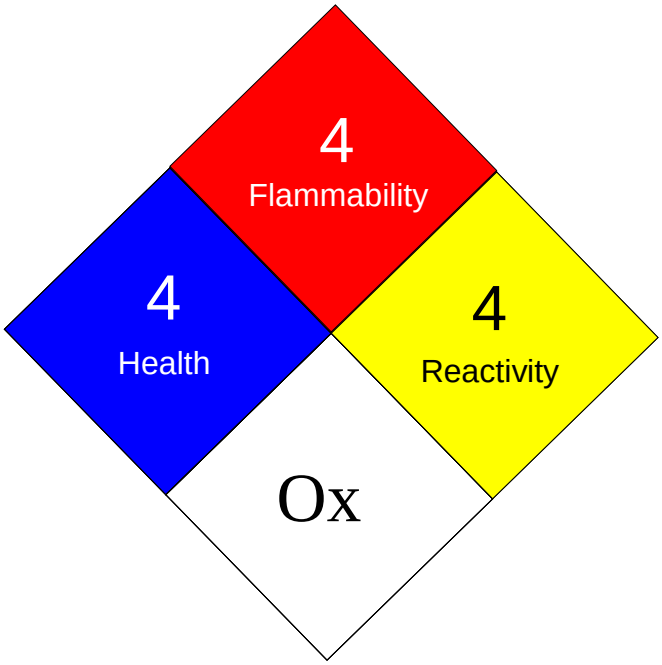
WVU Share Research Facilities

EMF Lab (within BNRF) Chemical Hygiene Plan

G75E2 Engineering Science Building

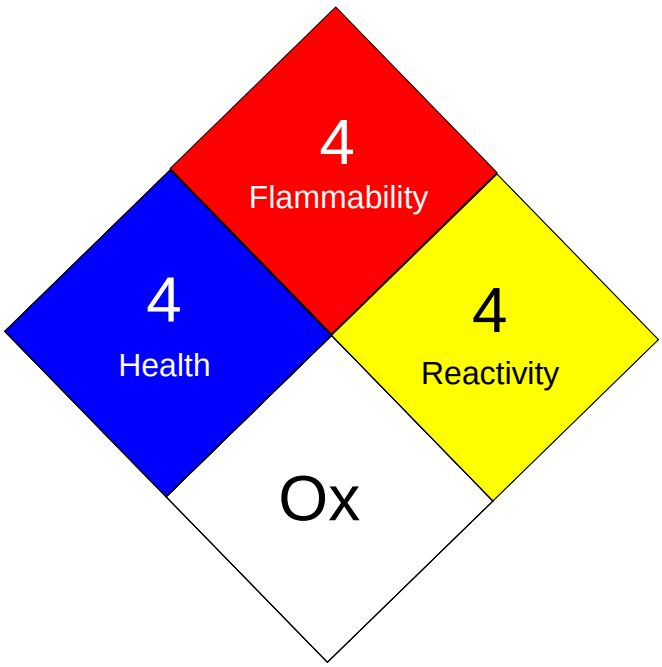


Emergency Contact:

Marcela Redigolo, Ph.D.
Office: G75D Engineering Sciences Building
Cell Phone: (304) 680-3007

SECTION 5: G75E2 - CHEMICAL HYGIENE PLAN

The following safety information is specifically for ESB G75E2. This lab is within the space of the BNRF Cell Culture Lab (G75E) and a complete Chemical Hygiene Plan for the entire space and rooms within is available under the BNRF CHPs.



Emergency Contact:

Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Room: **G75D ESB**

In case of FIRE, INJURY, or EMERGENCY ASSISTANCE, contact in the following order:

9-911 from any campus phone
or
Campus Security
Phone: **(304)293-3136 (293-COPS)**

Then Call

Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Room: **G75D ESB**
or

Huiyuan Li, BioNano Research Facility Manager
Cell Phone: **(304) 906-5368**
Room: **CRL 381**
or

Kolin Brown
Cell Phone: **(304)366-6551**
Office Phone: **(304)293-9683**
Room: **G75D ESB**

If no one responds to any numbers above, then contact:

Kenny Claudio
Cell Phone: **(304) 216-4858**
Office Phone: **(304) 293-4091**
Room**: 373A MRB**

Royce Watts,
Cell Phone: **(304) 288-6762**
Office Phone: **(304) 293-4124**
Room: **377A MRB**

For non-emergency assistance please contact:

Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Room: **G75D ESB**

SECTION 5.1: SHUTDOWN PROCEDURES

If a dangerous situation is evident (smoke, fire, sparks, etc.), ONLY if it is safe to do so, should a user attempt to shut down a system. The user should then notify all other persons in the lab to evacuate immediately. After evacuation, a user should contact proper emergency personnel from a safe place.

If no one is available and a machine is not acting as expected then the user should attempt to put the machine in its default mode; do not leave the machine running in an abnormal state! If the machine cannot be placed in its default mode, the user should stay by the tool and contact one of the WVU Shared Facilities Staff Members. If it becomes necessary to leave the tool then the user should leave a large, legible note on the machine stating that the tool is down, and the user's contact number.

Listed below are the procedures to place the BioSample Prep Lab equipment into a safe default mode, or to shut down. Shutdown procedures may also be found on the cover of each instrument's logbook or in the Standard Operating Procedures found in Appendix A.

To place the CO2 Critical Point Dryer in default mode or turn OFF the instrument:

- **Turn OFF the power switch located on the back of the instrument**
- **Turn OFF the controller power switch.**
- **Close the CO2 bone dry cylinder valve.**

To shutdown (this emergency shutdown will turn OFF power to the entire instrument):

- **Close the CO2 bone dry cylinder valve.**
- **Unplug the power cords from the wall.**

To place the Ultramicrotome in default mode:

- **Turn OFF the power switch on the back of the instrument**

To shutdown (this emergency shutdown will turn OFF power to the entire instrument):

- **Unplug power cord from the wall.**

To place the Furnace(s) in default mode:

- **Turn OFF the furnace by rotating the temperature knob to the Zero position.**

To shutdown (this emergency shutdown will turn OFF power to the entire instrument):

- **Unplug cable from the power outlet on the wall.**

SECTION 5.2: LAB OVERVIEW AND DESCRIPTION

The BioSample Prep Laboratory (G75E2) in the Engineering Sciences Building (ESB), is located inside the Bio-Nano Research Facility lab (G75E). While the BNRF is a different facility under the management of Dr. Huiyuan Li, the BioSample Prep Lab is part of the Electron Microscopy Facility (EMF) under management of Dr. Marcela Redigolo.

Both facilities are part of the WVU Shared Research Facilities (SRF), and provide students and postdoctoral researchers with the opportunity to learn how to use cutting-edge materials science and engineering equipment. The facilities are open to all researchers, including researchers at government laboratories and industries.

Because the BioSample Prep Lab is inside a BNRF space, any person working in the lab will need to follow all safety protocols and procedures requested by both labs, to ensure a safe working environment.

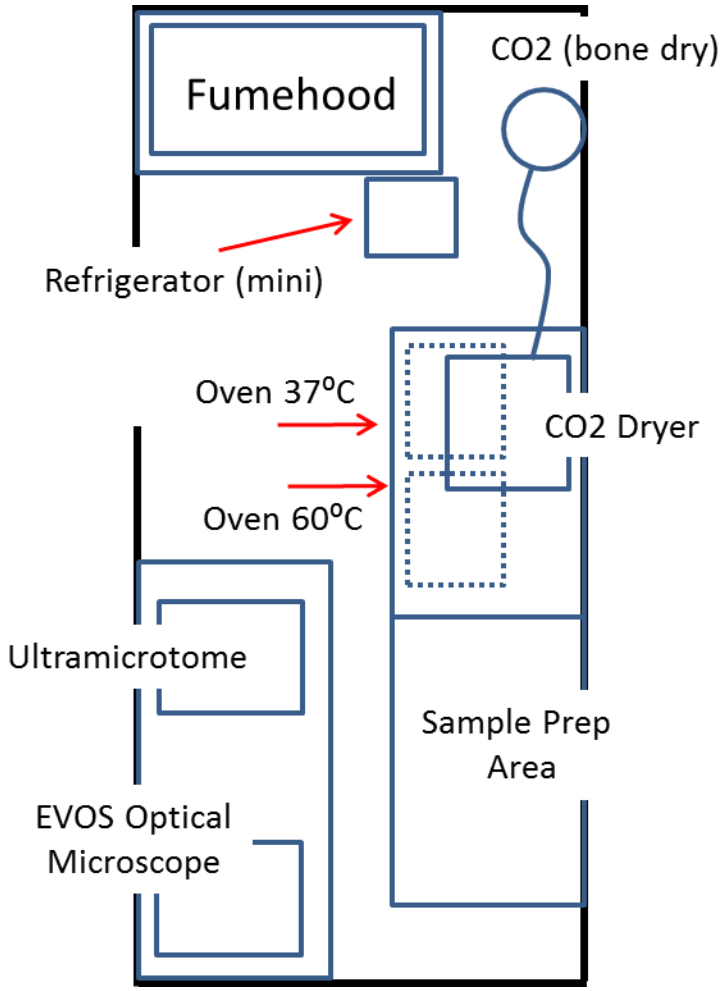


Figure 4: Representation of the instruments distribution within room G75E2.

The BioSample Prep Lab contains specific chemical and physical hazards related only to this room. They are addressed in this section of the Chemical Hygiene Plan. All hazards related to all rooms within lab G75E are addressed throughout this Chemical Hygiene Plan.

SECTION 5.3: CHEMICAL SAFETY

The following chemicals are always stocked in room G75E2:

- Acetone
- Ethanol
- Isopropanol
- Glutaraldehyde
- Dubecco's Phosphate Buffered Saline (PBS)
- Dodecany Succinic Anhydride (DDMS)

- Nidic Methyl Anhydride (NMA)
- ER-SCI Scipoxy 812 Rsh
- DMP-30 DiMethylAminomethyl
- Osmium Tetroxide
- Propylene Oxide

This list represents the standard chemicals used inside the BioSample Prep Lab. A current chemical inventory may be found in Appendix B. This inventory is updated yearly.

SECTION 5.3.1: CHEMICAL STORAGE

Chemicals in G75E2 are stored under the fume hood in its flammable cabinet or inside a minirefrigerator, depending on chemical requirements.

When a chemical order arrives, an approval chemical label that is dated and signed by a SRF staff member is applied to each chemical container. The chemical is then stored in the appropriated storage space.

The approved chemical labels are color coded to quickly identify proper chemical storage locations. The following color code is used:

- Green for acids and etchants
- Yellow for solvents
- Pink for polymers
- Blue for cell cultures
- Orange for bases

The minirefrigerator is used to store:

- Osmium Tetroxide
- Polymeric beads for TEM calibration
- Flammables cannot be stored in the minirefrigerator

NOTE: Osmium Tetroxide is stored within two enclosed and sealed containers, the external one serving as a secondary container. Only a small amount of the prepared solution of Osmium Tetroxide is stored in this lab.

SECTION 5.3.2: AUTHORIZING CHEMICAL USAGE

The WVUSRF Electron Microscopy Facility (EMF) only purchases chemicals that are general use. Often, research projects require the use of chemicals that are specific to a project. Any user, who wishes to bring a new chemical into the lab, must first obtain permission from the facility manager. The user must submit a signed material tracking form and a material safety data sheet(s) (MSDS) for each chemical container to the EMF Manager for approval. A copy of this

form can be found on the shared research facilities website (http://sharedresearchfacilities.wvu.edu/info/forms/). This form identifies the chemical, proper storage and proper disposal methods. Material tracking forms are kept in a binder in the SRF EMF Manager's office. The MSDSs are added to the MSDS file kept in G75E2 ESB and to this Chemical Hygiene Plan, appendix B.

All containers must be brought to a SRF staff member to receive an approved chemical label, which is signed and dated by the SRF staff member. The chemical container must be appropriate for the chemical and it must be labeled appropriately with the full chemical name to receive an approved chemical label. Only containers with approved chemical labels may be taken into the lab.

A chemical must be approved before being brought or stored into the lab, even if the chemical is only going to be used one time.

SECTION 5.3.3: CHEMICAL HANDLING

All wet chemistry or any work with open chemical vessels must be performed in the fume hood. Users should be working with all chemicals at arm's length in the back half of the hood for their protection.

Users working in G75E2 are required wear lab coat and nitrile gloves. Lab coats protect from splashes and nitrile gloves are resistant to some chemicals. However, users are required to wear additional personal protective equipment (PPE) when working with specific chemicals.

When working in the fume hood:

- Check for appropriate chemical ratios. Improperly mixed chemicals may create dangerous reactions or dangerous fumes.
- Use the appropriate PPE and type of gloves for chemical usage
- Wear splash goggles when working with chemical processes
- Keep the work area inside the hood clean and free from obstructions.
- Always use appropriate vessels.
- Never heat solvents in closed beakers.
- Do not store items in the hood.
- Do not leave unlabeled chemicals unattended in a hood.
- Always properly label beakers.

Users working at the fume hood are required to wear the appropriate level of PPE determined for their process.

When working with Osmium Tetroxide, users must wear splash goggles, buttoned lab coat and double nitrile gloves, making sure there is no exposed skin between gloves and lab coat sleeves at any time.

First, remove the doubled container that has the Osmium Tetroxide from the refrigerator. Bring it to the fume hood. Do NOT open the containers under any circumstance while it is not under the fume hood. Once under the hood, the doubled container can be opened and the Osmium Tetroxide vial can be removed. Just keep the vial opened long enough to remove the amount of liquid you need for your process. Then, close the vial and put it back inside the containers. Close the doubled container and place it back in the refrigerator.

SECTION 5.3.4: ACCIDENTAL CHEMICAL EXPOSURE

Users are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately. If a user has been exposed to a chemical, the following procedures should be followed immediately.

For eye exposure:

1. Remove all contaminated clothing and gloves.
2. Initiate water flow at eyewash station by either pressing on the pedal or the hand lever.
3. Hold eyes open with fingers and lower face into eyewash bowl, so that water is rinsing the eyes.
4. Keep eyes open and rotate.
5. Flush eyes for a total of 15 minutes.
6. Seek emergency medical attention.

For exposure to any part of the body:

WARNING: Do not wipe off the chemical; you will only increase area of contact! Increasing area of contact will increase absorption through the skin and may result in faster or more sever reaction or poisoning.

1. Remove all contaminated clothing and gloves.
2. Initiate water flow at safety show by pulling down ring.
3. Flush contaminated area for a total of 15 minutes.
4. Seek emergency medical attention

For exposure to Osmium Tetroxide:

1. Remove all contaminated clothing and gloves.
2. Flush contaminated area for a total of 15 minutes.
3. For eye exposure: Flush opened eyes with water for at least 15 minutes.
4. Inhalation exposure: if osmium tetroxide vapor has been inhaled from a spill, move the victim to fresh air immediately.
5. In all cases, seek emergency medical attention!

In case of MEDCIAL EMERGENCY contact:

9-911 from any campus phone
or
Campus Security
Phone: **(304)293-3136 (293-COPS)**

If going to the hospital for medical assistance, make sure you:

- Take a copy of the MSDS with you
- Inform medical personnel if you have been working with or been exposed to Osmium Tetroxide.

SECTION 5.3.5: CHEMICAL SPILL RESPONSE

If at any time a user is unsure of how to clean up a spill or is uncomfortable in trying to clean up the spill they should immediately evacuate the area and seek assistance. Users should attempt to only contain the spill and then seek assistance from the SRF staff. Personal protective equipment should be worn at all times.

If a chemical has been spilled in the lab G75E2:

A spill kit is kept in the room. Users should only attempt to contain the spill if it is less than one gallon.

To contain the spill:

1. Open the spill kit and follow its instructions.
2. Do not discard the spill kit.
3. Once the spill has been contained, seek SRF staff assistance.
4. The SRF staff will contact EH&S for pickup.

If Osmium Tetroxide has been spilled in the lab G75E2:

In the event of a spill, take appropriate actions to prevent exposure of osmium to everyone in the room, and to avoid the spread of contamination. If the spill is small and manageable (less than 2 ml):

1. Alert everyone in the immediate area to evacuate.
2. Isolate the area to prevent the spread of contamination.
3. Wear appropriate PPE (double nitrile gloves, buttoned lab coat and safety splash goggles)

4. Contact SRF staff personnel immediately.
5. SRF staff will cover the spill with inert absorbent (from spill kit or kitty litter) that has been infused with vegetable oil (corn oil preferred)
6. Scoop the contaminated material up and place it in a glass or plastic container (jar or pail) with a tight fitting lid.
7. This container will be put inside the solid waste container in the lab.
8. Wash the area of the spill with an aqueous solution of sodium sulfite.
9. Clean the area again with detergent solution.
10. Remove contaminated PPE carefully and place it in the solid waste container.
11. Label the waste container with a properly completed hazardous waste label and notify EH&S immediately for pickup.

At no time, will the BioSample Prep Lab have Osmium Tetroxide in amounts larger than 2 ml.

In case of an ACUTELY HAZARDOUS SPILL contact in the following order:

Environmental Health & Safety
Phone: **(304)293-3792**

Then Call,
Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Office Phone: **(304) 293-9973**
Room: **G75D ESB**

or

Kolin Brown, SRF Chemical Hygiene Officer
Cell Phone: **(304)366-6551**
Office Phone: **(304)293-9683**
Room: **G75D ESB**
or

If no one responds to any numbers above, then contact:

Kenny Claudio
Cell Phone: **(304) 216-4858**
Office Phone: **(304) 293-4091**
Room**: 373A MRB**

Royce Watts,
Cell Phone: **(304) 288-6762**
Office Phone: **(304) 293-4124**
Room: **377A MRB**

SECTION 5.3.6: CHEMICAL DISPOSAL

All chemicals inside room G75E2 must be captured in waste jars.

To dispose of Osmium Tetroxide:

Osmium Tetroxide disposal should only be performed by the laboratory manager. To reduce hazards involved in discarding osmium tetroxide, the following neutralizing procedure will be used:

1. All these steps should be complete in the fumehood and wearing proper PPE.
2. A 2% solution of osmium tetroxide can be fully neutralized by twice its volume of vegetable oil (corn oil is preferred because of its high percentage of unsaturated bonds). For example, for every 10 ml of 2% osmium tetroxide solution, 20 ml of corn oil is required.
3. Pour the corn oil into the osmium tetroxide solution, and wait for the oil to completely turn black.
4. To test if the osmium tetroxide is fully neutralized, hold a piece of filter paper soaked in corn oil over the solution. Blackening indicates that osmium tetroxide is still present and more corn oil should be added.
5. Aqueous solutions contaminated with osmium tetroxide can be fully neutralized by adding sodium sulfide or sodium sulfite to reduce osmium tetroxide to less hazardous forms.
6. Dispose of neutralized solutions and all materials used for the procedures as hazardous waste.

All solid materials used to manipulate osmium tetroxide must be disposed in the solid waste container for osmium tetroxide. This includes gloves, tissues, manipulation tools such as wood sticks, wipes, plastic pipettes, etc. The solid container is clearly labeled and dated. This container will be inspected weekly by an SRF manager, and the facility manager will request EH&S pickup if the container has any waste.

To dispose of all other chemicals or chemical mixtures:
Used chemicals must be captured in chemical waste jars. All chemical waste is captured and stored in an appropriately chosen waste jar which is properly labeled with the word WASTE. The jar label should clearly list all contents of the waste jar. Ideally, the waste jar should be an empty original container of the same chemical being disposed of. Each chemical or mixture should have its own waste jar. The only waste jar that should be used for multiple chemicals is the alcohol waste jar; this jar may be used to dispose of methanol, isopropanol, and ethanol.

Waste jars should be closed when not in use and properly stored.

When a waste jar is full, it will be marked with a label stating "Hazardous Waste" and dated. SRF staff will contact EH&S to have it removed.

WARNING: Do not pour a hot liquid into a waste jar! Allow the hot chemical or chemical mixture to cool before adding it to a waste jar. Sealing a hot liquid in a waste jar may cause the jar to explode.

WARNING: Do not seal a waste jar if the material is highly reactive! If the waste jar heats up, or if it fumes, keep the jar open in the fume hood and contact a SRF staff member immediately for assistance. Sealing a chemical reaction in a waste jar may cause the jar to explode.

SECTION 5.4: SHARPS AND BROKEN GLASS DISPOSAL (PHYSICAL HAZARD)

A broken glass disposal box is kept in the lab, room G75E2. This box is for the disposing of broken glass, used glass knives from the ultramicrotome and Pasteur (glass) pipettes. SRF staff members inspect this box monthly. When full, the box is sealed and disposed with the laboratory trash.

Sharps disposal boxes are kept on top of the workbenches in the lab. The boxes are primarily for the disposal of razor blades, though any sharp may be disposed of here, including needles. SRF staff members inspect this box monthly. When full, the box is sealed and properly disposed.

WARNING: Do NOT recap used needles for disposal.

In the case of a sharp that is contaminated with osmium tetroxide, it should be discarded into a solid waste container designated for osmium tetroxide, not a sharps disposal box.A SRF staff member should be alerted immediately.

SECTION 5.5: LIQUID NITROGEN

Liquid nitrogen (LN_2) is inert, colorless, odorless, non-corrosive, non-flammable, tasteless, extremely cold, and has no warning properties. Special care must be taken by persons who handle or work in areas where liquid nitrogen is used. The hazards associated with LN_2 include:

- Over-pressurization and explosion due to LN_2 vaporizing to nitrogen gas (700x expansion ratio) in unvented containers.
- Severe burns caused by exposure to cold temperatures.
- Asphyxiation due to displacement of oxygen in the air in confined work areas.

Humans cannot reliably detect the presence of nitrogen. Liquid nitrogen has a 700x expansion ratio which may create physical hazards and injuries from the explosion of unvented containers. Extensive tissue damage or burns can result from exposure to LN_2 or cold nitrogen vapors.

Asphyxiation may result from the displacement of oxygen in the air with nitrogen to levels where there is insufficient oxygen to support life. Inhalation of oxygen deficient air can cause dizziness, nausea, vomiting, loss of consciousness, and death.

When working in the lab the body must be protected with pants and closed-toe shoes as required by the dress code for G75D entrance.The following personal protective equipment is required when handling or using LN_2:

- **Water proof thermal insulated gloves** (e.g., cryo-gloves):- Hands must be protected with water proof thermal insulated gloves that can be quickly removed if LN_2 is spilled on them. Insulated gloves are not intended for submersing hands into LN_2.

- **Cryo-aprons**:- Thermal insulated aprons are available and should be worn.

- **Safety goggles**:- Eyes are most sensitive to the extreme cold of LN_2 and its vapors. Over-pressurization may result in the explosion of improperly vented equipment. Chemical splash goggles must be worn when handling LN_2.

- **Full face shield**:- Face must be protected against splashes and spills of liquid nitrogen by a face shield.

People handing liquid nitrogen are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately.

Although Liquid Nitrogen is not physically present in this lab, in case of certain specific samples, the protocol for preparation for electron microscopy study may require the use of a small quantity (less than 1L) of liquid nitrogen. A SRF staff member should be notified when liquid nitrogen is being brought into the lab. In these cases, users should follow all safety protocols to work with it.

SECTION 5.5.1: ACCIDENTAL EXPOSURE

Users are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately. If a user has been exposed to liquid nitrogen, the following procedures should be followed immediately.

For frostbite exposure:

1. Remove all contaminated clothing and gloves, and any clothing item that may restrict circulation to the frozen area.
2. Do not rub frozen parts, as tissue damage may result.
3. Place the affected area in a warm water bath that has a temperature not exceeding 105°F (40°C).
4. Never use dry heat!!

In case of MEDCIAL EMERGENCY contact:

9-911 from any campus phone
or
Campus Security
Phone: **(304)293-3136 (293-COPS)**

If going to the hospital for medical assistance, make sure you:

- Take a copy of the MSDS with you
- Inform medical personnel you were exposed to liquid nitrogen.

SECTION 5.5.2: LIQUID NITROGEN SPILL RESPONSE

Personal protective equipment should be worn at all times. If a significant quantity of liquid nitrogen has escaped, or been spilt, the area affected will not contain adequate oxygen to support life. The area should be immediately evacuated and the following personnel should be contacted:

Environmental Health & Safety
Phone: **(304)293-3792**

Then call,

Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Office Phone: **(304) 293-9973**
Room: **G75D ESB**

Or

Kolin Brown, Cleanroom Manager
Cell Phone: **(304)366-6551**

Office Phone: **(304)293-9683**
Room: **G75D ESB**

If no one responds to any numbers above, then contact:

Kenny Claudio
Cell Phone: **(304) 216-4858**
Office Phone: **(304) 293-4091**
Room**: 373A MRB**

Royce Watts,
Cell Phone: **(304) 288-6762**
Office Phone: **(304) 293-4124**
Room: **377A MRB**

If a person is showing symptoms of mild or severe asphyxia, they should be moved to an area with fresh air. If they are not conscious, security should be called immediately and resuscitation started by a qualified first aid officer or physician.

Cold burns from liquid nitrogen should be immediately and continually flushed with tepid or unheated tap water.

Professional medical advice should always be sought urgently for significant cold burns and asphyxia. Persons affected may need urgent medical treatment at a hospital.

SECTION 5.6: GAS SAFETY

The following gas cylinder is typically used by the CO2 Critical Point Dryer inside the biosample prep lab room, G75E2:

- Carbon Dioxide, bone dry.

Users should not have to open, close, or make adjustments with the regulators. Adjustments to regulators should be done only by SRF EMF staff.

The cylinder in use is mounted securely against a wall. All cylinders are transported using a cylinder hand truck. Eye protection or face shield is to be worn when changing cylinders. Users should communicate if a cylinder empties while running the critical point dryer. A new cylinder will be installed by a SRF staff member.

APPENDIX A

STANDARD OPERATING PROCEDURES

BNRF LAB – rooms G75E and G75E1 ESB
EMF LAB – room G75E2 ESB

APPENDIX B

CHEMICAL INVENTORIES AND MATERIAL SAFETY DATA SHEET (MSDS)

BNRF LAB – rooms G75E and G75E1 ESB
EMF LAB – room G75E2 ESB