Safety guide

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Faculty of Agriculture
Graduate School of Bioresource and Bioenvironmental Science
School of Agriculture
Institute of Tropical Agriculture
Biotron Application Center
Bio-Architecture Center
Material Management Center

Foreword

In order to carry out research projects, teachers, researchers, and graduate and undergraduate students in Faculty of Agriculture, Graduate School of Bioresource and Bioenvironmental Science, and School of Agriculture should understand the risk of chemicals and devices, and the research activity environment. It is necessary to secure one's own safety, and social confidence of the university. "Safety Guide Working Group", which is designed under the safety and health department in Faculty of Agriculture, created a safety guide called "Safety Guide in Faculty of Agriculture." It shows compliance rules for safety when doing experiments and practice. It requires that not only teachers but also students should have enough knowledge of various laws about safety and try to prevent accidents, and comply with the rules because accidents which happen in the University became eligible for criminal punishment, after transformation into an independent administrative institution in 2004. This booklet was newly rewritten based on the "Safety guide in Faculty of Agriculture in 2008 (Japanese version)." First of all, we reorganized the contents of the chemical list to be more understandable. The contents of the chemical list has been severely restricted by various laws such as Fire Defense Law, Ordinance on Prevention of Organic Solvent Poisoning, Ordinance on Prevention of Hazards due to Specified Chemical Substances, and the Poisonous and Deleterious Substances Control Law. Moreover, because of the new content, we tried to add security precautions in physics system laboratory, biological system laboratory, and chemistry system laboratory and outdoor activities. It covers the basic part of research activities done at the agriculture research academy. We would like you to share enough understanding concerning security with others by reading the related part of this booklet when doing a research project. Moreover, teachers and students should always keep this booklet alongside when performing research and try to do their best to follow security management procedures. In order to revise the previous edition, we referred to the chemical coterie publication "Conducting safety experiment, seventh edition in 2006", chemical coterie publication in Tokyo "Chemical experiment safety guide for students in 2003", Graduate School of Engineering, Division of Advanced Science and Biotechnology, Department of Biotechnology, Osaka University "Basic operation for experiment of applied biology -to experiment safely and correctly-", Graduate School of engineering, Department of applied chemistry, Kyushu University "Guidance of Safety and health, environmental management in 2007 ", Graduate School of Science , Kyushu University "Guidance of safety in Faculty of Science in February, 2006", Laboratory for waste water treatment, Kyushu University "Utilization Guidance in February, 2008." Furthermore, although the English version of the safety guide was already published online in 2009, it has finally become a printed booklet with the cooperation of the G30 Lecturers of the Faculty of Agriculture. Lastly, we would like to express our appreciation to the teachers who contributed to this booklet.

February, 2011

Safety and health department, Faculty of Agriculture

CONTENTS

1. General preparation for safety working every day

For activities in school, it is necessary to pay attention to avoiding the disaster and accident, and create secure surroundings. This chapter contains (a) general preparedness for daily school life, (b) preparedness for laboratory operation, (c) protection against fire, (d) countermeasures against earthquake, (e) preparedness for traffic safety, (g) countermeasure against burglar (h) overseas traveling, (i) adequate insurance.

1.1. Preparation for school life

(1) Check the name tag of the section or laboratory, and clarify whereabouts at arriving and leaving.

(2) Check electricity, gas, water service and air-conditioning every day. If you finish your work or leave laboratory for a long time, you must put the power switch off and close the main cock of the gas and water service, and confirm the safety of locking the door.

(3) Confirm the installation location of the fire extinguisher, the fire-plug, the fire alarm, the emergency telephone, and the fire escape equipment, and well informed of these operation methods.

(4) Smoking is limited in the specified places and deposit cigarette butts in the proper containers. Moreover, never smoke in "Fire strict prohibition" place.

(5) Fix the laboratory equipments that are likely to fall during the earthquake.

(6) Obtain permission from your professor when you go to the laboratory 10:00 PM or later. (Sunday, Saturday and national holiday are all days).

(7) Lock the doors when going in and out building and laboratory. Moreover, when you leave laboratory for a long time, doors should be locked to prevent things within the room from being stolen.

(8) Keep all the room clean and organized.

1.2. Preparation for laboratory works

This section contains a general preparation for doing research experiments in a correct and safe way.

(1) Well-organized, Checks, and Settlement in mind
   (a) Inessential things should be not put around the laboratory units, and things you need for experiment are prepared in order.
   (b) Keep the doorway of the laboratory for shelter, walkways, distribution board clean.
   (c) Check the security in the laboratory regularly, and confirm no abnormality.
   (d) Put up warning signs (eg. ”Danger Zone”) and alert people to take precautions against the

hazardous experiment device and its surrounding.

(e) Chemicals are kept in a specified place, and never left on laboratory table. Dangerous chemicals must be managed safely and kept in a specific cabinet, and taken out only the amount necessary for the experiment.

(f) Fix a chemical shelf and a large-scale equipment as not to fall down in case of earthquake.

(g) Use a table tap or an outlet that endures the electric power used in the laboratory. Don't connect several table taps to one power source. Moreover, set up a breaker if necessary, and replace the corrupted or old electric wire with new one.

(h) Put on appropriate working wears and safety shoes for experiment. If necessary, wear the hard hat, gloves, eye protector, earplugs, and gas masks for safety.

(i) After experiment, make a final safety check for gas, electricity, and water service. The waste fluid and products are safely disposed. Appropriate working clothes and protective equipment are used.

(2) Careful preparation for experiments

Experiments with incorrect design and incomplete devices likely cause the accidents. Before doing experiments, check the normal operation equipment and make careful preparations. Experiment should not be conducted alone especially at the risky experiment and nighttime. If you make an experiment with an all-night unattended operation, please obtain the teacher's approval in the experiment beforehand. Moreover, take care of worker's health because worker's loose power of attention may cause an accident.

(3) Sincerely manage to do an experiment

While doing an experiment, it is necessary to stay calm and work on experiment seriously. Moreover, you always pay attention to surrounding circumstances so as not to cause an accident.

(4) Confirm the measures you will take when an accident occurs before starting experiment.

In preparation for an emergency situation, it is necessary to know location of the main cocks, switches, fire extinguishers, and escape routes. Furthermore, first aid measures, emergency contact number and how to use the fire extinguisher also should be aware.

1.3. Fire prevention

The disaster with high possibility of encountering in experiment and daily life is a fire accident. Here, fire prevention methods that should be noted in normal circumstances are summarized

(1) Check the following matters regularly.

(a) Fire extinguishers (P. 3-4), fire-plugs (Handling method: P. 5), protective gear (gas masks, etc), and emergency equipment (emergency ladder and survival kit, etc).

(b) Keep the following places clean: emergency staircase, fire door, fire-plug, corridor and balcony.

(c) Table for the products causing fire or smoke should be made by noncombustible materials, and be separated from surrounding combustible matters.

(d) Use undamaged rubber pipe and vinyl chloride tube for experiment.

(The one with bends and cracks are improper.)

(e) Separate electric code and gas pipe.

(f) Do not use non-standard fuse for power supply. Moreover, do not make many electric wiring loads on the same electrical outlet.

(2) Make a suitable arrangement of devices and chemical shelves in laboratory for emergency escape whenever an accident occurs. Furthermore, keep the laboratory always clean and tidy.

(3) For chemical experiments, use only necessary amount of combustible solvent.

(4) Notes

(a) Do not put the inflammability, flammable and combustible materials near the fire.

(b) Confirm the action taken when igniting. Make sure of installation location of gas valve, which should be turned off and power supply and fire extinguishers and escape routes, etc.

(c) Keep the escape routes clear when it breaks out.×

(d) Put out fire after doing experiments.

Fire extinguishers

×There are no effective extinguishing methods for all kinds of fires, so that an appropriate extinction method is adopted according to the kind and the level of a fire.

Knowing fire extinguisher's location, kinds and handling methods is always helpful for you. Moreover, participation in the fire practice regularly and learning the manner of operation are necessary. The types of fire extinguisher corresponding to the kind of a fire are shown here.

A Fire (Ordinary Fire)　B Fire(Oil Fire)　C Fire(Electric Fire)

The fire extinguishers correspond to the above-mentioned fire.

There are three kinds of fire extinguishers in Department of Agriculture in the following.

×

Powder ABC fire extinguishers 10 (it is being put by each laboratory)

Powder ABC fire extinguishers 50 (it is being put by each building electric room)

Carbon dioxide fire extinguishers 5 (it is being put by a part of laboratory)

ABC fire extinguisher

How to use a fire extinguisher

- Stay about 2 m away from flame and use, please. Especially, in the case of oil fire, it is possible to scatter oil and enlarge the range of a fire when approaching too much.
- Straightly target the bottom of fire and slowly approach to the fire to extinguish.
- Extinguish fire from the windward side when a wind existence.
- Radiate water without stopping to the last minute even if the flame disappears.
- Keep fire extinguisher upright when using.
- Grab the lever of the fire extinguisher firmly. When you cannot grasp the grip due to weak-handed, once you put it on the ground or floor, and grasp firmly while strongly pushing the lever.

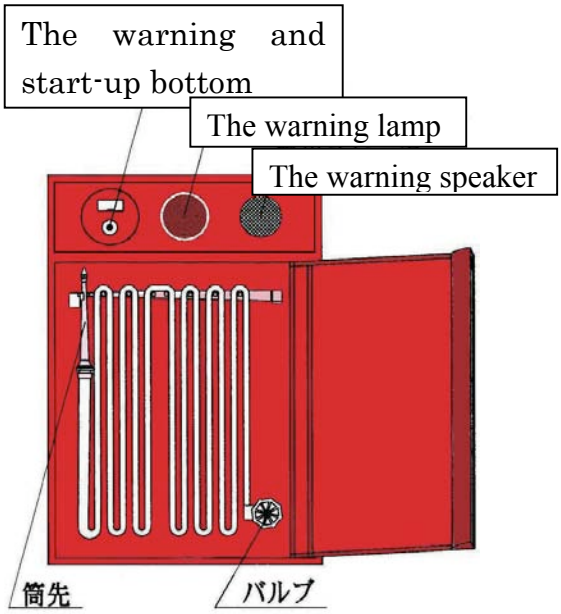
Attention after using a fire extinguisher

1. Turn off gas pipe immediately after extinguish a blaze related to city gas or propane gas.
2. In case of fire related to electricity, switch off the power supply immediately and consult a qualified person of the electric installation.
3. Confirm a fire whether there is an ember after extinguishing fire.
4. After extinguishing fire, ventilate the room promptly and evacuate to outdoor not to inhale neither chemicals nor generated gases due to a fire.
5. Contact a doctor immediately when you feel unpleasant or abnormally in your body by inhalation of fire-extinguisher.
6. Do not eat foods attached to fire-extinguisher.
7. Request dealers to refill the fire-extinguisher promptly after using, even if a little volume used.

8. Consult manufacturers or dealers to dispose fire extinguisher. Do not throw away or radiate it recklessly since it is very dangerous.

The indoor fire hydrant operation points

1. Confirm a fire.
2. Push the warning and start-up button.
3. Open the box and pull out the hose.
4. Extend the hose so as not to be twisted.
5. Open the valve and turn on water.
6. Hold the nozzle head and aim at the base of the fire.



1.4. What to do in an Earthquake

Earthquake is unpredictable. Moreover, there is no perfect measure against earthquake because it is hard to know the scale and it causes an unexpected secondary disaster. However, conducting an emergency drill against the unpredictable disaster and establishment of the measures might help the damage lightened. In this section, the measures taken when the earthquake happened in the laboratory are shown.

(1) Storeroom for books

Do not put a tall thing in surroundings of the desks usually used. Put the heavy load on the lower of the storage. Storerooms which are not frequently used are locked as much as possible. Moreover, enforce the resistance to earthquake about the computer monitor.

(2) Chemicals

Chemical bottles are kept in the steel shelf that lock system is installed. The shelf of two or more is firmly fixed between upper and lower part. Chemical storage room is attached to the wall and fixed firmly. When the chemical shelf is set up on the board of an experiment, the bridge is built across the chemical shelves, and each laboratory table is connected mutually or fixed with the frame firmly.

The sliding door should be installed in the shelf. To prevent the chemical bottle from falling, wooden or pipe crosspiece of which width is several centimeters is installed under the front side of the shelf in each shelf. Chemical storage rooms with lock systems installed strongly withstand earthquake.

(3) Cylinder

The chain for the gas cylinder fixation comes off foundation in case of the severe earthquake, and it falls with the support stand of gas cylinder. When fixing with the chain, wrap not only one place near the head, but also the middle part to fix the bottom with iron belt. The chain was tied to the clasp buried under a concrete wall. There is no space between the cylinder and the wall. Don't fix few gas cylinders together. Each cylinder is fixed individually. When the gas cylinder is connected with Gas Chromatograph, it fixes so that both should not slip down at the same time. The gas cylinder which is not being used is always covered with the cap.

(4) Glassware and sample

Glassware which is being used for distillation should be installed in the frame which fixed firmly on the laboratory table or the wall. When keeping it in the shelf, it puts in the shelf with the sliding door as well as the case of the chemicals and it packs it so that there is no space. Use a wide shelf board so that there is no space between doors with the shelf board. The sliding door must be closed after use, as well as the chemical shelf.

(5) Measuring instrument

Measuring instrument should put on the rubber mat or it's foots are covered with rubber. Do not put it on a slippery concrete stand. Surroundings of a fixed stand are raised a little. It is efficient to use prevention tool for slip drop. It often moves to the heavier one at the earthquake.

1.5. Traffic safety measure

Regardless of school or outside school, it is necessary to note the traffic safety enough, to observe a traffic rule, and to try to prevent the transportation disaster.

It is necessary to observe the following matters about the traffic of vehicles such as the car, motorcycles, and bicycles in school to secure safe traffic of the pedestrian and, keeping a tranquil environment in school.

(1) Follow the traffic sign, and pay attention to an excessive speed.
(2) Do not park except the place designated as the parking. Especially, do not park near the fire-plug and other no parking places so as not to disturb urgent activities such as fire engines and ambulances.
(3) Do not do obstructive activities such as rumbling engine noise or a horn. It obstructs the research and educational activities. Practicing about the motorcycle and the car, idling away and idling for a long time also disturb the neighbors.
(4) When causing a traffic accident in school, it first gives priority to the injury person's treatment, and it notifies the police afterwards. In addition, inform advising teachers, and the student in charge.

1.5.1. Attention concerning driving car

Please join voluntary and liability insurance when you drive a car. There are two kinds of insurance in Japan. One is compulsory automobile liability insurance, the other is voluntary insurance. It doesn't suffice in the insurance of the compulsory automobile liability insurance at a lot of accident amends. The parking lot for the car is also necessary.

1.5.2. Avoidance of bicycle accidents

There are so many bicycle accidents reported. There is a case to have to pay a large sum of indemnity money when becoming an assailant, and take care not to cause the accident not meeting with an accident, please.

-Prohibition of drunken driving
-Prohibition of driving with no lamplight at night.
-Prohibition of double driving.
-Do not use umbrella when you bike.
-Do not use a cellular phone when you bike.
-Prohibition of excessive speed.
- It takes a ride on a bicycle while breaking down.

1.6. Theft and burglary

(1) Don't walk alone as possible for the prevention of accident.
(2) Contact a police at once when becoming a victim of the theft or the burglar by any chance. In the case that happened in school, please contact a related school officer such as the Komatsu gate post, the advising teachers, and the officers in charge of general affairs for school. Moreover, it is useful for the arrest when assailant's characteristic is remembered at that time.
(3) Contact the Komatsu gate post when you find bike gangs to crowd in school.

Komatsu gate post ・・・call 642-2196 extension 8888
General affairs in Faculty of Agriculture・・・call 642-2796

1.7. Overseas travel notes

As provided in the admission rules of Department of Agriculture, students should give notice to the student office beforehand in spite of its purpose or reasons when students go abroad. When going abroad as a group of Department of Agriculture, the representative should inform. When you hope to study abroad, you should submit appropriate document to give permission from the president beforehand. Please refer to the details of Chapter 10.5 in this booklet.

1.8. Disaster and accident insurance

1.8.1 Disaster and accident insurance for student education and research

Kyushu University recommends for students to sign up for disaster and accident insurance for students education and research. This insurance covers the injury and the disaster happened in the class, during an experiment, a practice, and outdoor activities and various university events including the internship and the volunteer work. Moreover it covers an accident of movement between the university facilities. Students have to join insurance positively because the number of accidents increases every year. Students who are employed as a research assistant could not be covered by insurance. (They will be covered by the workers accident compensation insurance.)

(1) Insurance fee

Duration of insurance	Insurance fee（Science and technological）
One year	¥1,200
Two years	¥2,100
Three years	¥3,050
Four years	¥3,900

(2) Procedure to insurance

Junior year or more and graduate student must inquire of the student life division in school affaires section, and receive a payment form at the office. Pay it in the nearest post office.

Inquiries The students life division in school affaires section

Call: 642-2261

1.8.2 General liability insurance

This insurance covered the liability for damage and accident happened by making others injured, and other's fortune destroyed during school events.

(1) Insurance fee ¥340 a year

(2) Procedure to insurance

Bring insurance fee to the student life division in school affaires section.

You must join above-mentioned insurance (disaster and accident insurance for student education and research) beforehand.

2. Laboratory emergency plan

2.1. Preparation for emergency and contact system

It is important to ensure personal safety and settle down.

2.1.1. Occurrence of emergency situation

(1) When an accident occurs, notify the accident with full voice to the person who is close to you.
(2) You should not take measures against an accident by yourself.
(3) The injured person should be moved to the safe place.
(4) If possible, try to minimize the accident.
- Switch off electric power.
- Close the main cock of the gas.

(5) In case of fire
- Mind an appropriate initial extinction.
- Push the button of fire alarm.
- If it is an uncontrollable fire, take shelter promptly and ensure emergency contact.

If the flame reaches the ceiling, it is uncontrollable.

(6) In case of gas poisoning
-Do not enter the area carelessly.
-Close main cock of the gas
-Open windows.

(7) In case of electric shock
-Switch off power supply.
-Do not touch the person getting an electric shock, carelessly.
-Use dry stick, clothe or insulation gloves.
-If necessary, give artificial respiration and heart massage (Call ambulance?).

(8) In case of massive bleeding
-Raise the hemorrhage part more than the heart.
-In order to stop bleeding, press an artery that is closer to the heart than the wound.

2.1.2. Emergency contact

(1) Fire station (Higashi-ku) 119 or 641-1307
(a) When
(b) Where
(c) What kind of accident. Fire or an accident resulting in injury or death.
(d) How's situation
-Situation of the occurrence
-Possibility of expansion
-Casualties presence
(e) Caller's name and phone number

(2) Emergency contact in the campus
  (a) Komatsu gate: Emergency call 8888 or 642-2196
  (b) Person in charge or laboratory: Professor, Associate Professor, Assistant Professor.

2.2. Action plan when fire starts

(1) Call out loudly when there is a fire.

(2) Keep away surrounding combustible materials such as curtain from fire. Close the main cock of the gas and switch off power supply. Close the main cock of the supply source of the fuel such as gas cylinders.

(3) Calm down and try an initial extinction with a fire extinguisher or a water bucket. Be careful not to water when chemicals catch a fire.

(4) Try an initial extinction with a fire extinguisher if wall burns. When a ceiling burns, extinction with the fire extinguisher is difficult.

(5) Take shelter promptly and call an emergency phone when fire reaches to ceiling or your initial extinction becomes difficult.

(6) In case someone's clothing catch fire, do not let him/her run or keep standing. It will make the flames higher. Take off his/her clothing immediately, or drop down to the floor and roll his/her body (otherwise, other people must help to do so.) Then douse the fire directly or over the blanket rolled around the body to extinguish a fire.
  Remove heat with cool water or ice packs until tissue around burn feels normal to touch. Cover injured person to prevent shock.

(7) In case of a fire in the fume hoods, generally speaking, stop the ventilating because of the prevention of fire expansion to the upper side and the effects of extinction of fire. However, when a fire generates smoke and/or poisonous gas, ventilation should be kept. Judge the ventilation based on the materials burned and the situation.

(8)When the ignition by the gush of the flammable gas from gas cylinder happens, surrounding combustible materials should be removed without extinguishing fire. Next, pour water to the gas cylinder until it cools.

(9)When the flammable gas gushes without fire, remove the ignition source such as cutting the main cock of the power supply and the gas. Next, open the windows. If possible, close a nozzle of gas.

(10) In case of fire accompanying poisonous fumes, use protective equipment such as hazard masks for extinguishing the fire or extinguish it from the windward side.

(11) When you find a fire in other laboratory, first secure your safety and then run to the room with a fire extinguisher.

2.3. First-aid treatment

In proportion to the accident's size, treat it as follows until carrying to the hospital.
Please refer to guidelines of this web site: http://www.healthcentral.com/

2.3.1. Gas poisoning

Do not enter a contamination area carelessly.
In case of gas leak, close the main cock of gas with caring fire and open the windows. Get the individual out into fresh air and ventilate the area. Call an ambulance immediately. Inhale oxygen into the individual as a first-aid treatment. Artificial breathing may need to be administered if individual appears to stop breathing.

Occurrence of following poisonings is considered.
（1）Carbon monoxide poisoning
（2）Halogen poisoning
（3）Hydrocyanic acid poisoning
（4）Sulfur dioxide poisoning
（5）Hydrogen sulfide poisoning
（6）Organic solvent poisoning

2.3.2. Accidental ingestion of poisonous substance and absorption to skin

The countermeasure is different according to swallowed material. Please refer to "Safety guide of chemical experiment for students", or "To make an experiment safety." Those booklets are listed in reference. When consulting a doctor, you should bring MSDS (Material Safety Data Sheet) of the originating chemical.

(1) Ingestion of poisonous substances

First of all, vomit a poison by putting your fingers in throat when you have a clear sensorium. However, the submucosal lesion may be promoted when forcibly vomiting in the case of ingestion of strong acid, alkali, gasoline, kerosene, insecticide for atomization, and bleach. Do not let vomit.

In such cases, try to protect mucous membrane by giving milk or albumen.

(2) Chemical Exposures to the Body

Quickly remove all contaminated clothing and footwear. Immediately flood the affected body area in cold water for at least 15 minutes. Remove jewelry to facilitate removal of any residual material. Wash chemical off with clean water only. Do not use neutralizing chemicals, cream, lotions or salves.

Get medical attention promptly and call for assistance.

It should be noted that some chemicals (phenol, aniline) are rapidly adsorbed through skin. If a large enough area of skin is contaminated an adverse health effect (systematic toxicological reaction) may occur immediately to several hours after initial exposure depending on the chemical. If more than 9 square inches of the skin area exposed to a hazardous chemical, seek medical services after washing the material off. If the incident involves hydrofluoric acid (HF), immediately seek medical attention. Provide the physician with the chemical name.

(3) Chemical Splash in Eye

Irrigate the eyeball and inner surface of eyelid with plenty of cool water for at least 15 minutes. Use eyewash or other water source. Forcibly hold eyelids open to ensure effective wash. Check for and remove contact lenses. Get medical attention promptly.

2.3.3. Getting burned

Wash in volumes of clean water and cool for over 30 mins to 2 hours with ice-wrapped wet towel. Do not cool the same part too much. In case of slight burn: Aerating a slightly burnt part, the victim might cause pain, thus this part should be pasted with Vaseline, the sterile gauze, or the first-aid adhesive tape. For the medium degree of burn: Wash in clean water, cover with the clean towel or sheet, and medical attention should be received. In case of airway burn occurred, ice should be held in one's mouth, examination of the medical institution should be received.

2.3.4. Injuries

If hemorrhage is a little, wash the wound parts with clean water and with 3% hydrogen peroxide. If the wound parts are dirty with oil, oil should be wiped off with alcohol. The wound is disinfected with antiseptic solution at a slight injury, and covered with sterilized gauze, a first-aid adhesive tape, or an application bandage.

A vicious wound and a deep wound must receive doctor's treatment early.

The hemorrhage part is promptly maintained higher than the heart in case of the serious loss of bloods, and the part of an artery that is nearer the heart than the wound should be pressed with the finger or gauze. Stopping the flow of the blood with the bandage is not recommended, since the strong binding with it might destroy organization of the wounded parts. A bruise is painted with iodine tincture and covered with a cold compress. When you hit the head, you must see a doctor, even if outside abnormality is not found.

In the case of fracture, a splint should be applied to the fracture and seek a doctor. The fixation of the splint bandages at the top and bottom of the fracture site, and avoids the fracture part.

2.3.5. Electrical shock

When someone gets an electric shock, turn off the switch or liberate person from electric circuit by destroying apparatus. If removal of the electric wire is necessary, use a completely dry stick, cloth, or insulation gloves. When victim's breathing stops, start artificial respiration. Next, if pulse does not start, do the heart massage for a long time. For the heart massage, lay the victim down on his back, put your both hands on the victim's heart, and push by the rhythm of once per second while putting weight.

2.3.6. Cardiopulmonary resuscitation

When an unconscious person doesn't react to calling, the following measures should be done. Automatic external defibrillators (AED) are set up in the main entrance hall of 1st, 4th, and 6th buildings of Faculty of Agriculture. In case of emergency, use this.

(1) Artificial respiration

1) Securing the airway

Place your hand on the victim's forehead and tilt the victim's head back as far as it will reasonably go, essentially securing of the airway. Bring your face close to the chest of the patient with the airway secured. Bring your cheek close to the patient's mouth and nose, confirm the sound of breath, and feel the breath. Pay attention to vertical motion of the patient's chest and abdomen. This examination should be conducted within ten seconds.

2) Artificial respiration

Begin artificial respiration if the patient does not breathe. Secure the airway of the patient and blow your breath twice into the patient's mouth with closing the patient's nose and paying attention to leak of air. If impossible, close the patient's mouth and do artificial respiration by mouth to nose.

When you cannot blow smoothly, lift the neck of the patient, keep the airway, and blow in again. If you hesitate to do artificial respiration by mouth to mouth or nose, you may use handkerchief for the artificial respiration. It is convenient to carry the simple type of artificial respiration mask. If the patient has hemorrhage around mouth and nose, you may apply only heart massage.

(2) Cardiopulmonary resuscitation (CPR)

Close your ear to the patient's mouth, and then check the following symptoms.

- Does the patient breathe? (Check the movement of the chest or listen the sound of breath)
- Does the patient cough?
- Does the patient have any movement in the body?

Examine the above within ten seconds.

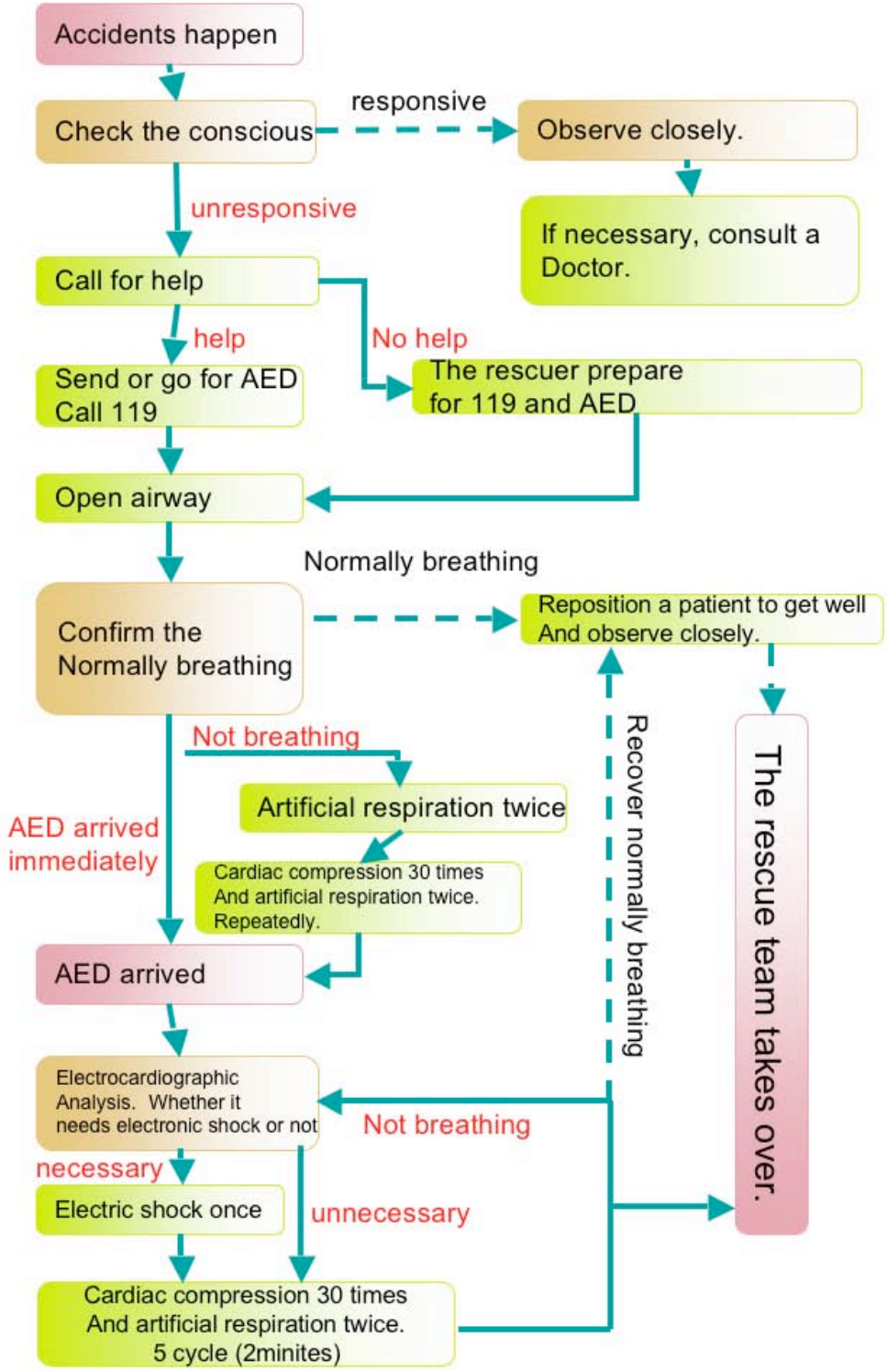
When these symptoms are not seen or they are not clear, begin the cardiopulmonary resuscitation soon. When either of symptoms is seen, the circulation is maintained, and it is judged that it is not an arrest cardiac. When the circulation is not maintained, begin the heart massage at once.

If any signature of the circulation is not seen after it practices artificial respiration twice, the heart should be massaged. Trace the edge of the lib of the patient using forefinger and middle finger and place your hand at the center of a right and a left lib. The hand that has traced the rib is piled up with the other hand. Extend the elbows, weigh straightly, and press the center part 15 times in a palm root. Press with 3.5-5 cm depth at the speed of 100 times per a minute.

(3) Automatic external defibrillator（AED）

AED is a machine of the heart electroshock that can judge the state of the heart for which the electroshock is necessary. Most of the sudden death is due to a heart failure. It is called a cardiac sudden death, and the sickness of ventricular fibrillation is a major cause of the death.

Ventricular fibrillation cause convulsion of heart and the heart would not play as the pump. In such a case, survival is missed by about 10% per 1 minute and the almost person will die in ten minutes. The only method of restoring this ventricular fibrillation to normal state is a defibrillation (electrical shock to the heart). AED judges whether the defibrillation is necessary, and directs a lifesaving procedure by the voice. AED is made to easily do a lifesaving act including the defibrillation.



Japan Red Cross Society : http://www.jrc.or.jp/study/safety/kinkyu/index.html

2.4. Evacuation Procedure

(1) When escaping from the danger, close the main cock of gas. If possible, after processing dangerous substances and confirming that there is no person, leave the room and close the door. But, do not lock it.
(2) Run away to the windward seeing the movement of smoke when there is no information on the announcement for the selection of the escape route in the passage.
(3) Do not use an elevator because it will be stopped in the case of an emergency such as fire.
(4) The stairs become the passages of smoke, and there are a lot of dangers. Think about the escape route usually, and examine the construction of a building and the fire exit.
(5) When there is a lot of smoke, cover the mouth with the washcloth and take shelter by low posture. Act calmly because it will take considerable time for smoke to fall to the floor level.
(6) When you could not utilize the escape stairway and the shelter ladder, please escape from the window in the terrace. However, please note that there might not be handrail in the terrace.
(7) Even though rooftop is a safe place, do not use it as an evacuation place except for emergency. Confirm that there is no person inside before closing the fire protection door. You can open it by pushing or pulling it strongly.
(8) When issued evacuation advisory due to fire or earthquake, the evacuation route is Kaizuka playground.

Method of operating

□foreside□

1)

Remove the cabinet

2)

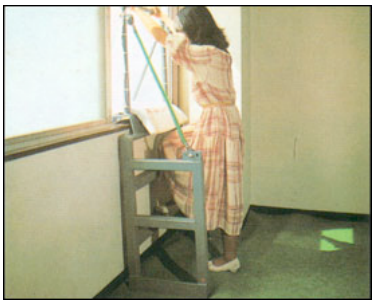
Let down the main body of bag

3)

Pull out the entrance frame

□downside□

4)



Please descend from the foot.

5)

Bend knees a little

And go down the slide on the hips

6)

Go above the ground without any impact

Perpendicular escape equipment

Method of operating

□foreside□

1)

Remove the cabinet and throw a rope

2)

Throw the main body of bag and pull out entrance frame

3)

Descend from the foot.

□downside□

1)

Eliminate the rope and fix the catch of the forehead of bag to anchor point.

2)

Hitch the catch to anchor point and pull out block line to extend the bag

3)

Fix the block line.

Descent escape equipment

3. Safety handling of chemicals

3.1. Precautions

3.1.1. General rules

A careless experiment accompanies risks. Whenever you do experiments, do not lose your guard. As for the shock of accident, it does not abide in material, in the person concerned physically, and the influence is large also in mental respect. In addition, the influence is exerted to surrounding people. For avoidance of damaging yourself and rolling in others, it is necessary to carry out an experiment with full attention to the given instructions.
Researchers should bear the following points in mind upon the experiments.

(1) To prevent an accident and use laboratory efficiently, keep laboratory bench clean and arrange stuffs orderly inside and outside.

(2) All the things kept in the laboratory must be secured safely to be prepared for unforeseen circumstances like earthquakes. Flammable materials such as organic solvents which are more than restriction amount, and hazardous substances designated under the law must be classified. Keep these materials in the dangerous material-keeping-cabinets specified under the responsible person in an orderly manner and lock the cabinet.

(3) Laboratory custodian should handle a deleterious substance, a poison and an explosiveness material. The custodian should confirm the operating suggestions carefully before use. Not only deleterious substances, but also many materials strictly managed by other regulations (Deleterious Substance Control Law, PRTR law, Ordinance on Prevention of Organic Solvent Poisoning, Ordinance on Prevention of Hazards Due to Specified Chemical Substances) should be carefully handled and have the use records using records.

(4) When handling a dangerous or harmful material, first investigate risk of ignition, explosion, combustion (fire point, flash point, and range), and hazardous properties (tolerable quantity and fatal dose) enough. Second, examine assumed measures or treatment provided against emergency, and then prepare necessarily available internal- and external-medicines and apparatus beforehand.
Distributors of the chemicals must be obligated to present MSDS to their customers. Therefore, you can obtain the MSDS（Material Safety Data Sheet）beforehand. You can check it anytime. MSDS is often converted into CD version in each reagent company. Moreover ”Vermont Safety Information Resources, Inc” also provides the following things.（http://siri.org/msds/index.php）
Regarding Japanese domestic laws and regulations for chemicals, you should ask Japanese members in your lab.

(a) Fire fighting measures against flash point
(b) First aid measures, accidental release measures
(c) Purifying method of volatile bad odor and acrimonious materials

(5) Use an explosion-proof type when you keep a low boiling point-inflammable material in the refrigerator.

(6) Notes when other materials are used.

(a) While doing experiments, protective spectacles (safety glasses) should be used without fail. Moreover, use the protective equipments such as gloves, disaster prevention masks, hazard masks, wire nets, and screens appropriately.

(b) Confirm location of the fire extinguisher in preparation for emergency. Do not conduct an experiment with materials which have a possibility of igniting in an ordinary laboratory.

(c) When chemical contacts to eyes and skins, start flushing the area immediately with running water over 15 min.

(d) In the experiments with generation of a smell malodorous substance or an irritating material, observe the followings.

a. Perform the experiment in a draft, and use devices blocked outlet.

b. Lead the smell malodorous or the irritating material exhausted from the experimental device to the outside through appropriate wet scrubbers.

(8) Working with two or more people whenever you conduct risky experiments.

(9) When you handle the chemicals, wear the white robe and fasten a button (Prevent it from blowing down to the equipment). The white robe contacted to the chemicals should be washed. Do not go outside laboratory with the white robe on.

(10) Conducting an experiment with wearing sneakers will help to evacuate easily in emergency situation. Prompt actions cannot be taken with slippers.

(11) Carefulness when you conducting experiments with a gas burner there are possibility to explosions occurrence and serious burn occur. Fix your hair carefully before doing an experiment.

(12) The following website will be helpful to handle the chemicals and make an experiment safely.

Health and safety

The University of Melbourne: http://safety.chemistry.unimelb.edu.au/Chemsafety.php

MIT: http://web.mit.edu/environment/ehs/about_ehs.html

University of South Carolina: http://ehs.sc.edu/

Harvard University: http://www.uos.harvard.edu/ehs/

Yale University: http://www.yale.edu/oehs/

3.1.2. Notes on the Industrial Safety and Health Law

The universities in Japan receive the application of the Industrial Safety and Health Law. When an accident happens in the laboratory, penalty (penal servitude, penalty, and experiment stop and emergency measure instruction, etc.) is imposed on people who are in charge of the safety management.

This law has several ordinances including Ordinance on Prevention of Organic Solvent Poisoning and Ordinance on Prevention of Hazards Due to Specified Chemical Substances as rules that should be considered in conducting experiments of the chemistry system. Pertinent reagents are provided about each rule. Moreover, when these are used, the working environment measurement is applied.

Industrial Safety and Health Law ⇔ Working Environment Measurement Law

↓

Enforcement Order of the Industrial Safety and Health Act Cabinet Order

↓

Ordinance on Prevention of Organic Solvent Poisoning

Refer to 1st, 2nd, 3rd, Table 3-10

Ordinance on Prevention of Hazards Due to Specified Chemical Substances

Refer to 1st, 2nd, 3rd, Table 3-9

3.2. Hazardous substances leading to fires and/or explosions (Fire Service Law object chemicals)

The substances with ignited danger are numerous. Therefore, their classification is based on several Japanese domestic laws as shown in Table 3-1. In general, there are two types of combustible substances, one ignited by heat or impact, and the other ignited by contact and mixture. Besides, these substances are classified into 6 groups by their characteristics.

Table 3-1. Hazardous materials under several laws

Characteristics of chemicals			Hazardous materials under the fire defense law	Under the high pressure gas safety law	Under the industrial safety and health law	Under the gunpowder law
Oxidation nature	Flammable materials are oxidized and the fire explosion is caused.	Solid	1st group	−	Oxidation nature	−
		Liquid	6th group	−		−
		Gas	−	○		−
Flammability	It ignites in air, and the fire explosion is caused.	Solid	2nd group	−	Flammability	−
		Liquid	4th group	−	Flammability	−
		Gas	−	○	Flammability gas	−

Pyrophoric, Water reactive	React to water or air	Solid	3rd group	−	Flammability	−
		Liquid	3rd group	−		−
		Gas	−	○		−
Autoreactive	The explosion are caused by heat and the impact.	Solid	5th group	−	Explosive nature	○
		Liquid	5th group	−		○
		Gas	−	○		−
Other		Inactive gas	−	○		−

Table 3-2. Hazardous materials categorized by the Fire Service Law

Type	Aspect	Chemicals
1st Group	Oxidizers (solid)	Salts of following acids (chloric acid, perchloric acid, chlorite, bromic acid, nitric acid, iodic acid, bichromic acid, permanganic acid), inorganic peroxide
2nd Group	Combustible solid	phosphorous sulfide, red phosphorous, sulfur, iron powder, metal powder, magnesium, solid alcohol
3rd Group	Pyrophoric liquid or solid	metal potassium, metal sodium, alkylaluminum, alkyllithium, yellow white phosphorus, alkali (earth) metal, metal hydride, metal phosphide, calcium carbide, alminum carbide
4th Group	Flammable liquid	highly flammable substances, petroleum substance (class 1-4), alcohols, animal oil and plant oil
5th Group	Explosive liquid or solid	organic peroxide, nitrate esters, nitro compound, nitroso compound, azo compound, diazo compound, hydrazine dericative
6th Group	Oxidizers (liquid)	perchloric acid, hydrogen peroxide, nitric acid, bromine trifluoride, bromine pentafluoride

3.2.1. Pyrophoric chemicals (3rd group hazardous materials under Fire Service Law)

Pyrophoric substance is easily ignites or generates heat only by air touching. The representative of these are metal organic compounds such as alkylaluminum, alkyllithium or nickel and palladium reduced.

3.2.2. Water reactive chemicals (3rd group hazardous materials under Fire Service Law)

Water reactive chemicals react with water and release a gas that is either flammable or presents a health hazard. The representatives of these are Alkali metals such as natrium and kalium, metaric carbide, lithium, and many organic metallic compounds.

Table 3-3. Representative substances of pyrophoric substances/ Water reactive chemicals

Substances	Compound	React number, gas, toxic, corrosive	Reaction
Alkali metal	Li Na, K	【4】, H_2 gas 【1，2】, H_2 gas, corrosive to skin.	【1】spontaneously catch fire on exposure to air. 【2】react with water to catch fire, blow up at times. 【3】There is a possibility to blow up when mixing with oxidizing agent. 【4】There is a possibility to catch fire when reacting with water to produce combustible gas 【5】Produce poisonous gas during combustion.
Alkali earth metal	Ca, Ba	【2】, H_2 gas	
Alkylaluminum	$(CH_3)_3Al$, $(C_2H_5)_3Al$, $(C_3H_7)_3Al$ $(C_2H_5)_2AlCl$, $(i\text{-}C_4H_9)_3Al$, $C_2H_5AlCl_3$, $(C_2H_5)_2AlH$	【1，2】, C_nH_{2n+2} gas 【4】, C_nH_{2n+2} gas	
Alkyllithium	CH_3Li, C_2H_5Li	【1，2】, C_nH_{2n+2} gas	
Zinc alkyl	$(C_2H_5)_2Zn$	【1，2】, C_2H_6 gas	
Metal hydride	LiH NaH,CaH_2	【4】, H_2 gas 【3，4】, H_2 gas, have toxic consequence.	
Metal phosphide	Ca_3P_2	【2】, PH_3 gas, have toxic consequence	
Metal carbide	CaC_2 Al_4C_3	【2】, C_2H_2 gas 【2】, C_2H_4 gas	
Phosphorus	P_4, yellow white phosphorus	【1，5】, toxic, corrosive	
Chlorinated silicon compounder	$SiHCl_3$	【1，3，4】, HCl gas, toxic	

3.2.3. Potentially explosive chemicals and water reactive chemicals (2nd group hazardous materials under Fire Service Law)

Of low degree ignition materials, metallic powder and phosphorus pentasulfide such as magnesium and aluminum are well known. Generally, they are strong reducing reagents and ignite by heating, impact and friction when mixing with an oxidizing substance.

Table 3-4. Low degree ignition materials

Substances	Compound	React number, toxic corrosive	Reaction
Phosphorus	P_4S_3, P_4S_7 P_2S_5	【3，5】 【5】	【1】There is a possibility to blow up when mixing with oxidizing agent. 【2】There is a possibility to blow up when igniting dust powder. 【3】Catch fire by strong friction. 【4】spontaneously catch fire on exposure to air, oilcloth or cutting trash. 【5】React with water or produce poisonous gas during combustion.
Metallic powder	Fe, Al, Zn, Mg（including ribbon base）	【1，2】	
Others	P_n, red phosphorus S_n	【1，2】, toxic corrosive 【1．2，4，5】	

3.2.4. Oxidizers (Solid)(1st group hazardous materials under Fire Service Law)

Oxidizing agents include chloric acid, perchloric acid, permanganic acid, nitric acid, inorganic peroxide, and organic peroxide. Generally, they are easily decomposed by heating, impact and friction and release oxygen to burn off inflammable materials. Oxidizing agents can blow up or catch fire when mixing with organic substances or reducing materials, especially when mixing with strong acid. Organic peroxide itself has possibility to blow up to react with heating, impact and friction.

Table 3-5. Oxidizers solid

Substance	Compound	React number	Reaction
Salt of Chloric acid	$NaClO_3$	【1,2,3,5】	【1】substance itself is unstable, it blow up because of heating, impact and friction. 【2】It blow up when mixing with substances which are susceptible to oxidation. 【3】add strong acid to blow up 【4】React with water and heat up. There is a possibility to blow up. 【5】It is a deliquescent material. There is a possibility to blow up after penetrating into paper or tree and dry.
	$KClO_3$	【1,2,3,toxic】	
	NH_4ClO_3, $Ba(ClO_3)_2$, $Zn(ClO_3)_2$, $Pb(ClO_3)_2$, $AgClO_3$, $HgClO_3$	【1,2,3】	
Salt of Perchloric acid	$NaClO_4$, $KClO_4$, NH_4ClO_4	【1,2,3】	
Salt of Chlorite	$NaClO_2$, $KClO_2$, $Cu(ClO_2)_2$, $Pb(ClO_2)_2$	【2,3】	
次 Chlorite	$Ca(ClO)_2$, bleach powder	【2】	
Salt of Bromic acid	$KBrO_3$	【1,2】	
	$NaBrO_3$, $Mg(BrO_3)_2$, $Ba(BrO_3)_2$	【2】	
Salt of Iodic acid	$NaIO_3$, KIO_3, $Ca(IO_3)_2$, $Zn(IO_3)_2$	【2】	
Salt of periodate	$NaIO_4$	【2】	
Salt of Permanganic acid	$NaMnO_4$, $KMnO_4$, NH_4MnO_4	【2,3】	
Salt of Bichromic acid	$Na_2Cr_2O_7$, $K_2Cr_2O_7$, $(NH_4)_2Cr_2O_7$	【2】	
Salt of Nitric acid	$NaNO_3$, KNO_3, $Ba(NO_3)_2$	【2】	
	$AgNO_3$	【2, corrosive】	
Salt of Nitrous acid	NH_4NO_3	【1,2】	
	$NaNO_2$, KNO_2	【2】	
Inorganic peroxide	Li_2O_2, Na_2O_2, K_2O_2	【2,4, corrosive】	
	MgO_2, CaO_2, SrO_2, RbO_2, CeO_2	【2】	
	BaO_2, PbO_2	【2,toxic】	
Salt of Peroxosulfuric acid	$(NH_4)_2S_2O_8$	【2】	
Peroxyborate	NH_4BO_3	【2,3】	
Oxidated products of Cr・Pb・I	CrO_3	【2,3, corrosive】	
	PbO_2,	【toxic】	
	I_2O_5	【1,2】	
Others	$C_3N_3O_3Cl_3$, trichloroisocyanuric acid	【2】	
	$HIO_4・2H_2O$, metaperiodic acid	【2】	

3.2.5. Oxidizers (liquid)(6th group hazardous materials under Fire Service Law)

As a strong acidic substance, fuming nitric acid, fuming sulfuric acid, concentrated nitric acid, concentrated sulfic acid, and chromic anhydride are known. These materials might generate heat, ignite when mixing them with organic materials and reduced materials. There is a possibility to explode when mixing it with strong acid.

Table 3-6. Oxidizers liquid

Compound	React number	Reaction
$HClO_4$	【1b, 2a, 3, corrosive】	【1】(a) metallic powder, (b) inflammable material such as alcohol, (c) catch fire or blow up when mixing it with amine and hydrazine
H_2O_2	【1a, 1b, 4, corrosive】	
HNO_3, fuming sulfuric acid	【1c, 3, toxic, corrosive】	【2】react with water, (a) generate heat, (b) produce oxygen
BrF_3, BrF_5	【2b, 5, toxic, corrosive】	【3】Spontaneously ignite when touching organic substances such as sawdust.
		【4】hydrogen fluoride reacts with many substances to produce fluoride.

3.2.6. Flammable materials (4th group hazardous materials under Fire Service Law)

Flammable materials burn easily when there is an ignition source near by though it does not ignite only by touching air. Note that a material having a low flashing point is dangerous and that even if a material has a high flashing point, when you heat it more than the flash point is dangerous, too. Among the flammable materials, there are special flammable materials, high degree inflammable materials, medium degree inflammable materials, low degree inflammable materials. The handling method is described about each category.

3.2.6.1. Special flammable materials

The aspects of these materials are liquid at 20°C or liquid at from 20 to 40°C. The ignition temperature of the materials is below 100°C. Their flashing points are below -20°C, and their boiling points are below 40°C. The typical materials are follows: diethyl ether, carbon disulfide, acetaldehyde, pentane, iso-pentane, propylene oxide, divinyl ether, nickel carbonyl, and alkyl aluminum. Bear in mind that a fire is the most frequent accident in a chemistry laboratory. When these materials are handled, the following attentions are necessary.

(1) Take care of discharge of gas and steam.
(2) Know the flash point, the fire point, and the explosion limit.
(3) Do not put needless solvent on the bench.
(4) Turn off the nearby naked light when you use the materials. Because their ignition temperature and flash point are extremely low, they ignite easily.
(5) Do ventilation well because the boiling point is low and the explosion limit is wide.
(6) It will be continued explosively when igniting once and fire is not extinguished easily.
(7) The space over the solvent container is often in the range of explosion. The container must be sealed up. Take extra care of the ignition source when you use a little solvent.
(8) Do not keep voluminous solvent in the laboratory for a long time.
(9) Confirm location of a fire extinguisher. Especially, prepare it beforehand near yourself when there is fear of the ignition.
(10) The powder extinguisher, the carbon dioxide extinguisher or sand for fire prevention are good for extinction. Although pouring water is not suitable for inflammable materials, it is good for the extinction of a peripheral fire.

3.2.6.2. High inflammable materials (class 1 petroleums, alcohols)

Substances which have high flammable characteristics at room temperature and their flash points below 20°C are considered as high inflammable materials. The typical materials are petroleum ether, gasoline, petroleum benzine, ligroin, hexane, heptane, octane, pentene, and benzene, toluene, and *o*-xylene, alcohols (methylic-, pentyl-), dioxane, acetone, methyl ethyl ketone, ester of formic acids (methylic-, pentyl-), acetic esters (methylic-, pentyl-), and acetonitrile, pyridine, and chlorobenzene.

When these materials are handled, the following attentions are necessary.

(1) Know the flash point, the fire point, and the explosion limit.
(2) Do not put needless solvent on the bench.
(3) Because the inflammability of these materials is high, do not place the materials nearby fire, electric spark, and burners nearby, and do not heat the materials by direct flame.
(4) Be sure to do ventilation and not to stay in a low place because specific gravity of steam is heavy.
(5) The powder extinguisher, the carbon dioxide extinguisher or sand for fire prevention are good for extinction.

3.2.6.3. Medium inflammable materials (class 2 petroleums)

Substances which have high flammable characteristics at intermediate temperature range and their flash points between 20°C and 70°C are defined are medium inflammable materials. The typical are as kerosene, light oil, turpentine, xylene, styrene, aryl alcohol, cyclohexanol, benzaldehyde, formic acid, and acetic acid. When heating it in the container with the opened mouth, it is necessary to note the accumulation of steam of these materials within the container. The powder extinguisher or the carbon dioxide extinguisher is good for extinction of flame caused by these types of materials.

3.2.6.4. Low inflammable materials (class 3 petroleums, class 4 petroleums, animal fat and vegetable oils)

Substances generating inflammable gas while being heated at high temperature, and their flash point over 70°C are considered as low inflammable materials. The typical materials are crude petroleum, creosote oil, spindle oil, turbine oil, transformer oil, tetralin, ethylene glycol, diethylene glycol, ethyl acetate, ethanolamine, nitrobenzene, aniline, *o*-toluidine, heavy lubricant (gear oil and motor oil, etc.), and animal- and vegetable oils (canola oil, soybean oil, palm oil, etc.). These substances are not easily ignited, because their flash points are high. However, it will be difficult to extinguish easily once they catch fire. Be sure not to heat them over their flash points and take care not to flash off steam that generated from the heated materials. The powder extinguishers or the carbon dioxide extinguisher are good for extinction.

Table 3-7 shows properties of general solvent that is flammable or combustible.

Table 3-7. Properties of general solvents

	Boiling point ℃	Flash point ℃	Explosion limit Upper limit %	Explosion limit Lower limit %	spontaneous ignite point ℃	Vapor ratio (air)	Permissible level consistency mg/m^3	Toxic potency
Pentene	30	–49	1.4	8.0	309	2.48	1800	(N)
Hexane	69	–23	1.2	6.9	260	2.97	180	(N) (R)
Heptane	98	–4	1.2	6.7	233	3.45	1600	(N) (R)
Benzene	80	–11	1.4	8.0	538	2.77	80	(S) (R)
Toluene	111	4	1.3	7.0	552	3.14	370	(ND)
Xylene	138	25	1.1	7.0	496	3.66	435	(N) (R)
Methylene	40	－	12.0	19.0	662	2.93	1740	(N) (ND)
Chloroform	61	－	–	－	－	4.12	50	(N) (ND)
Carbon tetrachloride	77	－	–	－	－	－	65	(N) (R)
Ethylenedichloride	82	21	6.2	15.9	449	3.35	200	(ND) (S)
Methanol	65	18	6.0	36.5	470	1.11	260	(N)
Ethanol	78	16	3.3	19.6	399	1.59	1900	(N)
2－propanol	82	21	2.5	5.2	456	2.07	980	(N)
Diethyl ether	34	–45	1.8	48.0	180	2.55	1200	(N)
Tetrahydrofuran	66	–14	2.3	11.8	321	2.50	590	(N)(ND)
Dioxane	101	12	2.0	22.0	180	3.03	180	(ND)
Acetone	57	–18	2.6	12.8	538	2.00	2400	(ND)
Ethyl acetate	77	4	2.7	11.5	482	3.04	1400	(ND) (R)
Acetic acid	118	43	4.0	16.0	426	2.07	25	(ND) (S) (R)
Acetic acid anhydride	140	54	3.0	10.0	380	3.52	20	(ND) (S) (R)
	80	6	4.0	16.0	524	－	70	(ND) (S) (R)
Acetonitrile	46	–30	1.0	44.0	100	2.64	30	(ND)
Carbon bisulfide	95	95	2.6	42.0	215	–	–	(S)
DMSO	58	58	2.2	15.2	445	–	30	(ND) (S) (R)
DMF	105	105	–	–	–	6.18	–	(ND) (S)
HMPA								

（R） Respiratory obstruction
（N） Narcotic action
（S） Skin lesions
（ND） Nervous disorder

3.2.7. Potentially explosive substances (5th group hazardous materials under Fire Service Law, Explosives Control Law)

Potentially explosive substances are ignited or exploded by fire, impact, and friction. Nitric ester, nitro, nitroso compound, diazo compound, diazonium salt, and compound, hyperoxidation combination thing, and halogen acid conductor and acetylene heavy metal salt agitation are known.

In general, risk becomes high like the one to have a lot of uniting materials such as N－O,N＝O,N－N,N＝N,O－O,O－halogen, N－S,N－halogen. Moreover, substances itself are not dangerous to explode. There is a reagent that can produce peroxide while storing it and causes the explosion. You

have to be careful to handle dioxane, tetrahydrofuran, and diethyl ether that have been preserved for a long time, or used. These substances should not be concentrated without discretion.

Table 3-8. Self-reacted materials

Substances	Compound	React number	Reaction
Organic peroxide	$C_6H_5CO•OO•COC_6H_5$, benzoyl peroxide	【1, 2, 3】	【1】It explodes by heating, the impact, friction, and light 【2】It explodes by contact with the strong acid 【3】It explodes by the mixtures such as the organism, the halogen, and sulfur. 【4】The spontaneous decomposition might be caused to ignite and explode.【5】The cracked gas might cause the explosion. 【6】The inflammability is large, and it is likely to explode during burning.
	$CH_3COC_2H_5•O_2$, methyl ethyl ketone peroxide	【1, 4】	
Nitric ester	CH_3NO_3, $C_2H_5NO_3$	【6】	
	$C_3H_5(NO_3)_3$ nitroglycerin	【1, explosive】	
	$Cell—(NO_3)_{2\sim3}$ nitrocellulose	【1, 4, explosive】	
Nitro compound	$(NO_2)_3C_6H_2OH$ picric acid, $(NO_2)_3C_6H_2CH_3$ trinitrotoluene	【1, 3, 6, explosive】	
Nitroso compound	$C_5H_{10}N_6O_2$ N,N-dinitrosopentamethylenetetramine	【1, 2, 3】	
Azo compound	$C_8H_{12}N_4$ azoisobutyronitrile	【corrosive】	
Diazo compound	$C_6H_2N_4O_5$ diazodinitrophenol	【1, explosive】	
Hydrazine	$NH_2NH_2•H_2SO_4$ hydrazine sulfate	【corrosive】	
Hydroxylamine and salt	H_2NOH hydroxylamine, $H_2NOH•HCl$, $(H_2NOH)_2H_2SO_4$ hydroxylamine sulfate		
Metallic azide	NaN_3 sodium azide	【5, corrosive】	
Others	$(NH_2)_2C=NH•HNO_3$ guanidine sulfate	【1】	

3.2.8. Regulation for maintaining hazardous materials

Chemicals described above are categorized as “hazardous materials” according to the Fire Service Law. Based on their potential hazardousness, “Specified Quantities” of them are defined (Table 3.9.). Each material can be stored less than 20% of the “Specified Quantity” in a compartment partitioned by firedoors. In case any item listed on “hazardous material” is required to be kept more than 20%, it must be stored in a specified storage under the supervision of safety department staff.

Table 3-9. Specified quantities of hazardous materials

Group	Item	Property	Specified quantity
1st Group		Class 1 oxidizing solids	50 kg
		Class 1 oxidizing solids	300 kg
		Class 1 oxidizing solids	1,000 kg
2nd Group	Phosphorus sulphide Red phosphorus Sulphur		100 kg
		Class 1 combustible solids	100 kg
	Iron powders		500 kg

		Class 2 combustible solids	500 kg
	Flammable solids		1,000 kg
3rd Group	Potasium Sodium Alkyl aluminium Alkyl lithium		10 kg
		Class 1 spontaneously combustible materials and water prohibitive materials	10 kg
	Yellow phosphorus		20 kg
		Class 2 spontaneously combustible materials and water prohibitive materials	50 kg
		Class 3 spontaneously combustible materials and water prohibitive materials	300 kg
4th Group	Specified flammable materials		50 L
	Class 1 petroleums	Non-water soluble liquid	200 L
		Water soluble liquid	400 L
	Alcohols		400 L
	Class 2 petroleums	Non-water soluble liquid	1,000 L
		Water soluble liquid	2,000 L
	Class 3 petroleums	Non-water soluble liquid	2,000 L
		Water soluble liquid	4,000 L
	Class 4 petroleums		6,000 L
	Animal fats and vegetable oils		10,000 L
5th Group		Class 1 self-reactive materials	10 kg
		Class 2 self-reactive materials	100 kg
6th Group		Oxidizing liquid	300 kg

3.3. Poisonous substances which need strict management

There are a lot of poisonous (toxic) substances of which are not defined the allowable concentration. It is difficult to obtain information about toxicity of new compounds caused by reaction. Thus, you should be careful when you handle reagents or new compounds. There are two cases of the symptom when you touch toxic substances. One is that the symptom appears in a short time. The other is that the symptom appears after repeating exposure to small amount of the substances. When directly affected the eyes or skin, there are cases to receive stimulation with rapid pain such as acid or alkali to eyes or skin, or to have skin irritation later.

Among known chemicals, there are toxic gas (permissible density is less than 200 mg/m^3), toxic substance (oral fatal dose is less than 30 mg / kg body weight), and deleterious substance (oral fatal dose is over 30 to 300 mg / kg body weight). Besides these acute toxicities, be careful to handle the chronic toxicity and the carcinogen. The following attention in handling is necessary. Note: The fatal dose is not necessarily a standard of classification for toxic and deleterious substances.

(1) Investigate the allowable concentrations and recognize them beforehand.
(2) From the general knowledge of chemicals, deleterious inorganic substances are easily figured out whether they are harmful or not, however, organic substances are very difficult to figure out. Some of them have strong toxicity without anticipation. Use them after you examine them thoroughly.
(3) Wear the protection tools such as protective spectacles, the disaster prevention masks, hazard masks, and gloves.
(4) Experiment has to be in the draft. Do not leave the toxic or deleterious substances in the draft after experiment.
(5) Be careful not to leak the materials with the low allowable concentration.
(6) Use materials after obtaining approval from the person in charge of the laboratory. Moreover, let other people in the room know the use.
(7) Be careful especially when you handle a large amount of the substance.
(8) Even if you handle the deleterious materials wearing protective equipments, have first-aid immediately in the case of accidental ingestion or adhesion to the eyes or skins. Generally, wash at least 15 min or more with a large amount of stream when adhering to eyes and the skins. Have a medical examination depending on the situation. In the case of inhalation of the substances, move to another place and keep the body warm with the blanket.
In the case of difficult breathing, artificial respiration or oxygen inhalation should be done. At the same time, arrange the medical examination promptly by calling 119.
(9) When you use deleterious substances which are kept in the locked cabinet labeled with “poison”, please receive permission from the teacher in the laboratory. Moreover, keep a record of the date, the name of the user and the quantity consumed on the user’s list.

3.3.1. Poisonous and Deleterious Substances Control Law

For handling poisonous or deleterious substances, it is necessary to obey "**Poisonous and Deleterious Substances Handling Rules in Kyushu University** " besides "Poisonous and Deleterious Substances Control Law" and other laws. (Rule Article 1) The obligation of the administration manager in the department, the management representative, and the users provided in rules of this University are written as follows.

(1) Obligation of the administration manager in the department (Rule Article No. 3)
The management head in the department must summarize the management of poisonous and deleterious substances and take steps to prevent any accidents.

(2) Obligation of the management representative (Rule Article No. 6-11)
1) The cabinet for poisonous and deleterious substances should be hard, made of metallic, and be separate from one for general chemicals.
2) It is necessary to take measures to prevent the cabinet from falling down and to prevent the containers for poisonous and deleterious substances from dropping from the shelf of the cabinet, due to disaster such as earthquake.
3) It is necessary to lock the cabinet surely. The management representative has the responsibility to manage the key.
4) It is necessary to label the character “poisonous substance quasi drugs” on the cabinet and

bottle. In the case of poisonous substances, write “Poisonous” by white line on red-colored base. In the case of deleterious substances, write “Deleterious substances” by red line on white-colored base.

5) The poisonous or the deleterious substances kept for a long term and without prediction of use should be discarded promptly.
6) When the poisonous or the deleterious substances are discarded, you must obey to the standard of the abandonment provided by laws, ordinance, and the Kyushu University exhaust water and waste management rule.
7) It is necessary to provide with the use list of the attached paper style, and to understand the volume of inventories and the quantity consumed.
8) The user’s list should be crosschecked regularly with the quantity of poison and deleterious substances kept in bottle.
9) In case of loss or theft of poisonous or deleterious substances, notify the administration manager immediately.

(3) Users’ obligation (Rule Article No. 5, 9, 11)

1) People who use the poisonous and deleterious substances must obey to the instruction by the administration manager in the department or the management representative. Have enough knowledge of the security and be careful to prevent an accident.
2) Every time you use the poisonous and deleterious substances, you should record in the user’s list.
3) If you use the web-system “chemical design for laboratory ver.3.0 (http://chem.jimu.kyushu-u.ac.jp/default.aspx)” and records on the web-system, you do not have to write the use on the paper. However, user should obey to instruction by the management representative.
4) If you find a loss or theft of poisonous or deleterious substances, you should notify the administration manager immediately.

Poisonous and deleterious substances user list　　No.

Substance				Unit	g ・ mL ・ (　)
Storage area					
When	Total		Remaining amount	User name	Purpose for use	Others
	Purchased	Used				

3.3.2. Specified chemical substances (Ordinance on Prevention of Hazards due to Specified Chemical Substances, Industrial Safety and Health Law)

As for the trouble by the Specified Chemical Substance, the organ (target internal organs) in which substances accumulated was dependent on the kind of substances. As for the symptom, cancer, hepatic damage (icterus, hepatic cirrhosis), kidney problem (kidney inflammation, hematuria), lesions of hematopoietic organs or blood problem (hemolysis, anemia) are known. The hazardous properties of main specified chemical substances and all specified chemical substances are listed as follows. The use of type 1 and type 2 specified chemical substances are subject to the Working Environment Measurement Law. Parenthetic values show the management density. Refer to MSDS for other hazardous properties.

Moreover, when the Specified Chemical Substance is used, the application exclusion regulations seen in Ordinance on Prevention of Organic Solvent Poisoning are not admitted. Thus, the limited exhaust ventilation must be used during handling the Specified Chemical Substance.

Type 1 substances

Seven kinds of chemicals specified for the type 1 substance that is confirmed to be carcinogenic should basically refrain from the use. Consider the use of the alternative.

Type 2 substances

The substances must be used in the limited part exhaust device.

Table 3-10. Specified Chemical Substance classification

Type 1 Substances	
1.dichlorobenzidine and its salts	5 dianisidine and its salts
2 ∂-naphylamine and its salts	6 beryllium and its compounds
3 chlorinated biphenyl	7 benzotrichloride
4 ortho-tolidine and its salts	
8 Substance contains 1-6 materials over 1% by weight. Or substance contains 7 over 0.5% by weight.	
Type 2 Substances	
1 acryl amide	20 methyl bromide
2 acrylonitrile	21 diachronic acid or its salts
3 alkyl mercury compounds	22 mercury or its inorganic compounds
4 asbestos	23 trilene-diisocyanate
5 ethleneimine	24 nickel carbonyl
6 vinyl chloride	25 nitroglycol
7 chlorine	26 *P*-dimethylaminoazobenzene
8 auramine	27 *p*-nitrochlorobenzene
9 *o*-phthalodinitrile	28 hydrogen fluoride
10 cadmium	29 ß-propiolactone
11 chrmic acid or its salts	30 benzene
12 chloromethyl methyl ester	31 pentachlorophenold (PCP) or its sodium salts
13 vanadium pentoxide	32 magenta

14 coal tar	33 manganese or its compounds
15 arsenic trioxide	34 methyl iodine
16 potassiumcyanide	35 hydrogen sulfide
17 hydrogencyamide	36 dimethyl sulfide
18 sodiumcyanide	37 formaldehyde
19 3-3'-dichloro-4-4'-diaminodiphenyl methane	38 Substance contains 1-37 and is designated by Department of Labor.
Type 3 Substances	
1 ammonia	6 phenol
2 carbon monodioxide	7 phosgene
3 hydrogen chloride	8 sulfuric acid
4 nitric acid	9 Substance contains 1-8 and is designated by Department of Labor.
5 sulfur dioxide	

3.3.3. Organic solvents (Ordinance on Prevention of Organic Solvent Poisoning, Industrial Safety and Health Law)

Although approximately 500 kinds of the organic solvents are used industrially, 54 kinds of those receive the application of "Ordinance on Prevention of Organic Solvent Poisoning" as listed in Table 3-9. These solvents are confirmed to harmful against human body and used comparatively widely. "Ordinance on Prevention of Organic Solvent Poisoning" is providing the rule to prevent poisoning and the troubles by organic solvents for workers.

(1) Notes in general characterization of organic solvent and handling

Organic solvents have generally high volatility. It is easy to stay in a high density when handling it in an insufficient place to ventilate because the specific gravity of steam doesn't diffuse easily more greatly than air.

If you might not notice a high-density drift in experimental room, there is a case that it caused exposure when you are sitting down.

Thus, if you handle the organic solvent which is over acceptable concentration, you should make an experiment in the limited part exhaust device (the fume hood). Furthermore, it is desirable to ventilate an experimental room by taking enough fresh air from outside to reduce the concentration of organic solvent.

Moreover, most organic solvents are inflammable and become explosive gas by mixing volatilized solvent with air as described in the paragraph of "Fire Service Law." Therefore, when handling organic solvents, you should pay careful attention not only to the health but also to the fire risk.

(2) Acceptable consumption of organic solvents

Health problems by organic solvent depend on concentration in the air. It is rare for university to consume large amounts of organic solvents unlike private factory.

About type 1 organic solvent:

It is excluded that the consumption of organic solvent in laboratory is below the "permissible

consumption" calculated by the next expression.
"Permissible consumption" for type 1 organic solvent (g/h): (1÷15)× laboratories capacity (less than 150 m^3)

About type 2 organic solvents:

It is excluded that the consumption of organic solvent in laboratory is below the "permissible consumption" calculated by the next expression.
"Permissible consumption" for type 2 organic solvent (g/h): (2÷5)× laboratories capacity (less than 150 m^3)

As far as the chemicals under "Ordinance on Prevention of Organic Solvent Poisoning" go, you do not have to handle the chemicals in the limited part exhaust device when the chemicals are used within permissible consumption. However, the chemicals with a strong smell or with a dangerous should be handled in the fume hood naturally.

(3) At the risk of harming human body

The trouble with an organic solvent often chiefly appears in the central nervous system, the liver, and the kidney. The hazardous property of the main solvents is shown in the following. The management density is shown within the parentheses. The hazardous property of the class 1 solvent is higher than that of the class 2 solvent as understood from the management density.

Moreover, the Working Environment Measurement (refer to paragraph 3.5.) is applied to the use of class 1 and class 2 organic solvent. But it is not applied that of the class 3.

Class 1 organic solvents

-Chloroform (3 ppm) →Strong narcotic. Liver and renal lesion.
-Carbon tetrachloride (5 ppm) →Cell poison (cutaneous absorption) to liver and kidney.

Class 2 organic solvents

・Methylene chloride (100 ppm) →Skin and mucosal irritation. Opiate potency.
・Aceton (750 ppm) →Skin and mucosal irritation, headach, and vomiting.
・Methanol (200 ppm) →Loss of sight due to optic nerve disease.
・1-butanol (25 ppm) →Irritaing odor. Stimulation of respiratory organ. Liver and renal lesion. Percutaneous absorption.
・2-butanol (100 ppm) →Few odor. Dermatitides. Keratitiss
・isopropanol (400 ppm) →Skin and mucosal irritation, Liver and renal lesion
Ethyl ether (400 ppm) →＞Dizziness (over 2000 ppm)＞lie unconscious (over 35,000 ppm)＞Dead (over 100,000 ppm)
・Ethyl acetate (400 ppm) →Skin and mucosal irritation Anesthetic action
・xylene (100 ppm)・toluene (50 ppm) →Anemia, trouble of hematopoietic system
・DMF (10 ppm) →Skin and anterior eye mucosal irritation. Liver problem
・THF (10 ppm) →Mucosal irritation in skin, eye, nose, throat. sick feeling,dizzy.
・ *n*-hexane (50 ppm) →multiple neuritis (sensory paralysis, difficulty in walking)

Table 3-11. Industrial Safety and Health Act appendix 6.2 and Ordinance on the Prevention of Organic Solvent Poisoning 1st.

Class 1 organic solvents	Chloroform Carbon tetrachloride 1,2-dichloroethane 1,2-dichloroethylene 1,1,2,2-tetrachloroethane Trichloroethylene Carbon disulfide
Class 2 organic solvents	Aceton Isobutyl alcohol Isoproryl alcohol Isopentyl alcohol Ethyl eter Ethylene glycol monoethyl ether Ethylene glycol monoethyl ether acetate Ethylene glycol monobutyl ether Ethylene glycol monomethyl ether O-dichlorobenzene Xylene Cresol Chlorobenzene Isobutyl acetate Isopropyl acetate Isoamyl acetate Ethyl acetate *n*-butyl acetate *n*-propyl acetate *n*-amyl acetate methyl acetate Cyclohexanol Cyclohexane 1,4 dioxane Dichloromethane N,N-Dimethyl formamide Styrene Tetrachloroethylene Tetrahydrofuran 1,1,1-trichloroethane Toluene *n*-hexane 1-butanol 2-butanol Metanol Methyl isobutyl ketone Methyl ethyl ketone Methyl cyclohexanol Methyl cyclohexane

	Methyl-*n*-butyl ketone
Class 3 organic solvents	gasoline coal tar naphtha petroleum ether petroleum naphta petroleum benzin oil of terebinth mineral spirit
Mixtures consisting of the chemicals listed in above column	

3.3.4. Pollutant Release and Transfer Register (PRTR) Law

The PRTR law has aimed at "The improvement of independent management of chemicals by the entrepreneur is promoted, and the obstacle in the environment maintenance is prevented beforehand".

In PRTR law, about the chemicals of one ton or more a year (The first designated specific chemical substances are 0.5 tons or more) among the first designated chemical substances (354 items), each campus should calculate the amount of emission to the atmosphere, the public water area, the soil and the sewers. It reports to the Minister of Education, Culture, Sports, Science and Technology.

Our university record and calculate the transaction volume, the amount of emission, and displacement about 7 substances of the following 9 substances except for ethylene oxide gas used in specific place and dioxin.

Table 3-12. Chemical substances applied to the PRTR law in Kyushu University

PRTR number	CAS number	Substances	Specific	Toxic or deleterious chemical	Ratio	Steam pressure mmHg	Solubility g／L
12	75-05-8	Acetonitrile		Deleterious	0.79	88	High
63	1330-20-7	Xylene（*o-, m-, p-*）		Deleterious	0.87	8	0.1
95	67-66-3	Chloroform		Deleterious	1.48	197	8.0
145	75-09-2	Dichloromethane			1.33	435	13.0
227	108-88-3	Toluene		Deleterious	0.87	28	0.5
299	71-43-2	Benzene	○		0.88	95	1.8
310	50-00-0	Formaldehyde		Deleterious	Formalin fluid		
42	75-21-8	Ethylene oxide	○	Deleterious	−	Gas at normal temperature	High
179	—	Dioxides	○		–		

Notes

(1)“specific”：The first specific chemical substances

(2)“steam pressure”, “solubility”：25°C property. Pay attention to the emission into air about the substances that have high steam pressure, and the displacement into sewerage about the substances that have high-solubility.

（3）Ministry of the Environment website（http://www.env.go.jp/chemi/prtr/risk0.html）describes the PRTR law and property.

3.3.5. Globally Harmonized System of Classification and Labeling of Chemicals (GHS)

In Japan, hazard classification of several chemicals is carried out based on the domestic laws described above. In other countries, several laws or regulations exist. Through variations in definition of hazards, a chemical may be considered flammable in one country, but not another. A lot of chemicals are traded globally and imported into Japan. Different aspects of hazard for a chemical are not preferable.

In 2003, United Nations Conference on the Environment and Development proposed adoption of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) for all countries (http://www.unece.org/trans/danger/publi/ghs/ghs_rev00/00files_e.html). Through the GHS, we are able to recognize physicochemical hazards of chemicals and hazards of chemicals to health and environment. Even if one chemical is not designated as substances regulated by Japanese laws or regulations, we should pay attention to the following GHS pictograms labeled on the chemical bottles or MSDS.

GHS pictograms and hazard classes
(Physicochemical hazard)

GHS pictogram				
Hazard statement	Explosives Self-reactives Peroxides	Flammables Self reactives Pyrophorics Self-heating Emits flammable gas Organic peroxides	Oxidizers	Gases under pressure

(Hazard to health and environment)

GHS pictogram					
Hazard statement	Irritant Dermal sensitizer Acute toxicity (harmful) Narcotic effects Respiratory tract	Acute toxicity □Category 1-3□	Corrosives	Carcinogen Respiratory sensitizer Reproductive toxicity	Environmental toxicity

Acute oral toxicity

	Category 1	Category 2	Category 3	Category 4	Category 5
LD_{50} (mg/kg)	< 5	< 50	< 300	< 2,000	< 5,000
GHS pictogram					—
Signal word	Danger	Danger	Danger	Warning	Warning
Hazard statement	Fatal if swallowed	Fatal if swallowed	Toxic if swallowed	Harmful if swallowed	May be harmful if swallowed

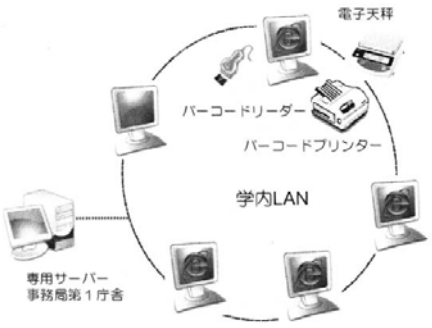
3.4. Measurement of working environment

××Experimental laboratory which handle the chemical substances under Ordinance on Prevention of Organic Solvent Poisoning (OSP) or Ordinance on Prevention of Hazards due to Specified Chemical Substances (SCS) becomes the subject of the measurement of working environment as a rule. Measurement of working environment is that measure concentration of the substances twice a year in experimental laboratory which certified as indoor working area which handle harmful substances.
Measurement of working environment follows the working environment measurement standard that the health minister defied the method of gathering the samples. It is necessary to preserve the record of the working environment measurement for three years.

Table 3-13. Substances applied to measurement of working environment

Law	Chemical substances	Management density		Boiling point□□□	Stream pressure □hPa×25□□
		ppm	mg/m^3□25□□		
SCS Type2	Formaldehyde	0.1	0.12		
OSP Class2	Methanol	200	262	65	154
OSP Class2	Acetone	500	1,186	56	304
OSP Class1	Chloroform	3	14.7	62	267
SCS Type2	Acrylamide	—	0.1	193	1.2
OSP Class2	Isoproryl alcohl	200	491	83	58.7
OSP Class2	n-Hexane	40	141	69	200
OSP Class2	Ethyl eter	400	1,212	35	713
OSP Class2	Ethyl acetate	200	720	77	129
OSP Class2	xylene	50	217	140	10.7
OSP Class2	Dichloromethane	50	174	40	581
OSP Class2	Toluene	20	75.2	111	37.0
OSP Class2	Tetrahydrofuran	50	147.5	65	243
SCS Type2	Benzene	1	3.2	80	125
OSP Class2	N, N – Dimethyl formamide	10	30	153	4.5

3.5. Online chemical control system



Chemical control system is to manage chemicals by registering personal computer, and it has been used in whole university since 2006.

Control system is used only in the computer of university as right-chart indicates. If you register the chemicals on the system, you get unique chemical number. You can manage chemicals by writing the number on the bottle or attaching bar-cord to the bottle.

The advantages of control systems are not to buy the extra chemicals by searching in stock online. This leads to decrease the amount of stock. Moreover, regulation of chemicals and safety information (MSDS) can be obtained

(1) Installation Guide

1. Personal computers which connected with LAN on campus are needed. Bar-code reader and bar-code printer are not necessary.
2. You need “attending Login ID” provided each laboratory. If you don’t know it, please ask the Facilities Affairs Section, Faculty of Agriculture (Nogakukei Hozen-kakari).
3. You can download first step guide and operating manual from website http://chem.jimu.kyushu-u.ac.jp/.
4. Refer to the first step guide and the operation manual, and resister user information and initial setting of your attending (laboratory, minimum unit for chemical management).
5. Log on by user ID, and register the chemicals.

(2) The method of control

1. If you buy new chemicals, you should register it on the control system before use.
2. Every time you use poisonous and deleterious substances, you should register the amount of its use on the control system. This called “usage control.”
3. Other chemicals except for poisonous and deleterious substances are managed by “usage control” or registering the date of purchase and the end of usage date. This called “number control.” “Usage control” is desirable.
4. You may manage the box that contains a lot of ampoules and small bottles or a variety of kits in units or in kits.
5. The system doesn’t cover medical products, radioactive substances, and fuel such as heavy oil, and other materials in retention tank.
6. Synthetic materials and decomposing materials are also managed in this system.

(3) Other notes

1. If your research department is divided into two campuses, you handle it as another department.
2. When you move to other campus, you should ask the Facilities Affairs Section, Faculty of Agriculture (Nogakukei Hozen-kakari) to change contents you registered.

4. Handling liquefied gas and high-pressure gas

High-pressure gas and liquefied gas are sometimes used in research works in University. We use dry ice, liquid nitrogen or liquid helium to decrease the temperature of devices and samples. These substances generate high-pressure gas if someone handle them wrongly and cause rapid evaporation. It is possible for high-pressure gas to be poisonous or combustible such as chlorine gas or hydrogen gas. Therefore the safety measurement against high-pressure gas needs multiple especially in University.

4.1 **"Gas Property"** indicates main gas types used in laboratory and physical and chemical properties of gas. It presents to pay attention to gas property such as heavy or light, combustible or the susceptibility of substances to burn, poisonous, and deficiency of oxygen. Table 4-1 shows properties of gases.

4.2 **"Handling high pressure gas"** indicate a manual how to handle a cylinder in laboratory. When you handle high-pressure gas, you should have a certain level of gas savvy. For example, it is necessary to check the container and the seal of cylinder for before using high pressure gas. Close a valve tightly after use.

4.3 **"Handling low temperature liquefied gas"** indicate the notes on liquefied gas used as cryogen.

4.1. Gas property

Various gases are used in laboratory. Information on chemical properties, pressure, low temperature and liquefied of gas are needed depending on the intended use. Gas exists in gaseous state, liquid state, and solid state (only dry ice is used practically). Table 4-2 shows the characters of compression gas in common use.

Table 4-1. The characters of gases in common use

Gas	Formula	Molar weight (g/mol)	Gravity (air＝1)	Flammability (vol %)	Toxicity	Odor	Others
Hydrogen	H_2	2.016	0.070	○4～75	×	None	
Helium	He	4.003	0.148	×	×	None	
Methane	CH_4	16.04	0.555	○5～15	×	None	
Ammonia	NH_3	17.03	0.60	○5～27	○25 ppm	Yes (irritant)	
Neon	Ne	20.18	0.696	×	×	None	
Acetylene	C_2H_2	26.04	0.91	○2.2～85	△	None	
Hydrogen cyanide	HCN	27.03	0.969	○5.6～40	○10 ppm	Yes (almonds)	
Nitrogen	N_2	28.01	0.967	×	×	None	
Carbon monoxide	CO	28.01	0.966	○12.5～74	○50 ppm	None	
Ethylene	C_2H_4	28.05	0.975	○3.1～32	△	Yes (banana flavor)	
Oxygen	O_2	32.00	1.105	△	×	None	Liquid is powder blue
Fluorine	F_2	38.00	1.31	△	○0.1 ppm	Yes	Wear protected gear
Chlorine	Cl_2	70.90	2.46	△	○0.5 ppm	Yes (irritant)	Yellow green

Argon	Ar	39.95	1.38	×	×	None	
Carbon dioxide	CO_2	44.01	1.52	×	△	None	By 0.5%
Propane	C_3H_8	44.10	1.55	○2.2～9.5	△	None	
Ozone	O_3	48.00	1.65	△	○0.1 ppm	Yes (irritant)	Liquid is dark blue
Butane	C_4H_{10}	58.12	2.16	○1.8～8.4	△	None	
Krypton	Kr	83.80	2.82	×	×	None	
Chlorofluorocarbon	CCl_2F_2	120.93	13.8	×	×	None	
Xenon	Xe	131.30	4.53	×	×	None	
Sulfur hexafluoride	SF_6	146.05	5.10	×	△	None	By 0.1%

(1) Gravity･･･Show at 0℃ and 1 pressure because ratio of air by gas leakage is connected with safety. Ventilation technique will be decided by this.

(2) Flammability･･･Flammability gas is indicated by ○circle sign, and flammability range is indicated by mix proportion （vol %）in the air. It will burn if there is a gas in the range of this mix proportion. Triangular sign △ indicates the susceptibility of substances to burn.

(3) Toxicity･･･Gas with toxicity is indicated by ○circle sign. These numbers show the limit you can work in this atmosphere for 8 hours a day and 40 hours a week. Small numbers have stronger toxicity. Narcotic is indicated by triangular sign △.

4.1.1. Combustibility

Table 4-1 shows combustible gases. Combustible substances are marked by ○ on the table. It shows flammability range (%) when leaking to air. This range is called “detonation” when burning speed is over acoustic velocity. For example, in case of hydrogen, the range of burning is 4~7%, detonation is 18~59%. There is danger of ignition in the empty-container. Once igniting, gas decreases and blows up.

In order to avoid an accident of combustible gas, it is necessary to be aware of the range of burning, avoid leaking gas, and ventilation. In case of gas leak, open the window immediately and dilute it with carbon dioxide or nitrogen. For ventilation, please be aware of gas gravity. Ventilate a room in upper side in case of light gas and lower side when gas is heavy. Do not leave a fire or spark near the place where you handle gas. Once igniting, extinguish by fire extinguisher and water, (notes on chemical reaction), then close the main cork of the gas.

4.1.2. Poisonous property

Carbon monoxide and ammonia are known as poisonous gases in laboratory. Be aware of ventilation, and use a gas mask to avoid taking a breath directly. In case of people losing consciousness, take them out the room and lay them on their side, then call an ambulance. Please consult a doctor immediately when poisonous gas was inhaled.

4.1.3. Oxygen’s susceptibility of substances to burn

Oxygen itself is not combustible and poisonous. But pure oxygen is very dangerous. Almost everything ignites in pure oxygen. A little grease attached to the cap of oxygen cylinder causes a fire when gas is leaked. As nitrogen having low boiling point evaporates first in liquid air and then enriched oxygen evaporates, we should notice the concentrated oxygen. Pouring liquid nitrogen into open bottle,

oxygen seeps in and reaches a high concentration.

4.1.4. Oxygen starvation

Leaking gas other than oxygen into room causes oxygen starvation. It leads to breathing difficulty. Permissible limit of oxygen for people is 18%. People become unconsciousness when available oxygen is fewer than 7%. It is necessary to ventilate a room when leaking a large amount of gas into room and in case of liquid gas leakage evaporation is required. Pay attention to lack of oxygen with an oximeter. In case of losing consciousness, take victims out of the room immediately, and call an ambulance or consult a doctor. Be aware of further accident. Actually, there was an accident in which two students died in University because a large amount of liquid nitrogen was handled in a bad ventilation condition.

4.2. Handling high-pressure gas

It describes the case of using a cylinder filled with high-pressure gas to reduce the compression. Gas is filled in steel bottle called cylinder with reduced pressure. Usually gas is bought from the agency. Gas is very dangerous and causes major accidents when mistakenly handled.

4.2.1. Container indication

According to High Pressure Gas Safety Act, it is mandatory to put a punch mark on container. If it is difficult to put it, use a seal. Possessors should indicate it very clearly and put their name, address and phone number. Change marking color to distinguish different kinds of gas. (Refer to Table 4-2)

4.2.2. Transportation

Use a special transportation car to transport cylinders with cap. Handle it very carefully during transportation. Take off the adjuster of cylinder and put a cap to protect valve. Container of acetylene gas and liquid gas should not be lay down.

(1) Use a special hand barrow for carrying cylinder.
(2) Take off adjuster and close valve tightly with protect cap on valve before moving.
(3) When you do not use a hand barrow, tilt cylinder slightly and transport it carefully.
(4) When taking stairs, the cylinder should be carried by two persons or more. Do not cover the cap with rope.

4.2.3. Storage

When fixing cylinders in laboratory, shield direct light and fix it with special trestle in well-ventilated space. If you do not use a special trestle, you should fix it in the laboratory table or the pole with chain or band. Keep it away from heater to avoid danger of blowing up safety valve. If the safety valve blows up, keep the cylinder in a safe place and leave it as it is until the pressure decreases. In case of combustible gas, be aware of surrounding fire and ventilate a room immediately.

(1) Cylinders should be fixed on the container trestle.

(2) If you don't use a trestle, fix it in the laboratory table or the pole with chain or belt.
(3) Keep liquid gas or acetylene cylinders stand vertically to use.
(4) Shield the direct light and avoid a moisture place.
The temperature should be under 40□.
(5) Keep cylinders away from electric cable or earth cable.
(6) Keep cylinders away from emergency stairs.
(7) Close a valve tightly and cover it with a cap when you don't use.
(8) Store cylinders separately for combustible and poisonous, as well as for filled and empty.

4.2.4. Confirmation of container

Confirm the display of the gas container before opening the valve. As shown in Table 4-2, container is separated into different colors depending on the type of gas. The name of filled gas and its properties and address are also displayed. □□means combustible gas, " "means poisonous gas. The holder's name and address is also shown. As shown in Fig. 4-1, there is a punch mark on the upper side of cylinder, and it shows the details of cylinder and filled gas.

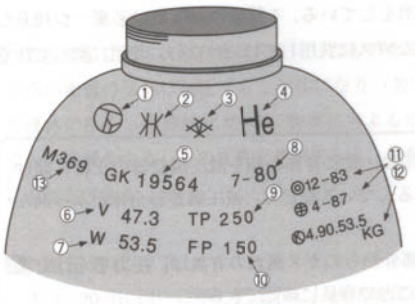


Fig. 4-1. Punch mark on cylinder

Punch mark and code

1. The symbol of a specific container.
2. The symbol of passing an inspection or the name of inspector.
3. Container manufacture or its code
4. Name of filled gas i. e., "He"
5. Container code or serial number i. e., "GK19564"
6. Volume i. e., "V 47.3"
7. Weight i. e., kg "53.5"
8. The date of pressure proof test i. e., month-year (two digits of the year) "7-80"
9. Pressure proof test pressure i. e., kg/cm^2 TP250
10. Maximum filled pressure kg/cm^2 i. e., FP150

-In the case of passing pressure proof test.

11. The code or the name of inspector and the date of inspection. i. e., 12-83 and 4-87

12. Confirmation of mass and variance of mass i. e., 4.90.53.5KG □90 □ 4 □ 53.5 kg□
13. Holder registry number i. e., M369

Table 4-2. Color-coding and display of properties depending on the type of gas.

High-pressure gas	Container color	Character color indicates gas type.	Character color indicates properties of gas.	Gas exit screw
Oxygen gas	black	white		right
Hydrogenous gas	red	white	□□ □white	left
Liquefied carbon dioxide	green	white		right
Liquefied ammonia gas	white	red	□□ □red□□□ □black	left
Liquefied chlorine gas	yellow	white	××××××□□ □black	right
Acetylene gas	bark	white	□□ □white	joint without screw
Combustible gas	gray	red	□□ □white	
Combustible, Poisonous	gray	red	□□ □red	
Poisonous gas	gray	white	××××××□□ □black	
Other gas	gray	white		
Nitrogen gas	gray	white		right
Helium gas	gray	white		left

××□ □Combustible gas ,□ □poisonous gas

4.2.5. Notes on handling pressure regulator and valve

(1) Pressure regulator (Fig. 4-2.)

(a) Use a specific regulator for gas. Do not use it for another purpose. Especially use “no-oil” only for oxygen gas.

(b) Understand the handling method because each regulator has different methods depending on their types.

(c) Keep the connection between valve and regulator clean and use appropriate screw. Use new packing appropriate for the connection.

(d) When installing hexagon cap nut of regulator, use a specific screw wrench. Install it carefully not to break the packing or screw.

(e) Pressure meter is used 1.5-3 times as much as normal.

(f) Do not lubricate the regulator with oil too much. It causes a fire.

(g) Close valve tightly and take out all gas from the regulator before taking off the regulator.

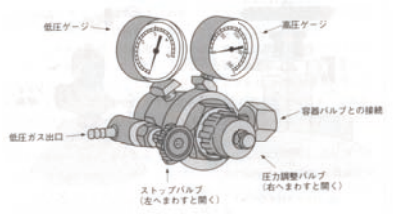


Fig. 4-2. Pressure regulator

(2) Handling methods of valve (Fig. 4-3)

(a) Valve has two screws, right and left. (Fig. 4-2)

(b) Do not touch a safety valve.
(c) When opening and closing valve, stand on the side of pressure meter. Quickly opening cause the breakage of regulator. At this time, use a special handle or wrench.
(d) Open valve fully two and half turns when using it. (Acetylene is one and half turns)
(e) When leaking gas from valve, move its container into safe place.
(f) Handle the valve carefully. Do not displace gas in the container to another container.
(g) Close the valve with leaving a little gas in the cylinder and take off regulator, and put a protect cap to the valve after you've done with it.

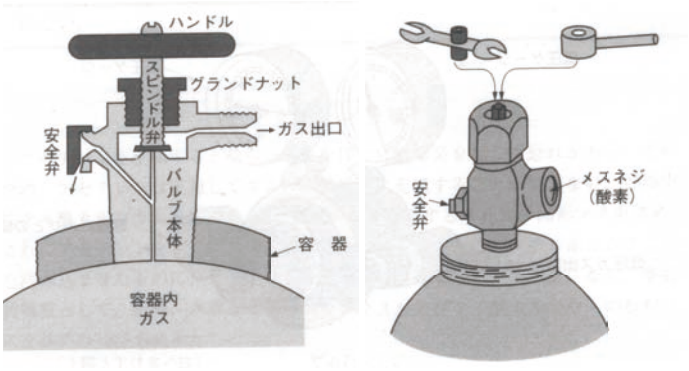


Fig. 4-3. The structure of valve and valve with wrench

(3) Other notes
(a) Close not only regulator but also valve of cylinder, and take off the connection between laboratory units and regulators when suspending use of gas.
(b) In the case of heating up regulators and valve, use heating pad or hot water the temperature under 40□.
(c) Return cylinders immediately after you've done with it.

4.2.6. Other notes

(1) Combustibility/susceptibility of substances to burn
(a) Do not use a fire within 5m from facilitates in which you use combustible gas or oxygen. Do not put inflammable and combustible substances at a nearby site.
(b) Remove combustible substances such as oil from devices before using oxygen.
(c)The susceptibility of substances to burn (oxygen) should not be used in the room where× combustible gas is used.
(d) Oxidized ethylene should be used after replacing its contents with N_2 or CO_2.
(e) To prevent from decomposition explosion, acetylene should be used under 1 kg/cm^2 pressures.
(2) Poisonous gas
(a) To prevent from inhaling poisonous gases□H$_2$S, CO, Cl$_2$□one should conduct an experiment in the draft. In the case of inhaling cyanide gas (hydrogen cyanide: HCN, sodium cyanide: NaCN), it will cause some danger of cyanide intoxication (headache, dizzy, respiratory paralysis). Silane gas□SiH$_4$□is combustible and has the capability of causing a danger of fire easily.
□b□Make poisonous gas harmless with alkali absorbent before being disposed off.
(c) Poisonous gas should be handled carefully because it effects on human health.

(d) To use poisonous gas, wear appropriate clothes, glasses and gas masks.
(e) To use poisonous gas, it is better to use a small amount of it as much as possible in terms of safety.

4.3. Cryogen (cryogenic liquefied gas)

(a) When using elevator for transportation, you should not take on it with liquid gas containers and cylinders. If the valve of cylinders would be broken, gas will be leaking in the elevator and people might choke to death. In the case that elevator stops by blackout when gas may not be leaked but one may choke to death from the vaporizing gas.
If you find cylinders or containers in the elevator, you should not get on the elevator.
(b) Do not touch cryogenic liquefied gas directly. Please use it in a well-ventilated place and handle it near floor surface.
(c) Do not attach oil to the devices of cryogenic liquefied gas.
(d) Wear a leather glove when using cryogenic liquefied gas. Do not use a cotton glove.
(e) It is harmless that you inhale a little amount of inert gases (carbon dioxide, nitrogen, argon gas) and cryogenic liquefied gas. However, if you inhale them in large amount, it may cause the absence of oxygen. One may be faint by the decreased level of oxygen fewer than 10%. When handling large amount of them, use a well-ventilated place.
(f) When using liquefied gas such as liquid nitrogen, open the window even in the winter. Do not use it in the place without window.
(g) After taking a lecture "**cryogen user training session**" presented by low temperature center and understanding the guideline that gave out in the lecture, you can handle liquefied gas by yourself.

5. Electric safety planning

5.1. Treatment for electric shock

Electric shock means that electric current run through the human body. It has a high fatality rate and causes several problems. It is highly dangerous for electric current to run through nerve center of human body such as brain and heart. According to past statistics, over 40-60 V of electricity causes death.

5.1.1. Electric shock result from indoor distribution line

(1) Be aware of working on floor surface which allows electric current through easily.
(2) Do not touch a distribution line directly with wet hand.

5.1.2. Electric shock result from high voltage equipment

(1) Connect electric discharge without touching.
(2) Do not use inappropriate insulation (for example, phenol or resin).
(3) Work with two or more persons near high voltage equipment. One person has to monitor.
(4) To prevent from exposing high voltage, shut the devices tightly with metal cover.
(5) Emit a warning sound and lighting when generated high voltage.
(6) Earth the edge of high voltage equipment when touching it or not.

5.1.3. Electric shock result from electric/information devices

(1) Some devices contain high voltage circuits
(2) Capacitor is charged even though the power supply is cut.
(3) Many series of connected batteries are dangerous.

5.2. Electric disaster and its countermeasure

5.2.1. Overheat

Short circuit, excess current, earth leakage may cause a fire. Pay attention to the following points.
(1) Overloading an electric circuit
(2) Bad connection and partial disconnected by heavy load.
(3) Use an electric line endured rated current.
(4) Use an appropriate fuse or a breaker.
(5) Set up ground fault interrupter.
(6) Use a heat-resistant code for conversion heater.

5.2.2. Electric spark

Combustible gas or vapor of inflammable liquid causes blowup or a fire by electric spark. Liquidization of insulator or fast fluidization of powder cause spark and lead to blowup accident.

5.3. Notes on electric devices

5.3.1. OA equipment

(1) Follow the rated current of power code and power strip.
(2) In the case of putting power code on floor surface people walk, protect it with a code protector.
(3) Cut the power supply when you are out.

5.3.2. Equipment in pause

If you reuse the equipments that had not been used for a long time, you should check the conditions before using because it is highly likely that such an equipment has lack of lubricating oil or insufficient insulation.

5.3.3. Blackout, all-night service

When the power comes back on, turn off all the equipment because the electric equipments are automatically working when returning from blackout.
Confirm the schedule of blackout by regular checking.

5.4. Check for accident avoidance

To avoid the accidents, check the following points regularly.
(1) Feel a shock when touching the devices or pipe: It is very dangerous. Stop using it immediately and contact agricultural maintenance personnel.
(2) Breakage of covering codes: Be aware of old codes.
(3) Loosing connected screw: It causes short out or heat.
(4) Abnormal sound: Electric leak and discharge or defect of motor.
(5) Abnormal odor: Electric leak and discharge, degradation of insulator by heat.
(7) Run off ground lead: Confirm whether you forget to put ground lead when transported or not.
(8) Confirm whether the equipments and hard wiring are covered with water, gas or chemical liquid.
(9) Confirm whether power codes are covered with heavy load or not.

6. Handling glassware

6.1. Safety precaution

There are many cases of injury caused by carelessness of handling glassware. Check the following points to avoid accidents resulting from handling glassware.

(1) Dispose of glassware cracked.

Cracked glassware are easily breakable. Broken glassware causes injury and it is likely to leak organic solvent and poisonous substances. If you find cracked glassware in the laboratory, you should dispose it immediately.

(2) Handle cracked glassware appropriately.

If the edge of glassware is cracked, it should be burned to smooth down the edge. If you do not take care of it, someone might be injured.

(3) Purchase plastic containers for large amount of capacity.

Glass beaker and measuring cylinder more than 1 L are easily broken. So, it is recommended to use plastic containers unless there are special circumstances.

(4) Well-organized

Doing experiments in a narrow place is not only decreasing working efficiency but also exposing you to danger. Troubles may be caused by knocking the container and spilling its contents or breaking the glassware. Keep the laboratory table clean and take enough working space when you make an experiment.

(5) Tip-resistant

Do not leave materials at the edge of laboratory table. Those materials may cause accidents when somebody passing through. If you find something may cause an accident, you should move it to a safe place.

(6) Magnetic stirrer

When putting magnetic stirrer into glassware, skew the container and slide it. As stirrer has a strong magnet, glassware would be broken when it move with a high speed.

6.2. Insert glass tube into a rubber plug (pipetter)

(1) Do not try to insert thick glass tube into a narrow hole.
(2) Dab water on glass tube for sliding well before inserting into rubber plug. However, do not use water or vaseline when inserting measuring pipette into pipetter.
(3) Grab the point in 2 cm from the edge of glass tube with thumb, forefinger, mid finger and carefully insert it while twirling.

Breakage of glass tube may occur in just a couple of centimeters from the edge of rubber plug. If you grab the points far from the edge of rubber plug and try to insert glass tube, glass tube will be subjected to bending stress. If you grab the glass tube with five fingers, bending stress is more increased. Grab the point in 2 cm from the edge of glass tube with thumb, forefinger, mid finger. If you don't use fourth finger and little finger, you can avoid accidents of breaking glass tube. Please try to create an appropriate hole again if you could not insert with three fingers because the hole is too small.

For a bad example, Fig. 6-1 indicates. Glass tube will be broken if a force in the point of arrow applied to it. According to the left-fig 6-1, when squeezing the glass tube into rubber plug, a force acts in a vertical direction by the little finger. Breakage of glass tube occurs in the base of rubber plug. According to the right figure, glass tube will break between mid finger and fourth finger.

×

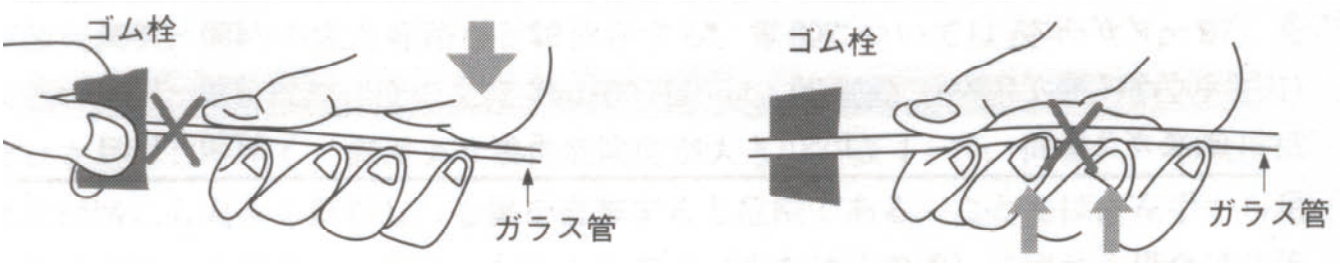


Fig. 6-1. Bad examples (Insert glass tube into a rubber plug)□□

6.3. Remove glass tube from a rubble plug

Accidents occur when removing glass tube from a rubber plug.
To prevent from an accident, some cautions are needed as follows.

-Grab the glass tube with three fingers and rotate it softly. It is helpful to drop little amount of water between glass tube and rubber plug, and knead the rubber plug.

-If you could not remove the glass tube from a rubber plug, cut a part of rubber plug.

6.4.□Hold a large glass container or dangerous reagent bottle with one's hands

(1) Do not hold strongly a beaker over 500 mL in one hand. There is a case of cutting the nerve endings and it will take one year to cure it.

(2) Hold a gallon bottle with one's both hands. If you hold only a part of handle, the handle may often torn by its weight. Handle is not for carrying.

6.5. Opening the lid of glass container

There is a need to be more cautious for handling vial container, test tube, and thin glass container.

Remember that glass will be broken by strong force. To chill a glass or warm the lid are effective for opening it. Even though jelly bottle has enough thickness to avoid an accident, vial container and test tube doesn't have enough thickness. Those are easily breakable.

6.6. Opening ampoule

Think the reason why test reagents are inside the ampoule. There are test reagents unlikely oxygen or water, having strong odor and poisonous. Consider the reason why those reagents are inside ampoule, and prepare for handling it.

(1) If you do not use all reagents you opened, prepare for the appropriate container to preserve it tightly.
(2) In the case of volatile materials, chill it with icy water and take off the liquid.
(3) File down
(4) Open it carefully. Grasp an ampoule with one's left hand and break off it with right hand. Take care of your hands. You can wrap up it with a dry cloth before breaking off.
Consult an experienced teacher regarding opening of poisonous reagents such as poisonous materials, special flammable materials and explosive properties or ampoule over 5 ml.

6.7. Clean up after damaged

When the glassware is damaged, use a broom or vacuum cleaner. Do not use your bare hands because broken piece of glasses are very dangerous. When you break glassware filled with solution, you wipe off the solution with a paper towel. If you wipe it with a cloth, you will be injured when wringing out the rag.

6.8. Other notes on handling glassware

(1) Glassware.
1. Containers should fit in with the size of the cover. The cover and container should be numbered.
2. Do not keep the cover dry when screwing it. Glassware would be damaged.
3. Put a strip of paper between the container and cover after drying.
4. Do not use glassware for preserve of alkaline solution because glassware will be melted by alkaline.

(2) Magic marker
Do not write anything in a part of white letters and fogged glass.

(3) Take off a label and magic marker.
Take off a label and magic marker before putting it into drying machine. Pulling it by force leads may cause accidental breakage.

6.9. Notes on cleaning

(1) Secure working space.
Secure enough working space when cleaning. Start working in the half of sink with nothing on it.

(2) Do not leave any devices in the sink.
Professional household workers wash dishes immediately after use because washing dishes after long time requires a lot of time and effort. If you could not wash dishes immediately after use, you soak the devices in water and put it on your laboratory table. If you leave it in the sink, you will bother others in the following reasons.

1. Other people do not know whether the device handled dangerous reagents or not.
2. Other people can not use enough space to wash.
3. There are no devices when other people would want to use.

(3) When leaving a measuring cylinder in the sink, please lay it down.
Measuring cylinder is easy to be damaged by a high gravity point.

(4) Take off a tape and magic marker.
Take off a tape and magic marker before putting it into drying machine. Pulling it by force leads may cause accidental breakage.

(5) Do not use a cleanser.
Do not use a cleanser for disposal of grime and magic marker. Even though you use a cream cleanser, it scratches the surface of glass.

(6) Proper brushing
Two or three times brushing is not enough to wash. Look at the shape of brush and confirm whether the devices are brushed enough or not. Use a sponge for open-mouthed containers like a beaker. It is better to use special glassware for quantitative determination of reducing sugars by using such as phenol-sulfuric acid method and for micro scale analysis such as fluorescent analysis. For example, put the devices in a large stainless-steel container, and wash them cleanly with mild detergent (0.5%).

(7) Notes on alkaline detergent.
When using alkaline detergent, wear the eye protector and glove. Glassware can be melted by strong alkaline. Using alkaline detergent for a long time and heating lead to melting the surface of glass.

(8) Wipe water off on the floor.
Floor become slippery when wet. Wipe water off immediately with mop.

7. Notes on physical system laboratory

Accidents occur when machines and tools are handled carelessly or by disorganized workers. When using tools and machines, follow the rules and pay attention to the foreseen accidents.

7.1. General notes

(1) Workers should be trained and understand how to use machines beforehand. .
(2) Confirm the range of work and eliminate a blockage before boot.
(3) Suspend the machines immediately when you feel abnormal.
(4) Switch off the machines before maintenance.
(5) Cover a rotating part of gear, belt, shaft, and rubber.
(6) Do not stop machines run by inertia with bare hands, tools, and bar.
(7) When the power went down, switch off the machines.
(8) After working hours, check the machines completely before switching off all machines.

7.2. Tools

Place emphasis on the general tools such as driver, spanner, puncher, and file.

(1) Treat tools with care.
(2) Use appropriate tools for work.
(3) Dispose of defective tools.
(4) Set tools to rights.
(5) Use an eye protector.

7.3. Safety handling on wood processing machine

Wood processing machines such as band saw and circular saw are used for sawing or grinding woods. Understand that wood processing machines are very dangerous tools because the knife rotates at high speed. Pay attention to the following points.

(1) Wear a long-sleeve tighten clothes. Do not wear clothes which have risk of getting sucked into such as a white coat.
(2) Wear a pair of athletic shoes. Do not wear a pair of slippers or sandals.
(3) Do not use a pair of gloves.
(4) If necessary, use an eye protector and other protectors.
(5) Confirm the fixing of cutting blade before work.
(6) Turn on the switch and confirm the speed of rotating becomes constant, then start working.
(7) If it is difficult to insert woods into machine, please do not force them into.
(8) Dispose of wood chips after machine stopping completely.

7.4. Safety handling on agricultural machines

People who work on the farm should take careful note of safety and daily check of agricultural machines to avoid an accident. When using machines or tools, check the safety device and protective cover in advance and understand how to use them. If you find some problems on the machines and tools, please take necessary steps to settle this issue.

7.4.1. Work environment

(1) Wear an eye protector and a dust mask when working under an environment where dust occurs. When working indoors, enclose source of release with curtains or suck in with suction machines. When working outside, stand in the wind.
(2) The noise may disturb the acknowledgement of alarm or contact between workers, and cause an accident of agricultural work. Moreover a hearing loss and compromised physical function may be caused by the noise.
(3) Vibration for a long time may cause an accident and compromised physical function. Take a rest or take turns properly. Prevent workers from working continuously.
(4) Evacuate to a safer place immediately at the bad weather.

7.4.2. Vehicle

(1) In an emergency, confirm how to stop an engine or operating machine.
(2) Adjust your seat and wheel position and seat suspension for your physical constitution. In the case of tilt steering wheel, column is needed to be fixed.
(3) Handle a machine with power steering carefully because its handle is very lightweight and you may sway dangerously.
(4) Understand that crawler machine change the axis of rotation and radius of turn due to the evolution system.
(5) There are many accidents caused by falls. Use seatbelts and safety frame and protective cab.
(6) When you start a machine, confirm back and forth and around. Keep people away from the machine. Get in the driver's side and confirm gear lever, PTO lever, each control lever be in neutral position, and brake before driving.
(7) When you drive a heavy working machine direct equipped at the back, go slow down and use a balance weight if necessary. A machine with a right and left brake should be connected with right and left brake pedal.
(8) As there is a possibility of going out of control, let out the clutch and maintain gear neutrality on a steep down-grade.
(9) A machine with right and left brake should be connected with right and left brake pedal.

7.4.3. Walking machine

(1) Each machine has a different operation procedure. Please understand how to operate it beforehand.
(2) Neutralize control lever or turn off it before starting engine.
(3) Do not get your feet under the rotary casually. Do not move with machine rotating.

(4) When going back, there is a risk of getting people caught in the operation machine. Pay attention to the rear things or the floor condition.
(5) On the road or steep ground, it is better to use steering than to use clutch.

7.4.4. Fixed mount type machine

(1) Engine type machine.
Engine type machine should be used in well- ventilated space because there is a risk of being poisoned by gas when you use it indoors. Be aware of a fire when taking on fuel. Spilled fuel should be cleaned away.
(2) Motorized model machine
The breakage or corrosion of consent, plug for power supply, code of power supply, earth cable, and switch box should be repaired immediately. Keep liquids away from power supply.
(3) Fixing should be in a flat and strong space. Consult a person who has a specific knowledge about moving and fixing. Cover a moving part or set up a guard fence.
(4) Use an adequate fuel tank. Fuel tank should be connected with machine to avoid leaking fuel.
(5) To avoid being poisoned by gas and imperfect combustion, fix an air hatch when using it in a blocked space. Use it in well-ventilated space.
(6) Keep an enough space around the machine because heat generated may influence on around.
(7) Keep control board away from water and dust. Secure an enough power supply for machine. Fix an earth cable and short circuit breaker to avoid a short circuit.
(8) Power supply code should not be bundled because it may generate heat. Install wiring to keep away from water, oil, and high temperature place, and sharp corner. Keep the aisle clear to avoid disconnected wiring by stomp. Cover wiring with metal pipe in which there is a risk of the breakage by mouse.

8. Notes on biological system laboratory

8.1. Introduction

Ethidium bromide is a carcinogen and usually used at high concentration for equilibrium density-gradient centrifugation (consider that it is more dangerous than RI). **If it is spilled, be sure to report it to the instructor and conduct proper decontamination**. In particular, the Beckman ultracentrifuge in the common equipment room in the basement can easily become contaminated at the time of heat sealing the centrifuge tube. Check whether there is contamination using a built-in ultraviolet (UV) lamp before and after sealing. Under short-wavelength ultraviolet irradiation in a dark room, an orange glow indicates contamination. Of course, short-wavelength ultraviolet must not be irradiated directly onto the skin or looked at with the naked eye.

8. 2. Experimental equipment

8. 2. 1. Autoclave

8.2.1.1. Autoclave structure

Among the many possible injuries that can occur in the laboratory, a burn caused by the use of an autoclave is common as well as laceration from glass. Use the autoclave bearing in mind that its operation has risks.

Figure 8-1 shows the structure of the most common autoclave in the field of biochemistry. The time to maintain the obtained temperature after the temperature reaches the set value (the range indicated by the arrow in Figs. 8-2(A) and (B)) is set on the timer and the standard time is 10 – 20 minutes at 121°C.

8.2.1.2. Operation

(1) Check that the autoclave is filled with water up to the bottom plate level.
(2) Check that the drain valve is closed.
(3) Fasten the lid moderately.
If the lid is fasten insufficiently, the vapor will leak, and if fastened too tightly, the packing will deteriorate fast, also causing vapor leakage. It is better, as a rule, to turn the handle using the forefinger to the end, and then fasten it by turning the handle by 90 degrees with both hands.
(4) Set the time and start the sterilization.
(5) Wait until the temperature decreases to at least 70°C.
(6) Check that the temperature is 70°C or less and the pressure is zero. Actually, there was a case in which the thermometer was broken and the spewing vapor caused a heavy burn on the upper body of the victim. Be sure to check both values.

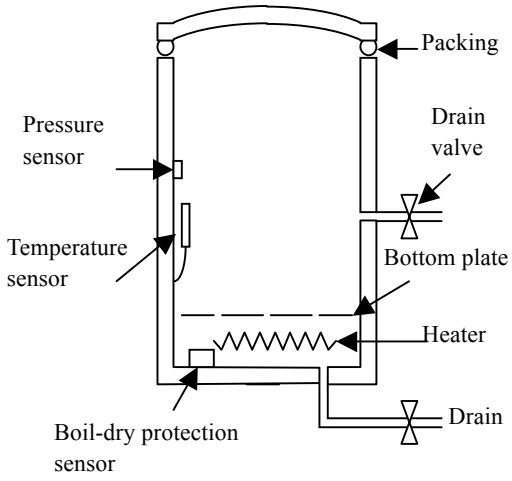


Fig. 8-1. Structure of autoclave

(7) In addition, open the drain valve and check that no vapor spews, and then open the lid (some autoclaves have no drain valve or automatic open and close system).

8.2.1.3. Safety precautions

Do not leave the laboratory unattended until the timer cuts out. This is because no-water burning will be caused if the vapor leaks caused by the aged deterioration of the packing. If vapor leaks, the sample will continue to be heated up because the temperature cannot reach the set value and the timer cannot start. Finally, all of the water will be exhausted, causing no-water burning (refer to Fig. 8-2(C)).

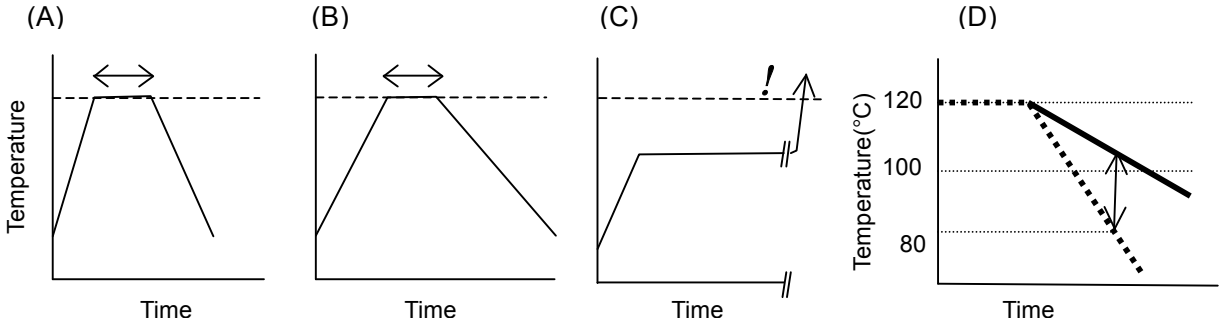


Fig. 8-2. Change in temperature in autoclave with time

(A) A small amount of medium is sterilized, (B) a large amount of medium is sterilized, (C) when the vapor leaks, and (D) the deviation of the real temperature of difficultly cooled liquid (solid line) from the value indicated on the thermometer (dashed line)

(1) **Do not leave the laboratory before the timer is shut off**. **This rule should be strictly applied when everyone leaves the laboratory for a magazine meeting or seminar meeting**. **Do not place too much confidence on no-water burning protection sensor because it is not assured to always correctly function and whether the sensor correctly functions or not cannot be checked**.

(2) There is a deviation (difference) of the real temperature of the autoclaved liquid from the value indicated on the thermometer (the thermometer part is cooled earlier than the liquid because the outside of the autoclave is first cooled). **The real temperature of agar medium, antiforming agent, highly viscous liquids, such as sugar at high concentration and glycerin, and a large amount of solution may be more than 100°C even though the thermometer indicates a temperature less than 80°C** (Fig. 8-2 (D)). When taking out such a sample, sudden boiling may occur, causing a burn. When it is autoclaved, wait until the value indicated on the thermometer decreases to 60 – 70°C, take it out to avoid sudden boiling, and **never shake up the solution just after it is taken out**.

(3) Do not put agar medium into a container exceeding half the container's volume. For example, when using a conical flask with a volume of 500 mL, the agar medium limit is 250 mL. If more agar medium is used, the risk of sudden boiling increases, causing problems when mixing after autoclaving (the concentration of agar around the bottom after autoclaving is high because agar sinks). If agar is mixed just after autoclaving, sudden boiling may occur. Leave it to cool at room temperature for a few minutes.

(4) Do not autoclave a sample with a low boiling point, such as organic solvents. To do so may cause fire or explosion; in addition, the bad smell produced will disturb others. The concentration of the

sample will markedly change because of evaporation.

8.2.1.4. Other precautions

(1) If the apparatus is to be autoclaved, be sure to check that it can be safely autoclaved by referring to the catalogue and other information sources. For example, the Pipetman of Gilson cannot be autoclaved. Many plastic apparatuses cannot be autoclaved.
(2) A volatile strong acid (hydrochloric acid, nitric acid) can damage the autoclave. Sterilize it using a filter made of appropriate material.
(3) If a strong alkali is autoclaved, it will dissolve glass. If a glass container is repeatedly autoclaved many times, the glass will progressively become thinner, causing breakage (pay attention because glass components dissolve in alkalis).
(4) If a polyallomer centrifuge tube carrying organic solvent such as phenol and chloroform after DNA or RNA extraction is autoclaved, it will be melt. Remove the solvent completely before autoclaving.
(5) If the jar fermenter is autoclaved, do not open the lid until the temperature decreases to 60°C or less as a rule. It is difficult to cool a large amount of medium and it not only has a risk of sudden boiling, as shown in Fig. 2 (C), but also may damage the pH sensor and dissolved oxygen (DO) concentration sensor owing to the change in its pressure.
(6) Be sure to loosen the lid of the container to be autoclaved. If sealed, the pressure outside the container increases prior to the inside as temperature increases, and decreases as temperature decreases. This pressure difference may cause the breakage of a glass container or the deformation of a plastic container.
(7) If a plastic container, such as a centrifuge tube, is autoclaved, the lid will be pressed closely against the body when the container is cooled having a negative pressure after autoclaving, and will be deformed when the container is further cooled having a lower inner pressure. Therefore, sterilize the container with the lid removed, or wrap the container with aluminum foil while the lid is being sufficiently loosened so that the lid cannot be pressed closely against the body.
(8) An uncracked medium bottle resistant to approximately one atmospheric pressure can be sealed and autoclaved. However, it cannot be sterilized unless a drop of distilled water is put in the bottle (it takes at least three hours at 160°C to sterilize the sample under a dry condition).
(9) Overheating may transform the sample. If compounds with amino groups and reducing sugars, such as yeast extract and glucose, are autoclaved at the same time, the Maillard reaction occurs; therefore, the medium turns brownish and the sample strain may be prevented from growing. The longer the heating time and the higher the pH (pH>6), the more marked this reaction. Moreover, this reaction occurs more markedly when a large amount of medium is autoclaved (Fig. 8-2 (B)) than when a small amount of medium is autoclaved (Fig. 8-2 (A)).
(10) If the sample is spilled (boiled over) in the autoclave, clean it up. Even if no sample is spilled, change the water once a week and clean up the autoclave every two to four weeks.

Examples of accidents caused by using the autoclave are as follows.

- A bottle, 90% filled with agar medium, was autoclaved with the lid closed. When it was taken out at 90°C or less, sudden boiling occurred and the flying lid caused a burn on the face of the victim.
- When a jar fermenter was taken out at 90°C or more, sudden boiling occurred, causing a heavy burn on the upper body of the victim (a keloid remains).

An antiforming agent was autoclaved in a conical flask. Although the antiforming agent filled a quarter of the flask, sudden boiling occurred when it was taken out at nearly 90°C, causing a burn that required

a month for recovery.

(1) Do not leave the laboratory unattended until the timer shuts off.
(2) Do not open the lid until the temperature decreases to 70°C or less.
(3) A sample with a low heat conduction (high viscosity or a large amount) has a risk of sudden boiling even at 70°C or less.
(4) No organic solvent, strong acid and strong alkali should be autoclaved.

8.2.2. Centrifuge

First, please answer the following questions (the answers are on the next page).

Q1. Is it permitted to use a combination of differently shaped centrifuge tubes if they are balanced with the scale?
Q2. When there is only one sample, can the sample be balanced with another centrifuge tube of the same shape filled with water?
Q3. When there is only one sample, can the sample be balanced with another centrifuge tube of the same shape filled with a solution, such as sucrose, that has the same specific gravity as the sample?
Q4. Is it unnecessary to switch on the cooler when centrifuging at room temperature?
Q5. Why is the moisture wiped before balancing when the outside of the centrifuge is wet?
Q6. What might happen when a sample leaks from a tube broken during centrifugation?
Q7. Why should the centrifuge rotor be left upside-down after use?
Q8. Why is the cover of the chamber closed during cooling condition and open after use?

Did you answer all the questions correctly? The centrifuge is rotated at a high speed and its kinetic energy is high. If used in a wrong way, a serious accident may result and the lifetime of the machine may be markedly decreased. Know the correct method of use and understand the reasons why such a method is used.

A1. No (There is no balance of moment.)
A2. No (There is no balance of moment.)
A3. No (If separated, the sample will have an imbalance of moment.)
A4. No (There is an increase in temperature due to the heat generated by the motor and the friction between the rotor and air.)
A5. Water on the outside of the centrifuge tube is transferred to the bottom of the rotor by centrifugation, causing an imbalance of moment.
A6. (1) Machine damage (spindle, motor and rotor) due to imbalance.
(2) Biological contamination, chemical contamination, corrosion and fire (explosion) caused by wet sample.
A7. To avoid imbalance owing to dust dropping on the rotor and precipitating water.
A8. Frozen ice in the chamber falls off by the wind pressure generated during centrifugation and damages the rotor.

8.2.2.1. Precautions concerning maximum rotating speed

Use a centrifuge at 80% or less of the maximum allowable rotating speed as a rule. Because each rotor of the centrifuge has a specified maximum allowable rotating speed, be sure to check the speed before use. **Never use the centrifuge at a speed exceeding the maximum allowable rotating speed for any reason.** The maximum allowable rotating speed indicates the allowable rotating speed of the rotor, which has no contamination or scratches, used correctly with regular maintenance. Therefore, normally use the centrifuge within 80% of the maximum allowable rotating speed of the rotor.
If the rotating speed is decreased by 20%, an almost equivalent centrifuging effect can be obtained by increasing the running time by 50%.

8.2.2.2. Precautions concerning centrifuge tube

(1) Check the allowable centrifugal force and proof-solvent performance

Be sure to check to what extent the centrifuge tube to be used can withstand centrifugal force. If solvent or a strong acid is centrifuged, also check whether the material of the centrifuge tube can withstand them. Refer to Table 8-2 and the web sites below (it is advisable to bookmark them). The allowable maximum centrifugal force is the value obtained when the rotor shape and centrifuge tube shape are properly fitted. For example, a centrifuge tube with sharp edge is used with a round-bottom rotor, the centrifuge tube may be broken even with centrifugal force within the allowable range.
http://www.hitachi-koki.co.jp/himac/support/m-tube.htm: endurances of centrifuge tubes made by several companies (in Japanese)
http://www.nalgenunc.co.jp/html/info.shtml: endurance, proof-solvent performance, cleaning method, sterilization method (in Japanese)
http://www.assist-sar.co.jp/: endurances of several plastic products (in Japanese)

(2) Do not use a deformed or cracked centrifuge tube

The centrifuge tube inevitably deteriorates as it is used. Do not use a deformed or cracked centrifuge tube. Note that some centrifuge tubes may be deformed when a small amount of sample is centrifuged even at less than the allowable centrifugal force, as is the case with Teflon centrifuge tubes (80 - 90% of the centrifuge tube must be filled with solvent). Note that a disposable centrifuge tube is not designed for repeated use, as its name suggests.

8.2.2.3. Precautions when balancing

For centrifugation, not weight balance but moment balance (weight × turning radius of center of gravity) is required. This can be easily understood by comparing the case of a piece of clay attached to the center of a string (a) with that of a piece of clay with the same weight on the end of a string (b) shown in Fig. 8-3. It is clear that the force given to the arm when swinging the string is higher in (b).

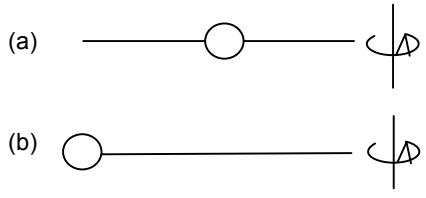


Fig. 8-3. Gravity balance and moment

Table 8-2. Maximum allowable centrifugal force of disposable centrifuge tube

Manufacturer	Volume (mL)	Material	Maximum centrifugal force (g)
Assist	50	Polypropylene	4,000
		Polystyrene	4,000
	15, 13	Polypropylene	4,100
		Polystyrene	1,800
Corning	50	Improved polystyrene (Cat.No.430304)	1,800
		Polypropylene (Cat.No. 430290, 430291, 430828, 430829, 430522)	6,000
	15	Improved polystyrene (Cat.No.430053, 430055, 430788, 430789)	1,800
		Polypropylene (Cat.No. 430052,430766, 430790, 430791, 430630)	6,000

(1) There is no balance of moment between centrifuge tubes with different shapes (different centers of gravity).
(2) There is a balance of weight but no balance of moment between a solution with a specific gravity of 1.2 and water with 1.2-fold volume (specific gravity: 1.0) (the distance from the center of gravity to the rotating axis is different).
(3) For example, when a 50% suspension of fungus with a specific gravity of 1.2 (gravity as a suspension: 1.1) is balanced using saline with a specific gravity of 1.1, there is a balance of moment before centrifugation. After centrifugation, however, the fungus with a specific gravity of 1.2 will be concentrated on the bottom of the tube (the center of gravity moves away from the rotating axis), meaning that there is no balance of moment.
(4) If the centrifuge tube is balanced with the moisture on the outside of the tube or with water precipitated in the rotor, there is no balance, either.

Correct balancing involves **equally dividing a sample into centrifuge tubes of the same shape (material), the lid of which should also be of the same shape**. The moment imbalance caused by the specific gravity is practically allowable to some extent (for example, the case that *Escherichia coli* culture solution is balanced with water). However, if a sample is centrifuged at the maximum allowable rotating speed, and ammonium sulfate, sucrose and glycerol solution at high concentration or a fungal (cell) suspension at high concentration are centrifuged, the same sample is equally divided for balancing.

8.2.2.4. Precautions concerning sample leakage

In the case of an angle rotor, the liquid surface becomes vertical (parallel to the rotational axis) during centrifugation (Fig. 8-4(A)). If the liquid surface is over the edge of the centrifuge tube, the sample will leak with poor sealing between the centrifuge tube and the lid. If the sample leaks, imbalance will result, which will not only damage the machine and rotor but also cause biological contamination (strain harmful to the human body and phage harmful to other experiments), chemical contamination (for example, carcinogens, and poisonous and deleterious substances), corrosion (for

example, ammonium sulfate, if left, heavily corroding aluminum alloy rotors), and at worst, fire and explosion (in the case of organic solvent). Therefore, considering the case of the incomplete sealing of the lid, **do not put a sample into a centrifuge tube so that the liquid surface during centrifugation is over the edge of the centrifuge tube. In particular, when a sample containing solvent with a low flash point (for example, ethanol) is centrifuged, never completely fill the tube to a level where the liquid surface during centrifugation (rotation) is above the edge of the centrifuge tube. In addition, never use a cracked or deformed centrifuge tube. If a dangerous reagent is spilled, be sure to report it to the instructor and then, treat the spill**. Even if the sample does not leak when the closed centrifuge tube with the sample is pressed by the finger, there is no assurance that the lid has been sealed completely. Assume that the liquid surface is 1 cm nearer the side of the rotational axis from the edge of the centrifuge tube in Fig. 8-4(B). The pressure of a 10 m water column is 1 atmosphere under the condition of 1 *g*. In the case of centrifugation under the condition of 10000 ×*g*, the pressure of a 1 cm water column corresponds to 10 atmospheres.

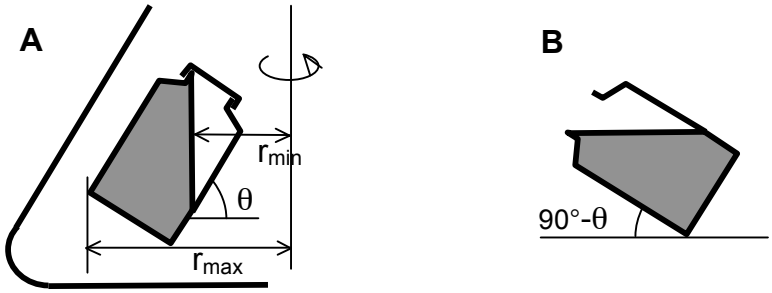


Fig. 8-4. Liquid surface during rotation (A) and calculation of safe amount of liquid (B).

(1) Examine the angle θ of the rotor to be used.

(2) Tilt the centrifuge tube full of water by 90 - θ.

(3) The remaining amount of liquid is the maximum allowable.

8.2.2.5. Precautions during operation

(1) Attach the rotor completely

Check the meshing direction of the tab on the bottom of the rotor with that of the rotating axis, and then set the rotor completely (some centrifuges have no tabs). Take care not to bend or break the tab. After setting, check that the rotor is completely set by slightly turning it by hand. If you remember how many turns the lid of the rotor requires for fastening it, you can find the incorrect setting when you turn the lid less than usual.

(2) Check the attachment of packing

Set the packing between the rotor and lid. The screw of the rotor lid is cut in the direction in which the screw is fastened by acceleration. Conversely, it is loosened when the rotor is decelerated (the rotor is decelerated, but the lid tries to rotate by inertia force). If packing is not correctly attached, the screw cannot be sufficiently fastened and the lid may become loose and fall off during deceleration. If the lid flies off during rotation, it is generally very expensive (more than a few hundred thousand yen) to repair it. To our knowledge, there were three accidents presumed to have resulted from such a default previously in our department.

(3) Monitor the centrifuge until the running state becomes steady

Do not leave the centrifuge unattended until the rotating speed increases up to the preset value and becomes steady. Otherwise, appropriate action cannot be taken if there has been a mistake in setting the centrifuge tube or in balancing, or if breakage of the centrifuge tube should

occur. If something abnormal (abnormal sound) occurs, immediately push the stop button or set the timer to zero. Take refuge and keep anyone away from the centrifuge until it completely stops.

(4) Never open the cover during running

Do not open the cover carelessly during running even if there is an abnormal sound. If the centrifuge tube has been broken, the broken pieces will scatter at a high speed and can cause serious injury, such as blindness.

(5) Never stop the rotor by hand

The hand may get caught in the rotating rotor, causing a serious accident, such as a fracture. The rotational axis may be bent and the lifetime of the centrifuge may be decreased markedly. "Because I am in a hurry" is not a valid excuse. Be patient and wait until the centrifuge stops completely.

(6) Always keep the cooler switched on

The temperature of the centrifuge will be increased by the heat from the motor and from the friction between the rotor and air. Therefore, keep the cooler switched on even during centrifugation at room temperature.

(7) Cover of the chamber

While the cooler is switched on, keep the lid of the centrifuge closed to prevent condensation and freezing in the chamber. Inversely, when switching off the cooler, open the lid to dry the inside of the chamber. If a large amount of ice is generated, ice crystals may fall off with draft pressure during centrifugation and scatter, damaging the rotor and chamber.

(8) Rotor after use

Remove the rotor and check that there is no contamination by possible sample leakage. Lay the removed rotor downward to prevent losing balance the next time centrifugation is carried out, owing to falling dust and condensed water collecting in the rotor. If the sample leaks, rinse the rotor immediately. The rotor contamination causes not only imbalance but also corrosion of the rotor and breakage. **If a dangerous sample (recombined organism, carcinogen, poisonous and deleterious substances, corrosive substance, and flammable substance) leaks, be sure to report it to the instructor and then, treat the leakage properly.**

8.2.2.6. Precautions concerning ultracentrifuge use

(1) Use the ultracentrifuge in the presence of an instructor

The ultracentrifuge rotates at an ultrahigh speed of around 100,000 rpm at maximum. Take special care as the rotor may fly out and cause a serious accident if balancing is forgotten or the sample leaks. If the rotor crashes through the chamber, the rotor will fly around the laboratory at a high speed and destroy the laboratory. If we consider the normal centrifuge as analogous to a normal car, the ultracentrifuge would be a racing car (F1 car). Even a small mistake may directly lead to a serious accident. **Use the ultracentrifuge in the presence of an instructor until you sufficiently acquaint yourself with its use. Do not use it alone until you are recognized to be sufficiently familiar with its use by the instructor.**

(2) Precautions concerning plasmid purification

When purifying a plasmid DNA by cesium chloride – ethidium bromide equilibrium density-gradient centrifugation, **take special care of the temperature setting**. If a density gradient is formed, the concentration of cesium chloride at the bottom of the centrifuge tube will increase. Therefore, if the temperature is wrongly set at '**4°C**,' the concentration of cesium chloride will exceed the saturated concentration and its crystal will precipitate. Because the density of the crystal is much higher than that of the solution, **the moment balance will be lost and the rotor will fly out. Strictly observe the setting of temperature and time designated in the protocol**.

Ethidium bromide is a carcinogen and usually used at high concentration for equilibrium density-gradient centrifugation (consider that it is more dangerous than RI). **If it is spilled, be sure to report it to the instructor and conduct proper decontamination**. In particular, the Beckman ultracentrifuge in the common equipment room in the basement can become easily contaminated at the time of heat sealing the centrifuge tube. Check whether there is contamination using a built-in ultraviolet (UV) lamp before and after sealing. Under short-wavelength ultraviolet irradiation in a dark room, an orange glow indicates contamination. Of course, short-wavelength ultraviolet must not be irradiated directly onto the skin or looked at with the naked eye.

(3) Heat sealing

When sealing the centrifuge tube by melting its rim through heating, any solution adhering to the rim will cause incomplete sealing (increase in inner pressure by the centrifugal force → liquid leakage → imbalance → very serious accident). **Carefully read the instruction manual** and pay attention to the following when sealing.

1) Pour a sample up to the line shown in Figure 8-5. If a larger amount is poured, the tube will tend to be sealed incompletely, and if smaller, the centrifuge tube will be deformed by the pressure due to the centrifugal force, also causing imbalance.
2) Completely wipe the sample solution adhering to the rim of the tube with Kimwipe. Because the temperature at the part of the tube with the attached solution will not increase even when heated by a heater (the tube will not melt), the tube will be incompletely sealed. When the solution adhering to the rim dries up, the solute dissolved in the solution will remain in a dried-up state, also causing incomplete sealing.

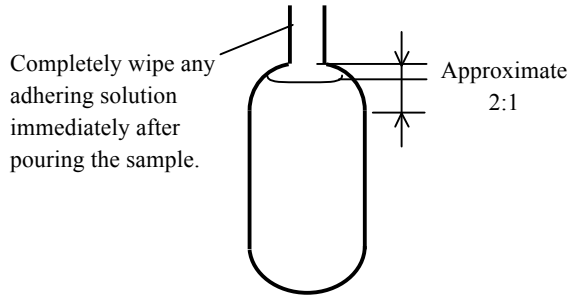


Fig. 8-5. Appropriate sample

8.2.2.7. Precautions concerning swing-type centrifuge (clinical centrifuge)

When running a swing-type centrifuge, in which the test tubes can be centrifuged, mount the same assemblies in all four arms. Even if centrifuging one sample (two samples for balancing), mount four identical assemblies. In general, when using an assembly in which eight test tubes can be placed, the maximum rotating speed is 2,000 rpm (be sure to check the value yourself because it depends on the type of centrifuge).

8.2.2.8. Know-how for centrifuging

(1) Points for deriving the centrifugation effect from the theoretical formula

Assuming that the Stokes radius and specific gravity of the particle to be centrifuged are R and ρ, respectively, the rotation speed is ω, the specific gravity and viscosity of the solvent are ρ_0 and η, the distance between the rotational axis and the liquid surface is r_{min}, and the distance between the rotational axis and the bottom of the centrifuge tube is r_{max}, the time for the deposition of the objective particle is given as

$$t = \frac{9}{2}\frac{\eta}{\omega^2 R^2(\rho - \rho_0)}\ln\frac{r_{max}}{r_{min}}.$$

Therefore, the following apply.

1) The centrifugation effect is proportional to the product of centrifugal force (rotating speed squared) and time. Therefore, if the rotating speed increases by 1.2-fold, 1.44-fold the effect is obtained (here, strictly observe the maximum allowable rotating speed). If the rotating speed cannot be increased, rotation time can be increased.
2) When particles in a solution of salt or sugar at high concentration are centrifuged, it takes a long time to centrifuge because $\rho-\rho_0$ is low. It is desirable to centrifuge such solutions after diluting with water or buffer solution if possible. For example, when a sample with a specific gravity of 1.21 in a solution with a specific gravity of 1.20 is centrifuged, the difference in specific gravity between the two is 0.01. However, if a solution is diluted to a specific gravity of 1.10 using an equivalent amount of water (specific gravity: 1.00), the difference in specific gravity will be 0.11 and the sample will be centrifuged for a time ten-fold shorter than the original. Moreover, dilution has an effect of decreasing the viscosity of a solution.
3) The viscosity of a solution decreases as its temperature increases. When increasing preset temperature or diluting a solution, the viscosity of the solution will decrease, which enables shortening of the centrifuging time.
4) Using an angle-type rotor with a low r_{max}/r_{min}, a sample is centrifuged more rapidly than when using a swing-type rotor with a high r_{max}/r_{min}.

(2) If there are problems with suspension of precipitate

In most cases, the objective is not centrifugation at a given rotating speed but precipitate collection; therefore, it is desirable to decrease rotating speed. Moreover, when resuspending a precipitate, loosening (softening) the precipitate to which the solution has not been added yet using a vortex mixer will facilitate suspension.

(3) Precooling of rotor

In principle, mount the rotor, set the temperature and precool it for 0.5 – 1 hour before use. If the rotor is required to cool rapidly, it is desirable to properly mount the rotor and lid and then to idle the rotor at 1,000 – 2,000 rpm for 5 – 10 min.

About normal centrifuge:

(1) Balance the centrifuge with respect to moment not weight.

(2) Strictly observe the maximum rotating speed and usually use the centrifuge at 80% or less.

(3) Check that the centrifuge tube withstands the given centrifugal force and solvent.

(4) Sample leakage = risk (imbalance, biological contamination, chemical contamination, explosion and fire).

(5) Be sure to use a cooling device if equipped.

(6) Mount the same assemblies to all the arms of a swing-type rotor.

(7) Supervise the rotor until its rotation becomes stable.

(8) Do not open the cover of the chamber during rotation.

(9) Never stop the rotor by hand.

(10) Report sample leaks to the instructor.

About ultracentrifuge:

(1) Use an ultracentrifuge in the presence of an instructor until sufficiently acquainted with its use.

(2) Strictly observe temperature during equilibrium density-gradient centrifugation.

(3) Take special precautions not to contaminate the sample with ethidium bromide.

8.2.3. Thermostat Bath (Incubator)

The thermostat bath is generally used without special care but has a risk of causing large-scale accidents such as fires. In our department, there have been small fires caused by boiling the water bath dry and by heating of the oil bath, and foul odor emissions of melting plastic on the verge of fire in the dryer. An incorrect operation of the thermostat may cause inaccurate control of temperature, resulting in the failure of the experiment even though no accident is caused.

8.2.3.1.Water bath incubator

(1) Caution concerning overnight running and unattended running

Never run a water bath overnight without the boil-dry protection device. Even in the daytime, unattended running is prohibited. At each use, check that the boil-dry protection device is functioning correctly before carrying out the experiment. In the case of the boil-dry protection device, which stops the current flow when the float descends to a certain level due to a decrease in water level, if water residue accumulates on the float, it will not move and will fail to function properly when the water level descends. Do not neglect the maintenance of the bath to prevent residue accumulation around the float.

(2) Water supplement

Water in the incubator (thermostat bath) evaporates. Be sure to maintain a sufficient amount of water in the bath. Because there is a large difference between room temperature and water temperature and a low air humidity in winter, pay particular attention to increases in evaporation. When running the bath all night, devise an automatic water supply system or prevent evaporation by covering the bath with aluminum foil. In particular, when using the bath at a high temperature, examine the water level decrease per unit time and check whether a safe water level can be maintained until your arrival at the laboratory the next day. It is dangerous to place plastic balls or foam polystyrene on the water surface. They may cause fire if they come into contact with the heater.

(3) Check of water level

The last person leaving the laboratory must check the water level of a water bath left to run overnight and add water if necessary even if it is not their own experiment.

(4) Do not overly rely on the safety device

In the user instruction of the water bath equipped with the boil-dry protection device, there is a note “check the operation of the safety device at each use.” The operation is not assured unless checked properly. **If the safety device is activated, the power will be shut off and the temperature will not be maintained, resulting in the failure of the experiment**. The presence of a safety device is not an excuse to neglect the addition of water even for a water bath equipped with the safety device.

(5) Caution concerning shaking

When using the water bath with a shaking apparatus, adjust the water level and shaking speed so that water is not scattered during shaking. In particular, when a sample requiring sterilization is shaken, devise a way to keep dirty water (more than 10^7 bacteria/mL is not unusual) away from the

cotton stopper and silicon stopper.

8.2.3.2. Incubator, dryer and dry heat sterilizer

(1) The incubator, dryer and dry heat sterilizer have no explosion-proof construction.

Never put organic solvent or flammable gas into them.

(2) Do not put any flammable materials into the dry heat sterilizer

Do not put combustible material, such as plastic and paper, into the dry heat sterilizer. The only exception is the cotton plug.

(3) Fix the temperature setting dial with tape

If the thermostat bath has a temperature setting dial, fix the dial with tape so that the preset temperature cannot be shifted by contact with other materials. In our department, there has been a case in which a plastic instrument melted due to an inadvertent shift of the temperature dial (fortunately, fire was prevented because of detection of a foul odor).

(4) Check that any instrument used in the dryer or dry heat sterilizer can withstand the preset temperature

In general, plastic instruments (including the Pipetman) cannot withstand the temperature in the dry heat sterilizer (160°C). If a chip, Eppendorf tube or plastic beaker is dry-heat-sterilized, it will melt and flow into the high-temperature heater, causing a fire.

(5) Do not block air circulation

Do not place the sample or instruments on the outlet of the fan. Do not place large objects in the machines and do not fill the machines with too many objects. In general, the temperature sensor is installed in the upper part of the machines and the heater in the lower part. If air circulation (convection) is prevented, the temperature cannot be controlled. The heater continues working until the temperature in the upper part reaches the preset value, which causes the temperature in the lower part to become too high. There has been an incident of fire due to a plastic instrument melting in the high-temperature heater and flowing into the heater part.

(6) Do not remove the bottom plate

The board at the bottom of the machines separates the bottom plate (the temperature of which will become high) and the sample to ensure air circulation. Do not remove the bottom plate even when attempting to put in a tall sample, otherwise fire may result.

(7) When the sample is spilled

If the sample is spilled into the incubator, wipe it off immediately. Otherwise, it may corrode the incubator and cause contamination.

Basic precautions

(1) Display the user name and scheduled termination time for overnight running.

(2) Unattended (overnight) running of the machines without the safety device is prohibited.

(3) Check that the temperature has attained a steady state.

(4) Never use a socket multiplier.

(5) Pay attention to the electric capacity when using an extension cord.

(6) The control unit must be kept dry.

Water bath

(1) Is the amount of water sufficient?

(No water + nonfunctional safety device = fire)

(2) At each use, check that the boil-dry protection device functions correctly.

(3) The last person leaving the laboratory must check the water level regardless of whose experiment it is.

Incubator, dryer and dry heat sterilizer

(1) Do not use organic solvent or flammable gas.

(2) Do not block the air circulation in the machines.

(3) Do not place combustible material in the dry heat sterilizer.

8.2.4. Reducing Pressure

8.2.4.1. Cautions concerning apparatuses

(1) Do not draw up benzene or halogen-containing solvent directly with the water-jet pump.

Do not draw up benzene or halogen-containing solvent (chloroform, dichloromethane and chloroethylene) with the water-jet pump. Otherwise, halogen-containing solvent will be discharged into the sewage and cause environmental contamination. Neither should they be drawn up with the circulation water-jet pump (when replacing water in the circulation water-jet pump, water with the halogen-containing solvent will be disposed of into the sewage).

(2) Do not draw up acidic gas directly with the vacuum pump.

For example, hydrochloric acid, acetic acid and trifluoroacetic acid will corrode the inside of the pump and considerably reduce its lifetime. Use a special Teflon-coated pump or a pump with a trap containing sodium hydroxide or other chemicals.

(3) Do not draw up organic solvent or solution directly with the hydraulic vacuum pump.

Organic solvent and solution are less detrimental than acidic gas, but also reduce the lifetime of the pump. In addition, a sufficient vacuum level cannot be attained because of the vapor pressure of the drawn solvent or water. Draw them up through a cold trap cooled with liquid nitrogen or dry ice - ethanol (methanol). If drawing up a solution, the silica gel column can be used alternatively. In this case, replace the silica gel when its color changes (the gel becomes tinged with red).

(4) Break the vacuum condition before stopping the pump

Before stopping the pump, break the vacuum condition by using the three-way cock or detaching the hose. Some vacuum pumps have no backflow valve. If such a pump is stopped before breaking the vacuum, oil will flow back and ruin the sample. (Cleanup is very difficult.) The water-jet pump normally has no backflow prevention valve. If it is operated incorrectly, water will flow back and ruin the valuable sample. In particular, for drying at a low pressure with a desiccator, backflowing water will react strongly with the drying agent (phosphorus pentoxide, calcium chloride, concentrated sulfuric acid, etc; silica gel is no exception), causing an accident. In fact, the use of the water-jet pump for drying at a low pressure is incorrect (the sample cannot be dried completely because of the vapor from the water flow).

8.2.4.2. Notes on operation for reducing pressure

(1) A container exposed to a low pressure must be resistant to a low pressure

Use only thick containers designed for use under a low pressure, such as a pressure-reduction bottle, an aspiration bottle and a desiccator. Before each use, check that the bottle is not cracked or

chipped. Be aware that a conical flask may break when the pressure is reduced. The broken pieces of the flask and contents scatter violently, which is very dangerous.

(2) Be ready for quick response to 'sudden boiling'

When reducing the pressure of the solvent, attach a three-way stop cock to the pump **to enable immediate cessation of pressure reduction in the event of sudden boiling**. Sudden boiling tends to occur particularly just after starting pressure reduction, when starting to heat the sample, and just before the sample solvent is completely exhausted. Therefore always monitor the pump and be prepared to take immediate action, such as keeping your hand on the stop cock in the event of sudden boiling. Never leave the pump unattended just after starting pressure reduction.

8.2.4.3. Rotary evaporator

(1) Start the exhaust slowly and carefully

Start the exhaust, keeping your hand on the stop cock to immediately break the vacuum condition in the event of bubbling or sudden boiling. Never leave the pump unattended just after starting pressure reduction.

(2) Do not regularly use the vacuum pump for pressure reduction

Generally use the water-jet pump, not the vacuum pump. If using the vacuum pump, draw up solutions through the trap cooled with liquid nitrogen or dry ice – ethanol (methanol), as noted above.

(3) Do not heat until the pressure has become sufficiently low and stable

Start heating after a sufficiently steady low pressure is obtained and no bubbling or sudden boiling occurs.

(4) Stopping the flow of cooling water may cause a fire

Check that sufficient cooling water flows 5 minutes after starting pressure reduction and at the time of leaving the evaporator unattended. The solvent may be not collected but exhausted, which may cause an explosion and fire.

(1) Use a container designed for use under a low pressure.

(2) Stopping the flow of cooling water in the rotary evaporator causes explosion and fire.

(3) Drying agent + backflow water = danger (stop the pump after all operations)

(4) The use of benzene or halogen-containing solvent with the water-jet pump is prohibited.

(5) Attach the proper trap to the vacuum pump.

8.2.5. Ultraviolet Lamp

Ultraviolet radiation, in particular, short-wavelength ultraviolet radiation, is harmful to the human body. When staining and observing DNA, short-wavelength ultraviolet radiation will damage the DNA, for example, by causing thymine dimer formation and DNA breakage.

(1) When turning on the lamp, even for a short time, always wear protective glasses.

(2) For operations taking more than a few seconds, such as collecting DNA fragments from agarose gel, wear long-sleeved clothes (lab coat), gloves and a full-face mask.

(3) The ultraviolet lamp attached to the clean bench emits harmful short-wavelength (normally 245 – 254 nm) ultraviolet radiation. Although ultraviolet radiation can be blocked by glass or acrylic plates, direct exposure of the skin or direct viewing with the naked eye must be avoided. Furthermore, it differs from the long-wavelength lamp (generally 312 – 365 nm) used in the

transilluminator. Care must be taken not to mistake them when exchanging the lamp.

(4) When using the transilluminator, the wavelength of which can be varied, set the switch to the long wavelength for DNA excision (pay attention to H and L on the selector switch, which refer not to the output power but to the wavelength).

8.2.6. Clean bench/Safety cabinet

Clean bench is a device which makes a space separated from the surrounding air. If you perform experiments with the bench, you can avoid contamination of several organisms or substances from outside. Before experiments, the clean bench kills saprophytic bacteria with equipped ultraviolet lamps in the device. During experiments, the device keeps positive pressure inside by incorporating clean air filtered with a HEPA filter, leading to avoidance of bacteria's invasion into the device. You should handle reagents or perform experiments for cell culture in the clean bench or safety cabinet in order to avoid contamination. (The safety cabinet keeps a negative pressure inside to avoid leakage of biological materials such as virus etc. to the outside).

(1) Management of Bunsen burner

When you have done experiments or leave the clean bench even for a short time, you must extinguish put out the fire of a bunsen burner. You should also keep the bunsen burner off the wall surface of the clean bench. Keep it away from the place where the wall surface becomes hot.

(2) Notice for sterilization with ethanol

When you fire a spreader for sterilization, you should realize the risk of fire and solvent at the same time. Ethanol should be kept in the metal box. The lid should be kept handy during operation. If by chance, ethanol ignites, you should put out the fire by covering the lid on the box.

(3) Even if you wash your hands, your hands are still the most dirty thing in the clean bench. Consider the sequential order of the operation and arrange the components so as not to pass your hands over the non-contaminated components.

- Non-contaminated items should be put in the inmost part of the operation space and possibly-contaminated items should be put nearest your side.
- As a general rule, you should put items, which are treated on the right hand, in the right side, and ones which are untreated by the left hand, on the left side.
- An operation, such as opening the lid with your both hands, should be finished before starting experiments.

(4) Keep the inside of clean bench clean

In the case of spilling medium, you should immediately wipe it up. The spilled medium might lead to growth of bacteria and contamination of the experimental system.

(5) Wear clean lab coat during experiments and roll the sleeves up to your elbow.

Before experiments, you should sterilize your hands with 0.05-0.1% benzalkonium chloride solution or 70% ethanol. Of course, you should sterilize apparatus used in the bench with the solution or ethanol.

(6) Do not leave unnecessary items in the bench.

Do not leave apparatus such as an unused laboratory dish, a medium, a pipette or the mixer in the clean bench. The following problems occur:

・Work space becomes small.

・Parts under shadow and not exposed to the UV lights increase and those parts might not be sterilized.

・Plastic parts deteriorate in particular, and the apparatus will be damaged by UV lights.

8.3. Handling Reagent

When using reagent, always wear protective glasses. If you get dangerous reagent (solution) in your eyes, immediately (within a few seconds) wash the eyes with generous amounts of water for more than 15 minutes, and consult a physician. In particular, if you get alkali or organic solvent in your eyes, wash the eyes vigorously without delay. Every second you delay the higher the risk of blindness. It is more important to sufficiently wash the eyes than to quickly consult a physician.

Before using reagent in your experiment, understand the handling instructions for all reagents. Refer to a textbook, such as "*Carrying Out Experiments Safely*" (KAGAKU-DOJIN) to acquire general basic knowledge about "what substances are dangerous in what ways," and "what should be done in the event of an accident." In addition, consult the Merck Index and make sure to acquire sufficient background knowledge for each reagent to be used with respect to, for example, the following points:

(1) How harmful is the reagent to the human body?
(2) Are there any risks of fire or explosion?
(3) What procedure should be followed when the reagent is spilled?
(4) Proper method of treating waste liquid
(5) Proper storage method (refrigerating, freezing, protecting from light, nitrogen substitution, etc.)

8.3.1. Examples of reagents to handle with care

Some reagents, which are normally used without special care, may be unexpectedly dangerous. Some familiar examples of reagents that are rarely used but should be handled with special care are given below.

(1) Special flammable substances

Ether is an example. According to the Fire Defense Law, its danger level is ranked the highest, and therefore, all open flames in the room must be extinguished before it can be handled. Here, "handling" includes "holding" the solvent bottle. Ether is flammable because of its extremely low ignition temperature. Once ether catches fire, the fire expands explosively, is difficult to extinguish, and therefore, utmost caution is required. **The centrifugation of these solvents is strictly prohibited** (a special explosion-proof centrifuge, which is unavailable in our department, is required).

(2) Highly flammable substances

Ethanol, methanol, acetonitrile, hexane and acetone are examples. Similar to special flammable substances, extinguish all open flames, such as that in a stove, before "holding" the bottle, in case the bottle is dropped and broken. It is dangerous to dispose of these solvents in a sink even though they are soluble. Because the amount of solvent vapor increases with liquid surface area, these solvents may catch fire from the water heater or a spark from the motor in the centrifuge and

explode.

(3) Poisonous and deleterious substances

Potassium cyanide, sodium cyanide, sodium azide, mercury compounds and arsenic compounds are examples. Phenol, chloroform, acrylamide, sulfuric acid, hydrochloric acid, sodium hydroxide, potassium hydroxide and acetonitrile are also examples. Store them in their designated lockable storage cabinet and manage their mass by preparing a log. **Upon recognizing a lost or incomplete form in the log, immediately report it to the instructor**.

When the ordered poisonous and deleterious substances are delivered, be sure to record them in the log. When borrowing or lending such substances between laboratories, be sure to obtain approval from the instructor.

(4) Other familiar dangerous substances

- **Hydrogen peroxide solution**

If metal is mixed into this solution, it may react explosively (recall the science experiment to generate oxygen from hydrogen peroxide solution using potassium permanganate as a catalyst, performed in elementary and junior high schools). There has been a case of explosion due to the contamination of a small amount of rust. **Never use a metallic container or injector**.

- **Ammonium persulfate**

This is used as a polymerization initiator of polyacrylamide gel. **Because it occasionally may react explosively with metal, do not use a metallic medical spoon when weighing the ammonium persulfate**. Use a plastic or bamboo spoon. When using the ammonium persulfate for the preparation of polyacrylamide gel, the following procedure is recommended (10% ammonium persulfate solution for electrophoresis, although slightly more expensive, is commercially available):

1) Purchase ammonium persulfate in units of 1 g.
2) Add 3–5 mL of pure water directly into the reagent bottle and dissolve the ammonium persulfate.
3) Transfer the solution into a disposable tube with a volume of 15 mL and dilute to 10 mL.
4) Dispense an amount suitable for one-time use in the range of 100 – 150 μL into several Eppendorf tubes.
5) Store it in the freezer below –20°C.

In most protocols, ammonium persulfate should be prepared at the time of use. However, there is no problem with the use of ammonium persulfate dispensed from a stock kept in the freezer. The error in the preparation by this method does not cause any problem when using ammonium persulfate as a polymerization initiator of acrylamide gel.

- **Acrylamide**

This is neurotoxic. If it comes in contact with the skin, no problem arises if the skin is washed immediately, but it may cause paralysis if the skin is left untreated.

- **Phenol**

This is a corrosive substance. If it comes in contact with the skin, it causes burns. Precautions during the extraction of nucleic acid are given in 8.3.6.

- **Chloroform**

This is deleterious and corrosive and is designated as a specified toxic substance. If it comes in contact with the skin, although less harmful than phenol, it may still cause burns. Chloroform is considered to cause liver cancer if inhaled over a long period. Be sure to use it within the draft chamber. Precautions during the extraction of nucleic acid are given in 8.3.6.

- **Ethidium bromide, nitrosoguanidine, ethyl (methyl) methane sulfonate**

These are highly carcinogenic agents. Consider them more dangerous than isotopes. Use them under the instructor's guidance until you acquire full handling expertise. Ensure full

knowledge on the treatment for spills and the disposal method.

- **Phosphorus pentoxide, quicklime (calcium oxide), concentrated sulfuric acid, silica gel**

  These are also used as drying agents and reagents of these four substances react violently with water. Even silica gel is dangerous because it bursts upon contact with water. Exercise sufficient caution when disposing of the contents of a desiccator.

8.3.2. Cautions in purchasing

(1) Acquire proper knowledge about the reagent to be purchased

Be sure to acquire knowledge about the reagent to be used, using information sources, such as the Merck Index. It is necessary not only to obtain the proper knowledge concerning toxicity and explosiveness but also not to fail in the experiment. For example, useful information, such as the preparation method of the solution of a difficult-to-dissolve reagent (shifting its pH slightly or dissolving it initially in another solvent) and the stability of the reagent, can be obtained.

(2) Purchase the reagent only in the smallest necessary amount

It is not uncommon for a reagent disposal fee to be tens or hundreds of times more expensive than the purchasing cost. Recognize that the remains of excessive purchases are not only wasteful but require additional disposal fees.

Many reagents deteriorate in storage. Purchase reagents requiring special storage methods (hygroscopic reagent, reagent in ampoule and reagents to be protected from light, stored under nitrogen, refrigerated or frozen) in only the smallest necessary amounts. For example, 1-g bottles and 10-g bottles are commercially available; if 2 g of the reagent is required, it is often less expensive to purchase two 1-g bottles than one 10-g bottle.

8.3.3. Precautions for use

(1) Label check

Check the label three times so as not to use an incorrect reagent. First check: when retrieving the reagent bottle from the reagent cabinet (always check the label). Second check: when measuring the reagent (if placing numerous reagent bottles together near the balance, always check the label before measuring the reagent). Third check: when returning the bottle to the reagent cabinet (always check the label when replacing the bottle back on the shelf). The following are examples of commonly confused reagents.

1) Polyvalent anions, such as phosphoric acid, citric acid, EDTA and ATP
   For example, tetravalent EDTA has five types of salt, namely, free acid and mono-, di-, tri- and tetra sodium salt. Phosphoric acid has several salts, such as sodium salt, potassium salt and ammonium salt, and each salt has three types.
2) Number of bonded water molecules
   For example, Na_2HPO_4 has three types of salt, namely, anhydride, heptahydrate and dodecahydrate. For mol concentration, the amount to be measured can be calculated from each molecular weight. However, note that the weight concentration is different depending on the type of salt used (the number of bonded water molecules in the reagent used must be accurately recorded in the experiment note).

(2) If reagent is spilled

If the reagent is spilled during measurement, be sure to clean it up. Otherwise, the spilled reagent will come into contact with the back of the powder paper and contaminate the next reagent being measured. **Only the person who spilled the reagent knows its identity. If left untreated, it**

cannot be properly cleaned up.

(3) Medical spoon

Before using the medical spoon to measure reagent, ensure that the spoon is washed well, rinsed with pure water and dried completely. If a wet medical spoon is used, the reagent may deteriorate due to moisture, and will not be measured accurately. It is not uncommon for experiments to fail because of a very slight amount of impurity contamination. Note that a metallic medical spoon should not be used for some reagents, such as ammonium persulfate.

(4) Be sure to return the reagent

Be sure to immediately return the reagent used with the label facing forward. If the label is peeling off or worn away, take proper steps, such as reattachment or rewriting of the name. It is highly expensive to dispose of reagents containing unknown substances.

(5) Opening container of refrigerated or frozen reagent

When using a reagent stored in a refrigerator or a freezer, **be sure to bring it to room temperature before opening the lid.** If the lid of a cold bottle is opened, vapor in the air will condense, making the reagent wet. When wet, the reagent may deteriorate and will no longer be measured accurately. The deterioration of a cold reagent, caused by exposure to room temperature for a short time, is negligible compared with that caused by storing under a wet condition at a low temperature for a long time. However, (6) and (7) are exceptions.

(6) Ammonium water, hydrogen peroxide, corrosive carbohydrate solution

The pressure in a bottle of ammonium water and hydrogen peroxide may be high. If the temperature in the bottle is high, cool it with ice water before opening the lid. When a medium containing sugar corrodes in a sealed bottle and the pressure within a bottle filled with carbon dioxide is high, be aware that the contents may scatter explosively if the lid is carelessly loosened. **Always wear protective glasses without fail.**

(7) Opening an ampoule

Open an ampoule after cooling it with ice water. If the total amount of reagent is not used in the experiment, prepare a container of suitable volume and material and which can be sealed, before opening the ampoule. Because there is a reason for sealing the reagent in an ampoule (for example, extreme sensitivity to oxygen or moisture, or a foul odor), it must again be sealed for storage after opening. Note that Parafilm cannot be used to seal an ampoule, because of its breathability.

8.3.4. Handling of carcinogens

Ethidium bromide, nitrosoguanidine, ethyl (methyl) methane sulfonate are highly carcinogenic and should even a slight amount of these substances be spilled and left untreated, all those present, not just the experimenter, will be exposed to long-term risk of disease. **Consider these substances more dangerous than isotopes because spilled substances are difficult to detect** (ethidium bromide can be detected by ultraviolet irradiation). When using these substances, as with the case of isotopes (or more than with isotopes), exercise particular caution, as described below. **Be sure to use them under the instructor's guidance** because procedures differ depending on the reagent and the experimental aim**.**

(1) **Always wear protective glasses and gloves**. Never touch these substances with bare hands. **Consider used gloves to be contaminated, and do not carelessly touch other materials when wearing gloves**.
(2) **Request the presence of the instructor when measuring these substances**. Take sufficient care not to spill any amount of the substance.
(3) Store the reagent bottle in a transparent plastic bag with the top closed.
(4) Dispose of the old bag used to store the reagent by the method described in (8).
(5) Place polyethylene-coated filter paper of sufficient size on the laboratory table to be used.

(6) **If the substance is spilled outside the area of the filter paper, report to the instructor and immediately neutralize it properly and adequately.**
(7) Be sure to perform neutralization for all the instruments used (containers such as test tubes, chips, medical spoons and powder paper).
(8) Dispose of used gloves, filter paper on the laboratory table and reagent bag, keeping them away from people (not only those in the laboratory but also those involved in waste disposal). For example, hold the bag to be disposed of while wearing gloves, then take the gloves off inside out such that they envelop the bag and then bind them at the top. Moreover, put the entirety into another bag, bind the top of the bag and dispose. Be sure not to include any sharp objects, such as a tip, which may tear the bag. Dispose of the tips after separate neutralization.

8.3.5. Treatment of carcinogens

The following are general precautions and not to be considered complete. Study and devise proper suitable treatments by yourself.

(1) Ethidium bromide

This is often treated with sodium hypochlorite solution, however, its carcinogenicity cannot be completely neutralized, and it is also said that its toxicity may in fact be increased. The oxidation treatment with potassium permanganate described in "Molecular Cloning (2nd Ed., E8)" is also incomplete. Therefore, hire a professional disposer to dispose of dilute solution, such as gel stain solution, and concentrated solution used for equilibrium density-gradient centrifugation, treating them both as halogen-containing waste liquid. The dilute waste should be discarded as "H-b waste" described in Chapter 11.4.

A spot contaminated with ethidium bromide shows orange luminescence when ultraviolet light is irradiated in the dark. Although the detection sensitivity is higher when using short-wavelength ultraviolet light, such light is itself carcinogenic. If ethidium bromide comes into contact with the skin (or there is a possibility of it having come into contact with the skin), check for orange luminescence using the long-wavelength ultraviolet lamp and wash the skin with a brush and soap until the orange luminescence is no longer seen.

(2) Nitrosoguanidine

Nitrosoguanidine is decomposed upon heating in an autoclave as an acid or alkali solution. Take special caution never to spill this solution in a space where heat treatment is prohibited.

(3) Ethylmethane sulfonate (EMS)

Neutralize this solution with sodium hypochlorite or sodium thiosulfate solution. According to "Microorganism Genetics Experiment Method," 9.8 mL of 6% sodium hypochlorite solution is used to neutralize 0.2 mL of 3% EMS. If EMS is spilled, wipe it thoroughly with sodium hypochlorite solution wearing gloves.

8.3.6. Extraction of nucleic acid with phenol/chloroform

(1) Wear protective glasses and gloves when treating with phenol/chloroform

When extracting nucleic acid with phenol/chloroform, use a tube made by a manufacturer with a reliable sealing level. Depending on the manufacturer, some tubes that are insufficiently sealed and cause sample leakage may be included in the provided supply, which will require special attention when mixing tubes using a vortex mixer. **Be sure to wear protective glasses and gloves**.

(2) When phenol or chloroform comes into contact with the eye or the skin

If it enters the eye, immediately (within a few seconds) flush the eye with water for more than 15 minutes, and then, see a doctor. **If it comes in contact with the skin, never wipe it with ethanol**. Skin oil will be removed by the solvent, which will cause an even worse burn. Immediately wash it away with water, and then with soap.

(3) Collect the waste liquid

Be sure to collect waste liquid and hire the proper waste disposal firm for its disposal. Do not dispose of it in the tube. Tubes are disposed of as nonflammable substances, which are usually used as landfill. Therefore, tubes with waste liquid will contaminate the environment and pose a danger to the worker.

8.4. Disposal of wastes

Materials including bacteria or touched by similar things are at risk of pathogenic infection. Sterilize all of them before disposing of them appropriately. Artificial recombination of genes should be treated in the same way. Autoclave treatment (121°C, 20 minutes) is effective.

8.4.1. Main wastes and waste disposal method (Refer to 11.6.2.)

-Containers for culture, aluminum foil, glass fragments.

Treat materials used for culture by autoclaving and dispose of as noncombustible. When liquid or solid media are left in the container, separate and wash the container before disposing of it.

Example for waste: Containers used for cell culture and pipettes (plastic) are put in a plastic bag for autoclaving and given an autoclave treatment before disposing of them as noncombustible materials.

-Liquid wastes such as medium

Sterilize liquid wastes if possible in an autoclave container. If necessary, dilute them or neutralize acids and alkali. They should be treated with fungicide including chlorine. Treated by autoclave, it is necessary to neutralize them with sodium thiosulfate beforehand. Solutions which include chlorine give off poisonous chlorine acid gas. Handle them with care.

-Materials designated as an industrial waste, such as injection needles and syringes, are subject to special control. Dispose of them as quasi hospital wastes following the regulations of Kyushu University.

8.5. Guidelines for Research Involving Recombinant DNA, Microorganisms, Animals, Radioisotopes and Radiation generator

8.5.1. Recombinant DNA

(1) According to the safety control regulations of Kyushu University http://www.kyushu-u.ac.jp/university/office/kikaku-bu/kenkyusenryakuka/dna/index.htm), it is necessary for all researchers planning Recombinant DNA experiments to submit their safety plan document to the Recombinant DNA experiments committee in Kyushu University and to undergo screening by the committee and receive the approval of the President of Kyushu University.

Based on the combination of host and donor of nucleic acids, there are two categories. They are institutional approved experiment and ministerial ascertained experiment, and require affirmation by the Minister of Education, Culture, Sports, Science and Technology.

(2) According to the safety control regulations of Kyushu University, researchers who perform recombinant DNA experiments are required to be provided education and training and go through a medical checkup before the experiment. They should use appropriate equipment depending on the experimental level and considerations of safety in order to prevent accidents.

(3) By departmental regulations, it is required that researchers prevent proliferation of recombinant organisms by transfer to research workers and other persons or proliferate to outside within facilities (**Physical containment**). Researchers must carry out their experiments in special facilities classified as follows; P1, P2 and P3 for microbiological experiments; LS-C, LS-1 and LS-2 for large-scale culture; P1A, P2A, P3A and specific rearing compartments for animal experiments; P1P, P2P, P3P and specific isolation chambers for plant experiments. For all laboratories and facilities except P1, advance approval must be obtained for the chief researcher and facilities must bear a P2 (or other) level testing sign.

(4) All researchers must have regular medical checkups complying with a regulation prescribed by the University.

(5) Principal investigator must submit a report to the President at the end of an experiment.

(6) For transfer of recombinant organisms, researcher must report to the President. In the event of any assignment of recombinant organisms, advance approval of the safety director is required.

(7) The department for Recombinant DNA in the Faculty of Agriculture is the Strategic Planning Section (Senryaku-kikaku kakari).

8.5.2. Microorganisms for research

(1) When handling microorganisms for research one is required to give consideration to ensuring safety and to acquiring the knowledge and skills necessary to handle pathogenic microorganisms.

(2) When handling microorganisms for research, the researcher is required to give notice to the head of a department (level 2) or to receive the President's approval for use of those organisms through the Dean (level 3, 4). These levels are governed by the management rule of the University (http://www.kyushu-u.ac.jp/university/office/kikakubu/kenkyusenryakuka/dna/index.htm).

For microorganisms categorized as level 1 which have no risk of causing severe disease in humans and animals, the researcher is not required to notify, however, organisms having a risk of opportunistic infection or disease should be treated based on level 2.

(3) According to the safety control regulations of Kyushu University, researchers who perform microbiological experiments are required **to receive education and training** and to go through a medical checkup before the experiment. They should use appropriate laboratories depending on the experimental level (P2 laboratories for level 2 microorganisms; P3 laboratories for level 3 microorganisms).

(4) Microorganisms used for research must go through sterilization procedures depending on the level.

(5) All researchers must have regular medical checkups complying with the regulation prescribed by the University, and they must have an extraordinary medical checkup in case of an accident.
(6) For conducting research using microorganisms categorized as level 3 or 4, a researcher must receive the President's approval for use through the Dean.
(7) The department for Recombinant DNA in the Faculty of Agriculture is the Strategic Planning Section (Senryaku-kikaku kakari).

8.5.3. Animal experiments

(1) According to regulations for animal experiments at Kyushu University (http://www.kyushu-u.ac.jp/university/office/kikaku-bu/kenkyusenryakuka/animal/index.htm), all researchers performing animal experiments are required to attend lecture(s) concerning animal experiments, to be certified, receive a registration card, and then to register themselves as animal experimental professionals. They should refer to the "Manual of animal experiment, Kyushu University" when performing experiments using animals.
(2) All researchers are required to submit an application for animal experiments and to receive the President's approval for performing the research through the Dean.
(3) The rearing of experimental animals must be pursuant to the conditions that are approved. The researcher must understand the physiology, ecology and habits of the animals, and they must carefully and humanely rear animals and make efforts to contribute the body scientific knowledge. The researchers must also house the experimental animals responsibly and must prevent damage to human life, personal injury, assets and contamination of the environment that might be caused by experimental animals.
(4) Animal experiments using recombinant animals, infection experiments with pathogens and experiments using dangerous chemicals must follow the guidelines for recombinant DNA experiments, handling of microorganisms for research and this handbook, respectively. And these experiments must be carried out in an authorized animal room or animal experiment laboratory that is available and provides physical containment for prevention of pollution and escape.

8.5.4. Radioisotopes and Radiation generator

(1) It is impossible for us to perceive radial rays and X rays without using devices which will detect the rays. We know, however, that a large amount of exposure can pose a serious health risk and gene disturbance. Therefore, use of this equipment must adhere to guidelines and rules to prevent the exposure to the user and third persons.
(2) **Only a researcher who is provided education and training and goes through a medical checkup before the experiment and is registered in handling the facility (radiation controlled area)** can use radioisotopes (RI), ionization radiation and the radiation generator. **In case of multi-year usage, the research is required to receive education and training and to go undergo a medical checkup.**

(3) Every handling facility, RI caddy and radiation generator for ionization radiation or X-ray carry the radioactive sign (Red trefoil on yellow triangle).
Any persons who have not been registered as a user are not permit to enter to the controlled area or use equipment indicated by the radioactive sign. It is prohibited to use the sign without permission.

(Use of unsealed radioactive material)

(1) In general, a person who belongs to Faculty of Agriculture, Graduate School of Bioresource and Bioenvironmental Sciences and School of Agriculture must use unsealed radioactive material at the Radioisotope Center (Laboratory of Hakozaki campus or Semi-high level laboratory). The research must be performed according to the rules concerning the prevention of radiation hazards due to radioisotopes and others of each facility.
(2) Because nuclear species and amount used are settled with respect to each facility, the user should register a suitable laboratory according to each research plan.
(3) All users are required to apply for registration and receive the acceptance of the Dean and to certify registration by the Director of the Isotope Center. The user planning to use these materials and facilities during the next year should complete an application for renewal within the annual period.
(4) When a registered user of RI, he/she must obtain permission from the Director of the Isotope Center before use. Because the maximum amount used per day is set with each nuclear species at facilities, total usage in the facility must be kept at less than the max amount.
(5) When users are using RI, the user must wear a dosemeter and carry out the experiment under the regulations of the facility to keep exposure at a minimum. User should perform the experiment with an understanding of the principles for preventing exposure, which are distance, time and shielding.
(6) At the time of your first experiment, it is recommended that the user perform the experiment without RI (perform a cold run) before undertaking the real experiment and confirm operating procedures, abilities of exposure and contamination. After the simulation, the user should devise countermeasures for problems for using during the real experiment.

(Use of sealed radioactive material)

(1) A person who belongs to the Faculty of Agriculture, Graduate school of Bioresource and Bioenvironmental Sciences and School of Agriculture is permitted to use the equipment containing sealed radioactive material (sealed source) only with permission from the education minister. The research must be done according to the faculty rule concerning the prevention of radiation hazards due to radioisotopes.
(2) All users are required to apply to register for acceptance from the Director of the Department and to certify a registration by the Dean of Agriculture. The user who plans to use these materials and equipment in the next year should complete the application for renewal within the annual period.
(3) For use, permission of the Dean of Agriculture is required. User should submit the experimental plan document and receive permission before use.
(4) Inexperienced users must not independently use the facilities but must carry out their experiment

with an experienced person. Admission of visitors who are not included in the experiment is restricted.

(5) In use, a user must wear a dosemeter and carry out the experiment so as to keep exposure under a minimum. The registry must be supplied.

(6) Because the installation location of the equipment containing sealed source is registered, allowing the equipment and radiation source to move freely is prohibited. In case of redeployment or temporal ablation of a radiation source is caused by repair, the user must get the permission of the Dean through General Affairs Section (Shomu kakari) before moving the equipment.

(7) If you find or presume you have a risk of ionizing radiation injury, you must report this to the office of Faculty of Agriculture or guard's room at Komatsu gate (after office hours). You should refer to the network indicated near the equipment.

(Use of X-ray generator)

(1) Persons who belong to Faculty of Agriculture, Graduate school of Bioresource and Bioenvironmental Sciences and School of Agriculture are permitted to use the X-ray generator in a designated area. Research must be performed according to the faculty rule concerning the prevention from radiation hazards due to radioisotopes.

(2) All users desiring to use this equipment are required to apply for registration with acceptance from the Director of the Department and to certify a registration by the Dean of Agriculture. The user planning to use it during the next year should complete the application for renewal within the annual period.

(3) For use, permission of the Dean of Agriculture is required. A user should submit the experimental plan document and receive permission before use.

(4) Chief administrator concerning the prevention from Radiation Hazards due to X-ray and others is appointed for each service space of the X-ray generator. Authorization by the chief X-ray inspection engineer is set for the equipment and is required for internal operation.

(5) Researcher must use the X-ray generator under the guidance of the authorized chief. User must indicate the machine they will work with prior to use in front of the area. If a lamp for indication is equipped, user must turn on the lamp during use.

(6) When user operates in the controlled area, user must wear dosemeter and carry out the experiment in order to keep exposure at a minimum. The registry must be supplied.

(7) During running of the X-ray generator, admission of visitors who are not included in the experiment is restricted.

(8) The registry must be supplied when running the X-ray generator.

(9) If you find or presume you have a risk of ionizing radiation injury, you must report to the office of Faculty of Agriculture or guard's room at Komatsu gate (after office hours). You should refer to the network indicated near the equipment.

9. Notes on chemical system laboratory

It is possible to cause unforeseen accidents in chemical experiments, so that please pay attention to all operations you do. You should study basic knowledge about chemical completely to do safety experiment. Each basic operation of chemical experiment has meaning, so you examine it before doing experiment. We will explain necessary knowledge on chemical experiment and indicate instructions to avoid an accident. To avoid an accident, you should acquire the following knowledge before doing experiment.

9.1. Clothes

(1) If you do not wear glasses usually, please wear an eye protector because you may lose sight if chemicals are put into your eyes. Do not perform experiments with the naked eyes in the laboratory because chemicals might splash into your eyes from other tables. Moreover, avoid wearing contact lenses because it is possible that contact lenses melt and stick to your face.
(2) When using dangerous reagents, you should wear appropriate protecting gloves to reagents.
(3) Wear comfortable shoes. Do not wear a pair of slippers, shoes with high heels, and boots.
(4) Wear a white coat. Do not wear fancy clothes.
(5) Do not wear accessories.

9.2. Notes on experiments

Organic chemistry experiments require sensitive handling because you use a large amount of inflammable organic solvents such as ether, ethanol, and hexane.

9.2.1. Glassware

(1) Check the cracks of glassware you use. Even though there is a small crack, it causes breakage when you expose it to a vacuum.
(2) Do not expose thin and flat-base glassware to a vacuum.
(3) An accident by glassware is caused when you insert glass tube and temperature gauge into cork stopper and rubber plug. Please refer to chapter six for the operation.

9.2.2. Organic solvent and reagent

(1) Most of organic solvents are inflammable. These may catch fire even for lighter or little sparks and there is a risk to cause a tragedy. Pay attention to hot organic solvents in the flask because there is a risk for boiling and catching fire immediately. Organic solvents may catch fire even quite far from a fire on laboratory board because the ratio of steam solvents is heavier than that of air. Especially, carbon disulfide is dangerous.
(2) There is a case that volatile solvents such as dichloromethane and ether (boiling point is under

40°C) may blow out the plug by the evaporation even in sunlight.

(3) Dry the paper absorbed inflammable solvents and desiccating agents such as sodium sulfate and calcium chloride in the draft before disposing of it in the specified area.

(4) Try not to inhale the vapor of solvents. If you feel dizzy when using solvents, you should stop the experiment and take a rest in the fresh air. Chemicals should not be attached to the skin. Organic solvents are easily absorbed from the skin. Pay attention to the sodium cyanide because it is absorbed from a wound site. Do not take any chemicals in the laboratory into your mouth.

(5) Handle toxicity gas and fuming reagents in the draft. Devices are treated appropriately after using.

(6) Concentrated sulfuric acid and concentrated sodium hydroxide should be washed by a large amount of water because these damage not only clothes but also laboratory board. When you spill it in large amounts, you make it cold and diluted, and neutralized. In the case of acid, it is treated by $NaHCO_3$.

9.2.3. Device/Reaction

(1) The devices should be assembled with instruction and firmly fastened after placing center of gravity down.

(2) When heated, make a hole to prevent the pressure inside from increasing.
It is better to use a pressure buffer such as air ball. Moreover, when putting calcium chloride drying tube on the reflux tube, be careful not to be aggregated by damp.

(3) When liquid boiled, boiling stone should be added. If you forget to add it, you should add it after cooling the temperature under the boiling point. If you add a boiling stone at high temperature, there is a risk that liquid may come to the boil and explode intensely. Moreover please do not add an activated charcoal to the heated liquid. If it comes to the boiling point, liquid is ejected to the ceiling.

9.2.4. Others

(1) In the case of an accident, you rely on a reliable person who has judicial type of mind.
You should do experiments with others at all times.

(2) Keep your laboratory table clean. You should care about second accidents.

(3) You should predict an accident caused by chemicals you use before doing experiments.

10. Measures for safety in the field work

There are various research and practical training in the field work of agricultural department. Measures which are different for safety depend on their aim and content. Sometimes, field work distracts attention away because you are in a different environment from a daily life. There is a risk that causes tragedy by the lack of experience. Even though you have enough experience in the field work, you should not count on yourself.

10.1. General rules

(1) Guide of practical training

In the case of field work, you should follow the instruction of teachers. Practical training has a broad range of contents. The guidelines you should follow are different between practical training in the farm, forest and oceanfront training. If you have any questions, please do not hesitate to ask teachers something to know before training.

(2) Clothes

You should wear a long sleeve shirt and trousers to protect against sun burn and insects even in summer and wear a hat to protect against heat. It is effective to cover your neck by wearing a towel to protect against solar heat. If you go to mountains in the hunting season, you should wear brightly-colored clothes to prevent a misfire incident. In winter, you should dress warmly. In the extremely cold season, it is better to use a jacket or coat than to wear several layers of clothing. Moreover it is necessary to wear long socks to protect your ankle from dangerous animals.

You should not wear high heeled shoes or pumps. It is better to wear trekking shoes. Athletic shoes or tennis shoes should not be worn in the rocky place or mountain stream.

There are bees or woolly worms in the mountain and needles which hurt you. In the beach, there is a risk of fall and hurt your hand by oyster shell. Therefore, you should wear gloves.

(3) Baggage list

During the field work, you should keep your hands empty. If you have belongings, you use a backpack. Do not carry a handbag or haversack. Even though you have research equipments, you should use only one hand and keep your foot. When it is raining, you should wear a rain suit or have a folding umbrella. In summer, it is better to carry water to avoid heat stroke. Moreover it is better to carry medical goods such as band-aids and antiseptic substance.

(4) Act in the field work

You should follow the instruction of teacher during the field work. If you go from teacher's sight, you should act with others. The most important thing for fieldwork safety is acting slowly. Please be aware of the importance of safety on each training program by confirming with your supervisor.

(5) Lodgment

Observe lights-out time to prepare for working of next day and do not cause trouble with neighborhoods

There is a risk that causes lack of sleep, hangover and heat exhausted. It is better to avoid sleeping in a tent. If you stay outside, you should research well beforehand.

(6) Dangerous animals

Please do not touch wild-animals in the field work without reason. There are lots of precious animals that are in danger of disappearing. Also there are dangerous wild-animals such as hornet, adder, tiger keelback and akajei. Please follow the instruction of teachers to protect such animals. You should keep off carcass because there is a risk of catching infection diseases such as bird flu.

Fig. 10-1. Venomous creature

(7) Graduation thesis research/Graduate school research

When you do a field work research in your graduation thesis, you have to pay attention to the safety and act in a level-headed manner. Please do not overestimate yourself. Consult with a teacher and consider adequacy and safety of the research plan. Moreover you should make an application for special permission about collection of wild animals if necessary.

Observe rules in the laboratory-level and guidelines of each academic society. In the case of using an adjunct facility, you make an application for special permission.

10.2. Research in the sea and river

(1) Clothes

It is necessary to wear a wet suit (Fig. 10-2 left) or life jacket. It will keep certain force of buoyancy in deepwater. High boots make you go to deep water level for researches. Using a dry suit (Fig. 10-2 right) makes you stand on your head in the water because of allowing air into the foot area. So, you should evacuate the air before you go into the water. Shoes with felting bottom such as Japanese style socks (Fig. 10-3 AYU-TABI) are effective and can avoid danger from the bottom of river. Wearing gloves is also effective. It is necessary to wear a helmet in the cliffy area.

Fig. 10-2 Wet suit and dry suit

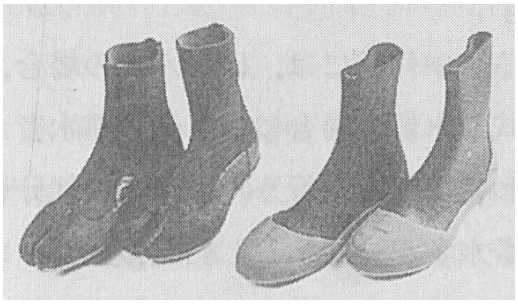


Fig. 10-3. Japanese style socks (AYU-TABI)

(2) Pay attention to safety ways in the river

Even in the shallow river, you can be swept away. Even in the slow river, it is possible that the depth of water is increased. It is necessary to make preparations for safety.

You have to confirm the weather condition before starting research and doing researches even if the weather is clear. When there is a dam in the upper of river, there is a risk of high water. Pay attention to watering alarm.

(3) Pay attention to safety ways in the sea

In the sea, pay attention to the condition of waves. In high waves, it is not only dangerous but also affects the working efficiency. You may be swept off your feet by waves on the beach or ledge. In case of an earthquake, there is danger of tsunami. Bring a radio in the place where you can not listen to community wireless system by autonomy. In case of an earthquake, you have to return to the beach and confirm tsunami information and evacuate if necessary.

(4) Sea-tide

Pay attention to tides in the sea. Make a research plan in consideration of the tides condition. Incoming tide makes waves lead from off-island to shore, and falling tide makes waves lead from shore to off-island. At the dry beach, there is a risk that the tide comes in. If you research off-island, you have to act with time to spare.

(5) Watch where you walk

Some of the rocks in the mountain stream can be slippery by the moss. Shingle bottom in the middle of river can be not only slippery but also may make you tumble over because shingle bottom is easily crumbled by your weight. You may get in the sand in sand-clogged place just after work of construction. Your feet may sink in the sand in silt-clogged estuary. If you get in the mud, you should fall on your knees. Rocky shore can be slippery by marine plants. There are many dangerous and harmful things in the bottom of river and sea such as glass chip, oyster shell, and acorn shell.

(5) Others

There are many dangerous wild animals in Kyushu such as hornet, adder, and tiger keel back of the river, and akajei, plotsus lineatus of the coast and estuary. Be careful of lightning. There was a case that some people were dead in the river and sea during research. You should not do research by yourself, and you should call for help immediately in case of an accident.

10.3. Shipboard, dive, snorkeling

(1) Shipboard

Wear a life jacket that has a certain amount of buoyant on shipboard and wear safety boots and cotton work gloves if necessary.

While the vehicle is moving, hold the handhold tightly. Do not get seated on the side of the ship. It is necessary to be cautious when going by an oncoming ship due to the ship swings.

Unfixed observation equipments, brought from outboard, may be moving. Moreover, hat and towel may get blown. While you work, be cautious not to get your hands, feet and clothes caught in wire, cable and rope. Do not throw your body overboard when you use a rope and cable.

While you work on the shipboard, you should follow the instruction of captain and confirm the information of weather condition and ocean weaves. When going by an oncoming ship, the big wave may come, so you should be cautious about handling. If you get sea sick, you should take turns

working. Using the ship that each university owns, you should get permission of the university office. Please contact the office beforehand.

(2) Dive and snorkeling

Diving operation needs a lot of experience. It is necessary not only to take a license of scuba diving and diver but also to have a lot of experience of dive. Buddy system is necessary. Snorkeling does not need a license, so you should have self-management for safety because there are not enough rules about snorkeling. It is better to have a license of scuba diving or diver. Diving is very special operation, so read through “Kyushu University Diving Regulation” carefully.

10.4. Research at the mountain

10.4.1. Research at the mountain

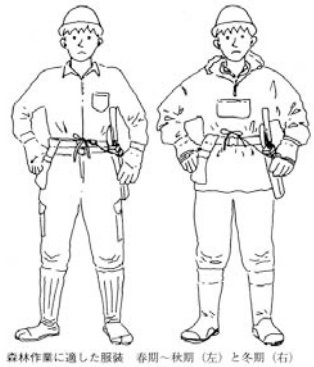
(1) Clothes

Wear a hat or helmet and long sleeve shirts and trousers to protect your head from fall of rock in the mountain. Moreover wear mountain climbing boots or trekking shoes and carry rain apparel. Heavy-duty backpack is recommended. It is necessary to carry leather gloves or cotton work gloves. Cell phone, map, handy GPS, and field note are needed. According to circumstances, water, food, flash light are needed. In case of camping or climbing a snow mountain, choosing appropriate clothes with extra caution is required.

As an emergency contact tool, cell phone is effective. But there are many places you can not use a cell phone because of outside cell phone communication area in the mountain. You have to research the area if you can use a cell phone beforehand. In case of an emergency contact by cell phone, it is necessary to inform the disaster area clearly. Make sure the cell phone has not run out of battery.

(2) During research, before and after research

Contact an administrator of the mountain beforehand and get permission to enter the mountain. If you are late to arrive at lodgment, please contact an administrator of lodgment. Entering the mountain, please carry an emergency contact number such as administrate office number and hospital number.



森林作業に適した服装　春期～秋期（左）と冬期（右）　（川辺書林「森を育てる技術」より）

Fig. 10-4.　Clothes for working in the mountain

Collecting information beforehand and communicating with an administrator of mountain are needed. Only persons who are well-seasoned can use a chainsaw for cutting trees or grass cutter. You should learn how to use cutting machines before using them. You should enter a mountain with others. If you have no other choice but to go into a mountain by yourself, you have to contact the management office or research education institute often. Going by car to a research area, keep away from road shoulders on the forest road because there are undeveloped crash barrier or collapse prone road shoulders. Excessive speed may cause an accident. It is easy to slip on the dirt road and animals may dash to the road. Do not drive at night if you drive on the forest road for the first time. Be cautious to walk on the forest road because there is a cliff or steep terrain on the side of the road. It is easy to get lost in the forest so please do not walk on the road by yourself. If you get in the forest at night for animal research, please get permission of forest management office. Confirm the dangerous point in the daytime beforehand and avoid walking alone. There are lots of dangerous animals in the forest, please avoid getting in the bushed area. There are many cases to be dead from the bite of a hornet. Wasp venom allergy must be checked beforehand and you should know how to deal with the problem in case of the bite from a hornet.

Your skin may get poison ivy from anacardiaceous such as Rhus ambigua. This disease may be preventable to cover your skin carefully. Get a permission of a forest management officer before working in high place where you climb up a tree or steel tower. Wear a safety belt, helmet and harness if necessary. When going up or down a slope, you grasp a rock or tree. Do not keep your weight on the dead wood or float stone. When going up or down a slope, do not stand on the slope vertically. Especially when going down a steep slope, you go down slowly with facing a slope. Going down a slope is more dangerous than going up.

Please be cautious not to throw a stone on the upper side of a steep slope or a cliff. If there are workers, houses and roads at the bottom of the cliff or steep slope, give your full attention to it.

If there are falling rocks, you should shout “Rakuseki” (Falling rock) .

Fig. 10-5. Vespa ducalis

Weak aggression and poison comparatively.

Strong threatening conduct

(Left : on the leaf, right: killed by insectcide)

Fig. 10-6. Rhus ambigua

Left: mixed community with other leaf, right: juvenile leaf)

Pictured by 山と渓谷社「樹に咲く花」

10.4.2. Research in urban neighborhood and farming and mountain village

There are many accidents in urban neighborhood and faming and mountain village. Depending on the condition of land or the purpose of research, clothes or devices you should wear for safety can be changed. For example, you are recommended to wear comfortable shoes when walking for a long time for the research. In the construction area or river area, it is better to choose clothes that may become dirty. At the hearing research in farming and mountain village, wearing high heels or skirt is not appropriate even though meeting place is an office because the research could be conducted in a field, mountain forest, or plastic greenhouse. Sometimes, people working at the food processing institute or cattle farmer do not like perfume and long nails. So, you should pay attention to your appearance. Recently, as bird flu is especially prevalent in the world, researchers from outside are considered as a possible cause of flu. Please follow the instruction wearing a mask, hat and white robes and sterilizing hand and shoes. You should not give neighborhoods troubles. As you meet with other people in the research circumstance, it is assumed that your research devices may be stolen or you may hurt other's devices. Pay attention to your safety and other's safety and prepare for accidents before doing research.

10.4.3. Research in the disaster

It is necessary to have portable GPS, helmet, and cotton work gloves in the research of volcano or slope collapse area. Moreover you should have a gas mask, gas detector, and goggle and wireless application in the area of crater or eructation. Volcano gas is heavier than air and remains in hollow. Please avoid going to the hollow when there is no wind.

In the research of national park or quasi-national park, please get permission from the environment ministry or park management office beforehand. When doing research in the national forest, file proper records with forest management office.

In off-limits area, you should get permission from each autonomous community and collect

information about the weather and volcano beforehand. Deploy people in the safety area and contact them when you enter and leave there. When you handle the electricity at the disaster site in the district of residence, you should wear rubber gloves. Wearing trekking shoes is better. When you carry heavy things, you should wear safety shoes. Especially, collapsed buildings and wasted materials contain edged tools, so it is better to wear safety shoes with iron in the shoe sole. During disaster-relief service, you should not do research in the area. In case of an earthquake or sediment disaster, there may be a second disaster. Collect enough information before going to the research area. In case of sediment disaster, if you are not specialist of it, you should be away from a dangerous slope. During the summer, you should be careful of heat stroke.

10.4.4. Other notes

Be careful of lightning strike in a mountain or large grass field during the summer. When lightning is coming, bend forward and wait the lightning go away. It is safe to go into the car. It is important to collect the information about lightning from the weather news on the radio. Working environment sometimes influences your health. For example, you become heat stroke under the scorching sun, and become hypothermia in the cold area. Do not drink spring water without permission. Pay attention to the disease such as Japanese fever river and echinococcus, and be careful of the dangerous wild animals such as wild bears and feral dogs. In case of unforeseen accidents, you have to be committed to your safety primarily, and all participants, and contact a responsible person and your college. When getting lost, you should go up to a mountain ridge you can look around and try to find a way out. Use a cell phone. If you get lost completely, refrain from moving around. It is better to stay there. Do not move at night. There are many cases where the mountain stream is shut by dam or waterfalls. By walking a lot, you are needlessly exhausting yourself. Do not go down along a mountain stream without reason.

Refer to the following website.

"Field Research Safety Manual"
Edited by Ecological Society of Japan, Field Safety Management Committee
http://www.esj.ne.jp/safety/manual/

10.5. Overseas Research

10.5.1. Introduction

In the overseas research and field working, it is possible to be killed by accidents or diseases because there are dangerous animals, diseases such as endemic, and public health problem due to poor hygiene. Collect information of target areas or regions as much as you can and be cautious about health management, hygiene control, and accident prevention. Instructors have responsibility for the student safety management and accident prevention, and students should observe general regulations even

though the teacher does not attend your overseas research. In case of an accident, you stay calm and make the best effort for the rescue and contact your instructor or institute. The instructor and institute should give support appropriately.

Notes on safety management and accidents prevention
-Make an appropriate plan and notification has to be done before going abroad.
-Change your plan depending on the conditions.
-Ensure an emergency contact number.
-In case of an accident, you stay calm and require for help in accordance with the emergency contact system.

10.5.2. Plan and notification

(1) Plan

Consult your instructor for your plan and write “oversea plan” before making your plan by yourself. Writing “oversea plan” makes you collect information, research the condition of your area, and predict dangerous matters. There is a case that overseas research can not progress as you think by change of the condition. Make preparations in good time and add some more research plans according to the situation. Moreover, ensure your emergency contact number in the field beforehand. Beginners should work with experienced people and learn the method of overseas research from them. To go to a dangerous research area, you work in a team centered on experience people.

(2) Notification

When students go abroad, they have an obligation to inform the Student Section of School of Agriculture. Teachers need to submit “Overseas travel notification” and its schedule to the General Affairs Section. “Overseas travel plan” and “Schedule” should be provided to your instructor, related offices and your parents beforehand.

(3) Preparation

Disease prevention

Collect information about disease prevention one month before going abroad. Receive a vaccination if necessary after consulting a doctor. There are quarantine disease vaccine, basic vaccination, and voluntary vaccination. When you go to an area where malaria is present, you can get a medicine for malaria prevention but it is necessary to confirm a period after injection whether there are side-effects or not. If you have previous disease, fill out your health condition on “Overseas travel plan.” Avoid lack of sleep and go abroad in good health. You carry personal medicine.

Insurance

It is the responsibility of everyone to have “personal accident insurance for student pursuing education and research,” however, the expense for medical treatment and hospital, rescue at the time of

accidents may not be covered enough. It is better to have “overseas travel accident insurance.” For the area of insurance coverage, make sure you include the expense for a disease, rescue, changing the plan of travel, emergency temporary return.

Other preparedness

Consult an experienced person about the necessity materials for research, equipments, and procedure. Adequate confirmation of operation capability is needed beforehand especially for carrying equipments required preliminary check. There is different danger lurking in overseas research from in domestic research. Contact a counterpart closely, and collect information about the field from the website (Foreign Ministry overseas security HP: http://www.pubanzen.mofa.go.jp/). In preparation for emergency, carry the phone number or address at your local embassy or consular office provided by Foreign Ministry HP. Moreover the copy of overseas plan, airplane ticket, and overseas travel accident insurance should be left to your family and laboratory.

10.5.3. Stay

(1) Secure security

Confirm the contact number to Japan or counterparts, and know the place, facilities, the contact number of local ambulance, police and hospital. Prepare for having local information by internet, newspaper and TV. Understand a foreseen risk and have a meeting about the safety beforehand. Change or reschedule research plan due to the weather condition. When you are caught in thunder storm during outdoors research, evacuate to a safer place immediately. Do not drive in a research area. If any, rent a car with a driver. Do not use a bike or bus. If you have no other choice but to use a bike, wear a helmet and be aware of a car accident and crime. Observe the local low and pay attention to the local religion. In an accident, get a document required by the insurance company. In case of a disease or injury, a medical certificate and receipt are needed. In case of a theft, a police certificate is needed.

(2) Health management

Time differences and different dietary life may damage your life and you may be easily infected with a disease. The management of dietary life and sleeping becomes very important. In some areas, there are many causes to damage people’s health such as hygienic condition, dangerous animals, and endemic disease so be careful of health damage. Pay attention to food, water, ice pool, and river thoroughly.

In poor physical condition, stop a research immediately. Oversea research requires a lot of energy and needs a rest and health management more than domestic research. In poor physical condition, contact a teacher or counterpart and go to hospital. At the same time, contact your laboratory immediately in Japan. A disease transmitted by a mosquito such as malaria, yellow fever, and dengue fever is spreading, so please be aware not to be bitten by a mosquito. Canine madness is also spreading all over the world except a part of area, so pay attention to wild dogs.

(3) Contact between teacher and student

Teacher and student should exchange the contact numbers and student contact teacher in the following cases.

-When disaster or serious accident happened.
-When a student is in an accident or disease.
-When changing a schedule.
-When arriving at the area.
-Around the time of leaving the area.
-Before the time of leaving the area.

Teachers should contact students constantly. If there is an emergency call from a student, the teacher contacts a department head immediately.

10.5.4. After returning to Japan

After returning to Japan, take care of your health. There are diseases you found developed one month after you returned such as malaria and type A hepatitis.

When you feel physical damage, fever, and digestive trouble, you should consult a doctor immediately. When you have a fever or diarrhea in airport, you go to the quarantine station to consult a doctor. Submit necessary documents immediately after returning to Japan.

10.5.5. Others

It is effective to use a cell-phone for long and short stay. Use a cell-phone rental service for travelers or carry a GSM mobile phone and buy a SIM card at the site. Moreover there is a SIM card having a universal number. Select an appropriate way depending on the term of research or the case of the field. In the case of long-stay or visiting the field many times, it is better to buy a cell-phone at the site. Contact an information center about traveler's insurance. Some credit card companies offer the overseas traveler's insurance, so it is recommended to contact a customer service in advance.
Refer to the following website.

http://www.anzen.mofa.go.jp/manual/index.html（Safety guide for Japanese living overseas）
http://www.anzen.mofa.go.jp/index.html（Foreign minister overseas safety HP）
http://www.forth.go.jp/（Ministry of Health For Traveler's Health）
http://www.cgiar.org/（CGLAR: Consultative Group on International Agriculture）
http://www.mobistar.jp/（cell-phone rental service for travelers）
http://www.mobell.co.jp/simcard（World SIM card）

11. Disposal of wastes

We use various chemicals in the University. When you conduct an experiment using chemicals in the laboratory, you must handle them and the wastes properly. Release of harmful waste solution to the environment causes pollution to groundwater and their accumulation.

As the Japanese law requires everyone to perform the proper waste disposal, you need to know the property of the wastes well. It is the responsibility of the person who generates the waste to dispose his/her wastes properly.

In Kyushu University, rules regarding effluent standards and ways of waste disposal have been established in the "Rule for effluent and waste managements." (http://www.kyushu-u.ac.jp/university/rule/zenbun/2004kisoku104.pdf)

11.1. Classification of the laboratory wastes

Except for some special substances such as PCB, the laboratory wastes can be processed using any method classified in Table 11-1. The liquid waste is classified into inorganic and organic wastes (Table 11-1, 11-2 and Fig. 11-1). Especially, inorganic liquid waste that is collected by Special Liquid Waste Processing Facility, Kyushu University once a month is called "special liquid wastes". As described below, other inorganic liquid wastes except for the special liquid wastes, or some organic liquid wastes and harmful solids that cannot entrust to the agents must be processed as "Chemicals being discarded" once a year.

Table 11-1. Classification of the laboratory wastes

Category	Group	Code	Collecting method, Collecting day
Particular liquid waste (inorganic)	inorganic mercury	A	20 L Specified plastic container First Tuesday in every month
	cyanide and arsenic	B	
	fluoric	C	
	heavy metals	D — a	
	heavy metals and organic substances	D — b	
	photographic fixer	E	
Organic liquid waste	Halogenated organic solvents	H — a	Drum can in the collecting place The day 10 in every month
	Other organics	H — b	
Regular pickup	Chemicals being discarded (dangerous and harmful solid and liquid)		November (submit the list in summer)
	Pseudo-medical wastes		June and December
Separated garbage in the lab.	burnable garbage (include the plastic wastes)		carry out to the garbage yard Entrusted agent collects them as needed
	harmful chemical-adhered matter (both burnable and nonburnable)		
	bottle (cleaned chemical bottles)		
	no burnable garbage		

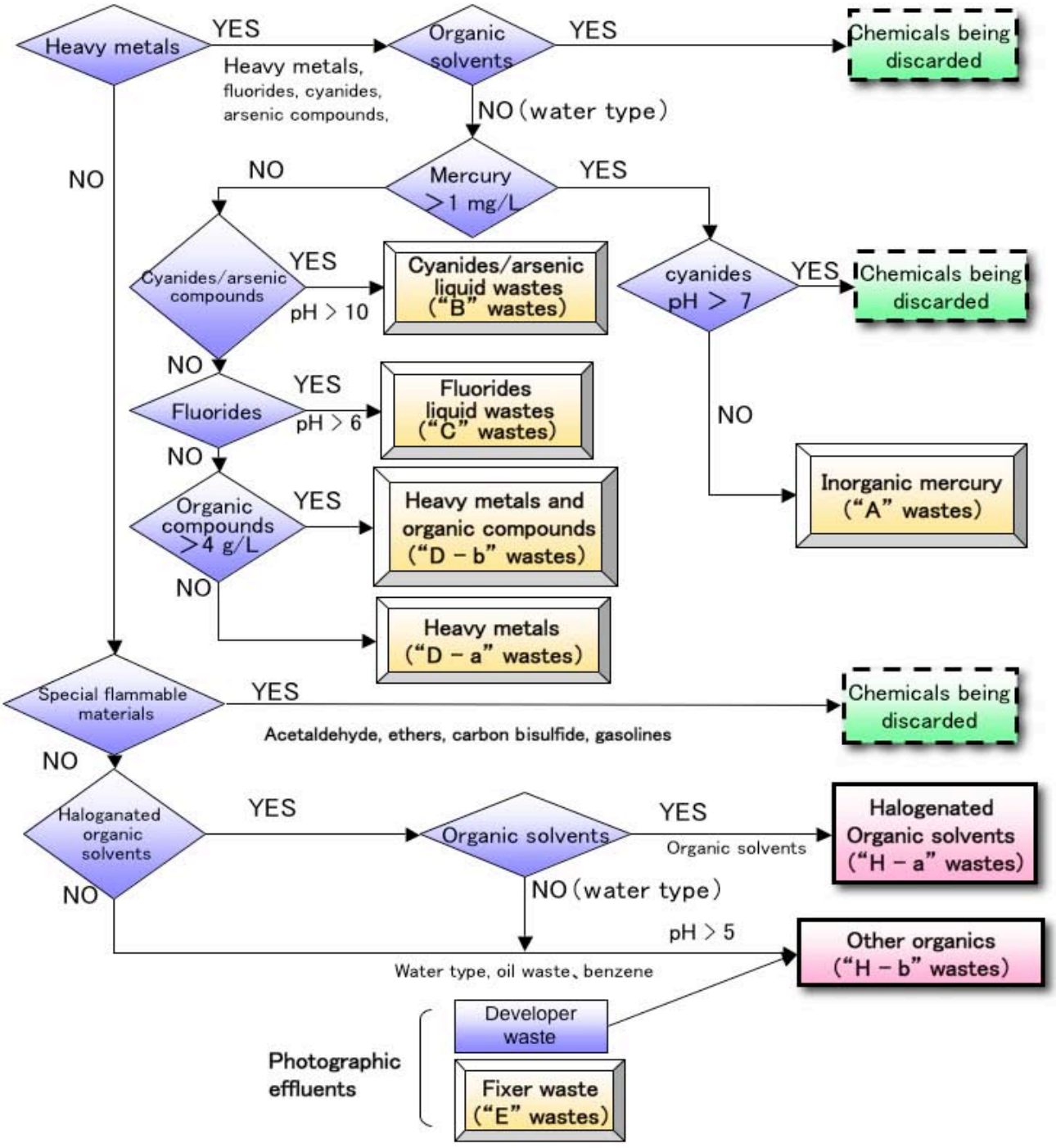


Fig. 11–1. Classification of liquid wastes

: Particular liquid waste

The special liquid wastes must be pooled in the rectangular 20 L polyethylene-made container. The collection is conducted at 10:00 to 10:30 on first Tuesday in every month.

: Organic liquid waste

The collection is conducted at 10:00 to 10:30 on the day 10 in every month.

: Chemicals being discarded

The collection is conducted in November.

11.2. Disposal of inorganic liquid wastes (special wastes)

To handle various kinds of liquid and solid wastes generated in the experiment in the laboratory safely, you need to prepare their separate containers appropriately according to their property, toxicity and hazardous natures. In addition, you must hand them over to the Faculty personnel of the Special Liquid Waste Processing Facility, Kyushu University or to an outsourced personnel.

11.2.1. Acidic and alkaline liquid wastes

You can release acidic and alkaline wastes that are free from a heavy metal or organic substance, by neutralizing them to pH 5-9. If it is strong acid or strong alkali, you can classify them as "heavy metal including waste solution (special waste solution)" when it is dangerous to do neutralization.

When the acidic or alkaline waste includes any organic substance, you can classify them as "other organic liquid waste" and neutralize it to pH 5-9, or "Liquid waste with heavy metals and organic substances" even if it includes no heavy metals when it is difficult to do neutralization.

11.2.2. Liquid waste with inorganic mercury (special liquid waste)

When liquid waste includes any heavy metal, it must be classified as "inorganic mercury liquid waste".

The mercury liquid waste with cyanogen or arsenic compound is classified into "Chemicals being discarded" when it is alkaline. Don't neutralize because of the danger of the hydrogen cyanide (cyanide fume).

The amount of soluble organic substances does not matter.

11.2.3. Organic mercury

The liquid waste with organic mercuric compound such as alkyl mercury and phenyl mercury must be commissioned to the outsourced processor as "chemical waste, etc" or classified into "inorganic mercury liquid waste (special liquid waste)" by pre-treatment as follows;

Alkyl mercury --> After adding HCl to change to acidic waste, reduce it to metallic mercury by adding aluminum powder or iron powder, and leaving it overnight.

Phenyl mercury --> Make an oxidative degradation by adding diluted sulfuric acid and potassium permanganate solution, and heating for 2 to 3 hours at 70 to 90°C.

11.2.4. Liquid waste with cyanogen and arsenic (special liquid waste)

The liquid waste with free cyanide and arsenic compound such as cyanide, cyanic acid, thiocyanic acid, thiocyanate, hydrogen arsenide and arsenic acid must be stocked after you make it more than

pH10. Heavy metal and organic substance can be included.

When heavy metal liquid waste includes cyanogen or arsenic, you must classify and store the waste as "Liquid waste with cyanogen and arsenic". On this occasion, the amount of mercury must be 1mg/L or less and that of fluorine must be 15 mg/L or less.

Do not mix the waste with acidic liquid waste.

11.2.5. Liquid waste with fluorine (special liquid waste)

The liquid waste of fluorine compound such as fluoride or silicofluoride must be stocked to make it pH 6 or more.

The amount of mercury, cyanogen and arsenic in the waste must be 1 mg/L or less, respectively.

11.2.6. Liquid waste with heavy metals (special liquid waste)

Toxic liquid waste with heavy metals such as berylium, osmium, thallium and selenium is treated as "waste chemicals for outsourced processor". When it can be determined as low concentration and low risk, you can classify it as "Liquid waste with heavy metals and organic substances".

The content of the following: mercury, cyanogen and arsenic must be 1mg/L or less, that of fluorine 15 mg/L or less and that of organic substance 4 g/L or less.

11.2.7. Liquid waste with heavy metals and organic substances (special liquid waste)

The liquid waste with more than 4 g/L organic metal compound, metal carbonyl, chelating agent such as EDTA and polyamine or organic substances is classified into this category.

The contents of mercury, cyanogen and arsenic must be 1mg/L or less, respectively, and that of fluorine 15 mg/L or less.

The organic substance is restricted to water-soluble. Separated oil layer in it must be removed before handing over to the Special Liquid Waste Processing Facility, Kyushu University.

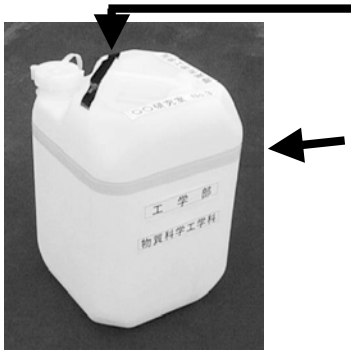
11.2.8. Photographic effluent

Fixing solution (special liquid waste) --> Argentine must be recovered from it. Set it aside from the development waste.
Photographic developer --> Store it as "other organic liquid waste".

11.3. Disposal of the special liquid wastes

11.3.1. Method for fractionation and pool

The special liquid wastes must be pooled in the rectangular 20 L polyethylene-made container (270×270×385 mm, over 1.7 mm in thickness). Contact a special liquid wastes facility regarding purchase of the container.



Attach the 15cm or more black tape here for organic substances 4 g or more and liquid wastes including chelate agent.

Yellow tape for liquid wastes

*Write the laboratory name, number of affiliation (medical management system login number), container number on the upper aspect.

example：○○laboratory 940401 No.3

*Write the name of department on the side of container.

(1) Each liquid waste is distinguished by the colored tape wrapped around the upper part of the container.

red: mercury
blue: cyanogen and arsenic
brown: fluorine
yellow: heavy metal
black: fixer
yellow and black: heavy metals with organic substances

(2) The volume of the waste in the container

The level of the liquid waste in the container must be less than the level of the colored tape described above.

(3) The storing of the container

Uncapped or uncovered container or broken container can not be used for the processing. You must make a check the packing and the inner cap to prevent spilling. In addition, you must always clean the outside of the container.

Deteriorated container should not be used and must be processed as "experimental burnable garbage" or "Harmful chemical-adhered material". Keep the container out of direct sunlight.

11.3.2. Method of disposal processing

(1) Inspection before the request

Check the following points in the laboratory before requesting for processing.

1. Whether or not the liquid waste undergone appropriate processing such as pH adjustment?
2. Is the volume of waste less than the level of attached colored tape on the container?
3. No leak of the waste?
4. Is the outside of the container clean?
5. Is the colored tape for the identification wrapped around the container?
6. Are the organic solvent, oils and fats separated form the liquid waste on the surface or bottom layer?
7. Is there any solid substance floating or precipitated in the container?

   If there is any solid substance in the container, you must clear it off with net or other appropriate tools.

(2) Preparing the forms for the request of special liquid waste processing and submission to the Facilities Affairs Section, Faculty of Agriculture (Nogakubu Hozen-kakari)

When faculty staff in the laboratory requests for waste processing, fill in the form and submit it to the Facilities Affairs Section, Faculty of Agriculture (Hozen-kakari). The form can be downloaded from the following webpage（http://kan-an.jimu.kyushu-u.ac.jp/download-muki-denpyo.htm）.

You have to fill in the login ID (6 digit number) of reagent management system since 2008.

The form must be written in Japanese. Usually, laboratory staff prepares the document. If you cannot use Japanese language and you need to prepare it, ask a Japanese staff in your laboratory for help.

無機系廃液 処理依頼伝票 ［センター保管用］

＿＿＿＿＿＿＿＿＿＿＿＿ 月 ＿＿＿＿ 日

部局・研究院		学科・部門		【内 線】	
講座名			所属等整理番号		
下記の廃液の排出責任を負いますので、処理方お願い致します。 【職　名】　　【取扱担当者】					

分別記号	廃液名	容器番号	含有物質名とその重量（g、mg）又は 濃度（%、g/L、ppm）	pH	缶数

				計	

農学系保全係へ、処理依頼伝票及び受入伝票とも提出して下さい
所属等整理番号:通常は薬品管理システムの「所属 Login ID(6桁の数値)」です。

(3) Collection of the special liquid wastes, pickup and return of the empty containers

The special liquid wastes form in your laboratory will be carried to the collecting place (P.108) according to the instruction by the Facilities Affairs Section, Faculty of Agriculture (Nogakukei Hozen-kakari). The collection is carried out morning of the first Tuesday every month. If the day is holiday, it is the next day. The empty container will be returned to the collecting place the next month, you must pick it up according to the Faculty personnel.

(4) Return of the liquid wastes that cannot be processed

The liquid wastes that cannot be processed, and did not meet conditions for disposal or have incorrect information in the form will be returned to you with the form along with the reason. You must take them back and request again after appropriate pre-processing.

Table 11-2. Special liquid wastes

Code	Liquid waste name Categorized tape	Conditions
A	Inorganic mercury liquid wastes Red tape	Inorganic mercury liquid wastes Include heavy metal or water soluble organic matter. Alkali liquid wastes including cyanide is categorized “wastes”
B	Cyanide or arsenic liquid wastes Blue tape	Cyanide or arsenic liquid wastes Heavy metal other than mercury and water soluble organic matter and cyanide. conditions: pH > 10、Hg < 1 mg/L
C	Fluorine liquid waste Brown tape	Inorganic fluoride such as fluorine、silicofluoride Heavy metal other than mercury, water soluble organic matter. conditions:pH > 6、Hg < 1 mg/L、cyanide・arsenic < 1 mg/L
D — a	Heavy metal Liquid wastes Yellow tape	Heavy metal including cadmium, lead, chrome, copper, zinc, iron, mangan, nickelic, cobalt, tin. conditions:organic substances < 4 g/L、Hg < 1 mg/L, cyanide, arsenic < 1 mg/L fluorine < 15 mg/L

D — b	Organic substances Heavy metal liquid wastes Yellow＋black tape	Organic metal, metal carbonyl compound, EDTA, chelate such as polyamine or heavy liquid wastes including organic substances 4 g or more. conditions：Hg < 1 mg/L
E	Photographic fixer wastes Black tape	Notes: Photographic developing wastes, ammonia water, diluted solution for slide processor “other organic liquid wastes”

［Notes］

1. Elimination of solid material: When there is a solid material or sediment in the liquid wastes, filter it with a mesh, like nets for catching insects. The sediment should be handled as wastes chemicals.
2. Removed oil: When special liquid wastes are separated organic solvent and lubricating oil, please remove the oil. Removed oil is processed as other organic liquid wastes (H-b).
3. Contaminant prohibited materials: nuclear fuel material, radioactive ingredient, and contaminated materials by pathogenic organism. Chemical materials caused odor or health disorder are handled as “wastes chemicals.” If it is diluted and probably not dangerous, it is handled as “Heavy metal liquid wastes including organic substances.

11.4. Disposal of organic liquid wastes

Organic solvent waste, hazardous organic solution, dense detergent waste and oil waste are classified as organic liquid wastes. Especially, you must handle the volatile organic compound such as dichloromethane and benzene because of the strict sewer standards by Fukuoka-city. The water containing the organic solvent must be processed as "organic liquid wastes, others".

11.4.1. Notes about the separated storage of the organic liquid wastes

When you store the organic liquid wastes in the same place, you must check whether it conforms to the Fire Service Law or not and follow the instructions by a dangerous object handler. Keep them from places where there might be sudden rise of temperature.

You need to use appropriate and safe container for the contents and do not use the polyethylene-made container for the inorganic liquid wastes or mix them.

When the waste is polymerizable compound, high polymer compound or tarry substance and it may form precipitate during the storage or by mixing with other wastes, do not mix other substances with the organic liquid wastes.

11.4.2. How to request the disposal of organic liquid wastes

Regarding the organic liquid wastes described below, bring them to the collecting place for organic liquid wastes and transfer them into the drum can according to the instruction of the Facilities Affairs Section, Faculty of Agriculture (Nogakukei Hozen-kakari) after the submission of the "application form for the disposal of organic liquid waste" by the end of the month. The form can be downloaded from

the website.（http://www.agr.kyushu-u.ac.jp/noujimu/）

Usually, the form is written by laboratory staff.　The collection is conducted at 10:00 to 10:30 on the 10th day of every month.

11.4.3. Notes at the request

-The acidic waste should be neutralized to avoid the corrosion of the drum can.

The organic solvent containing heavy metals whose concentration is more than the level of effluent standards should be classified as "Chemicals being discarded".

-Heavy metal solution with 4 g/L or more of organic substances should be classified into "liquid waste with heavy metals and organic substances (D-b)" in the special liquid wastes category.

-Special flammable materials that have ignition temperature of 100°C or less, burning point of -20°C or less or boiling point of 40°C are classified as "Chemicals being discarded".

有機系廃液　処理依頼書

提出日付　平成　　年　月　日

保全係担当者　殿

分　野　名	
分 野 責 任 者	
取　扱　者	
連絡電話番号	

下記の有機系廃液の処理を依頼します。

記

有機系廃液の種類		処理依頼　予定数量（ﾘｯﾄﾙ）
H-a	ハロゲン含有機溶剤	
H-b	その他の有機廃液	
計		

※ 依頼条件 ： 重金属濃度は排水基準以下

有機系廃液の分別

ハロゲン系　有機溶剤　　＝　　クロロホルム、四塩化炭素、クロロベンゼン等の脂肪族並びに芳香族ハロゲン化物で、希釈されていない廃ハロゲン化有機溶剤

その他の有機廃液	＝	非ハロゲン系有機溶剤、有機溶剤と接触した水、写真現像廃液、有害有機物質の水溶液、洗剤濃厚廃液、アンモニア水、油類（第3、4石油類）、エステル類、フェノール類、アルコール類、ポンプのドレン等

11.5. Chemicals being discarded

Concerning about the disposal of chemicals in the original container, if there is no danger of heat generation or explosion, you can reduce the processing costs by the following methods. The chemicals that are difficult to pre-process in the laboratory must be classified and store using the usual method in the laboratory until the day for disposal of "chemicals being discarded".

11.5.1. Chemicals being discarded (Class A)

-Organic solvent and water soluble organic chemicals: Take out them from the bottle or container and classify as "organic liquid wastes."

-Water-soluble heavy metal inorganic salt: If the amount of the substance is small, make a solution and classify as "heavy metal wastes". If there are several bottles of the same chemicals and they can be mixed together, and put them in a same bottle to reduce the number of bottles.

-Acidic and alkaline chemicals: Neutralize them and dispose. If not possible, classify as "heavy metal wastes".

11.5.2. Solid wastes with hazardous substances (Class B)

Muddy materials, precipitated and catalytic substances with mercury, cadmium, cyanogen, lead or chrome, or broken mercury thermometer are classified into "chemicals being discarded" and keep them safely until the day for the collection.

Notes regarding storage and moving:
Prevent the spillage, runoff and infiltration to the ground.
Pack carefully to prevent the leak when carrying the container.
Broken mercury thermometer should be put in a heavy-duty container that can be sealed.

11.5.3. How to request the outsourcing disposal of "chemicals being discarded"

The chemicals being discarded are processed by an outsourced company once a year, in November. The form of the list can be downloaded from the web site of the Special Liquid Waste Processing

Facility, Kyushu University (http://kan-an.jimu.kyushu-u.ac.jp/). The form is usually prepared by a laboratory staff. Ask staff in your laboratory as needed.

廃薬品等 処理依頼申込書 ［H○年度学外委託処理用］

H○年 ○ 月 ○ 日

申込者	部局･部門	○○○･○○○○○○		
	講座･分野	○○○○○○○○	ﾀﾞｲﾔﾙｲﾝ	123-4567
	職 名	○ ○ ○	氏 名	○ ○ ○ ○

下記リストの廃薬品について、排出責任を負いますので、処理方お願い致します。

排出責任者 ○○ ○○

A．廃 薬 品（購入時の容器に入ったもの）

TEL 下 4 桁	分別	No.	薬 品 名	容器容量	単位	本数	性状
4567	A	1	無水酢酸	500	mL	3	L
4567	A	2	内容物不明	25	mL	1	S
4567	A	3	フェノール	1000	mL	1	L+S
	A	4			mL		
	A	5			mL		
	A	6			mL		
					計	5	

B．廃液、汚泥、固形廃棄物、小分けした薬品等

TEL 下 4 桁	分別	No.	廃液・固形物等の品名	容量･重量	単位	個数	性状
4567	B	1	水銀入り実験器具(15×30×25cm)	15	kg	1	L
4567	B	2	重金属含有汚泥(ビニール袋入り)	5	kg	2	S
4567	B	3	不明廃液(ポリ容器入り)	20	L	1	L+S
4567	B	4	水銀温度計(5 本、ﾋﾞﾆｰﾙ袋)	0.1	kg	1	S
	B	5					
					計	5	

C．アンプル瓶、スプレー缶 **（注1）**

TEL 下 4 桁	分別	No.	品 名	容器容量	単位	個数	性状	ｱﾝﾌﾟﾙ長(cm)
4567	C	1	メチルメルカプタン	50	mL	1	L	15
4567	C	2	スプレー缶(テフロン)	300	mL	1	L	7Φ×20L
	C	3						
					計	2		

注1） 金属製容器に密封されたもので、容器の構造によっては処理不能のため集荷できない場合があります。ガスボンベ類は本処理では該当外ですので申し込まないで下さい。

11.6. Separated refuse generated in the laboratory and daily life

The solid wastes with hazardous materials such as mercury and cadmium should be handled properly. Clean empty bottles for chemicals and harmless wastes in the laboratory are considered as

industrial wastes or recycling materials and must be distinguished from daily refuse.

11.6.1. General wastes from business activities

The wastes from Kyushu University are divided into general waste from business activities and industrial waste. The latter is further classified into the industrial wastes subject to special control and the other industrial wastes.

When you process the wastes in the laboratory by yourself, you must ask the person in charge in the Faculty to confirm that the processing route is authorized. In Kyushu University, all of the wastes except for the burnable daily refuse are treated as the industrial wastes. This includes the laboratory wastes such as empty bottles for chemicals and glassware, empty beverage cans and bottles that are classified into "scrap metals" and "glass and ceramic garbage" according to the related law.

If the separation of the wastes is not done properly, the collecting agency's sorting takes time and can become dangerous. You must observe the precautions indicated in the poster or in following notes;

1. Do not mix different kinds of wastes in the same garbage bag.
2. Needles or sharp materials are dangerous. Put them in a container to avoid injury or accident.
3. Remove caps of beverage bottles, cans and plastic bottles and empty them.

11.6.2. Separated refuse in the laboratory

(1) Bottles (recyclable) [The backyards of 3rd, 4th and 6th buildings]

Washed chemical bottles of 500 mL or more (soft glass) are recyclable. The bottles that are not cleansed well will be classified as "non burnable", and those with heavy smudge are classified into "harmful chemical-adhered matter".

The bottles with frosted interface to the cap (hard glass, thermal glass) and glassware such as beaker and measuring cylinder are classified into "glass or ceramic garbage".

(2) Harmful chemical-adhered matter (both burnable and unburnable) [The backyard of 3rd, 4th and 6th buildings]

Harmful chemical-adhered in laboratory instruments (glass bottles, glass wastes, plastic wastes) and used silica gel must be put in the drum can with lid. Powdered or granular material such as silica gel must be enclosed in a bag or case with a label written on it.

(3) Laboratory burnable refuse [The backyards of 3rd, 4th and 6th buildings]

This includes plastic-made lab wares and empty bottles for chemicals. If possible, wash and reuse. Use a transparent garbage bag for them.

(4) Pseudo-medical wastes [West entrance of 2nd building]

Syringes and needles used outside of the University hospital and medical school are treated as

pseudo-medical wastes and are collected twice a year (June and December) with dry cells and spray cans. The date will be announced by the Facilities Affairs Section, Faculty of Agriculture (Hozen-kakari).

(5) Unburnable refuse [The backyards of 3rd, 4th and 6th buildings]

Non-recyclable bottles of chemicals, glass wares such as beaker and aluminum foil are processed as industrial wastes similar to daily unburnable refuse.

(6) Animal carcass [South side of 7th building, Practical room for anatomy]

The animal carcass except for large animals such as bovine, swine or goat is collected as infectious medical wastes once a month. You must freeze and store the carcasses and put them into a container designed for the carcass. When you bring the container to the room, you must prepare the request sheet for processing. Concerning about the carcasses of the large animals, you must contact with professional agency and do the processing individually.

(7) Floor mat for laboratory rodents and animal excrement [The backyard of 3rd, 4th and 6th buildings, University Farm in Hara-machi town]

The floor mat and excrement of rodents can be processed as non-infectious medical wastes if you can certify that they are non-infectious wastes. For the certification, you must perform a microbial monitoring paid by your research fund once a year, at least, and submit the certificate to Head Official of Faculty of Agriculture until 5th in every month. Paste the distributed "seal" on the garbage bag (double thick transparent, 10 kg or less), then take it out to the backyard of the 3rd, 4th, or 6th building on a twice-weekly basis: only in the afternoon on designated collection days. (The schedule is subject to change after April 2011.)　The cost is beneficiary's liability. In the case of infectious medical wastes, which do not have a certificate, laboratory staff should ask assistance from a special industrial waste dealer individually.

Floor mat for laboratory rodents and animal excrement can be also processed for the composting in the University Farm in Hara-machi town according to the "Agreement on the disposal of floor mat and excrement". At first, submit the " Application form of floor mat and excrement" to the Strategic Planning Section. Then, bring floor mat and excrement to the composting shed after writing in the sheet of the "Disposal record of floor mat and excrement" in the Farm office. Do not mix plastic materials or harmful substances in the wastes and bring back the plastic bag or container.

11.6.3. Daily separated refuse

(1) Used paper (newspaper, cardboard, magazine, miscellaneous paper, pieces of paper) [The backyard of 7th building]

-The materials that can not mix: tissue paper, thermal paper, coated paper, oiled paper, carbon paper, coated paper, photos, compound paper, cellophane, tape, fabric, pair, metal, etc.

-Pieces of paper: the amount of the pieces of paper mixed in burnable garbage bags is checked every year. These papers (scratch paper, cut paper shredder, envelopes, postcards, etc.)" are put into the clear

plastic bags. Cellophane tape, cellophane film or packing tape should be removed from envelopes and wrapping paper if it is put on the paper.

(2) Beverage bottles [The backyards of 3rd, 4th and 6th buildings]

Remove lids of bottles, empty them and put into the basket in the recycling yard waste. Reusable bottles (beer bottles, 1.8 L bottles) should be returned to the shop.

(3) Beverage cans [The backyards of 3rd, 4th and 6th buildings]

The cans should be made empty and put in a clear plastic bag.

(4) PET bottles [The backyards of 3rd, 4th and 6th buildings]

PET bottles should be made empty, squashed and put in a clear plastic bag.

(5) Styrofoam [The backyard of 3rd, 4th and 6th buildings]

Styrofoam that can be recycled is box- or board-form polystyrene only. Styrofoam has a mark of "PS" or "Recycle" on it. Other foamed materials such as polypropylene, tips-type, sponge-type and small pieces of packing materials are classified into "burnable wastes".

(6) Fluorescent tube [West entrance of 2nd building]

Fluorescent tubes contain mercury. They will be collected together with cells, battery and pseudo-medical wastes on June and December. Wait an announcement from the Facilities Affairs Section, Faculty of Agriculture (Hozen kakari).

(7) Dry-cell battery [West entrance of 2nd building]

Dry cells, lithium cells and batteries will be collected together with pseudo-medical wastes on June and December. Keep them in the laboratory and wait an announcement from the Facilities Affairs Section, Faculty of Agriculture (Hozen kakari).

(8) Spray cans [The backyards of 3rd, 4th and 6th buildings]

Spray cans must be punched a hole to make empty the gas. The empty can is classified into a scrap metal.

(9) Scrap metals [The backyards of 3rd, 4th and 6th buildings]

Small scrap metals can be recycled. The scrap metals equipped with plastic piece or others are classified into unburnable garbage (glass and ceramic garbage).

Needles must be put into a sealed can.

Empty 18 L can (oil tin) should be pressed if possible.

Aluminum foil is classified into unburnable garbage.

(10) Daily burnable garbage [The backyards of 3rd, 4th and 6th buildings]

Do not mix plastic wastes, food scraps in daily life, and the pieces of paper that can be recycled.

(11) Bulk trash or large garbage [The backyard of 4th building]

Large valuable metals such as steel rack or desk should be carried out according to the monthly instruction from the Supplies Section (Youdo kakari).

(12) Wood wastes [The backyard of 6th building]

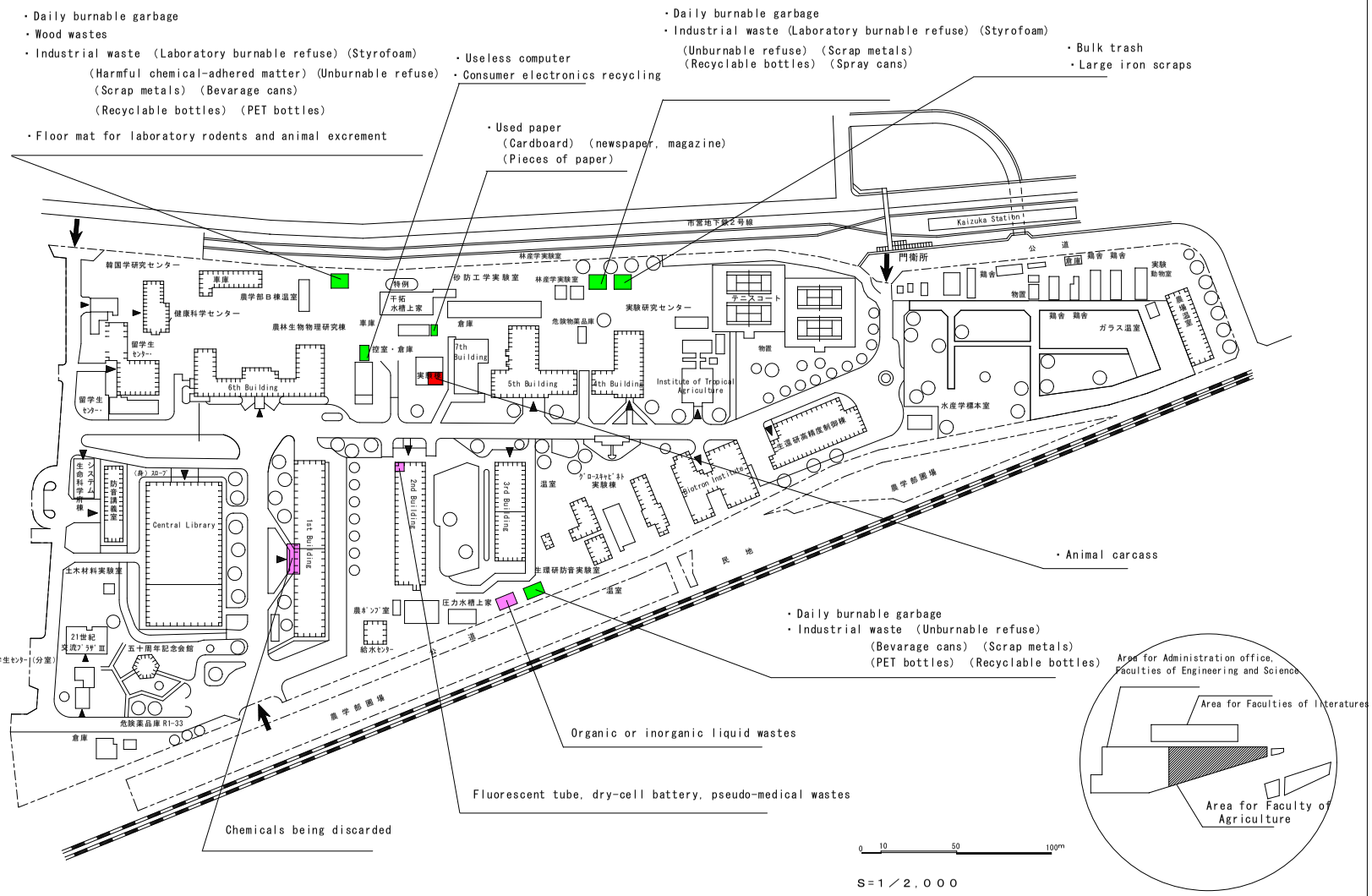
The large wooden desk or bookshelf that is difficult to destroy will be classified into the bulk trash.

(13) Useless computer, Consumer electronics recycling [The backyard of 2nd building annex]

They should be carried out according to the monthly instruction from the Supplies Section (Youdo kakari).

Area for Faculty of Agriculture

Several wastes collection site



12. VDT operation and computer safety management, network security

12.1. VDT operation

VDT operation indicates that works used Visual Display Terminals such as computer display and keyboard. At the VDT operation, notes the following points.

12.1.1. Operation environment

(1) Adjust the room lightning to remove the distinction between light and dark.
(2) Set up a blind or curtain at the window if necessary to shed out the direct sunlight into the display.
(3) About the display, adjust the position of screen, tilt of back and forth, and aspect of right and left and try to prevent the glare.
(4) When there is an unpleasant noise from the VDT or peripheral equipment, take measures to reduce the noise.

12.1.2. Working hours

Do not exceed one hour in series for working hours. Set up the break from 10 to 15 minutes to the next work. Moreover set up ones or twice small break for the successive work.

12.1.3. VDT equipment

(1) Sit upright in a chair with your spine pressed against the back of the chair and with all the sole of your foot touched on the floor while at work.
(2) Keep your visual distance 40 cm or more from the display. If necessary, use glasses to correct your vision.
(3) To reduce the distance between a display, key board, and documents, keep all things you see within an appropriate sight.

12.2. Computer safety management and network security

Computer is one of very important tools for the research and has precise mechanism inside. Therefore it is necessary to handle it carefully. According to the prevalence of network system, many cases of infections caused by the viruses or worms are being

reported. To avoid the network failure caused by infection, appropriate knowledge is needed. For the safety of computer, indicate the following points.

12.2.1. Notes on handling the hardware of the computer

(1) As computer is very weak against shake and impact, please handle it carefully.
(2) If you do not shut down a computer in a predetermined manner, you may cause fatal computer error. When shutting down a computer, please complete a shut down procedure. If you have a plan to knock out power, please shut down your computer beforehand.
(3) When changing the memory or hardware of the computer or opening the hardware, there is a fear of being gotten a shock. Pull out power plug beforehand.
(4) Computer is weak against static electricity. Do not wear a sweater or touch the door knob and window frame to avoid the static electricity.
(5) Computer is weak against water and dust. Keep the laboratory clean.
(6) Dispose of used printer ink cartridge or toner in an appropriate way.

12.2.2. Precaution in using computer

(1) When setting up new computer and connecting with internal network, submit terminal attachment application form to the LAN administrator and connect a computer with a designated IP address by the administrator.
Moreover, when connecting local network in the laboratory, observe the instruction of the administrator in the laboratory.
(2) If you connect a computer without permission, there is a possibility that other computer may not connect the network.
(3) It is legally prohibited to use unauthorized computer with other's IP address or attack vulnerability on the security.
(4) When the computer failure happened, report it to the administrator immediately. At that time, report about the condition of failure briefly and simply (5W1H)to the administrator.
(5) When finding the vulnerability in OS (Windows, Mac) or software, there is possibility of attack by malicious programs such as worm. Suspend using the program immediately and use modification program.
(6) It is highly possible that Windows is infected by the virus. Install virus software and check a virus regularly.
(7) Make a back up copy of important file to the storage medium (hardware, CD-R) regularly.
(8) Do not install file swapping software (WinMX, Winny, BitTorrent) because it is possible to infringe a copyright. The virus (Antiny) infected by file swapping software is found. There is a fear that internal data may be leaked.

(9) When using wireless LAN, restrict access to your web pages to keep out the hackers.
(10) Learn to improve your ability of knowledge about hardware and software.

12.2.3. Precaution in using e-mail

(1) E-mail account received in school should be used for the purpose of research. If you want to use e-mail personally, make another account out of school.
(2) When sending e-mail, avoid using half size of character receiver may not read or machine dependence character such as ①,②, Ⅰ, Ⅱ, ｷﾞ, kg, ㈱.
(3) Avoid using HTML mail may become the route of infection of virus, send e-mail by text. Set up the mail software not to create HTML mail.
(4) Set up the mail software not to open a mail automatically because there is a mail infected with the virus.
(5) Avoid sending mails with attached large file. If you need to send a large file, you should send it separately or using a file transfer protocol. There is no problem in sending files of 1 MB or less. Be careful not to be over the limit of mail server.
(6) If you receive a suspicious e-mail attachment, you should not open it because there is high possibility that it is infected with the virus.
(7) Contact the administrator and terminate your account if you are not using your mail account anymore.
(8) There are many incidents of taking personal information such as card number, ID, Password by people posing as a credit company or bank official page. Do not click the URL in the mail and give your personal information easily even though the mail seems reliable.

12.2.4. Precaution in using Web browser

As a web browser, Internet Explorer, Netscape, Safari, Firefox, Opera are known. They sometimes cause computer failures. Please note the following points.

(1) When sending important information such as personal data with a web browser, you should confirm whether the site is reliable or not or if you need to send it or not. It is important to confirm whether it is SSL compatible site or not.
(2) Do not use Web browser personally. Do not use if for net shopping or seeing unrelated site to the research.
(3) As there is a malevolent program being run when you view the website, nullify the effect of Web browser such as Java-script, Java, ActiveX or set up the permission every time the computer starts.

12.2.5. Precaution in using server

When using supercomputer and servers used by more than one person, please note the following points.

(1) Avoid using easy password imagined from the user. Create a password with more than 8 characters and mix the capital and small letter, number and code. A part of software or OS have a function to record ID and Password, but it is better not to use it because there is a fear for others to log in by your password or ID.

(2) To report the problem immediately, you should confirm who the computer administrator is.

(3) Read the computer instruction published by the administrator carefully and observe it.

(4) When using charged machines such as supercomputer or database, contact the administrator beforehand and use it appropriately. While using it, take note of charged money, connection time and hard disk utilization.

12.2.6. Precaution as an administrator

When you become an administrator of the server used by more than one person, you hold a position of great responsibility if something does happen. Be careful of the following points and you should manage the servers very carefully.

(1) Confirm the condition of the computer and related networks on regular basis.

-User account (delete unnecessary account)

-The condition of CPU, memory, disk, peripheral device.

-Types of application installed and OS, version, other setting status.

-When connecting with network, confirm the setting items such as hostname, IP address, DNS address, gateway address.

-The constitution of network connected with computer, Firewall, its setting status

(2) Confirm who is administrator (Network, mail server, computer center etc) and contact them immediately if something happens.

(3) When leaving university for a long time, inform your contact number to the person involved in the laboratory, just in case.

(4) Turn off the power of the computer that will not be used for the next 24 hours.

(5) When using a server, do not run unnecessary service.

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