

SLU
Department of Biosystems and
Technology
(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 is updated in January each year.

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

Latest update: January, 2021

Table of Content

- 1. Introduction**
 - 1.1 Contact persons for safety issues**
 - 1.2 Further information about safety matters**
 - 1.3 Points of order**

- 2. General routines**
 - 2.1 Equipment for personal protection**
 - 2.2 Responsibility assignments**
 - 2.3 Broken instruments**
 - 2.4 Dishwashing**
 - 2.5 Purchasing and work order routines**
 - 2.6 Labelling of containers and material in the laboratory**

- 3. Handling of chemicals**
 - 3.1 Risks and dangers**
 - 3.2 How to find the chemicals you need**
 - 3.3 The most common hazardous chemicals**

- 4. Waste disposal**

- 5. Rules for working involving gene modification (GM)**
 - 5.1 Genetically modified microorganisms (GMM)**
 - 5.2 Genetically modified organism (GMO)**
 - 5.2.1 Laboratories**
 - 5.2.2 Biotrone**
 - 5.2.3 Greenhouse connected to the Horticum building**

- 6. Radioactivity**
 - 6.1 Working routines**
 - 6.1.1 Routines in general for working with radioactivity**
 - 6.1.2 Routines for work with ³²P and other high activity isotopes**
 - 6.1.3 Routines for work with ¹⁴C (low-energy isotope)**
 - 6.2 Radioactive waste**
 - 6.2.1 solid radioactive waste**
 - 6.2.2 liquid radioactive wastes**
 - 6.2.3 scintillation waste**
 - 6.3 Radioactive spillage**
 - 6.3.1 Minor radioactive spillage without personal contamination**
 - 6.3.2 Major radioactive spillage with or without personal contamination**

- 7. Rules for microbial work**

- 8. Rules for handling of human material**

- 9. Use of the ultracentrifuges and their rotors**
 - 9.1 Cleaning the rotors**
 - 9.2 Lubricating the rotors**
 - 9.3 Balancing the rotors**
 - 9.4 Change of overspeed discs/decals**
 - 9.5 Overloading**
 - 9.6 Miscellaneous**
- 10. In case of fire**
- 11. Eye safety**

Important phone numbers

Warning symbols

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. However, first you should know which rules apply to you during your various activities.

Working alone in the lab with dangerous procedures 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 this manual to acknowledge that you have been informed of the safety routines of the department.

The Safety Manual is stored in the lab and the signed paper is stored at the office of the Human Resources Administrator.

Also: Your supervisor/host must introduce you to all the members of the Department.

1.1 Contact persons for safety issues

Area	Person Ext.	Responsible for	
Head of Department	Hanna Sassner	040-415062	Vegetum – BT
Dep. Head of Department	Georg Carlsson	040-415254	Vegetum – BT
Inflammable Substances and Gases	Marianne Oraviita	040-415231	Vegetum – BT
	Göran Birgersson	040-415300	Vegetum – PPB
Head Safety Officer	Lars Hagtorn	040-415331	SLU Alnarp
Safety officers	David Hansson	040-415138	Vegetum – BT
	Göran Birgersson	040-415300	Vegetum – PPB
Isotope safety	Ida Lager	040-415348	Horticum
	Åsa Grimberg	040-415541	Horticum
Union Representative ST	Vakant		
Union Representative Saco	Andrus Kangro	040-415479	SLU Alnarp
Service technician	Göran Persson	0702-231706	Horticum/Vegetum
	Anneli Rydén	0702-226685	Horticum/Vegetum
GMO responsible	Mariette Andersson	040-415541	Horticum/Vegetum
Financial administrator	Gunilla Andersson	040-415556	Vegetum – BT
Human resources administrator	Johanna Strand	040-415366	Vegetum – BT
Environment responsible	Linda Engh	040-415263	Vegetum – BT
Fire protection coordinator	David Hansson	040-415138	Vegetum – Evacuation officer
Evacuation Leaders	David Hansson	040-415138	Vegetum – Parallel corridor
	Linda Engh	040-415263	Vegetum – Parallel corridor
	Marie-Claude Dubois	040-415035	Vegetum – Corridor A
	Madeleine Magnusson	040-415085	Vegetum – Corridor B
	Maria Ernfors	040-415145	Vegetum – Corridor E
	Lars Mogren	040-415376	Vegetum – Corridor G/N. workhall
	Helene Larsson Jönsson	040-415370	Vegetum – Corridor H/N. workhall
	Karl-Johan Bergstrand	040-415343	Vegetum – Food lab/Greenhouse

1.2 Further information about safety matters

Further information can also be found on the SLU home page www.slu.se under staff web. <https://internt.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 the building.
- **Eating, drinking or preparing food is absolutely forbidden in the labs.**
- **Lunch room:** in order to keep this room enjoyable it is important that everyone makes the effort to keep it clean and tidy. Do not leave dirty cups, plates or utensils. Make sure the microwave oven is clean after you've used it. Do **not** enter the kitchen or lunch room wearing your lab coat!
- **Last person to leave the laboratory must close all windows, switch off the lights and lock the doors.**
- **For lab work outside office hours:** (Monday-Friday 7-18.30, excl. public holidays), please refer to the working environment routine "Unaccompanied laboratory work outside normal office hours"

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
- Chemical waste store

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. Responsible persons are signed on the doors of each laboratory.

2.4 Dishwashing

Each group/lab clean their own dishes. Dishwasher is located at the Northern end of Vegetum.

2.5 Purchasing and work order routines

- The Department has 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.
- Karl-Erik Gustavsson is responsible for labs in the Cropping Systems Ecology group.
- Håkan Asp is responsible for labs in horticultural production physiology group
- Anna Karin Rosberg and Maria Karlsson are responsible for labs in the Microbial Horticulture group

2.6 Labelling of containers and material in the laboratory

All containers and material in the laboratory **must** be labeled with content and concentration, name of responsible person and date

3. Handling of chemicals

3.1 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 where special information on certain chemicals can be obtained.

All hazardous chemicals must be labelled with an appropriate pictogram (warning symbol, presented at the end of this safety manual). If such a chemical is transferred from the original package to another container or bottle, the correct symbol must to be attached to the new container. 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/>.

Username and password will be obtained from Karl-Erik Gustavsson. You may also find this and other useful information of chemicals on web pages of some of the larger companies that provide chemicals like Sigma Aldrich, Fisher Sci and VWR.

Chemicals classified A and B can only be handled by a person with a special permit from Arbetsmiljöverket (Swedish Work Environment Authority) or Läkemedelsverket (Swedish Medical Products Agency). This permit is limited in time. Dimetoxibenzidin is an example of chemicals that requires permission.

Specific points:

- **It is forbidden to store inflammable liquids, e.g. ether, in refrigerators because of the risk of explosion.** An explosion-safe refrigerator is located in the chemical room for this purpose in Horticum. An explosion-safe freezer is located in GC-room #1154 also in Horticum.
- Only small amounts of hazardous chemicals should be out in the labs. **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, refrigerators, freezers or open lab shelves. Larger volumes of this type of liquids (*e.g.* organic solvents) are stored in the chemical storage in the South end of Vegetum, room 2870. The key to this room is stored in Karl-Erik Gustavsson's office.
- Poisons and heavy atoms must be stored locked in the designated cupboard.
- **Organic 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 clothes 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, **since it can displace oxygen in the room and cause suffocation without warning**

- **Liquid nitrogen** is extremely cold (-196°C) 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 comes inside.

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 referred to Karl- Erik Gustavsson, 040-415392, 0705-568957

3.2 How to find the chemicals you need

All chemical are registered in **KLARA** (<https://internet.slu.se/verktyg/klara/>), where to find them, their CAS numbers and quantities.

3.3 The most common hazardous chemicals

Organic solvents (also referred to as halogenated organic solvents) such as Chloroform, Acetonitrile, Acetone, Methanol, Hexane and Phenol. These compounds causes irritation to the skin, eyes and throat. Further, inhalation of high doses of halogenated organics causes dizziness, nausea and can lead to unconsciousness and even death. These compounds are also carcinogenic.

Acrylamide is a colourless and 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 so 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.

Heavy metals 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 Rifampicin, Neomycin, Ampicillin, Kanamycin, Tetracyclin, Carbenicillin, Gentamycin, Chloramphenicol and Hygromycin. **They may cause harm to unborn child, impair fertility and cause sensitization. Be very careful!**

Chemicals registered as “CMR”; e.g. **Boric acid, Imidazole, Crystal Violet, etc.** These chemicals are proved carcinogenic, mutagenic, toxic to fertility, etc. If no reasonable substitute for the chemical is found, a form must be created and signed by head of department, group leader and each employee that will perform the experiment. The form must state that **a)** there is no reasonable substitute and **b)** that all necessary precautions are considered at all times. The form is to be sent to the registry at SLU and must be saved for a minimum of **40 years**.

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

4. Waste disposal

Any collection of waste must, at all times, be labeled with content, the correct pictogram and name of user.

Different fractions of waste must be kept separated and must never 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 “glass recycle” container. This includes also chemical bottles.
Cardboard boxes	Should be folded and taken to the “wellpapp”-container
Broken glass, sharp objects and syringes Pasteur pipettes	These <u>must</u> be placed in a container before discarding them. Bring to the “brännbart” container.
Needles and scalpel blades, etc.	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". Bring to the chemical waste storage room.
Infectious waste. Infected cultures, contaminated work material; infected biological waste, GMO and infected animal waste.	Autoclave as soon as possible in laboratory or put in designated plastic bag and cardboard box. In the latter case, mark the cardboard box carefully according to instructions (ask your instructor) and bring it to be stored in locked container next to the H-house greenhouse to be picked up by Ragn-Sells.
Organic solvents- non halogenated	Acetonitril (90%), aldehydes, butanol, ethanol, ethyl acetate, formaldehyde, glutaraldehyd, hexane, isoamyl alcohol, ketones, methanol, petroleum ether, toluene, xylene Bring to the chemical waste store and pour in correct barrel.
Organic solvents (incl. water)	HPLC mixtures, acetonitril (less 90%), TCA (max 5%) Bring to the chemical waste store and pour in correct barrel.
Organic solvent with halogens or sulfur	B-Mercaptoethanol, phenol with chloroform, chloroform. Bring to the chemical waste store and pour in correct barrel.

Non-organic acids	Dilute and neutralize with NaOH, dilute H ₂ O ₂ to 5%. Bring to the chemical waste store.
Gels	Dry in fume hood and put in boxes. Bring to the chemical waste store.
Chemicals (no mixes- single bottles)	Cacodylate buffer, colchicine, dichromate sulphuric acid, dinitrosalicylic acid, formalin, hydroxyquinoline, osmiumtetroxid, scintillation liquid, uranyl acetate. Bring to the chemical waste store.
Silver	Bring to the chemical waste store.

Radioactive waste	See chapter 6 for special instructions!
Heavy metal compounds	<p>Do not mix the different metals. A collection bottle for each type of heavy metal is kept in the fume hood and then transferred to the chemical waste store.</p> <p>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.</p>

Environmentally hazardous waste	<p>No reactive or inflammable compounds should be poured down the sink/drains. The same applies for poisons, heavy metals etc.</p> <p>To reduce the amount and risks associated with this type of waste, you must evaporate off solvents, neutralize acids and bases, convert poisonous components to less poisonous substances etc. All such treatments should take place in a fume hood.</p> <p>Batteries, broken fluorescent lamp tubes are collected and taken to disposal by Göran Persson.</p> <p>Further information about handling hazardous waste can be found in Klara (https://internt.slu.se/verktyg/klara/)</p>
--	---

Soil and plants	Discard in container marked “compost” Note! No plastic labels.
------------------------	--

Genetically modified micro-organisms (GMM)	<p>Solutions such as culture media that contain genetically modified microorganisms, should be disposed of in marked (for autoclaving of GM material) containers (flasks or plastic jars). The container and its content must be sterilized by autoclaving.</p> <p>Nucleic acids isolated from genetically modified microorganisms should be disposed of in designated bags. The bags are later sterilized by autoclaving, using the autoclave situated in room #1100 in the Horticum building, before disposed of as common waste.</p> <p>Solid waste (plastics, agar plates containing genetically modified microorganisms, etc.) and small liquid volumes (30 ml universals) should be disposed of in designated, marked boxes. The boxes are collected and stored in container next to the H-house greenhouse in order to be picked-up for destruction.</p>
---	--

Genetically modified organisms (GMO), e.g. plants	<p>Genetically modified organisms (GMO) e.g. plants. Plant materials and soil of all type from genetically modified plants are to be disposed in special plastic bags and cardboard boxes. The boxes should be stored in room #1205 “förbindelsegången” to await pick-up for destruction by external company. Ragn-Sells, Malmö will pick up for destruction. Contact Heléne Lindgren or Niklas Olsson for more information.</p> <p>In vitro plants and for the plants used growth medium and utensils should be disposed in special plastic bags labelled "for autoclaving" or specially provided autoclave jars. The bags are later sterilized by autoclaving, using the autoclave situated in room #1100 in the Horticum building, before disposed of as common waste</p>
--	---

5. Rules for work involving gene modification (GM)

5.1 Genetically modified micro-organisms (GMM)

Before starting working with GMMs you should confirm with the responsible person 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.

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".

5.2 Genetically modified organisms (GMO)

Before starting working with GMOs you should confirm with the person responsible, Mariette Andersson, 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 GMOs see #4 Waste disposal.

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 .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. Contact the person responsible for GMO permits at the Department to check what's available. (<http://www.jordbruksverket.se/swedishboardofagriculture.4.6621c2fb1231eb917e680002462.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, 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 include a number of plants and traits. For the full list please inquire with the GMO responsible person at the Department, Mariette Andersson. Seed material of any kind that is GM should be stored in the Horticum building and in a special locked cabinet in room #1121 in the basement of the Vegetum building. Cold storage of seed potatoes should be in room #1188.

5.2.1 Laboratories

GM plant material should be handled in the laboratory in a safe and contained way so that there is no 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 closed 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.

Non-GMO control plants grown in the same room/area must also to be treated as GMO. The growth chamber has to be labelled with a specific GMO sign with information about GM-crop and responsible person. Special lab coat, stored in the GM area, has to be worn during flowering and when seed has developed. A special adhesive mat shall be placed close to the exit. Deposition of GM waste should be done according to instruction under #4 Waste disposals

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.

Also non-GMO control plants grown in the same room/area has to be treated as GMO. The greenhouse chamber has to be labelled with a specific GMO sign with information about GMO-crop and responsible person. Special lab coat, stored in the GMO-area, must to be worn during flowering and when seed has developed. A special adhesive mat shall be placed close to the exit. The drain has to be covered with a filter, preventing plant material to be released into the environment through watering etc. The filter should be discarded as GMO material specified under # 4. A special vacuum cleaner, stored in the planting hall marked with GMO, shall be used when cleaning the chamber. Deposition of GM waste should be done according to instruction under #4 Waste disposals.

Our contact person at SLU if you need help and advice in case of accident and questions about radioactivity is: Stig Larsson, radiation specialist at Karolinska sjukhuset in Stockholm (e-mail: stig.larsson@karolinska.se , telephone: 08-511 79 443). General questions about radioactivity can be asked to the radiation safety committee at SLU (stralsakerhetsexpert@slu.se), where Stig Larsson is one of the members . The person responsible for radiation protection at SLU is Torbjörn Alwehammar (telephone: 018-67 10 39).



6. Rules for radioactive isotope work

All work with radioactive isotopes (except ^{14}C), must be carried out in the room for radioactive work in Horticum (#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!

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 Department.

The classic symbols of 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.
- You are advised **not** to 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 at the Department.
- 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 ^{14}C).
- 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, must 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. When filtering, apply suction, not pressure.
- 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 ^{32}P 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 must be reported immediately to the responsible persons and the Head of Department.

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

Compounds with ^{32}P -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"). A fridge and a freezer are available for this purpose in this room. Labelling using ^{32}P should be done in this room. This work should be performed behind a protective Plexiglas shield. In case of any risk for producing radioactive air-borne compounds; work in the fume hood!

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

Compounds with ^{14}C -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 ^{14}C 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 " ^{14}C ". 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 $^{14}\text{CO}_2$ 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 cartoon boxes for ^{35}S , ^{33}P , ^3H and ^{14}C waste should be available (if not, contact the responsible persons). Some solid radioactive waste 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 cardboard boxes. Boxes for radioactive waste containing highenergy emitters are placed behind Plexiglas shields. Inform the responsible persons when cardboard boxes 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 waste box:

$$^{32}\text{P}: 10^5 \text{ Bq} = 2.7 \mu\text{Ci} \quad ^{35}\text{S}:$$

$$10^8 \text{ Bq} = 2700 \mu\text{Ci} \quad ^{33}\text{P}: 10^8$$

$$\text{Bq} = 2700 \mu\text{Ci}$$

$$^3\text{H}: 10^9 \text{ Bq} = 27000 \mu\text{Ci}$$

$$^{14}\text{C}: 10^7 \text{ Bq} = 270 \mu\text{Ci}$$

$$^{125}\text{I}: 10^6 \text{ Bq} = 27 \mu\text{Ci}$$

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 litre 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 accident:

In case of spillage of more than 40 MBq (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 minor 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 ^{32}P 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 persons 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 person with soap and lukewarm water. **Don't scrub!**
- Try to only clean the area around the contaminated skin to prevent spreading.
- If there is 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 at 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. All microorganisms must be classified according to potential health hazards. In case of any uncertainties regarding the classification of an organism, the Public Health Agency of Sweden must be contacted and consulted. If the new organism is classified into Category 2, a permission from Swedish Work Environment Authorities, regarding the organism and the room where experiment is to be performed, must be obtained before any work can start. In addition to the permission, notices must be posted at storage and work areas. Microorganisms with higher classification are currently not present at 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 autoclave bags.
- 6 Other contaminated glassware must be disinfected before sending it to dishwashing. Disinfection is normally done with 70% ethanol.
- 7 Media and other solutions contaminated with bacteria, yeast or 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 (“The Ten Commandments”, as above), 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 (ultra)centrifuges and their rotors

Before you use the ultracentrifuge or high speed centrifuge for the first time you must ask someone for a proper instruction! It is your own responsibility to make yourself correctly informed. Do not forget to close the lid on top of the rotor!

There are three different types of rotors: carbon fibre, 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 create 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:

$$1\text{g} = 602\text{ kg!}$$

$$1\text{ ounce} = 18.8\text{ tons!}$$

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 misshooked 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 must 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 for that 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/cm^3 , and the vertical rotors not over 1.7g/cm^3 .

9.6 Miscellaneous

- Log books: 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.

- After centrifugation: wipe off the centrifuge chamber to remove dirt and humidity.
- Classification of centrifuges: 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.
- Stuck tubes: If a tube get 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 fire in electric equipment

- fire quenching blanket
- fire alarm • first aid kit

The smoke detectors and alarms are connected directly to the fire department.

In case of a larger fire you must handle according to the following:

- Make sure nobody is left in the lab
- Alert your colleagues
- Stop the fire from spreading by closing the lab doors. If possible, extinguish the fire using the fire extinguisher or fire blanket
- Evacuate the lab! The meeting place is at the parking lot at the South end of Vegetum. This is where you must go immediately in case of fire; this is for the purpose of determine no one is left behind and in order for employees to receive further information about the situation.

All employees must participate in a basic education in fire protection.

Participation will be registered.

You will get information about these courses on your SLU mail.

11. Eye Safety/ Emergency showers

- Always use eye-protection (glasses, screens) when working with dangerous procedures.
- Inform yourself beforehand where the eye-showers are. If you get spills, splashes or squirt something into your eyes, do the following:

<u>The victim</u>	<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. If you have NaOH (or other alkaline solution) in your eyes, definitely not less than 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.

Important Phone Numbers

Fire department and ambulance
Our address : Växtskyddsvägen 3, Alnarp

(00) 112

To get a line outside of SLU, first dial: **00**

Lund University Hospital 046-17 10 00 Malmö University Hospital
040-33 10 00 Toxic chemicals - hot line 08- 33 12 31

Building service (Akademiska hus) (office hours) 040-664 23 41 (such as electricity, heating, water or sewer)
(non-office hours) **046 - 31 13 10**

Security matters (Securitas) 040-415070
(for suspicion of crime, unauthorized persons in the building, etc.)

Non Emergency Medical Care:

Vårdcentralen i Arlöv 040-623 41 00

Vårdcentralen i Lomma 040-623 48 00

Medical care hotline 1177

Medical care hotline www.1177.se

Avonova (Företagshälsovården/corporate health care) 046-19 33 30






Web address to report work incidents and injuries:






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

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.

GHS – Hazard Pictograms and correlated exemplary Hazard Classes

Physical Hazards				
				
Explosives	Flammable Liquids	Oxidizing Liquids	Compressed Gases	Corrosive to Metals

Health Hazards				Env. Hazards
				
Acute Toxicity	Skin Corrosion	Skin Irritation	CMR ¹⁾ , STOT ²⁾ , Aspiration Hazard	Hazardous to the Aquatic Environment

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

Informed Receipt of the Safety Regulations

I have read and I have understood the information in the booklet "Safety Manual". The contents of the manual have been discussed with my responsible group leader in the presence of the safety representative and I have been shown the protection and safety devices in the laboratory. I accept to follow the rules and advice given in this booklet.

Signature Employee

Date

Clarification of Signature

Signature Group leader

Date

Signature Safety Officer

Date

Store original at administration section of Department of Biosystems and Technology (Johanna Strand). One copy to Safety officer.