monitor frequently, e.g. every time you leave the area.
- The whole laboratory must be checked with a monitor once a week and the results recorded in a special protocol to be found in the laboratory.
- Use a plexiglass box or lead container for transport between laboratories.

### **Disposal and cleaning-up after working with $^{32}\text{P}$ and $^{33}\text{P}$**

- *Liquid waste* must be diluted to  $< 2.7 \mu\text{Ci}$  (0.1 MBq) per litre and poured down the sink. Afterwards let water run down the sink for about 5 min. Liquid waste containing more than 0.5 mCi (20 MBq)  $^{32}\text{P}$  must be collected. The maximum monthly limit that can be disposed of in the sink is 1.35 mCi (50 MBq) per permit for  $^{32}\text{P}$  and  $^{33}\text{P}$  (radionuclide group 3).
- Liquid waste, which in addition to  $^{32}\text{P}$  contains organic solvents (phenol), should be collected in a fume cupboard (e.g. in a special plastic container together with other phenol waste) until the isotope has decayed. It is then disposed of according to the Departmental rules.
- *All solid waste* contaminated with  $^{32}\text{P}$  must be collected. Use the plexiglas containers in the isotope laboratory.

In the Science Park, when the work is finished, the waste must be transported to the waste disposal room for radioactive materials beside the ice machine in the basement of building 3131. The plastic bag must be put in a special hazardous drum which must be labelled with group name, room number, and type of isotope. In "Biokæden", both liquid and solid waste must be transported to room 320, building 1131 where solid waste is put into a blue UN-drum in the plexiglas box in the "hot" room. The person responsible for this room will transport the filled drum to the waste disposal room on the 5th floor of building 1130 and replace it with a new.

When the total radiation level is less than 1.35 mCi (50 MBq) and the radiation is under  $5 \mu\text{Sv}$  on the outer side of the drum, it can be disposed of in the usual manner for hazardous waste. Less than  $0.27 \mu\text{Ci/kg}$  ( $0.01 \text{ MBq/kg}$ ) is considered to be inactive and can be disposed of in the usual manner for hazardous waste. Very weakly contaminated things (gloves, etc.), can be thrown into the usual hazardous waste boxes. All fluid waste, marked with group name and date, must be cleared away after six months.

- *Contaminated objects* can be washed with Decon (use *only* the sink in the isotope laboratory). When there is no trace of contamination, they can be sent for washing. If there is still contamination, put them to soak in a decontamination bath overnight. If this is still not sufficient, store the objects for 10 half lives.
- *Cleaning-up* the work area: Check the work area with a monitor and decontaminate any contaminated places. Remember that an isotope laboratory does not necessarily have to be contaminated!
- $^{32}\text{P}$  waste from the isotope laboratory in building 1131 is stored on the 5th floor in 1130 in the locked room on the South side for approx. 10 half lives. The box is sent for incineration when the total radiation level is less than 1.3 mCi (50 MBq) inside and the radiation is less than  $5 \mu\text{Sv}$  on the outside.

The local Occupational Health and Safety representative/supervisor will make sure that the waste from buildings 1120 and 122 (the teaching laboratories) is correctly stored.

### **Guidelines for working with isotope $^{125}\text{I}$**

Before starting to work with this isotope you must contact the person responsible for radioactive isotopes in the various buildings to get the latest details buildings. The person responsible is Niels Sandal, the Science Park.

- All work with isotope  $^{125}\text{I}$  in its free form must take place in the fume cupboard of the isotope room (room 0.10, building 3131 in the Science Park) behind an extra screen of lead.
- One must be especially careful about personal contamination with  $^{125}\text{I}$ . Free iodine is particularly dangerous.

- Work involving use of iodinated molecules (proteins, etc.) must take place in a type B S2 room (3131-0.10) or in one of the established type C (=1/10 S1) laboratories.
- $^{125}\text{I}$ -ampoules must be stored in a lead container.
- Always have a gamma monitor standing beside you.
- It is an advantage to take iodine tablets, especially when working with free iodine.

### Disposal and cleaning-up after working with isotope $^{125}\text{I}$

- *All liquid  $^{125}\text{I}$ -waste* must be collected in bottles inside lead containers labelled "flydende  $^{125}\text{I}$ -affald" in the fume cupboard in room 3131.0.10 in the Science Park. The bottle must contain 1 M NaOH. Liquid waste containing less than 2.7  $\mu\text{Ci}$  (0.1 MBq) per litre can be thrown directly into the sink. The maximum monthly limit that can be disposed of in the sink is 0.13 mCi (5 MBq).
- *Solid waste* containing  $^{125}\text{I}$  must be put into special lead-lined, airtight metal tubes labelled "fast  $^{125}\text{I}$ -affald" in the fume cupboard in room 3131.0.10 in the Science Park. Solid waste containing less than 0.27  $\mu\text{Ci/kg}$  (0.01 MBq/kg) is considered to be normal hazardous waste.
- *Glassware*, etc. must be rinsed with 0.5 M NaOH, which is then poured into "flydende  $^{125}\text{I}$ -affald" or down the sink, depending on the state of contamination. Continue to rinse a couple of times with cold 1% NaI and wash with Decon. If the monitor then shows no trace of contamination, the objects can be sent for washing. Otherwise rinsing should continue until it is no longer possible to count any radioactivity.
- *Cleaning-up workplace*. Check fume cupboard and gloves with the monitor and decontaminate any contaminated areas with 0.5 M NaOH and 1% NaI.

Waste must be stored for at least one year in room 3131.0.10 in the Science Park. Each box or other container must hold no more than the maximum limit of 0.13 mCi (5 MBq), and the outside of the box must show max. 5  $\mu\text{Sv.}$ , when it is sent for incineration.

### Guidelines for working with radioactive substances while pregnant or breast feeding

The working schedule for pregnant women should be such that an unborn child is not subjected to more than 1 mSv. The State Department for Radiation Safety recommends the following maximum limits:

$^{32}\text{P}$	5MBq ~ 135 $\mu\text{Ci}$
$^3\text{H}$ , $^{14}\text{C}$ , $^{33}\text{P}$ and $^{35}\text{S}$	50MBq ~ 1.35 mCi

Pregnant women **must not** work with  $^{125}\text{I}$ , and dosimetry films must be changed once a month.

If a pregnant woman works in a laboratory where colleagues are using open radioactive sources, then the dosage and risk should be seen as the total exposure. The Department of Occupational Medicine (Arbejdsmedicinsk Klinik) can be consulted regarding an evaluation of the risk involved.

When a woman is breast feeding at the time she is working with radioactive substances, she should be aware that in the event of accidental spill, radiation may be transferred to the child through the breast milk. If the amount used is less than the limits for an S1-classification, then the risk is very small.

The Department of Molecular Biology and Genetics has produced an information booklet for pregnant women entitled "Tillykke" (Congratulations):  
[http://mbg.medarbejdere.au.dk/fileadmin/site\\_files/mb/internt/arbejdsmljoe/graviditetspjece-UK.pdf](http://mbg.medarbejdere.au.dk/fileadmin/site_files/mb/internt/arbejdsmljoe/graviditetspjece-UK.pdf).

## **Accidents with radioactive material**

### **Spill or loss of radioactive material**

It is the responsibility of the person who spills to make sure that the spill is cleaned up immediately and thoroughly. If the spill is considerable and over a large area, then an Occupational Health and Safety representative/supervisor and the isotope-responsible staff member must be contacted. A small spill of liquid radioactivity should be wiped up with absorbent paper (paper towel). Spills of powder or other dry material should be wiped with wet absorbent paper. Afterwards, wash with a carrier-solution, i.e., a non-radioactive solution of the labelled substance that was spilled. For  $^{32}\text{P}$ -spills, a potassium phosphate solution should be used, and for  $^{125}\text{I}$ -spills - a sodium iodide solution.

All paper as well as other materials used for cleaning-up should be treated as solid radioactive waste.

After cleaning-up, check the area for radioactive contamination.  $^{35}\text{S}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{125}\text{I}$  and  $^{14}\text{C}$  can be checked directly with a monitor, but because of the monitor's low sensitivity for  $^{35}\text{S}$ ,  $^{33}\text{P}$ , and  $^{14}\text{C}$  - beta radiation, an extra precaution for these is to wipe the area with a damp filter paper, dry the paper, put in a scintillation vial with 5 ml scintillation fluid and count in a scintillation counter. The same method is used for  $^3\text{H}$ .

### **Radioactive contamination of persons**

Persons, who frequently work with or nearby  $^{125}\text{I}$  and  $^{32}\text{P}$ , must wear a dosimetry film. The the Danish National Institute of Radiation Protection sends the dosimetry results every month and a yearly statement. The limit is 20 mSv/year, although during pregnancy, the limit is 1 mSv, (see Guidelines for working with radioactive substances while pregnant or breast feeding).

Gloves must always be worn when working with radioactive isotopes, and hands must be washed thoroughly afterwards. However, in the event of contamination of the skin, the area should be washed a number of times with a carrier-solution and then several times with soap and water. If there is still sign of contamination (monitor), you should go immediately to the hospital's casualty ward.

If the skin is damaged, as well as radioactively contaminated (corrosion or sores), rinse liberally with water, possibly opening the sore to induce bleeding and cleansing. Immediately afterwards go to the hospital's casualty ward.

Clothing which has become severely contaminated should be treated as radioactive waste.

### **Ingestion of radioactive substances**

If you accidentally swallow a radioactive solution, vomiting should be induced immediately (finger down the throat) and go immediately afterwards to the hospital's casualty ward.

In the event of an accident, the radiation responsible person must be contacted (in "Biokæden" Tinna Stevnsner, tel. 27782804, and in the Science Park Niels Sandal, tel. 20760042). The radiation responsible must immediately inform the Board of Health (the Danish National Institute of Radiation Protection, tel. 44943773, 24-hour service) on accidents that occur and which may have resulted in unintended radiation exposure of personnel or other persons on lost radioactive sources and major contamination of people, premises, equipment or the environment.

### **Scintillation counting**

At present, in the Science Park, OptiPhase “HiSafe” 3 from Perkin Elmer is used as scintillation fluid.

In ”Biokæden” Ultima Gold (Perkin Elmer) is used.

Disposal is carried out according to the departmental guidelines.

It is no longer allowed to use scintillation fluid containing toluene and xylene.

When counting  $^{32}\text{P}$ , the Cerenkov-method should be used whenever possible (i.e. counting  $^{32}\text{P}$  without scintillation fluid in the  $^3\text{H}$ -window).

### **Useful telephone numbers**

Radiation responsible persons:

- “Biokæden” Tinna Stevnsner, tel. 27782804
- Forskerparken Niels Sandal, tel. 20760042

The Danish National Institute of Radiation Protection: 24 hour service 4494 3773 must be contacted in the event of large dose accidents with radioactive isotopes.

When calling the 24-hour service number, an automatic answering machine will be asked will ask you to provide your name, describe what the call is about and give the phone number where you can be reached. The person on duty will then call back within 15 minutes.

The person on duty will be able to help to:

- assess the situation
- make / provide measurements
- provide medical care
- request measuring teams from the state emergency centers
- contact other relevant institutions / individuals

## 18) Rules for working with gene technology Class I

The rules are based on the Ministry of Employment, Act no. 910 of 11 September 2008“ The Act of Genetic Engineering and Healthy and Safety” (“Bekendtgørelse om genteknologi og arbejdsmiljø”, and correction of Acts no. 88 of 22 January 2010 and no. 1707 of 15 December 2010.

Reference is made to the Danish Working Environment Authority’s guidelines C.0.4, December 2009 - updated in October 2014: "Classification of laboratories, plants for the production, etc." (“Klassifikation af laboratorier til genteknologisk arbejde”) and C.0.5, April 2001 "Risk Assessment of genetic engineering research, etc.” “Risikovurdering af genteknologiske forskningsprojekter m.v.”

*Biologically active material* refers to living organisms, cells or viruses that contain genetically engineered DNA or RNA. Isolated DNA, RNA or protein, prepared from genetically engineered material is *not* biologically active.

Work with biologically active material *must* be carried out in areas that have been classified by the the Danish Working Environment Authority (“Arbejdstilsynet”) (Class I).

- Bags, outdoor clothing and other unnecessary items must not be taken into classified laboratories.
- It is forbidden to use private mobile telephones in Class I laboratories.
- Access to classified laboratories by unauthorized persons must be kept to a minimum.
- A buttoned-up lab coat must be worn in classified laboratories. This lab coat may be yellow, green, or white one with the yellow gene technology symbol on the breast pocket.
- In mammalian cell labs in ”Biokæden”, green operation gowns are worn in both Class I and 2 laboratories. This also applies to visitors and workmen.
- The yellow, green and white (with gene tech symbol) lab coats are collected for laundering in polyvinylacetate bags in a plastic container with a lid marked “Biohazard”.
- All standard laboratory hygiene must be adhered to: It is forbidden to eat, drink, or in the laboratory. Remember to wash your hands before leaving the lab.
- Pipetting by mouth is not allowed.
- All glassware, petri dishes, test tubes, etc., containing biologically active material must be labelled with a yellow marker.
- Biologically active material that must be transported outside the classified laboratories or areas must be in containers labelled with a gene technology warning sign (yellow labels or a yellow marker). Sealed Eppendorf tubes can be transported in marked racks, agar plates in marked bags or plastic trays. Large flasks can, for example, be transported in marked plastic trays on a trolley so that any spillage is collected in the tray.
- All waste containing biologically active material, i.e., living organisms, cells, or viruses that contain genetically engineered DNA or RNA, must be collected in suitable containers and disinfected. Solid material must be collected in the buckets marked for autoclaving. After disinfecting and autoclaving, solid waste can be disposed of in the usual way. Liquid waste must be disinfected with 1% Diversol or 1% Virkon overnight, after which it can be poured down the sink. Liquid waste can also be disinfected by autoclaving. The liquid waste must be autoclaved before disposal. Liquid GMO waste containing small quantities of chemicals or cytostatic agents must be desinfected with 1% Virkon or 70% EtHO. After that, the waste is treated as hazardous waste (see information on the fume cupboards).

- Replacement of filters in the LAF benches and in the ventilation system is carried out by the assistant engineers from the department. Replacement of filters in the ventilation systems is taken care of by the building management technicians. The staff are required to wear respiratory aids, special clothing and gloves. Used filters must be placed in autoclave bags and closed tightly. Laboratory personnel can then autoclave the bags and dispose of them as for inflammable waste.
- The linen bag used in the ventilation system must be taken down every six months by the faculty's workshop technicians. The linen bag must be put into a polyvinylacetate bag and sent to be laundered by the same company used for laundering yellow or special white lab coats.
- Glassware, etc., contaminated with biologically active material must be autoclaved or disinfected with 70% EtOH for at least an hour before they are sent to be washed.
- Virkon and 70% EtOH can be used for cleaning and disinfection of all types of surfaces, although Virkon can be corrosive in contact with some metals, especially aluminium.
- Waste for autoclaving must be collected and transported in closed, labelled stainless steel buckets or, as in "Biokæden", on a special autoclave trolley that is wheeled directly into the autoclave. In "Biokæden", the waste is collected by the department's glass washing staff.
- Injection needles, scalpel blades and other sharp objects that have been in contact with GMO must be put in a special waste container for sharp objects before autoclaving and disposing as clinical hazardous waste.
- When working with biologically active material, procedures should be limited as much as possible. Any procedure likely to result in aerosols must be carried out in the fume cupboard or at a vertical laminar flow bench.
- A workplace must be cleared, cleaned, and disinfected daily. Spilled material that is biologically active must be immediately wiped up and the area washed with 70% ethanol. In the event of large spills, the safety officer must be notified.
- Disposable gloves used when working with biologically active material in classified laboratories must be autoclaved.
- Wash hands frequently after contamination with biologically active material, as well as when taking a break, and at the end of the day.
- Paper must not be left lying about or hung up freely in the laboratory. Protocols, etc. should be covered with plastic. Cardboard boxes must not be kept in classified areas - use plastic boxes instead.
- Note-taking should be restricted to an especially taped-off area, a pull-out shelf or the window sill.
- Remember that pull-out shelves must be pushed in when not in use.
- Remember that paper, protocols, books etc., must be kept separate from apparatus.
- In the event of an accident, measures to be taken must be decided with the project leader or another competent person. An Occupational Health and Safety representative/supervisor must be notified as soon as possible.
- All accidents or near-miss incidents must be reported to the responsible occupational and health safety representative/supervisor and Line L. Dvinge at the departmental office. The OHS representative and the person involved in the incident must make a report, which should be sent to Line Dvinge, who takes care of the sending it to the relevant place.  
<http://medarbejdere.au.dk/en/administration/hr/workingenvironment/reportinginjuries/>



- Please note! Although students are self-insured, they must still report the accident at the department.
- At the entrance to a classified laboratory there must be a sign on the door reading: “Gene technology laboratory Class I”.
- The project leader responsible for a laboratory has a duty to instruct all staff and students in carrying out the project in a safe and responsible manner and in accordance with the current safety regulation for working in classified laboratories. It should always be possible to call on a professionally competent person. A list with phone numbers should be hung at the entrance to the classified laboratories.

## **Class II laboratories**

The above rules relate only to work in Class I laboratories. For Class II, please see the rules for Safety regulations for Class II laboratories at the Department of Molecular Biology and Genetics.

## **Procedure for upgrading from Class 0 to Class I**

- The Danish Working Environment Authority (“Arbejdstilsynet”) must be notified when a laboratory is upgraded. This contact is made via the department’s OHS organisation. Upgrading of a laboratory can only take place after permission has been given.
- A person is selected to have the overall responsibility in cooperation with the HSO representative and they apply to the Danish Working Environment Authority for a Class I approval. The person’s name is given to the Danish Working Environment and written in the log book for the laboratory.
- At the entrance to each classified room is a notice with the telephone number of the person responsible for that room.
- For cleaning purposes, classified laboratories/areas should contain only the most items. All items standing on the floor must be removable (on wheels).
- All persons working in a classified laboratory /area **must wear buttoned-up lab coats**.
- Use of the log book is a requirement from the **Act relating to gene technology**. The date of the upgrading is to be entered into the log book.
- A **warning sign** stating: “**Genteknologisk laboratorieområde - Klasse 1**” (Class I laboratory for Gene Technology) must be found at the entrance to the laboratory.
- Round plastic containers for solid hazardous waste, labelled autoclave buckets, and containers for sharp objects must be found in classified laboratories
- Bottles with 70% ethanol must be found in classified laboratories.
- Lab coats must be hung on hooks just inside the classified laboratories.
- Cleaning personnel must wear lab coats when working in Class I laboratories. The

person responsible for a laboratory must inform the OHS Committee (Secretariat) whenever an upgrading occurs. Laboratory personnel must carry out the daily cleaning and disinfection of the working area and the items used.

- Students working with biologically active material must be supervised by competent persons.
- **Stainless steel buckets for autoclaving** must be placed in upgraded laboratories. These buckets are to be used for the collection and decontamination of items used while working with biologically active material (disposable pipettes, centrifuge tubes, etc.), fluids containing GMO must be autoclaved separately. In "Biokæden" these fluids are autoclaved by the glass washing staff.

## **Procedure for downgrading from Class I to Class 0**

### **Permanent/shorter periods**

- Permanent downgradings must be reported to The Danish Working Environment Authority ("Arbejdstilsynet") This contact must be made by the OHS Committee. A downgrading can only take place after permission has been given. The date for downgrading must be reported in the log book.
- Short-term downgrading can be done by the local OHS representative. Downgrading must be recorded in the room logbook, and the GMO door plate must be covered.
- All **lab coats** from classified laboratories must be put in the polyvinyl acetate bags which must then be closed and sent to be laundered following the department's normal procedure.
- Containers with disinfected needles or other sharp objects must be closed tightly and put into disposed of as clinical risk waste. In "Biokæden" it is placed in the waste room.
- All round plastic containers must be emptied for solid H waste and sent for incineration.
- All **autoclave buckets** must be sent for autoclaving.
- LAF benches, including the filters, must be decontaminated by the company with which the department has a servicing contract for that particular type of LAF bench.
- Possible **contaminated instruments** (gyro shaker, tabletop centrifuges, mixers, micro-pipettes, etc.), must be washed with a disinfectant before they are removed from the room.
- All other utensils must be removed from the room.
- All **work surfaces** (lab benches, sinks and their surrounds, fume cupboards, etc.), must be disinfected with 70% ethanol and then washed with a neutral soap solution.
- The person responsible for the project must notify the cleaning staff that a

laboratory has been downgraded, after which the cleaning personnel will be required to clean it thoroughly according to the regulations for Class I.

- The warning sign must be taken down.
- Further use and cleaning of the downgraded laboratory follow class 0 regulations until a new upgrading has been accepted.

### **Cleaning instructions for classified laboratories**

The cleaning staff **must** wear a buttoned up yellow or special white lab coats with a yellow gene technology mark on the chest pocket when cleaning classified laboratories bearing a notice “Gene technology laboratory Class I”.

In the Science Park, a yellow lab coat labelled “Rengøring” (“Cleaning”) is to be found on each floor in all the buildings. The lab coat hangs on a hook labelled “Rengøring” and is to be used only in classified laboratories.

When the lab coat needs washing, it must be put in a polyvinyl acetate bag in the classified laboratory and then put into the laundry basket in building 3132-0.10. In “Biokæden”, the lab coat must be placed in building 1131, room 311 for laundry.

The cleaning staff’s daily cleaning routine consists of mopping the floor and removing ordinary trash. Note that the trash is removed together with the waste bag, and that waste must not be poured from one bag to another. The waste bags must be closed before they are removed from the laboratory.

In addition to the daily cleaning, the floor must be washed 2-3 times a week. Trolleys and hazardous waste boxes must be moved out when the floor is being washed.

In the Science Park, floor cloth, soapy water and bucket are only to be used in the classified laboratories and the water is to be poured in the sink. The bucket and floor cloth may thus not be used anywhere else. When the floor cloth is to be washed, it must be autoclaved first. This is done by putting the cloth in an autoclave bag, closing it with autoclave tape and labelling it with yellow tape. Hereafter it can be taken to the autoclave room in building 3131-0.12. After autoclaving, the cloth is washed in the usual way. *The cleaning procedures in “Biokæden” are a little different and can be found in the Department Office.*

The project leaders and the technical staff at “ST-BYG” are responsible for overseeing that the rules are obeyed.

In addition, the staff from ST-BYG is responsible for taking care of the following tasks:

- dusting and vacuuming elevated ventilation channels and electrical fixtures every six months
- cleaning the cooling systems in the laboratories every six months
- cleaning under refrigerators and freezers twice a year (laboratory personnel must pull them out and put them back)
- cleaning all stationary furniture /equipment below table/bench height.

The dates for the thorough cleaning must be entered into the log book.

The laboratory personnel are responsible for the daily cleaning of laboratory sinks, window sills, tables/benches, fume cupboards, sterile benches and other work places, as well as a

monthly cleaning of shelves, cupboards, bottles, electric supplies, free-standing apparatus and furniture.

In connection with the up- or downgrading of a laboratory, all the above cleaning procedures must be carried out.

In the event of an accident, a notice must be placed on the door to the laboratory and the person responsible for the laboratory must be informed. The name of the person responsible for the project is to be found at the entrance to the laboratory. The cleaning must be discontinued.

**Rules for gene technology work in Class II labs at “Biokæden” (Campus)**

Please contact Lene Pedersen in “Biokæden” before starting work.

**Rules for cleaning gene technology Class II lab at “Biokæden” (Campus)**

Please contact Lene Pedersen in “Biokæden” before starting work.

## **19) Rules for biological work Class II**

Three laboratories in “Biokæden” are classified as biological Class II. The safety level for Class II is equivalent to a GMO Class I. Therefore work must be carried out in the same way and follow the same rules and regulations as for GMO Class I.

These labs include room 415 in building 1131 and 312 in 1131. In the laboratory in room 312, work with biological Class II is not always being carried out. An agreement has therefore been made with the Danish Working Environment Authority that during the periods where biologically Class II work is carried out a sign is hung in the lab (in addition to the warning sign outside the door) to inform the staff working in the lab. In lab 405, this is not necessary since all staff is informed that work with biological Class II is constantly being carried out.

In addition to the three laboratories that are approved for biological Class II work, the shaking incubator at the 4th floor (building 1131, room 430) is also approved for biological Class II when the following conditions are respected:

- A biological Class II warning sign is placed on the incubator when it contains biological Class II.
- The bacteria are to be stored in a closed vessel in the incubator.
- The bacteria transported to and from the incubator must always be transported in a sealed container and on a trolley. During transportation, 70% ethanol and paper must always be present to be able to wipe up spills and wash with spirits immediately, should an accident happen.

Biological Class II work with bacteria and mammalian cells may cause diarrhea if taken orally. The diarrhea disappears by itself and for normal healthy adults, it is not dangerous.

## **20) Rules for gene technology work involving animals**

Before starting animal research, you must study the rules applicable. Rules, permits and courses are available on the website of the Danish Animal Research Authority under the Ministry of Environment and Food (in Danish only):

<http://www.foedevarestyrelsen.dk/Dyr/Dyrevelfaerd/Dyreforsogsomraadet/Sider/Dyreforsogstilsynet.aspx>

You need to get an authorisation to carry out animal research.

### **Handling of animals in Building 1131, room 521. Contact Ernst-Martin Füchtbauer in “Biokæden” before starting.**

Room 1131-512 is divided into two sections. To the left of the door is the animal section, where there is a fume cupboard, a ventilated animal hood and a laminar flow bench. The other section is an S1 cell culture section which includes the entire right side of the laboratory together with a table on the left side closest to the window.

Work in the two sections must be kept separate. You are not allowed to move S1 GMOs to the animal section. In the S1 section, all the rules for working in S1 laboratories apply, including waste disposal.

In the animal section, the rules described below apply. In the event of an accident, where the animal section becomes contaminated with S1 GMOs, all waste from this section must also be autoclaved.

The animals are transported in transport boxes, either from the commercial breeder or from the central faculty animal room of Aarhus University. When the animals arrive they must be put in cages with a top filter in the fume cupboard, after which they are moved to the ventilated animal cabinet (Scantainer isolater).

The animals are handled or killed either in the fume cupboard or in the laminar flow bench alongside the animal cabinet.

Dead animals must be put into a sealed plastic bag and stored in the freezer room in the central animal room in the Bartholin building of Aarhus University.

Bedding from the animal cages must be transferred to a plastic bag in the fume cupboard. The plastic bag must be tied tightly.

Empty cages are to be put into autoclave bags and taken back to the central animal room of Aarhus University and placed in front of the autoclave on the ground floor.

### **Handling zebra fish in rooms 3133.3.10, 3133.0.09, 3133.015**

See the above rules for animal research.

Before handling the zebra fish, you must have received training in internal procedures.

## 21) Instructions for work with human biological material

See the department's website for further information:

<http://mbg.medarbejdere.au.dk/en/working-environment/authorisations-and-instructions/human-biological-material/>

According to Danish law, all research projects in Denmark involving human beings or any kind of human tissue, cells, blood, etc. must have permission from a regional ethics committee to carry out the experiments. In the case of multi-centre trials, the investigator shall only apply for permission from the regional committee where the principal investigator carries out the research project. However, in the case of multi-national trial projects, permission from a Danish committee is always required. Guidelines about notification, etc. of a biomedical research project to the committee system on biomedical research ethics.

If material or information from a biobank (i.e. 'a structured collection of human biological material which is accessible under certain criteria, and where information contained in the biological material can be traced back to individuals') is used in a research project, the additional approval of the Danish Data Protection Agency is required.

### **Standards for the collection, storage, handling and disposal of human biological material at MBG**

The principal investigator is responsible for ensuring confidential, secure and appropriate storage of the tissue, ethical use of the tissue, respect for donor confidentiality and appropriate disposal of the tissue. It is recommended that you do not work with your own biological material due to the risk of transformation and lack of antigenicity.

*Collection of samples from living individuals:* Only registered physicians, nurses or certified technicians who are trained to take out human biological material for scientific or medical purposes, and who obtain samples while working under protocols and procedures approved by the relevant regional ethics committee, are authorised to extract human biological material. This regulation does not apply for the non-invasive collection of biological fluids such as semen, saliva, milk, etc.

*Storage:* All human samples must be stored in a secure location, which is clearly labelled on the outside with the universal biohazard symbol. You must place samples in secure, leak-proof containers and store them in a manner that will prevent decomposition or deterioration during storage. Each container must also be labelled with the name of the biological material, the user's name and contact information. Containers used for sample storage must be discarded as biohazardous waste after removal of the sample.

*Handling:* All human samples should be handled as potentially hazardous in terms of contamination and infection. Adequate personal protective equipment for handling potentially contaminating agents should therefore be chosen according to the risk of exposure. Personal protective equipment includes gloves, protective eyewear, masks, aprons, shoe covers and cap/hair covers, etc.

All work with human samples **MUST** be carried out in areas that have been classified as Class I.

*Disposal:* Human samples should be disposed of in closed, non-leaking containers and put in the yellow bags or autoclaved. Blood sampling equipment, scalpels and other equipment that can damage the skin should be disposed of in specified yellow needle boxes.

*Transport:* To avoid spill, human specimens/samples should be transported in unbreakable closed containers marked as biohazardous material.

### **Accidents**

Biological spills on non-working areas such as the floor should be removed immediately and cleaned normally. You should also remove large spills immediately and disinfect the affected area with an appropriate agent (1% Diversol or 70% ethanol in water, possibly supplemented with UV light for 30 minutes).

If you injure yourself or others with equipment that has been contaminated with either blood or tissue fluids:

- let the wound bleed
- wash carefully with water and soap
- brush the wound with 70% ethanol or 2.5% iodine ethanol

If you become contaminated with biological material in your mouth or a wound, you should carefully rinse the area with saline or normal tap water.

If you get biological material in your eyes, rinse them carefully using the eye rinsing bottles available in all laboratories.

You should contact the emergency room at the local hospital (Aarhus University Hospital, Nørrebrogade) immediately or within two hours of the accident for a risk assessment of infection with HIV, hepatitis B or hepatitis C. There is normally no treatment 24 hours after the accident.

Contact your local safety officer at MBG to make a claim report of the accident. It is important that you contact the safety officer no matter how minor the accident.



## **22) Instructions for cleaning staff, workmen and other unauthorised personnel**

When you enter a laboratory you should behave as though everything is hazardous.

Therefore:

- **do not move anything**
- **do not touch anything**
- **do not attempt to smell the contents of bottles**

If you accidentally knock something over, leave it as it is but inform one of the laboratory personnel.

The incident may appear to be harmless, but it can be dangerous, or the results of several days' work. A knowledgeable person may be able to salvage the pieces, whereas everything will be lost if you attempt to put things right.

If you break a glass bottle and the contents run out, you must leave the laboratory at once. Do not attempt to wipe it up because the substance may be corrosive or poisonous. If you spill some on yourself, leave the room, and quickly wash with lots of water. Inform someone who can evaluate the situation.

If you come across something which makes you feel uncertain, e.g. a strange smell, sound, or smoke, go out of the room immediately and inform someone who can decide whether it is hazardous or not. Remember that the Occupational Health and Safety representative/supervisors are here to be helpful.

### **It is better to ask too often than too seldom**

Do not work alone. If you must work in a room where nobody can see you, inform someone about what you are doing and tell them when you are finished.

### **In case of an accident, remember**

- **do not touch anything, but leave the room**
- **wash with lots of water if you spill something on yourself**
- **fetch laboratory personnel**

When you enter a laboratory which has a sign "Genteknologisk laboratorieområde - Klasse 1" you must wear a **buttoned up yellow lab coat or a white one with the special yellow gene technology symbol on the chest pocket**. Cleaning utensils that you use in these laboratories must not be used in other areas.

**Persons you can contact, please check the department's website:**

**<http://mbg.medarbejdere.au.dk/en/working-environment/health-and-safety-organisation/>**.

## 23) Waste disposal

In the fume cupboards both in the Science Park and "Biokæden", you'll find an overview of the current rules on waste treatment.

In you are in doubt, or if you have other types of waste, ask your OHS representative.

In "Biokæden", you can find the waste packaging in the closet labelled "Si-Udv." in the corridor on the 4<sup>th</sup> floor, bldg. 1131.

In the Science Park, you can find the waste packaging in a room in the basement of building 3130.0.

Remember that plastic cans must be marked "UN 3H1/X" to be legal. Filled plastic containers must state the group name, room number and type of content.

### **Yellow hazardous waste boxes**

Must only be used for waste from medical treatment of persons, animals or biological experiments that pose a risk of infection.

Injection needles, ampoules and such like must be put into the container for needles and closed tightly. The container can then be put into the hazardous waste box. When these are ready to be collected they must be closed correctly so that they will not open again.

Write clearly on the outside: Aarhus University, Department, Building number and the contact person's initials.

The yellow hazardous waste boxes can be obtained from your OHS representative.

## 24) Batteries

In "Biokæden" used batteries must be placed in the special boxes for batteries placed at the entrances to buildings 1130 and 1135. New batteries can be picked up at the room for goods reception on the 3rd floor, building 1130.

In the Science Park, used batteries must be placed in the special boxes for batteries in the copy room in building 3130 next to the luncheon room, where new batteries can also be picked up.

## 25) Working safely with electricity

These shortened guidelines are based on information from the department's website:

<http://mbg.medarbejdere.au.dk/en/working-environment/safety-with-electricity/>

where there are illustrations of the correct plugs and sockets as well as wrong and dangerous plugs.

Safety when working with electricity must be taken very seriously.

Strict regulations were imposed in 1993 regarding:

- Equipment safety
- User familiarity with safety conditions

*Discard any apparatus that fails to meet safety standards.*

When in doubt, do not use it and ask MBG's staff from the workshop to have a look at it.

### General information on the dangers posed by electrical equipment

- Electric shocks cause muscles to contract violently, resembling a cramp or seizure.
- The most serious electrical accidents lead to cardiac arrest, while a milder shock can cause significant discomfort. Never touch electrophoresis apparatus or the electric cables when the electricity is switched on. (Under proper conditions\* this should not be possible).
- Most liquids in the laboratories conduct electricity. Many of them are even very good conductors and correspond to a direct connection with the connected core.
- Electrical apparatus and cables must always be clean and dry, without salt deposits. Never handle electrical equipment with wet hands or gloves (the thin rubber gloves do not protect against high voltage).
- The cold room increases the risk of electrical failure and accident because of condensing water.
- The two most dangerous currents are 230 volt mains electricity and that from high voltage electrophoresis apparatus.

If in doubt whether the apparatus is safely set up, think about this basis rule:

*\* The apparatus must be protected against accidental touching of live current in any situation!*

### 230 volts mains electricity

All electrical equipment must be earthed via the mains cable. This provides better protection from electric shock and accidents even when there may be some leakage in the apparatus.

Therefore:

*All laboratory apparatus must have a Danish 3-pin mains plug that is earthed, 2 round pins and one flat "earth" pin below!*

- Mains cables with 2-pin plugs are not earthed and must NOT be used. Throw them away. The correct plugs can be obtained from the MBG's workshop.
- Plugs and cables must be in perfect condition. Users must make sure that the mains connections, i.e., mains socket and apparatus socket are not damaged (the plug pins are intact and the cable insulation is not torn, etc.).

Note also that:

- cables must be of robust quality, without holes or burn marks from hot plates, etc.
- cables must be attached firmly to the mains and to the apparatus.

*When in doubt about the quality of the mains cable, discard it and get a new one.*

Please note: Leakage circuit breakers cannot handle everything. They can only ensure against leakage from 230 volt mains connection to earth, and may thus prevent an electric shock. Contact with both “active” mains cables will give many kilowatts for hours.

Leakage circuit breakers do not protect against errors in the high voltage output.

### **High voltage connection to electrophoresis apparatus**

- Cables must be of high quality and always have approved “safety plugs”
- Cables and gel apparatus must have insulation against more than 1000 Volt (even 1500 Volt). In other words:
- Silicon rubber cables that give a continuous heat should be used
- Avoid rubber tubing (they crack) and thin plastic tubing that melts in contact with the edge of a hot plate.
- Never use transformers, adjoining cables or adaptors that can transform the approved safety sockets to those that are less safe. They are dangerous and must be discarded.
- Cables and gel apparatus must be capable of taking the same current as the attached safety plug.

### **Safety plugs and sockets of the correct type**

At the Department of Molecular Biology, we use only two types of safety plugs. Other types are not allowed in the laboratories.

Note that both types have *solid insulation sleeves* so that the plug pins cannot be touched directly.

- Ordinary 4 mm safety plugs that are standard for most equipment. They are internationally approved and are suitable for about 1000 volt when they are dry.
- 2 mm safety plugs with a longer, stronger *covering*, suitable for up to 1500 volt.

Please note that plugs with resilient coverings are unsuitable as they are only for low voltage (to minimise short circuit). Under no circumstance must they be used or found!

The same applies for old-fashioned banana plugs (as well as sockets and extension cables). They must be discarded as they do not meet the regulations and are far too dangerous.

### **Safe power supplies**

- Power supplies must of course have the correct sockets, corresponding to the correct safety plug.
- 4 mm safety sockets have mouldings suitable for the plug’s covering.
- 2 mm safety sockets are deeper, corresponding to the long plug’s bigger covering.
- Equipment with old-fashioned sockets for banana plugs must be discarded.
- Power supplies with high voltage current must be insulated with respect to “earth” or have a safety circuit that breaks when earthed (this can be checked by MBG’s staff at the workshop).

### **New equipment and MBG rules**

All new equipment must be CE-marked (this has been obligatory since 1997). However, a CE mark is not an approval (as many think), but merely the factory’s statement that the equipment is safe to use.

You must therefore be critical and evaluate the equipment before buying!

Note, that Bio-Rad, for example, uses a “longer, modified, banana plug” *without a solid covering*, with accompanying lowered sockets in their power supplies.

Maybe this looks reliable when their equipment is assembled, but it is especially dangerous when used together with other equipment having the correct safety sockets.

**The Occupational Health and Safety Committee and the Head of Department therefore stress that:**

- banana plugs with exposed electrical parts must be discarded immediately
- plugs with resilient coverings must not be used
- high voltage plugs and sockets must be the standard safely type with solid coverings
- all electric laboratory apparatus must be earthed, i.e., mains plugs must be Danish with 3 pins
- you should contact the Department's staff at the workshop before buying new equipment to make sure that it is in agreement with departmental rules.

## 26) Instructions for the use of large apparatus and centrifuges

*Contact persons* are chosen by the Head of Department. Names of contact persons are to be found by each apparatus. Ask them for advice about the use of apparatus.

Students, trainee technicians, and all other users who do not have a thorough knowledge about the use of the apparatus, *must* - before attempting to use it - have clear instructions. It is the responsibility of each supervisor or project leader to ensure that each student or new person is well-instructed in the use of the apparatus. Instructions regarding use can be found in the "Instruction Manual" beside the machine. If in doubt, ask one of the contact persons.

*In the event of breakdown:* Please notify the staff in MBG's workshop or one of the contact persons for the apparatus.

*Cleaning:* It is not a matter of doing a spring clean, but the obvious and necessary cleaning that must follow every time it has been used. ***Each and every user must make sure that the apparatus is cleaned.***

In the event of spillage of genetically modified material, the spill must be wiped up and the area disinfected with 70% alcohol.

*Rotors must not* be washed with ordinary soap but with "Neutral Extran", MA 02 in a 1-2% solution. A bottle of this should be found by each centrifuge.

Remember that centrifuges must be closed in such a way that the lid cannot be lifted while a rotor is in action. Rotors must never be stopped by hand.

## 27) The Occupational Health and Safety organisation

You can find an updated overview of the Occupational Health and Safety organisation at the Department of Molecular Biology and Genetics on the department's website:

<http://mbg.medarbejdere.au.dk/en/working-environment/health-and-safety-organisation/>

## 28) Literature

KIROS database (<http://www.kiros.dk/W/>)

See also the selection of workplace guidelines for specific substances, to be found in the laboratories.

Relevant links can be found on the Department's website:

<http://mbg.medarbejdere.au.dk/arbejdsmiljoe/>

The Danish Working Environment Authority's ("Arbejdstilsynet") guidelines and laws can be found on: <http://engelsk.arbejdstilsynet.dk/en>.

The EU list of harmonised classifications (the former list of hazardous substances):

<http://mst.dk/virksomhed-myndighed/kemikalier/stoflister-og-databaser/listen-over-harmoniseret-klasseklassificering/> (Danish version) or

<http://eng.mst.dk/topics/biocides/application-in-accordance-with-the-danish-authorisation-scheme/requirements-subsequent-to-the-decision/authorisation-is-granted/classification-and-labelling/>

The Danish Health Authority's: Guidance regarding protection when working with radioactive substances, 2005.

Useful books:

- The Merck Index, 14th edition, 2004.
- Hazards in the Chemical Laboratory.
- "Opbevaring af laboratoriekemikalier" (How to store laboratory chemicals) by Lene Hjerrild, Hanne Troen and Jørgen Stage Johansen, DL-F
- "Laboratoriesikkerhed" (Safety in the laboratory) 6<sup>th</sup> edition (Nyt Teknisk Forlag)  
ISBN: 978-87-571-2811-6  
ISBN: 978-87-571-3335-6 (e-book)