Laboratory Safety Manual

NICPB Laboratory of Environmental Toxicology

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1 General principles

The terms in the current manual are mandatory to all employees working at the National Institute of Chemical Physics and Biophysics (NICPB) Laboratory of Environmental Toxicology (LET) (hereinafter " Laboratory") by full term or part time contracts, graduate and undergraduate students, within cooperation agreements and users of the equipment in the Laboratory premises (hereinafter „Worker"). The manuals are implemented to guarantee safe working environment and safety to the colleagues and to the worker.

Only competent Workers who have read and accepted the terms of the Laboratory Safety Manual are allowed to work in the Laboratory. Minors (general school students) are only allowed to work under constant supervision of the supervisor. The training of all students, including postgraduate students, is the responsibility of their supervisor.

Any worker is not allowed to work in the laboratory before he or she has given a signature to the safety instructions registration sheet of the Laboratory Safety Manual (Appendix 1).

The Worker who is not trained cannot work with the physical, chemical or biological hazard. The Worker has the right to demand training/supervision or to refuse assignments when training is not enabled.

None of the Workers are allowed to permit the access to the machinery or to delegate experimental assignments to other people who have not read and accepted the terms of the Laboratory Safety Manual and given a signature.

Workers must read and accept the terms of the First Aid manual (located in room 113, First Aid cabinet) (contact person Imbi Kurvet).

Where necessary, the manuals of certain machinery and protocols of laboratory practices may dictate tighter regulations of the working processes.

1 General safety

Visitors to the laboratories at the first floor (including excursion groups), must wear labcoats and, if needed, blue shoe covers (located at the closet at the first floor of the Laboratory).

Before starting a working assignement, a Worker is obliged to verify that the machinery is working properly and the personal protective equipment is safe and sound.

Depending on the character of the work and the room, the protective equipment such as labcoats, gloves, masks, glasses, sleeve covers is mandatory. Working in rooms where chemical or biological hazards are handled demands wearing a laboratory coat. Wearing open-toe footware in Laboratory is prohibited. Footwear should cover most of the top side af a foot. It is advisable to wear clothing that covers the knees.

The Worker is obliged to maintain working place in a clean condition, to prevent the spilling of chemicals and test organisms on the desk and on the machinery and to remove all the unnecessary containers and equipment from the desk.

Only clean containers are used for conducting experiments. The containers must be cleaned after use (following specific instructions) or must be taken to the containers` washing area.

Worker must always consider the character of the work in the Laboratory in general and in the specific room; be aware of the possibility to come into contact with the physical, chemical and biological hazards and possible cross-contamination.

2.1 Technical use and biosafety levels at the first floor

Room number	**Purpose of use**	**Biological hazards**	**Chemical and physical hazards**
004	Ecotoxicology	-	Chemicals used in the experiments
103	Toilet	-	-
104	Storage	-	-
105	Ecotoxicology/machinery(chemical and physical measurements)	-	Chemicals used in the experiments
106	Cell culture laboratory	1.-2. hazard class organisms (incl. possible intracellular pathogens)	Chemicals used in the experiments, UV-C radiation
107	Office	-	-
108	Office	-	-
109	Office	-	-
110	Office	-	-
111	Microrbiology/molecular biology	1.-2. hazard class and/or GM organisms	Chemicals used in the experiments, UV-C radiation
112	Office	-	-
113	Resting area/Seminar room	-	-
114	Laboratory of toxicology/scales/machinery (biological measurements)	1.-2. hazard class and/or GM organisms „end-point“ measurements	Concentrated chemicals, chemicals used in the experiments, DNA-binding dyes, chemical waste
115/116	Darkroom/sample treatment/chemical storage (microscopy/nano-scales /sonicator/electrophoresis)	1.-2. hazard class and/or GM organisms	Nanopowders, concentrated chemicals, ultrasound, UV-radiation, lasers, ethidium bromide (other DNA-binding dyes).

3 Working with chemical and physical hazards

3.1 Working with physical hazard

The user manual of the machinery must be read to take into account the physical hazards of working with macinery (e.g. lasers of confocal microscopy, UV-lamps under flow hoods, sonicators, liquid nitrogen for tissue culture maintenance etc).

The UV-C lamp must be switched off before starting to work under flow hood. Prior to entering the tissue culture room it should be confirmed that the UV lamp is switched off. UV-C radiation damages eyes and skin and is mutagenic. **It is prohibited to switch on the UV-lamp when other people are in the room.**

It is not prohibited to work in the viscinity of **the UV-A radiation source**. The eyes and skin should be protected from inadvertent exposure. Eye contact with the radiation source should be minimised (wear gloves and do not work directly under the lamp).

It is prohibited to work with an **open fire** in the horizontal outflow cabinets and the open fire should be avoided when working in biosafety level 2 cabinets. The fire at any time should not be left unattended.

3.2 Working with chemical hazard

According to the Chemicals Act (§3; RT I, 10.11.2015, 2):

(1) **A chemical** is a substance or mixture for the purposes of the REACH Regulation[1]

(2) **A hazardous chemical** is a substance or mixture, that meets the physical, health or environmental hazard criteria set out in parts 2-5 of Annex I to the CLP Regulation.[2]

- explosives (category: instable explosives and subclasses 1.1-1.6 explosives)
- flammable gases (1. and 2. category)
- flammable aerosols (1. and 2. category)
- oxidising gases (1. category)
- gases under pressure (compressed gases, liquified gases, frozen liquified gases and dissolved gases)
- flammable liquids (1., 2., 3. category)
- flammable solids (1. and 2. category)
- several substances and mixtures (types A,B,C and D,E and F, G)
- pyrophoric liquids (1. categroy)
- pyrophoric solids (1. categroy)

[1] Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

[2] Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (OJ L 353, 31.12.2008, pp. 1‑1355) (hereinafter the CLP Regulation) applies to the classification, labelling and packaging of chemicals.

- self-heating substances and mixtures (1. and 2. category)
- substances and mixtures that emit water-reactive flammable gases (1., 2. and 3. category)
- oxidising liquids (1., 2. and 3. category)
- oxidising solids (1., 2. and 3. category)
- oxidising peroxides (types A, B, C and D, E and F, G)
- corrosive substances to metals (1. category)

Health risks (Part 3 of Annex I of CLP regulation)

- acute toxicity (1., 2., 3., and 4. category), with subdivisions:
    - acute oral toxicity
    - acute dermal toxicity
    - acute inhalation toxicity
- skin corrosion/irritation (1. category: corrosion, subcatecories 1A, 1B, 1C; 2. category: irritation)
- severe eye damage/eye irritation (1. category: irreversible effect on the eye; 2. category: eye irritant)
- sensitisation of skin and respiratory tract (1. category: sensitisers of respiratory tract, subcategories 1A, 1B; 1. category: skin sensitisers, subcategories 1A, 1B)
- germ cell mutagenicity (1A, 1B and 2. category)
- cancerogenicity (1A, 1B and 2. category)
- reproductive toxicity (1A, 1B and 2. category) including additional categories for nursing and for toxic outcomes with nursing
- toxicity to target organs- single exposure (1. and 2. category; 3. category includes only narcotic effect and respiratory tract irritation)
- toxicity to target organ – repeated exposure (1. and 2. category)
- aspiration hazard (1. category)

<u>Environmental hazards (Part 4 of Annex I, CLP regulation):</u>

Hazardous to the aquatic environment (acute hazard category 1; chronic hazard categories 1, 2, 3 and 4)

<u>Additional hazards (Part 5 of Annex I, CLP regulation):</u>

Hazardous to the ozone layer (1. category)

(3) **Handling of chemicals** is the preparation, manufacturing, processing, packaging, storing, transportation, facilitation and other activities with chemicals.

Substances of very high concern (SVHC) REAACH article 57) are considered chemicals according to the following criteria:

- 1A and 1B category substances that are carcinogens, mutagens and substances with reproductive toxicity
- persistent, bioaccumulating and toxic or very persistent and very bioaccumalating substances that fulfil the criteria of the REACH regulation annex XIII

- substances that can be very toxic to the environment or to the human health and that can cause similar toxicity as compared to the substances listed above (e.g. endocrine disrupters) based on the scientific experimental data and on the isolated incidence.

All hazardous chemicals in the Laboratory are signed and recorded (file: fs2→KT→Kogu grupp→Kemikaalid→KTL kemikaalide andmebaas+CLP_tabel_2019.xls). Very hazardous substances are kept separately (in a closed cabinet, in the emerging necessity please ask more information from Anne Kahru, Kaja Kasemets or Imbi Kurvet).

Safety cards of chemicals (Safety data Sheets, SDS) are in a folder and available to everybody (room 113, shelf). In case of missing SDS, the information of the safety of the chemical can be found on the web site of the European Chemicals Agency (ECHA): „C&L Inventory".

Before starting to work with the hazardous chemical, the SDS and/or the information from the ECHA „C&L Inventory" must be read.

Labelling of the chemical must include the following:

- EC or CAS number that identifies the substance or the preparation
- a list of hazardous substances in the preparation
- nominal quantity of the substance or the preparation
- when necessary hazard pictograms, signal words, hazard statements and additional information
- supplier(s) name, address and telephone number

NB! Simplified labels should include name (or formula) of the substance and the hazard pictograms

CLP regulation hazard pictograms

Pictogram	Label element	What to do, examples of hazard statements
 **Gas under pressure**	Consists of gas under pressure; can be explosive when heated; consists of frozen gas; can cause freezer-burn or damage.	Keep away from sunlight. Wear protective gloves/mask/glasses. Visit a doctor immediately.
 **Explosive**	Unstable explosive; Mass explosion hazard; flammable-, explosive- or danger to splashing over; When inflamed the hazard of mass explosion.	Read special guidelines (SDS) before use. Do not use before full understanding of the precautionary statements. Keep away from heating/sparks/flames/hot surfaces. Do not smoke. Wear protective gloves/clothing/glasses/mask. Wear necessary protective clothing. Explosion hazard in case of fire.
 **Oxidising**	Can be ignitable or induce flames. Can cause flammability or explosion.	Keep away from heat/sparks/flames/hot surfaces. Do not smoke. Wear protective gloves/clothing/glasses/mask. Prior to removing contaminated clothes, wash them and skin with plenty of water.
	Highly flammable or flammable gas, aerosol, liquid, vapour or solid.	Do not spray in flames or in ignition source. Keep away from heating/sparks/flames/ hot surfaces. Do not smoke.

Flammable		Keep the container tightly sealed. Keep cool. Keep away from sunlight.
 **Corrosive**	Can be corrosive to metals; causes serious skin corrosion and eye damage.	Do not breathe dust/fume/gas/mist/vapours/spray. Wash thoroughly after use. Wear protective gloves/clothing/glasses/mask. Keep locked. Keep in original packaging only.
 **Acutely toxic**	Toxic or deadly when swallowed, in skin contact or enhaled.	Wash thoroughly after use. Do not eat, smoke or drink during use. Contact Poison Information Centre (16 662) or doctor when swallowed. Rinse mouth. Keep in sealed container. Avoid eye, skin contact or spilling on clothes. Wear protective gloves/clothing/glasses/mask. Wash gently with water and soap from skin. Remove all contaminated clothing immediately. Wash all contaminated clothing before next use. Do not breathe dust/fume/gas/mist/vapours/spray. Handle in open air or in well-ventilated area. Use respiration protection. When enhaled, bring the victim to the fresh air and place in resting position that enables to breathe easily. Store locked up.
 **Longer term health hazards such as carcinogenicity**	Can be deadly when swallowed or enhaled; causes organ damage; hazardous to reproductivity or fetus; carcinogenic; can cause genotoxicity; can cause irritation or asthma symptoms or breathing difficulties when enhaled.	Contact Poison Information Centre (16 662) or doctor when swallowed. DO NOT induce vomitting. Store locked up. Do not breathe dust/fume/gas/mist/vapours/spray. Wash thoroughly after use. Do not eat, smoke or drink during use. Contact doctor in case of not feeling well. Call Poison Information Centre (16 662) or doctor when contacted. Read special guidelines before use. Do not handle before reading and understanding safety guidelines. Use protective clothing. Contact doctor when contacted. Do not breathe dust/fume/gas/mist/vapours/spray. Use respiratory protection when handled in not well-ventilated area. When enhaled bring the victim to the fresh air and place in resting position that enables to breathe easily.
	Can cause respiratory irritation; Can cause nausea or dizzines; Can cause allergic reaction to the skin; Causes serious eye irritaion;	Do not breathe dust/fume/gas/mist/vapours/spray. Handle in open air or in well-ventilated area. When enhaled bring the victim to the fresh air and place in resting position that enables to breathe easily.

Caution –used for less serious health hazards like skin irritation	Causes skin irritation; Hazardous when swallowed; Hazardous to the skin; Hazardous when enhaled; Damages public health and environment, hazardous to the ozone layer.	Contact Poison Information Centre (16 662) or doctor when swallowed. Wear protective gloves/clothing/glasses/mask. Wash gently with water and soap from skin. Wash throughly with water for several minutes from eyes. Remove conact lenses if possible. Rinse once more. Do not eat, smoke or drink during use.
 **Dangerous to the environment**	Hazardous or very hazardous to the aquatic environment, long-term effect.	Avoid releasing into the aquatic environment. Avoid releasing to the environment. Absorb spillage.

3.2.1 Laboratory practice when handling chemicals

1. In chemical laboratory avoid touching eyes and face (danger of substances getting to the eyes and mouth).

2. Highest precautions are necessary when working with Substances of Very High Concern (SVHC). Containers with SVHC must be labelled accordingly. When handling carcinogens/mutagens the working area should be covered with a table cover (e.g. aluminium foil or absorbent cloth). When handling SVHC wear always nitril goves and protective glasses where possible. These gloves should be handled as contaminated (do not use used gloves for touching door handles, do not open refridgerator etc. Displacing SVHCs should be done in fume hood. The displacing of SVHCs in laboratory area, should be done with airtight containers. Residues of substances and other waste that has been in contact with carcinogenic substances should be handled as dangerous waste and should be labelled accordingly.

3. Handling strong acids and alkalines demands the following safety precautions:

- acids and alkalines should be kept in bfume hoods where also their decanting should take place;
- concentrated nitric acid, sulfuric acid and hydrochloric acid are kept in containers with at least 2 cm thick walls under the fume hood on the plastic or porcelain cover;
- large-scale acids or alkaline containers decanting should be done using siphone or funnels;
- pipetting should be done using rubber balloons or special pipettes;
- large containers on the floor should be tilted only with the tilter;
- larger containers than 5 liters containing acids or alkalines are transported only in special baskets;
- dissolution of the concentrated acid should be done by pooring the acid into the water not *vice versa* for avoiding sprays.
- discarded acid and alkaline residues should be neutralised. It is not allowed to discard the concentrated acids and alkalines to sewage.

4.Flammable and easily ignitted liquids (acetone, dioxane, ether, ethanol, toluole, ethylacetate, carbon sulfide, bensene etc):

- keep on the desk only the daily necessary amounts;
- do not keep unsealed;
- work only in the fume hood;
- small amounts of liquids should be decanted away from fire and heating sources. Larger amounts of liquids should be decanted in a room where lights are swithed off.
- do not poor liquids into the wash basin (except small amounts of diluted acetone, dimethylsulfoxide, ethnanol, ethyleneglycol, methanol and propanol when there is not danger of ignition).
- do not heat liquids directly on the heating source. Do it on the water basin with a cooler and a reflux.

5. Working with a flammable, easily ignitable corrosive substances and vacum machinery should be done by wearing protective glasses, mask or protective screens. Read the safety precaution terms before working with them.

6. Mixing or dissolving substances that emit heat should be done in heat resistant or porcelain containers.

7. Procedures which cause emitting of hazardous or easily ignited vapours and gases need to be done in the fume hood.

8. Working under the fume hood demands the lifting of the screen 20-30 cm, no more so that only the hands are in the cabinet.

9. Avoid water contact with electrical gadgets. Hands should be dry when handling electrical gadgets. Water spills should be removed as soon as possible around the gadgets.

10. Worker when leaving the working machinery should assure that everything is working properly during the leave.

11. The desk and the work equipment must be cleaned after work is finished. The last one who leaves the room should assure that all gas and electrical gadgets are swiched off, taps, ventilation and windows are closed.

12. In case of fire, ventilation, electricity should be switched off, doors and windows should be closed.

13. Hazardous substances and nanomaterials should be weighed in the nano-cabinet in the room 116.

14. Working with ethidium bromide (EtBr) demands wearing nitrile gloves (EtBr permeates latex gloves) and all waste in contact with EtBr (incl gloves, used gels, napkins) should be collected and stored in special waste disposal (labelled „DNA stain waste“) and handled as dangerous waste. Working at the area with high risk of EtBr contamination (electrophoresis desk, area around the transilluminator) should be marked. It is not allowed to touch door handles, desks or other items with the contaminated gloves. Weighing of EtBr powder should be avoided (preferred is the use of tablets or liquids to make stock solutions).

15. DNA binding dyes that are used in microbiology or tissue culture practices (incl. EtBr, propidium iodide etc) should be handled as carcinogens and collected separately with other chemicals in the working area and stored in special waste disposal („DNA stain waste“) and treated as dangerous waste. When the materials are contaminated and contain microbes, the material should be first inactivated by autoclaving and disposed of as dangerous chemical waste.

16. All the containers in use in the laboratory must at all times be labelled at least with the chemical name according to the nomenclature, preparation date and Worker name or initials. All containers that contain unidentified substances should be handled as dangerous waste.

It is prohibited:

1. To eat and drink in the laboratory area. It is not allowed to store food in the laboratory refridgerators and in the area of laboratory.

2. To wash floors with easily ignited or flammable substances.

3. To store in the laboratory cloths, towels and clothing contaminated with flammable liquids.

4. To pipet with mouth.

5. To abandon trash on the desks, cabinets and on the floor.

4 Working with biohazards

Working with biological material including genetically modified (GM) and/or biohazard class 2 organisms demands quality control of the experiment and following of the safety precautions for Worker and to the environment. Large quantities of biohazard class 1 micororganisms, all mammalian cell cultures and all biohazard class 2 microorganisms should be handled as biohazards and should be treated as potential human pathogens.

It is not allowed to discard the uninactivated bacterial and fungal cultures and mammalian cell cultures or materials contaminated with them to the sewage, also, independent of their biohazard class they are not handled as solid general waste. All materials contaminated with the microbes are collected and inactivated together, independent of the biohazard class and of the GM composition. All materials contaminated with 1. and 2. biohazard class microbes and/or mammalian cell cultures are inactivated by autoclaving. Liquid waste from handling mammalian cell cultures is inactivated chemically (guideline located at the cell culture laboratory).

To achieve quality and safety of the work, it is necessary to follow the good microbiology practice. Additional questions are answered by using the WHO laboratory biosafety handbook (available at the WHO Laboratory Biosafety maual, available from http://www.who.int) and ECACC laboratory handbook.

Biohazards are divided into four biohazard classes depending on their infectiousness:

2) Class 1 biohazards typically do not cause human illnesses (for example non-pathogenic *E. coli* strain MG1655);
3) Class 2 biohazards can cause human illnesses and pose a threat to the human health but are not infectious to the general population; there are effective means for treatment and prevention agains them (for example *S. aureus*, *P.aeruginosa*, *C. albicans*, pathogenic *E. coli* strains, cell cultures of humans and primates)
4) Class 3 biohazards can cause severe human illnesses; can be infectious to the general population; there are effective means for prevention and treatment (for example *Bacillus anthracis*, *Mycopacterium tuberculosis*, *Salmonella typhi*, *Yersinia pestis*, verotoxin-producing *E. coli* strains).
5) Class 4 biohazards can cause severe human illnesses and they can pose a serious threat to the Worker`s health and can be infectious to the general population; efficient means for prevention and treatment are missing (for example the smallpox virus, hemorrhagic fever viruses such as Ebola virus, etc).

At the Laboratory of Environmental Toxicollogy only class 1 and 2 biohazards are handled.

A risk analysis is necessary for dividing organisms to the biohazard classes. The most common hazards (including fungi, bacteria, viruses, parasites) are classified in the Appendix 3 of the „Occupational Health and Safety Requirements for Workplaces Affected by Biological Hazards „.

4.1Techniques for handling biological material

1. Wear always protective clothing (laboratory coat) and disposable gloves when handling microbes. The employer shall enable the accessibility of the protective clothing and other protective equipment and is also responsible for their regular laundering/maintenance.

2. It is required to wear protective glasses and protective mask when conducting laboratory practices with danger of spraying (when not under the biosafety cabinet class 2). Protective glasses should be worn on top of the optical glasses. Note, optical glasses cannot replace by the protective glasses.

3. Working area is cleaned with 70% ethanol before and after the working asssignment. The corresponding spray bottle and the paper towels can be found next to the working area.

4. The bioafety cabinet should work at least 5 min before and 5 min after the working assignment. It is suggested to sterilise the cabinet using the UV lamp for 15 min (please note that only the area that is directly exposed to the light is affected). UV-lamp is not suitable for sterilising pipettes, racks etc that are not exposed entirely to the UV-lamp.

5. After finishing the assignment, gloves must be removed and before leaving the room the hands should be washed for at least 10 seconds with warm water and soap. The gloves are disposed of in the waste determined for inactivation containing contaminated solids with microbes, not in general waste.

6. All necessary consumables and reagents should be collected before working. It is not suggested to look for consumable with gloves on.

7. It is required to keep the laboratory area clean. All unnecessary items and old samples should be removed from the desk.

8. When working with microbes it is necessary to avoid aerosol and leakage and the use of glass containers if possible.

9. Microbes are transported from one room to another using tightly sealed leakproof containers. Class 2 biohazard or GM organisms should be transported using closed boxes or other leakproof containers that do not break or cause spill when dropped.

10. Disposable transfer loops are used for cultivating microbes. Special care is taken to avoid dispersal of the material.

11. The tops, bottles etc should be wiped with 70% ethanol and returned to the original place after work.

12. It is not allowed to store unopened packages of consumables, pipettes etc in the biosafety cabinets and in the cabinets with horizontal outflow. Opened packages of consumables are stored in a specified area next to the work place. The work area can be loaded only with a minimum set of half-empty tops containing pipette tips, vortex machinery and sealed disposal containers that can be easily cleaned using ethanol.

13. Front frills of laminar flow biological safety cabinets cannot be loaded with containers, pipettes, notes or other devices that impair the airflow. When the airflow is disrupted, the level 2 biosafety laminar flow is not working properly and the clean environment and safety of the Worker is in danger.

14. It is not allowed to place guidelines, work papers and lab books on the working area and they cannot be touched with the gloves. It is not allowed to take the potentially contaminated papers to the office. Lab books or work sheets can be placed on a chair and can be protected with plastic covers.

15. All the dishes, tubes, bulbs should be labelled with the name of the microbe, cultivation date, Workers` name or initials. The stacks of the petri dishes should be identifiable with at least one correctly marked item on top. All unlabelled items with the suspicion of microbial contamination are handled as biohazard class 2 waste.

16. All other containers should be labelled with substance according to the nomenclature or with other understandable formulation, with the date of the preparation, the name or the initials of the Worker.

17. In all rooms that are labelled with the sign of the biological hazard and where the biohazard class 2 organisms are handled, the precautions according to the biohazard class 2 organisms should be considered regardless of what material is being handled.

18. Ultrasound water bath and probe should be considered as high risk of spray contamination hazard. Micorbes should be placed to the ultrasound water bath only in sealed containers.

19.Glassware should be replaced with plastic if possible. Damaged glassware should be removed from use.

20. Working with genetically modified biohazard class 1 and 2 organisms (including sensor bacteria) is allowed in room 106 (tissue culture), 111 (microbiology), 114 (toxicology), 115/116 (microscopy). All manipulations with suspension cultures with GM strains should be done under the properly working biosafety level 2 laminar flow hood independent of the biohazard class of the test organism. The doors of the corresponding rooms are labelled with the biohazard pictograms.

21. Working with biohazard class 2 micororganisms is allowed only in rooms 106 (tissue culture), 111 (microbiology), 114 (toxicology), 115/116 (micorbiology). The doors of the corresponding rooms are labelled with biohazard pictograms.

22. It is allowed to use in the tissue culture room only protective clothing and replacement footware that are not used outside that room. At the entrance, the two feet should stand on the sticky mats that are used to decrease the contamination threat from the footwear.

23. Fungal and bacterial cultures are not handled in the tissue culture room to avoid contamination.

24. All doors of the laboratory should be closed at all times.

4.2. Restrictions

1. It is prohibited to pipette with mouth

2. All pipettes should have cotton plugs to reduce contamination of pipetting devices. When contaminated the plugs should be replaced. It is prohibited to use syringes instead of pipettes.

3. It is prohibited to dispose of waste over the edge of the laminar flow cabinets or desks. Waste is collected in the disposal container with the disposable autoclave bag.

4. It is prohibited to use open flame in the biosafety cabinet with the horizontal outflow and it is suggested to avoid open flame in the biosafety level 2 cabinets.

5. It is prohibited to use laboratory clothing or gloves outside the laboratory area (including rest room, office and toilet) when not invincible (e.g. transportation of samples between the rooms of laboratory).

6. It is prohibited to store labortory protective clothing such as laboratory coat together with street clothing or in the office rooms. Laboratory coats (not tissue culture coat) are stored in the special closet or in the laboratory where it is used. The laboratory coat for tissue culture is stored in the front room of the tissue culture laboratory and it is not allowed to use it in other rooms.

7. It is prohibited to touch door handles and taps with the gloves.

8. It is prohibited to touch face, hair and personal items such as mobile phone with the gloves.

9. It is prohibited to work with long untied hair.

10. It is prohibited to eat, drink, preservtion of food and use cosmetics in laboratory areas.

11. It is prohibited to touch lab book or working sheet with the gloves and to store it on the table, in the laminar flow hood or on other surfaces where micorbes are handled.

12. It is prohibited to use open-toe footware in the laboratory areas. Footwear should cover most of the foot angle. It is suggested to wear clothing that covers the knees.

13. It is not prohibited to use seats with textile covers in the laboratory areas.

4.3. Working with biohazard class 2 organisms

Only Workers who are wearing protective clothing and who have been trained to handle biohazard class 2 organisms are allowed to work in the rooms that are labelled with a biohazard pictogram and where biohazard class 2 and/or GM organisms are handled.

All laboratoy techniques with biohazard class 2 suspension cultures, e.g. pipetting and cultivation should be done in the biosafety level 2 laminar flow cabinets that are used according to the manufacturers` instructions.

It is prohibited to handle viable biosafety level 2 organisms in the biosafety cabinet with the horisontal outflow. The restriction is for avoiding contamination of the room and other people.

When handling the biohazard class 2 organisms, working with the closed Petri dishes and opening of the tubes for instrumental measurements is allowed outside the biosafety level 2 laminar flow cabinets in the appropriate laboratory areas (labelled with biohazard pictograms) but not in the biosafety horizontal outflow cabinets.

The generation of aerosols and spraying/dispersal of material should be avoided at all stages of the work done and the transportation of the organisms between the rooms should be done in leakproof containers that do not pose a threat of leakage when dropped.

Ice that has been used for storage of biohazard class 2 organisms should be handled as contaminated liquid and should be inactivated before discharge to the sanitary sewer. The ice container should be cleaned with 70% ethanol.

All unidentified cultures and liquids in the rooms where biohazard class 2 organisms are handled should be handled as biohazard class 2 organisms and should be inactivated by autoclaving.

Medical, veterinary or food origin biological material should be always handled as biohazard class 2 material. All mammalian tissue cultures in the Laboratory are considered biohazard class 2 hazards.

5. Waste disposal

5.1.Chemical waste

Hazardous chemical waste should be collected using waste containers labelled accordingly.

Liquid organic waste (for example paraquat, phenols, ethidium bromide) is collected in the laboratory of toxicology (room 114) in a bottle located in the fume-hood (the bottle is labelled „Orgaanilised jäätmed“).

Heavy metals:

Liquid nanowaste (for example CuO, ZnO, Ag suspensions, etc) is collected in the laboratory of toxicology (room 114) in a bottle under the fume-hood (the bottle is labelled „Nanojäätmed“). The bottle contains 20% HNO_3. When the bottle is full, the content should be discharged in the sanitary sewer and the bottle should be washed with a large quantity of water and ¼ of the bottle should be filled with the fresh 20% HNO_3.

Solid waste (plates etc) should be handled similarly to the general waste. This does not entail the samples that contain materials that need inactivation by autoclaving.

NB! All mercury (Hg), Cadmium (Cd) and lead (Pb) compounds should be collected separately from other heavy metals:

- Liquid waste should be collected in the bottle located at the laboratory of toxicology (room 114) under the fume-hood (the bottle is labelled „Hg, Cd ja Pb liquid waste“).
- Solid waste (microplates etc) should be collected in a bag located in the room 114 (labelled „Hg, Cd and Pb tahked jäätmed“).

Materials contaminated with ethidium bromide or other DNA-binding dyes are collected in waste disposals marked „DNA stain waste“ located at the working areas. When necessary, the waste containing microbes are inactivated by autoclaving and stored in room 115 until disposed as dangerous chemical waste.

Chemical waste is collected and transferred to the recycling companies. A contact person in the Laboratory of Environmental Toxicology for chemical waste utilisation is Dr. Irina Blinova (room 109; tel. 6398 373, irina.blinova@kbfi.ee).

5.2 Biological waste

All materials contaminated with microbes should be collected separately from the domestic waste and should be inactivated by autoclaving. Contaminated waste and containers should be autoclaved only in special containers and not in autoclaving metal baskets. Waste is always autoclaved separately from other equipment and liquids (for example broth, clean containers).

Solid waste and used gloves are collected at the working areas (at the desk or in the biosafety cabinets) and stored in the closed waste disposal bag, that is sealed with autoclave tape and placed in the

autoclave container. When the container or the container bag is full, it is autoclaved for at least 15 min at 121 degrees of Celsius. After autoclaving, the waste is considered general waste.

All Petri dishes used for bacterial reverse mutation test (Ames test) are autoclaved. Mutagenic samples, control chemicals (4 nitro-1,2-phenylenediamine, nitrofurantoin, 2-aminoanthracene) and Petri dishes are collected in a waste disposal container that is labelled „AMES test jäägid“ and is located at the Laboratory of Environmental Toxicology corridor niche and is given to the waste recycling companies.

Liquid waste should be decanted at the working area into the screw-capped container, labelled with an autoclave tape signed „Waste“. The containers should be collected in the same room and autoclaved with other waste. Liquid biohazard class 2 waste is allowed to decant only in properly working biosafety level 2 laminar flow cabinets. The full bottles are autoclaved for at least 15 min at 121 degrees of Celsius and discharged into the sewer.

After use, the disposable serological pipettes are placed back into the wrapping papers and taken to the autoclaving containers.

Microplates with liquid waste should be disposed of in the autoclaving bags and taken to the solid autoclaving containers. Waste containers, flasks, bottles should not be washed before autoclaving! These are collected and autoclaved for at least 15 minutes at 121 degrees of Celsius using waste autoclaving containers. After this, the containers are suitable for washing and reuse.

Autoclaving containers should be never filled over the top edge and should be kept sealed with covers.

Sharps contaminated with microbes, such as glass, should be collected in the sharp-proof disposal containers and autoclaved for at least 15 minutes at 121 degrees of Celsius.

5.3. Mixed waste

The waste contaminated with both microbes and hazardous chemicals is first autoclaved and then collected for recycling according to the hazardous chemicals instructions.

6 Procedures in the event of an accident

Broken machinery or other equipment should be labelled with a sign and a responsible person or Head of the Laboratory should be informed immediately.

6.1 Accidents with chemical and physical hazards

1. In case of a spill of a flammable liquid:

- all burners should be swiched off:
- doors and windows should be closed;
- the spill should be collected with a cloth into a wide container and decanted into a vessel with a screw-cap;
- ventilation should be stopped after the odour of the spill has totally disappeared.

2. In case of ignition of flammable liquid (e.g. the flask is broken):

- switch off the burner;
- remove all the dishes with the flammable liquid;
- cover flames with fireproof cloth or sand or use the fire extinguisher;
- when it is not possible to extinguish the fire, the fire brigade should be called (call 112). Water can be used as an extinguisher only for water soluble liquids (such as ethanol or acetone).

An accident at work with a severe health injury should be reported according to the Estonian laws for the labour inspectorate (Estonian Government Act: „ Registration, reporting and investigation of accident at work and occupational disease“).

6.2 Accidents with biohazards

A spill of liquids containing microbes should be collected with paper towel and the work area should be repeatedly cleaned using 70% ethanol or 0.5% hypochlorite (0.5% or NaClO 100 ml/l). Take into consideration that the spray from the spill can be diffused to a wider area. When the spill was caused by a broken glass, the glass should be collected with tweezers and not with hands. When using hypochlorite on metal surfaces, the surfaces should be washed after use.

Contaminated documents or notes should be copied or photographed and the contaminated paper should be autoclaved. All the items that need to be cleaned should be placed to the autoclave container. During the neutralisation of the spill the laboratory personnel should be warned and the entrance of the people to the room should be avoided. The footware and protective clothing should be cleaned from contamination and inactivated if necessary.

When the materials with the microbial contamination become in contact with protective clothing or footware, the items should be collected in autoclave bags and autoclaved for at least 15 min at 121 degrees of Celsius prior to laundering.

When the materials contaminated with microbes become in contact with the skin, the contaminated area should be cleaned immediately with 70% ethanol.

When sharps contaminated with biohazard class 2 organisms cut the skin, the contaminated area should be immediately cleaned with 70% ethanol and the accident should be reported to the Head of

the Laboratory immediately and if necessary call the West-Tallinn Infectious Diseases Clinic (number 659 8598).

It is possible to use shower at the 1st floor in the main corridor.

All leakages and accidents with the biohazard class 2 microbes should be reported to the Head of the Laboratory.

Leakages with biohazard class 2 organisms must be registered by writing in the Safety logbook (Appendix 2). Severe injuries and harmful effects to the health should be reported to the labour inspectorate (according to the Estonian Government Act: „ Registration, reporting and investigation of accident at work and occupational disease“).

6.3 First Aid

First aid boxes and manual is located in room 113, in the first aid drawer!

First-aider: Imbi Kurvet (room 108, phone 58145 476, imbi.kurvet@kbfi.ee)

All Workers should know the main procedures in case of easier injuries.

Workers should be enabled doctoral care for each injury. Calling a doctor or transportation of the victim to the hospital is the obligation for every Worker.

First aid for burns:

1.In case of flame burns, the victim should be transported to a safe place, the flames should be extinguished with a blanket or a carpet. Injured body should be released from clothes by cutting with scissors. Clothes that have been stuck to the skin should be removed carefully with easy movements. The first aid should be given carefully by avoiding the contamination of the wound.

2. Cool the body area that have been burned by cool running water for at least 10-15 minutes or by applying special cooling gel (located in room 113 refridgerator at +4C degrees). Cooling alleviates pain, decreases oadema. During the procedure avoid hypothermia!

3. NB! Ice, icebags or ice water are not suitable for cooling.

4. Offer drink for the victim.

5. It is not allowed to apply to the wounds substances that are not meant for the purpose! Inappropriate are the oils, creams, ointments, talc, baking soda, aloe, petroleum, butter, soured cream.

6. It is no allowed to open burn blisters!

7. Keep the wound clean. After cooling and applying the burn gel the wound can be covered with wound dressings.

8. The doctor should be contacted when the wound is extensive (over the size of a hand) or deep. When the wound is black, compact and painless, deep (III-IV degree burn) and after the first aid the victim needs hospital care.

Vapours from liquid and cold gases could cause similar injury to the skin as compared to the burns. Touching the unisolated parts of the machinery with bare hands can result in the sticking of the skin to

the metal and the resulting injury when detached. It is not allowed to rub the injured site. Wound should be rinsed with warm water, covered with sterile dressing and the doctor should be called.

Degrees of burns:

- I degree burn (redness, only injured epidermis, enlarged subcutaneous blood vessels)
- II degree burn (redness+blisters, detaching epidermis)
- III degree burn (deep skin injury, black or white skin surface)
- IV degree burn (deep tissue damage)

Chemical burns (acids, alkalines, household chemistry etc) first aid:

- Injured area should be rinsed with plenty of water. DO NOT USE neutralising substances! It should be determined that the victim has not swallowed or enhaled corrosive chemicals!
- **Acids or alkalines or solids in the eyes** should be rinsed off for at least 20 min with plenty of cool and clean water (by opening the upper and lower eyelids). The doctor should be contacted immediately.

Cut wounds should be rinsed with 0.9% NaCl solution (saline) or special antiseptic solution for the wound cleaning purpose (Asept, Braunol etc). It is not allowed to clean wounds with ethanol containing substances (irritating, drying), hydrogen peroxide (could damage the tissues in the wounds), coloured liquids (brilliant blue, iodide or $KMnO_4$) (they hamper the evaluation of the wound condition and could damage the tissues). Cutasept is not suitable because it is intended to disinfect skin not wound.

Detached solid should be removed carefully. It is not allowed to remove or to pluck stuck solids.

The wound should be bound and covered with sterile bandages or plasters.

It is not allowed to clean or pour something into the severe deep wounds!

When the skin or the cut wounds are contaminated with microbes the injured area should be immediately disinfected with a special substance or with 70% ethanol. When the cut wound is contaminated with biosafety level 2 microorganims, it should be reported immediately to the Head of the Laboratory and when necessary the West Tallinn Infectious diseases Clinic should be contacted (call 6598598).

First aid for poisoning:

- Poison Information Centre: 16662
- Swallowed toxic substance: when the victim has swallowed corrosive substance, e.g. strong acid or alkaline, the victim should drink plenty of water to dilute the toxic substance (the amount should be at least 240 ml for an adult). It is not allowed to neutralise the corrosive substance with chemicals. **It is prohibited to induce vomitting** because vomitting with toxic agent could damage other tissuses.
- Toxic substance on the skin: rinse the skin with running cool water for at least 15 minutes. Doctor should be called immediately in case of severe pain, burn wounds or inflammation.

- **Toxic substance on the skin:** rinse the skin with cool running water for at least 15 minutes. Doctor should be called immeditately in case of severe pain, burn wounds or inflammation.
- **Inhaled toxic substance:** take the victim to the open air. Avoid inhaling the substance yourself. The doctor should be called immediately when the complaints from the victim are not decreased, severe cough, shortness of breath or other symptoms have emerged. Rescuer should not enter the room with the lack of oxygen without special oxygen mask.

In case of **electric shock** when the victim is in contact with the conducting gadgets, the electricity should be switched off immediately (swiching off should take place from switchboard, if possible). When it is not possible to switch off the electricity, the rescuer should isolate him/herself with rubber gloves or with dry cloth or should step on the dry cover and prior to removing the victim from the electrical gadgets. It is not allowed to touch the victim with unprotected hands.

7 Machinery maintenance

All the machinery should be maintained on regular basis and according to the instructions. The maintenance is conducted by the Worker responsible for the machinery or a competent technician. The safety and efficiency of biosafety cabinets and autoclave is assured by regular check from a competent company. Such controls and maintenance should be documented and presented next to the machinery.

Additional material and regulatory acts

Occupational Health and Safety Act

Contained Use of Genetically Modified Micro-organisms Act

Communicable Diseases Prevention and Control Act

Chemicals Act

REACH. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

CLP. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance).

WHO Laboratory Biosafety Manual

ECACC Cell Culture Laboratory handbook