SLU Department of Plant Protection Biology (www.slu.se)

Safety Manual

Note: Suggestions for improvements and updates are necessary to keep the safety manual as a useful tool in the safety work. Safety work is an on-going process, if something is wrong or missing, please tell your Safety officer. Safety Manual updates in January every year.

February, 2018

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This is an updated safety manual originally developed by Department of Molecular Biology, SLU.

1. Introduction

Welcome to the Swedish University of Agricultural Sciences.

In this manual we will give you some information on how to maintain a risk-free environment in your work place. But first you should know which rules apply to you during your various activities.

Working alone in the lab with dangerous stuff is **not** recommended.

Do not start your work or using any apparatus before reading this manual and before you have had a discussion with your supervisor/host. You must sign the form at the end of the manual to acknowledge that you have been informed of the safety routines of the department.

The Safety Manual is stored at the lab and the sign paper is stored at the economy assistant's office.

Also: Your supervisor/host should present you to all the members of the department.

1.1 Contact persons for safety issues

Area	Person	Room no	Ext.	Responsible
11104	CISON	Koom no	L'AL.	for
Head of	Rickard Ignell	2501B	5311	Plant
Department	Rickard Ignen	2301B	0735-984871	Protection
Department				Trotection
Dep. Head of	Åsa Lankinen	1053	5367	Plant
Department			0706-417584	Protection
C 0 1 00t	77.1	11.71	070-2966660	
Safety officers	Helene Lindgren	1151		Horticum
(Skyddsombud)	Helle Turesson	1161	0705-562768	Horticum
	Kalle Gustavsson	2809	0702-345437	Vegetum
	Göran Birgersson	2502	5300 0705-53 14	Vegetum
			32	
Safety officer for	Lars Hagtorn		5331	Hela
Alnarp			070-3000702	SLU/Alnarp
Isotope safety	Ida Lager	1157	5348	Horticum
-	Åsa Grimberg	1153B	5541	Horticum
Union	Gary Nilsson (ST)		5008	SLU Alnarp
representatives	Andrus Kangro (Saco)		5479	SLU Alnarp
Service	Göran Persson	1078	0702-231706 5600	Horticum
technician	Anneli Rydén	2853D	0702-226685	Vegetum
Miliäangyania	Erland Liliarath	1098	5567	Horticum
Miljöansvarig	Erland Liljeroth			
	Elisabeth Marling	2807A	5309 0705-915309	Vegetum
GMO	Mariette Andersson	1153B	5541	Växtförädling
responsible				Vegetum
Evacuation	Kalle Gustavsson	2809 0702-	345437 H	Iorticum
11	C D.	2502 0705		7

leader Göran Birgersson 2502 0705-531432 Vegetum

1.2 Further information about safety matters

Further information can also be found on the department's home page www.slu.se under internal. And also https://intert.slu.se/en/services/university-security

1.3 Points of order

- **Children** must not be brought into the lab area.
- **Pets** are not allowed in the work place.
- Rules of conduct: Discuss the local rules in the laboratory and report to the safety representative if problems arise.
- Working hours: Plan your work so that especially dangerous steps in a lab protocol are conducted while you are not alone in the laboratory.
- **Smoking** is not allowed inside or in atrium gardens. Use designated areas.

- **Lunch room:** To make this room enjoyable it is important that everyone try to keep it clean. Do not leave dirty cups, plates, microwave oven *etc*. for someone else to clean. Do not enter the lunch room wearing a lab coat!
- Cooking or eating is not allowed in the labs!
- Last person to leave the laboratory should close all windows, switch off the lights and lock the doors.

2.General routines

In order to work in our laboratories the user must individually go through the safety regulations. The person responsible for this is the safety officer. This safety demonstration includes the following:

2.1 Equipment for personal protection

Each person working in a laboratory will be provided with a lab coat and safety glasses or other appropriate protective clothing and have a designated place for personal belongings. Demonstrate for newcomers the following:

- Eye showers
- Emergency showers
- How the fume hoods work
- How to use the fire protection equipment
- First aid
- Information about Klara
- How to evacuate
- Destruction room

2.2 Responsibility assignments

Each group within the department maintains a list of who is responsible for different rooms, instruments and routines. If you have any questions or problems, turn to this person first. Please note that this does not mean that they will clean the instruments after you have used them. YOU have a responsibility to clean up after yourself and to wipe up any spills.

2.3. Broken instruments

Malfunctioning of any apparatus should be reported immediately to the responsible person. It is not sufficient to simply write a note "broken" and leave it on the instrument in question. Responsible technicians for "konsortiet" is Niklas Ohlsson, Mirella Beganovic and Helene Lindgren and at chemical Ecology Göran Birgersson.

2.4 Dishwashing

Each group/lab clean their own dish.

2.5 Purchasing and work order routines

• The department have several persons in charge of ordering for the lab's general use materials. All orders should go through these persons unless specified otherwise. Whenever you take the last portion out of a bottle, notify the person in charge of ordering immediately. In this way we avoid running out of an important chemical that might be urgently needed by someone after you.

- Practice good economy. Look at the prices of the materials you use and consider how to economize.
- 2.6 All containers in the lab have to be labelled with content, name and date.

3. Handling of chemicals

3.1 Economy

Find out the routines for purchase and storage of the chemicals. Since we have limited resources, you should always try to find the cheapest alternatives. Do not use more than what is needed, use chemicals of lower purity (like technical grade methanol instead of p.a. grade) etc. when possible.

3.2 Risks and danger

The supervisors must make sure that new graduate students/project students/guest scientists/technicians are well informed about the health risks of the chemicals and about where special information on certain chemicals can be obtained.

All hazardous chemicals have to be labelled with an appropriate "warning symbol" for the chemical in question. If such a chemical is transferred from the original package to some other container or bottle, an appropriate symbol has to be attached. The symbol stickers can be obtained from the Safety officer.

Always read the labels on the bottles/packages to be aware of the potential danger of the chemical, many of which are classified as poisons. You can find all information about chemicals in **Klara** https://internt.slu.se/verktyg/klara/. You will get username and password from your supervisor. Also you may find this type and other useful information on web pages of some of the larger companies that provide chemicals like Sigma and VWR.

Make a risk assessment in Klara: https://internt.slu.se/verktyg/klara

Chemicals classified A and B, according to the Swedish national Board of Occupation Safety and Health (Arbetarskyddsstyrelsen) can only be handled by a person with a permit from the Labour Inspector (Arbetsmiljöverket) and (Läkemedelsverket). This permit is limited in time.

Hydrazine and DMS (dimethylsulfate) are two examples that require permits.

Specific points:

• It is forbidden to store inflammable liquids, e.g. ether, in the refrigerator because of the risk of explosion. An explosion safe refrigerator is located in the chemical room for this purpose. An explosion safe freezer is located in GC room, #1154.

- Only small amounts of hazardous chemicals should be out in the open lab. **Ethanol** (95% and 99.5%) or other **inflammable** liquids should be stored in ventilated fireproof cupboards, *i.e.* you are *not* allowed to store them on lab benches or open lab shelves. Larger volumes of this type of liquid (*e.g.* organic solvents) are stored in the chemical storage building (outside).
- **Poisons** and **heavy atoms** are stored in a locked cupboard.
- Solvents and anaesthetic gases must be handled in fume hoods, on fume benches, or in ventilated filling cabinets.
- **Dry ice** is extremely cold (-78.5°C). Always handle it with care and wear protective cloth or leather gloves whenever touching it. If touched briefly it is harmless, but prolonged contact with the skin will freeze cells and cause injury similar to a burn. **Do not store dry ice in a completely airtight container. The sublimation of dry ice to carbon dioxide gas will cause any airtight container to expand and possibly explode.** Store the dry ice in an insulated container with a 'loose' lid and wear safety glasses or a face shield when you open it. Keep proper air ventilation wherever dry ice is stored.
- **Liquid nitrogen** is extremely cold: 77.3K = -196C at atmospheric pressure. This can cause severe frostbite. On vaporization it expands by a factor of 700 which can cause **explosion** of a sealed container, or it can displace oxygen in the room and cause **suffocation without warning**. Nitrogen gas is colourless and odourless -the cloud that forms when you pour liquid nitrogen is condensed water vapour from the air, not nitrogen gas.
 - Take care not to allow liquid nitrogen to be trapped in clothing near the skin. Wear safety glasses or a face shield when transferring liquid nitrogen and wear gloves when touching any object cooled by liquid nitrogen. Gloves should be loose fitting, so they could be thrown off if liquid were to pour inside them. Use only approved **unsealed** containers, never seal it in any container (it will explode). When you dip items like cryo tubes into liquid nitrogen never hold your face close to the container; it may spurt liquid. Never use in small poorly ventilated rooms, and never dispose of liquid nitrogen by pouring it on the floor. **Elevators must be locked** with a key before transportation of liquid nitrogen.
- Any other questions regarding handling of chemicals should be asked to Anders Carlsson. 040-41 55 61, 0730-24 76 02

3.3 How to find the chemicals you need

All chemical are registered in **Klara**, where to find them, their CAS numbers and quantities. https://internt.slu.se/verktyg/klara/. You also find Safety data sheet.

3.4 The most common hazardous chemicals

Chloroform is an organic solvent. It can cause irritation to the skin, eyes and throat. Inhalation of high doses of chloroform causes dizziness, sickness that can lead to unconsciousness and even death. It is also carcinogenic.

Phenol is an organic solvent. It has a corroding effect on the skin and the eyes; the vapour can give rise to severe lung damage. If someone gets phenol on her/his skin, first use a lot of water then 30% PEG 400.

Acrylamide is a colourless, odourless powder or white crystals. In the lab you most often handle it in solution. If you inhale it or if you splash it on your skin it can cause burns in the respiratory tract, tiredness, affect the central nervous system etc. It accumulates in your body over time why it is particularly hazardous. Also the polymerized form may contain unpolymerized acrylamide and should be treated as toxic. Choose readymade gels if possible!

Formamide is a colourless - faintly yellow liquid. It is hazardous if it gets in contact with your skin or if you inhale it. Some symptoms are burns in the respiratory tract and skin, and dizziness. It may cause spleen and liver damages and allergic reactions. It may also have teratogenic effects.

Ethidium bromide binds to DNA molecules by intercalating between adjacent base pairs. It is a powerful mutagen and moderately toxic. It should be treated as a carcinogen. In particular, the dry powder should be handled with extreme care. Heating gives rise to poisonous and corrosive gases. Always use protective gloves and be extra careful to avoid contamination. Special rules for disposal of EtBr must be applied (see Waste below).

We recommend you to use GelRed instead.

Heavy atoms bind to proteins and should be treated with extreme care. Wear gloves and use the scales that have been set up in the fume hood for this purpose.

Antibiotics Check safety data before use of antibiotics.

Acetonitrile

Chemicals that may pose special risks to pregnant or breastfeeding:

- Organic solvents
- Some pesticides that may have hormone-affecting effects. This applies, for example, to some of the plant protection products used in greenhouses.
- Cytostatics (medicines that inhibit cell growth).
- Anesthetic gases (anesthetic gases).
- Tobacco smoke and carbon monoxide.
- Carcinogenic or reproductive disorders. These substances have the following hazard statements in the safety data sheet (safety data sheet):
- H340 May cause genetic effects
- H341 Suspected of causing genetic defects
- H350 May cause cancer
- H351 Suspected of causing cancer
- H360 May damage fertility or unborn child
- H361 Suspected to harm fertility or unborn child
- H362 May cause harm to babies

The above-mentioned labeling system was introduced in January 2009 (CLP Regulation (EC) No 1272/2008 on the classification, labeling and packaging of substances and mixtures). This labeling system replaces the Chemical Inspectorate's labeling (according to classification and labeling regulations, KIFS 2005: 7) but the Chemicals Inspectorate's labeling still applies to mixtures during a transitional period until June 1, 2017. The designations of the Chemicals Inspectorate are as follows:

R40 Suspected of giving cancer

R45 May cause cancer

R46 May cause inherited injuries

R48 Risk of serious damage during prolonged exposure

R49 Risk of inhalation of cancer

R60 May cause impaired propagation

R61 May cause birth defects

R62 Possible risk of impaired propagation

R63 Possible risk of birth defects

R64 May cause harm to infants during lactation

Infections

Some infections may in some cases affect pregnancy. Some examples include chickenpox, measles, red dogs, toxoplasma, tuberculosis and hepatitis B and C. The risk of infection is generally not greater at work than in society. Good hand hygiene is often a sufficient preventative measure.

If you plan to become pregnant, it is good if you look at your vaccination protection, especially if you often come into contact with infectious agents in your work.

All the chemicals above should be treated with care and be handled in the fume hood.

4. Waste disposalThe different kinds of waste must not be mixed.

Categories of waste and disposal methods

Type of waste	How to dispose of it
Glass	Ordinary, non-contaminated glass is not regarded as hazardous waste. Should be taken to the "brännbart" container.
Cardboard boxes	Should be folded and taken to the "wellpapp" container
Broken glass, sharp objects and syringes Pasteur pipettes, etc.	These <u>must</u> be placed in a container before discarding them. Should be taken to the "brännbart" container.
Needles and scalpel blades	All needles, contaminated or not, are placed pre-collected in a container with a lid, and placed in the yellow boxes, and ticked skärande och stickande. Should be taken to destructionroom
Contagious waste Infected cultures, contaminated work material; infected biological waste, GMO and infected animal waste.	Put waste in autoclavable bags – autoclave and put in "brännbart"
Organic solvents- non chlorinated	Acetonitril (90%), aldehydes, butanol, ethanol, ethyl acetate, formaldehyde, glutaraldehyd, hexane, isoamyl alcohol, ketones, methanol, petroleum ether, toluene, xylene Should be taken to destructionroom
Organic solvents (ink. water)	HPLC mixtures, acetonitril (less 90%), TCA (max 5%) Should be taken to destructionroom
Organic solvent with halogens or sulfur	B-Mercaptoethanol, phenol with chloroform, chloroform. Should be taken to destructionroom
Non organic acids	Dilute and neutralize with NaOH, H2O2 dilute to 5%

	Should be taken to destructionroom
Gels	Dry in fume hood and put in boxes. Should be taken to destruction room
Chemicals (no mixes- single bottles)	Cacodylate buffer, colchisine, dichromate sulphuric acid, dinitrosalicylicacid, formalin, hydroxyquinoline, osmiumtetroxid, scintilationliquid, uranylacetate. Should be taken to destruction room
Photo chemicals	Fix and developer in separate bottles. Should be taken to destruction room
Silver	Should be taken to destruction room
Plastic	Take out to plastic container
Aluminum foil	Take out to metal recycling bin

Ethidium bromide (EtBr)	Use ready-made destaining bags. After use put destaining bags in waste room. The treated solution can be poured into the sink. Gels containing EtBr dries in fume hood and then put in waste room.
Radioactive waste	See chapter 6 for special instructions!
Heavy metal compounds	Do not mix the different metals. A collection bottle for each type is in the fume hood and then put in waste room. When you make up a heavy atom derivative, try to do it in as small quantities as possible. For example, use a 1 ml Eppendorf tube. Indicate what is in the Eppendorf before you discard it.
Environmentally hazardous waste	No reactive or inflammable compounds should be poured down the sink/drains. The same applies for poisons, heavy metals etc. To reduce the amount of and risk with the waste, you should evaporate off solvents, neutralize acids and bases, convert poisonous components to less poisonous substances etc. All such disposal should take place in a fume cupboard. Batteries, electronics and fluorescent lamp tubes are

	collected and taken for disposal by Göran Persson and Annelie Rydén. Further information about handling hazardous waste can	
	be found in Klara. https://internt.slu.se/verktyg/klara/	
Soil and plants	Discard in container marked "compost" Note! No plastic labels.	
Genetically modified micro-organisms (GMM)	Solutions such as culture media that contain genetically modified micro-organisms, should be disposed in containers (flasks or plastic jars) specially marked for this content (>for autoclaving of GM material<). The container is later sterilized by autoclaving, using the autoclave situated in room #1149 in the Horticum building, before disposed as common waste. Solid waste (plastics, agar plates containing genetically modified micro-organisms etc) and small liquid volumes (30 ml universals) should be disposed in special plastic bags labelled >for autoclaving< or special autoclave jars provided. Nucleic acids isolated from genetically modified micro-organisms should as well be disposed in the same type of bags. The bags are later sterilized by autoclaving, using the autoclave situated in room #1149 in the Horticum building, before disposed as common waste.	
Genetically modified organisms (GMO) e.g. plants	Plant materials and soil of different type from genetically modified plants are to be disposed in special cardboard boxes. RAGN-SELLS pick up for destruction. Contact Heléne Lindgren or Annelie Ahlman for more information.	

5. Rules for work involving gene modification (GM)

5.1 Genetically modified micro-organisms (GMM)

The national rules on contained use of GMMs are based on EC Directives. The directives governing the legislation of the Member States on biological substances and contained use of GMMs are minimum Directives, which means that stricter rules are permissible. Implementation of Directive can vary somewhat from one legal system to another, depending on the individual legal traditions of the different Member States. A conspectus of Swedish legislation on gene technology can be accessed through the website of the Swedish gene technology authorities, http://www.gmo.nu/. A guidance on contained use of genetically modified microorganisms (GMMs) can be found at http://www.av.se/dokument/inenglish/themes/gmm.pdf.

A genetically modified micro-organism (GMM) is defined as: a micro-organism whose genetic material has been altered in a way not occurring naturally through mating or natural recombination." Micro-organisms include, for example, bacteria, blue-green algae, virus, viroids, prions, micro-fungi, micro-algae, protozoa and cell cultures of superior organisms.

In order to work with GMMs the Department and/or your research group should have a permit for such activity issued by the Swedish Work Environment Authority. GMMs may not be released into the environment without permission. Therefore, specific containment and other protective measures have to be applied in order to limit contact between the organisms and the general public or the environment. The degree of protective measures has to be decided on a case to case situation. This mean that for each activity involving GMMs you will have to do a risk assessment, so as to determine which containment and other protective measures are needed for the prevention of harm to human health and the environment.

Contained use is defined as:

an activity in which organisms are genetically modified, cultured, stored, used, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment.

Before starting working with GMMs you should confirm with the person responsible that a permit for the specific activity has been issued to the Department and/ or research group and that it covers for the locality you have chosen for your work. You should then take the necessary measures and plan your work such that it can be done in a safe way. For disposal of GMMs see #4 Waste disposal.

5.2 Genetically modified organisms (GMO)

GMOs involved in activities at the Department of Plant Breeding and Biotechnology are limited to plants. Like GMMs, the national rules for use of GMOs are based on

EC Directives. The rules state that before starting work involving genetically modified plants (GM-plants) a permit for such activities should have been obtained from the Swedish Board of Agriculture (http://www.jordbruksverket.se/swedishboardofagriculture.4.6621c2fb1231eb917e68 0002462.html). A permit is required for the activity involving a specific GM-plant as well a permit for the premises in which the work is carried out. Please inquire with the person responsible for GMO permits at the Department on what's available.

In general, at the Department of Plant Breeding and Biotechnology, we have permits to carry out GM work in a number of laboratories in the Horticum building (inquire with the person responsible for the details). These premises include the climate chambers located in the Horticum building. In addition, we also have a permit to handle and cultivate GM plants in the biotron (see specific permit) and in the greenhouse (see specific permit on growth facilities). Regarding permits on specific plant species on which we are allowed to carry out GM related work, they presently includes a number of plants such as Arabidopsis, Crambe, Oat, Sesame, Camelina, Brassica juncea, Brassica carinata, potato. For the full list please inquire with the GMO responsible person at the Department.

Seed material of any kind that is GM should be stored in a special locked cabinet in room #1121 in the basement of the Vegetum building

5.2.1 Laboratories

GM plant material should be handled in the laboratory in a safe and contained way so that there is now risk of releasing it to the environment. Deposition of GM waste should be done according to instruction under 4. Waste disposal. Measures should be taken so that when transport of plant material to the laboratories needs to be done, it should be done in a contained way. When climate chambers in the Horticum building is used for cultivation of plant material such work should then be done in sealed petri dishes or similar.

5.2.2 Biotron

We can cultivate GM plants in the Biotron. Before starting your activities with GM material in the Biotron you are required to obtain information about the rules directing such activities and to understand them. Information about this and the rules for carry out GM cultivation in the Biotron is received from Karl-Johan Bergstrand in the Biotron and from the GM responsible person at the Department.

5.2.3 Greenhouse connected to the Horticum building.

Cultivation of GM plants can be carried out in the greenhouse during non-field season i.e. October – April. Before starting your activities with GM material in the greenhouse you are required to obtain information about the rules directing such activities and to understand them. Information about this and the rules for carry out GM cultivation in the green house is acquired from the GM responsible person at the Department.

6. Rules for radioactive isotope work

Contact the responsible persons (Ida Lager and Åsa Grimberg) well in advance before you plan to start your experiment. In order to start work with radioactive isotopes, you will get a short training course leading to a permission signed by the responsible persons and the head of the department.

All work with radioactive isotopes (except for ¹⁴C), must be carried out in the room for radioactive work in Horticum (room H1124). You are obliged to make yourself correctly informed about routines and permissions for work with radioactive isotopes before you start your experiment. Be aware that ionizing radiation can cause severe injuries in your body like cancer if you do not protect yourself properly. It is your responsibility to know the risks with the type of radiation you work with!

Our contact person at SLU if you need help and advice in case of accident and questions about radioactivity is: Enn Maripuu, radiation specialist (e-mail: stralsakerhetsexpert@slu.se, telephone: 0730-340262). General questions about radioactivity can be asked to the radiation safety committee at SLU (stralsakerhetskommitten@slu.se). The persons responsible for radiation protection at SLU is Torbjörn Alwehammar (telephone: 018-67 10 39 or 070-36 92 636).



The classical symbol for radioactive radiation (left) and the new symbol (right).

6.1 Working routines

6.1.1 Routines in general for working with radioactivity

- It is absolutely forbidden to eat, drink, snuff or smoke in the lab. This is true at all times and not just when working with radioactivity.
- You are advised to **not** work with radioactivity during weekends.
- You can only buy radioactive isotopes through persons with such permission; ask the responsible persons who these are.
- Use as small quantities (activities) as possible and plan your work ahead to minimize the time spent handling the isotope.
- Distance yourself appropriately from sources of radiation. (Doubling the distance from the source quarters the radiation dose; the Inverse Square law).

- Use appropriate shielding for radiation. Be aware that different isotopes require different shielding.
- A dosimeter is optional (not compulsory) for everyone working in the departments.
- Always use lab coat, gloves, and protective glasses. Do not work with open wounds on hands and arms.
- All benches and working areas should be protected by protection paper (plastic side down).
- To detect contamination monitor the work area before and after you start (use the Geiger-Müller tube). If necessary, decontaminate and change the protection paper. Monitor yourself afterwards and don't forget your hands. Sign the logbook! (Logbook is not needed for ¹⁴C).
- The responsible persons will do additional contamination control of the room for radioactive work every third month. The results will be noted.
- Please observe that cleaning up in the isotope lab is NOT done by the cleaning personnel, but by you.
- All vessels/containers, in which radioactive compounds are stored, have to be properly labelled (type of isotope, activity, date, warning symbol as well as with your own name).
- Use disposable materials, like plastic bottles, plastic syringes etc. Try to avoid the use of glassware that has to be washed.
- Transport of all radioactive waste has to be done with utmost care. Never carry directly in your hands. Use lead boxes, plastic shields and trays for ³²P radioactive waste.
- Decontamination of contaminated lab instruments has to be done IMMEDIATELY after you discover it (see below for rules how to handle minor and major spillage).
- Incidents and accidents during work with isotopes have to be reported immediately to the responsible persons and the head of the department.

6.1.2 Routines for work with ³²P and other high-activity isotopes

Compounds with ³²P-isotope are strong beta-emitters with a relatively short half life (14 days). All high-activity isotopes are stored in the special isotope lab ("radioactive room", H1124). A fridge and a freezer are available for this purpose in this room. Labelling using ³²P should be done in this room. This work should be performed

behind a protective Plexiglas shield. In case of any risk for producing radioactive airborn compounds; work in the fume hood!

6.1.3 Routines for work with ¹⁴C (low-energy isotope)

Compounds with ¹⁴C-isotope are very weak beta-emitters and the radioactive radiation is not dangerous as long as you do not work with very high amounts, and if you do not inhale or swallow it; therefore you are allowed to work with such compounds even outside the radioactive room. However, the half life of ¹⁴C is very long (5730 years) so it is very important that you do not cause contamination that can be of danger to your colleagues and to yourself; make sure that no one can get it by mistake into their mouth or through wounds. You have to mark the working area and all equipment and waste bottles you use with special tape which is labelled with "¹⁴C". Carefully clean up any spillage immediately. Very important is also that you think about if you produce any significant amounts of gases in your experiments, for ex ¹⁴CO₂ through respiration.

6.2 Radioactive waste

The radioactive waste is discarded in cartoon boxes with appropriate tags. **NEVER** throw radioactive waste in boxes or bins without appropriate tags! By law there are special rules for labelling and documentation of radioactive waste; this you will learn during the training course (or ask the responsible persons if you are not sure about the rules).

6.2.1. Solid radioactive waste:

Specially designated carton boxes for ³⁵S, ³³P, ³H and ¹⁴C waste should be available (if not, contact the responsible persons). Some solid radioactive wastes of small size (pipet tips, small plastic tubes etc) and hazardous waste like razor blades and needles are first collected in shielded plastic boxes on the lab bench. When full, these are sealed and put in the cartoon boxes. Boxes for radioactive waste containing high-energy emitters are placed behind Plexiglas shields. Inform the responsible persons when cartoons are filled so they remove it properly before it is overloaded. When you put up a new box, make sure that the box is labelled with the correct type of isotope before people start using it.

Each individual waste box is not allowed to contain more than the radioactivity listed below. The total sum of radioactivity waste from a lab that is handed to combustion every month is not allowed to be more than ten times the values listed below. If the waste includes several different isotopes there are special rules how to calculate the total activity.

The maximum dose allowed in each box:

³²P: 10⁵ Bq = 2.7 μCi ³⁵S: 10⁸ Bq = 2700 μCi ³³P: 10⁸ Bq = 2700 μCi ³H: 10⁹ Bq = 27000 μCi

 ^{14}C : 10^{7} Bq = 27000 μC i

¹²⁵I: 10^6 Bq = 27 μ Ci

6.2.2 Liquid radioactive waste

Sinks specially designated for radioactive waste are situated in the Radioactive Room and certain labs. One can flush such waste as long as no other hazardous chemicals are present. Flush with plenty of water before and after. A maximum of 10 times the activity listed above is allowed to throw out in the sink in total every calendar month. The amount allowed to throw out in the sink at one occasion is the amount listed above. Larger quantities must be discarded after first absorbing the liquid, followed by disposal via the appropriate carton.

6.2.3 Scintillation waste

Scintillation vials should be placed in cartons together with about 1 Liter of absorbing material. Caps must be screwed on tightly and the cartons must be stored in a well ventilated area.

6.3 Radioactive spillage

In case of an accident:

In case of spillage of more than 40MBq (1 mCi), report to Strålskyddsmyndigheten, "SSM" (tel: 08-799 40 00). Spillage of this amount and higher should be cleaned by experts and not by us. In case of contamination of a person you must report to SSM, even if it's a little spillage.

6.3.1 Minor radioactive spillage without personal contamination

- Use lab coat, gloves and shoe protection.
- Mark around the contaminated area with a pen.
- Absorb the spillage with an absorbing paper or vermiculite without rubbing.
- Wash repeatedly with soap water until you can't measure the radioactivity. If
 it's ³²P you need to clean with an alkali solution (dishwashing solution) or
 diluted NaOH.
- Cover with a plastic absorbing paper with the absorbing part downwards.

Major spillage with or without personal contamination

6.3.2 Major radioactive spillage with or without personal contamination

- Interrupt all work in the room. Close all apparatus.
- People that are going to help should have protection clothes, gloves, and shoe protections.

- Cleaning of contaminated people must be done immediately. Take off and leave contaminated clothes and shoes in the laboratory. Verify the contamination by measuring the activity.
- Clean the contaminated persons with soap and lukewarm water. **Don't scrub!**
- Try to only clean the area around the contaminated skin to prevent spreading.
- If there are a contaminated wound let it bleed for a while and then wash it under water for a long time.
- Close the laboratory and write a big sign to put on the door that informs about the accident.
- Inform everyone in the department.
- Contact the Department for Radiation Physics in and they will help with the cleaning and measurement on the contaminated persons.

7. Rules for microbial work

By law, all microorganisms should be regarded as pathogens or allergens. This includes bacteria, protozoa, fungi (yeast) and viruses. It is not allowed to introduce new microbes into the lab without notifying the safety officer or Head of Department. All microorganisms have to be classified according to potential health hazards. If the new organism is classified into Category 2, special notices have to be posted at storage and work areas. Microorganisms with higher classification are currently not present in the department. This legislation does not apply to purified components.

The Ten Commandments for handling microorganisms are:

- 1 No mouth pipetting.
- 2 No eating or drinking in the labs (true for all kinds of laboratory work).
- 3 Button your lab coat and use gloves.
- 4 Wash your hands before leaving the lab.
- 5 Used pipettes are placed in buckets with disinfectant.
- 6 Other contaminated glassware must be disinfected before sending it to the dishwashing. Disinfection is normally done with 70% EtOH solution.
- 7 Media and other solutions contaminated with fungi (molds) should be autoclaved (preferably for an extended period) before disposal.
- 8 Surfaces are disinfected with 70% Ethanol.
- 9 Agar plates, plastic tubes and disposables are to be collected in autoclavable bags and then autoclaved. Sharp objects must be collected separately.
- 10 It is advisable to use specially designated areas for microbial work to minimize risks for cross contamination.

Rules for work in the Microbial Horticulture Risk Class 2-laboratories 2109, 2111, 2104

In these laboratories, work with bacteria risk class 2 and GM-bacteria risk class 1 is performed. In addition to the general rules for microbial work, you must also consider the following rules:

Wash hands with soap and water, followed by ethanol, upon entering and before leaving the laboratory

No scarves or hats in the lab. Extra clothing must be left outside laboratory.

No soil in the laboratories.

Lab coats: the lab coat with name tag must always be worn when inside the laboratory. Make sure not to bring the lab coat outside the laboratory. Do not bring the same lab coat to another lab or to the greenhouse.

Magnets: the tag that has your name and phone number on it must be placed on the board outside the lab when you are working in the lab. Make sure you take it off when you leave. You must bring your phone with you when working in the laboratory.

In each lab, there is a hand lamp that will light up in the case of power failure. If the power goes out, unplug the lamp and bring it with you in order to light the way.

Each lab room has an alarm button that can be used in case of emergency. If the button is pressed, there will be an alarm ringing in the corridor outside the lab.

Before you start working in the class 2 laboratory of Microbial Horticulture, you must also read the following booklets compiled by the Swedish Work Environment Authorities:

- Contained Use of Genetically modified Microorganisms (AFS 2011:2Eng) Or in Swedish:
 - Innesluten användning av genetiskt modifierade mikroorganismer (AFS 2011:2)
 - Mikrobiologiska arbetsmiljörisker smitta, toxinpåverkan, överkänslighet (AFS 2005:1)

You must also read and sign the documents *Risk assessment for risk class 2-organisms* and *Handling instructions for risk class 2-organisms*.

8. Rules for handling of human material

Before you start working with human material, you must make yourself correctly informed. The special rules for the blood hood are found below.

- All blood (from the blood central, patients, colleagues *etc.*) should be handled as infectious.
- All tissue samples (from patients, colleagues *etc.*) should be handled as infectious.
- All handling of blood and tissue should be done in a specially prepared hood.
- No other work is allowed in this specially prepared hood and further practical instructions should be posted on the hood.

• All users and supervisors are responsible for keeping informed about the risks and to follow the instructions.

9. Use of the (ultra)centrifuges and their rotors

Before you use the ultracentrifuges the first time you have to ask someone to give you a proper instruction! It is your own responsibility to make yourself correctly informed.

There are two different types of rotors: aluminium (Al) and titanium (Ti). The aluminium rotors' buckets are black inside, while the titanium rotors are shiny.

9.1 Cleaning the rotors

The Al rotors corrode if they are not treated properly. This means that they have to be cleaned and dried after each run. Of course this should be done to the Ti rotors as well. Clean the buckets with a special brush and special detergent (bought from Beckman especially for this purpose). Be careful not to scratch the buckets. Rinse them with water and dry them immediately. Never leave an Al rotor in the sink; you can obtain a galvanic cell! Don't forget to clean the inside hang-on of the swing-out rotors.

Never use metallic tools on any of the rotors! **Scratched surfaces will ruin the rotor!** Never use any alkaline solutions for cleaning!

9.2 Lubricating the rotors

Check that the o-rings and the caps are properly greased. They should never be dry. Use Vacuum grease for o-rings and caps and Spin-coat for buckets.

9.3 Balancing the rotors

Before each run, the tubes have to be balanced very carefully. Be aware that at 602,000g:

1g = 602 kg! 1 ounce = 18.8 tons!

Balance the rotor symmetrically. All buckets must be on when the swing-out rotors are used. Check that the buckets are not miss-hooked on the swing-out rotors. A miss-hooked bucket can come off at 1000 rpm.

When using the VTi rotors, you should not put on caps on empty holes.

9.4 Change of over-speed discs

If the over-speed disc is damaged or lost, it has to be replaced by a new disc. It is, however, very important that this replacement is correctly performed and that the new disc is the right kind to fit that kind of rotor. If not, the rotor may be destroyed. Because of this, the replacement should always be taken care of by the service technician.

9.5 Overloading

The rotor information sheet has to be consulted in order to find out the maximum speed for any given density. For example: for maximal speed a swing-out rotor should

have a sample density not exceeding 1.2g/cm³, and the vertical rotors not over 1.7g/cm³.

9.6 Miscellaneous

- <u>Log books</u>: The log-books should be filled in correctly for every run. Don't forget to specify the rotor serial number. This information is needed in order to de-rate the rotors in time. Al rotors have a life expectancy of 2.000 runs or 5.000 hrs, Ti rotors 5.000 runs or 10.000 hrs.
- <u>After centrifugation:</u> wipe off the centrifuge chamber to remove dirt and humidity.
- <u>Classification of centrifuges:</u> On the lid of every centrifuge a list of all compatible rotors is found.
- Quick-seal tubes: These tubes should always be filled all the way up.
- <u>Stuck tubes:</u> If a tube gets stuck in the rotor, apply some SDS solution and carefully jiggle it out (no metal tools!). If you don't succeed, contact the service technician.

10. In case of fire

In the corridor you will find the following:

Fire extinguisher (water)
Fire extinguisher (CO₂)--for electrical fires
Fire quenching blanket
Fire alarm
First aid kit

The alarm is connected to the fire department.

In case of a larger fire you have to do as follows:

- Make sure nobody is left in the lab.
- Warn others.
- Stop the fire from spreading by closing the lab doors.
- If possible, put out the fire using the fire extinguisher.
- Evacuate the lab! *The meeting place* (meaning the place where everybody has to go to right away in order get information) *is at the parking lot next to the Biotrone*.

IN THE EVENT OF FIRE

- 1. RESCUE people.
- 2. FIRE ALARM-Automatic
- 3. Close doors to CONTAIN the fire.
- 4. (If possible, put out the fire.)
- 5. EVACUATE the building.

It's compulsory for all employees to participate in basic training in systematic fire prevention.

11. Eye Safety/Emergency showers

- Always use eye-protection (glasses, screens) when working with dangerous stuff.
- Inform yourself beforehand where the eye-showers are.
- If you spill or quirt something into your eyes anyway, do the following:

The victim	<u>Co-workers</u>
Hurry to an eye-shower	Help your buddy to eye showers and start it for him/her.
Yell for help	Help to direct the solution into the eyes of the victim and keep his/her eyes wide open
Wash/shower your eyes for 20 minutes.	Call the hospital and tell them somebody is on their way. Arrange transport to the hospital.
	Drop sterile physiological (0.9%) NaCl-solution into the victim's eyes during transport to the hospital.

TELEPHONE NUMBERS

Lund University Hospital (Skånes Universitetssjukhus Lund) 046-17 10 00 Malmö University Hospital (Skånes Universitetssjukhus Malmö) 040-33 10 00

Important Phone Numbers

Fire department and ambulance (00) 112 Växtskyddsvägen 3, Alnarp or Sundsv 14, Alnarp

To get a line outside of SLU, first dial:	00
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Toxic chemicals - hot line 08- 33 12 31

Building service (Akademiska hus) (daytime) 046-31 13 00 (such as electricity, (other hours) 046-31 13 10

heating, water or sewer)

Security matters (Securitas)

(for suspicion of crime, unauthorized persons in the building, etc.)

0771-50 16 15

House code:
40 44 81 12 + common code

Non-Emergency Medical Care:

Vårdcentralen i Arlöv040-623 41 00Vårdcentralen i Lomma040-623 48 00Medical care hotline1177Medical care hotlinewww.1177.seAvonova (Företagshälsovården)046-19 33 30

https://www.avonova.se/

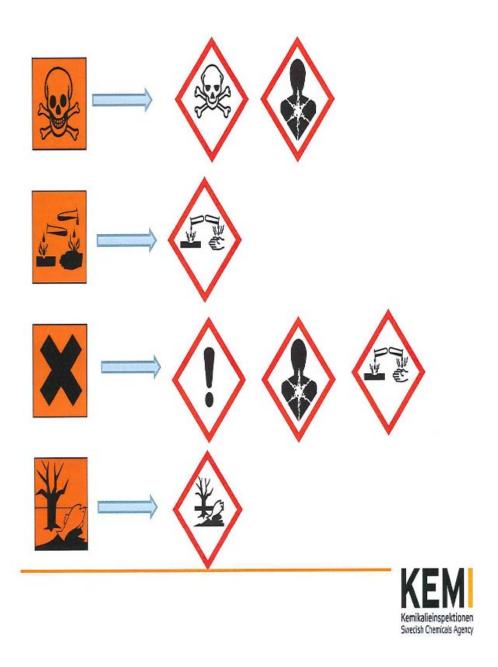
Web address to report working incidents

https://internt.slu.se/stod-service/admin-stod/sakerhet/kris-och-incidenthantering/rapportering/formular/

Web address to report injuries at work

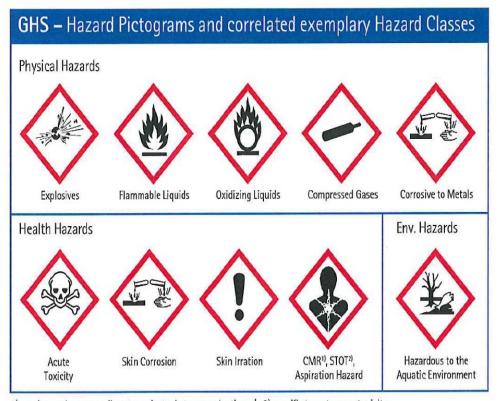
https://anmalarbetsskada.se/

Warning Symbols



GHS - Hazard and precautionary statements (H- and P-statements)

- The current Risk (R) phrases are transformed into a hazard statements (H-statements) under GHS.
- The current Safety (S) phrases are transformed into a precautionary statements (P-statements) under GHS.
- The number of H and P statements increases under GHS and the wording is revised.



¹⁾ carcinogenic, germ cell mutagenic, toxic to reproduction / 2) specific target organ toxicity

Informed Receipt of the Safety Regulations

I have received and read the booklet "Safety Manual" and	I will follow the
<u>instructions</u> . The contents of the manual have been discussed v	
group leader in the presence of the safety representative. I ha	ave been shown the
protection and safety devices in the laboratory.	
Signature Employee	Date
Clarification of Signature	
Signature Group leader	Date
Signature Group leader	Date
Signature Safety Officer	Date

It's compulsory for all employees to participate in basic training in systematic fire prevention

This receipt is stored by Elisabeth M