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Experiment Safety Review Form
Review Number: PS-ESR-1-163-2012

PRINCIPAL INVESTIGATOR: Mark Chance, Mike Sullivan
GROUP: Case Western Reserve
EXT: 3800 **E-MAIL: msullivan@bnl.gov** **LIFE NUMBER: S5438**

Project Title: Mass spectrometric analyses of X-ray irradiated samples using Beamline X28C	
Location(s): 0725	
Area(s): 0725-FIRST-1-163	
Proposed Start Date and Duration: 5/31/2011 - 1 years	

SIGNATURES:

Principal Investigator: Mark Chance, Mike Sullivan	**Date: 6/8/2011**
Experiment Review Coordinator: Lori Stiegler	**Date: 6/8/2011**
Co-PI or Alternate Contact (s): Michael Sullivan	**Date:**
Reviewer: Christopher Weilandics	**Date: 6/6/2011**
Reviewer: Frank Zafonte	**Date: 5/25/2011**
Reviewer: Deborah Bauer	**Date: 5/26/2011**
Reviewer: Brian Heneveld	**Date: 5/25/2011**
Approval: Lori Stiegler	**Date: 6/8/2011**
Review/Approval (ERC) Comments: 06/05/2012 1:50 PM No changes in scope or hazards. Extended for one year 06/08/2011 3:59 PM 06/08/2011 3:57 PM Added PI approval signature as per Mike Sullivan e-mail on 6/8/11	
Walkthrough Signature:	**Date:**
Expiration Date (max 1 yr.): 6/8/2013	
FUA Change Required? No	
Fire Rescue Run Card Changes Required? No	
Has a NEPA Review been Performed for this Project? No	
Required Approvals (i.e., IACUC, IBC, etc.):	
Project Termination Acceptance Signature: **Comments:**	**Date:**

I. Define the Scope of the Work

A. Description

1. Mass spectrometric analyses of X- ray irradiated samples using Beamline X28C General Purpose/ Experimental Scope: To resolve solution dynamics of macromolecular interactions and conformational changes at single amino acid residue or nucleic acid base level. Hydroxyl radicals generated using X- ray (at X28C) radiation cause oxidative modifications in protein solutions, which are analyzed using Mass spectrometry. Equipment/ Apparatus: Waters Alliance™ HPLC and Thermo Finigan LCQ™Deca XP Plus for LC/MS/MS analysis, Beckmann's DU 500 series UV Spectrophotometer for absorbance and protein concentration measurements, analytical balance, High Pressure Liquid Nitrogen Dewar (120L), Apparatus operating conditions: Typically, protein samples are prepared in micro molar concentrations in 10mM Sodium phosphate pH 7.0 buffer either by directly weighing milligram quantities of protein or measuring the concentration by UV Spectrophotometer. All LC/MS/MS analyses and UV spectrophotometric measurements are performed at room temperature (20 – 25 °C). X-ray irradiated and proteolytically digested protein samples (200 to 400 picomoles) are injected via automatic sample injector of the HPLC instrument for LC/MS/MS analysis. HPLC grade solvents are used for HPLC and Mass spectrometry performance, maintenance, standardization, calibration and actual experiments. Methanol, Acetonitrile, Water are used in varying proportions with TFA (Trifluoroacetic Acid). The solvent mixtures, usually 5- 95% Acetonitrile in water with 0.1% TFA are taken from 1-161 to the fume hood in rm. 1-163 where they are finally transferred to the HPLC solvent bottles. Typically, a single LC/MS run does not exceed 120 mins. The solvent flow rate used is 50ul/minutes. The helium from a compressed gas cylinder is delivered at a pressure of 40 psig. The HPLC solvent system and all other experimental parameters set up for calibration are similar to sample runs. The performance, maintenance tests for the HPLC and the LCQ Deca XP are performed on a regular basis either by the local staff or personnel sent by the concerned manufacturing company. LCQ Deca XP Plus tuning and calibration is done from time to time using a non- hazardous tetrapeptide MFRA. A High Pressure Liquid Nitrogen Dewar is periodically filled and connected with the LCQ™ Deca XP Plus to maintain a steady flow of nitrogen gas during data collection. Nitrogen pressure is delivered at 80 psig. Note: All protein samples used are non-hazardous. Sample volume for a single injection is not more than 60ul. Protein concentrations used are less than or equal to 5uM in dilute buffer solutions (10- 50 mM). 2. General Beam Line Support . Storage of spare beryllium windows.

Equipment manuals or procedures that are controlled documents:

N/A

B. Human Performance Factors

To save the staff time and to help go GREEN, we have installed RealVNC client software on the Mass spectrometer data collection and data processing computers. This enables the control of data acquisition and analysis from any computer that has internet access with the use of a BNL crypto card. We have also installed this software on the X3B beamline computers to allow staff to collect data and also troubleshoot problems that the general users might have during their beamtime. This helps us to be able to solve problems quickly without the need to travel to BNL during the nighttime hours, reducing the chance of accidents, lowering our carbon footprint and saving valuable beamtime. We plan to install camera to view HPLC operation and MS data collection status when the two instruments are ON overnight.

C. Waste Minimization/Pollution Prevention

N/A

D. Materials Used /Waste Generated

Materials Used	Disposal Method	Amount per Use	Amount per Year	Comments
				Combined waste disposed of as hazardous

Methanol (10% v/v) in water	Hazardous	3.00 ml	900.00 ml	waste
Acetonitrile (45%v/v) in water	Hazardous	0.10 ltr	1.00 ltr	Combined waste disposed of as hazardous waste
Trifluoroacetic Acid (0.1%v/v)in water	Hazardous	6.00 ul	1.80 ml	Combined waste disposed of as hazardous waste
Isopropanol	Fugitive	3.00 ml	500.00 ml	
De-ionized water 44.9	Hazardous	3.00 ml	900.00 ml	Combined waste disposed of as hazardous waste
Liquid nitrogen	Fugitive	1.00 ltr	300.00 ltr	
Proteins	Trash	0.50 mg	5.00 mg	
Enzymes (Trypsin, pepsin, V8)	Trash	0.00 mg	0.10 mg	
Ammonium bi-carbonate	Sanitary	0.10 mg	2.50 mg	
Sodium phophate dibasic (buffer salts)	Sanitary	0.10 mg	0.80 mg	
Helium	Fugitive	1.00 ft3	50.00 ft3	
Beryllium windows (stored)	Hazardous	0.00 g	0.00 g	

II. Identify and Analyze Hazards Associated with the Work

The following hazards were identified:

Physical Hazards:

- Cryogens (any substance or device capable of producing temperatures <= 170K) (Area: 0725-FIRST-1-163)
- Compressed gases (lecture bottles, cylinders, gas lines) (Area: 0725-FIRST-1-163)
- Flammable liquids (Areas: 0725-FIRST-1-163, 0725-FIRST-1-163)

Chemical Hazards:

- Flammable liquids (Areas: 0725-FIRST-1-163, 0725-FIRST-1-163)
- Storage or use of Beryllium or Beryllium articles. Attach Beryllium Use Review Form if checked. (Areas: 0725-FIRST-1-163, 0725-FIRST-1-163)

Ionizing and Non-ionizing Radiation Hazards:

- None

Biological Hazards:

- None

Offsite Work:

- None

Other Issues (Security, Notifications, Community, etc.):

- None

Significant Environmental Aspects

- Any amount of hazardous waste generation

III. Develop and Implement Hazard Controls and Assess Risk

A. Physical Hazards, Tasks and Controls

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Cryogens (any substance or device capable of producing temperatures <= 170K)** **Default Controls:** General Requirements: • Evaluate location oxygen deficiency • Store/transport only in approved containers (i.e. DOT/ASME or BNL LESHC)	**Negligible (0-20)**

• Never pour from above chest level • PPE: Long Sleeve Shirt (or Lab Coat), long pants (or skirt covering ankles) and closed shoes Pressurized transfer to open (vented) container; Or-Pouring > 5 liter volumes of LN2 between open containers: • Face shield along with either Safety Glasses (w/side shields) or Goggles • Gloves (Cryo or Heavy Leather) Pouring small (5 liters or less) volumes of LN2 between open containers: • Safety Goggles (face shield recommended if possible) • Gloves (Cryo or Heavy Leather) Work with samples immersed in LN2 in small (~1 liter) dewars: • Use Tongs (tools) to manipulate/handle cryogenic samples (do not touch with gloves)• Use insulated non-absorbent gloves with dexterity (cotton/nylon gloves under disposable nitrile gloves) • Safety Goggles	
Hazard: **Compressed gases (lecture bottles, cylinders, gas lines)** **Default Controls:** • Any systems >15psi must be SME Approved • Transport cylinders using a cylinder cart • Secure cylinders to a fixed object/wall • Use regulator, hoses, and components compatible with gas • Use hoses and clamps rated for maximum regulator output or use pressure relief device • Wear safety glasses with side shields when installing/removing/or adjusting regulator • Label piping/tubing	**Negligible (0-20)**
Hazard: **Flammable liquids** **Default Controls:** As for chemicals, plus Store large quantities in Flam. cabinets as required	**Negligible (0-20)**

B. Chemical Hazards, Tasks and Controls

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Flammable liquids** **Default Controls:** Use hazardous chemicals controls. Review large quantity storage with Fire Protection Engineer Note location on fire run card and hazard info. placard for storage of solids, greater than 40 pounds; liquids, greater than 5 gallons; gases, greater than 10 pounds	**Negligible (0-20)**
Hazard: **Storage or use of Beryllium or Beryllium articles. Attach Beryllium Use Review Form if checked.** **Default Controls:** Engineering Controls Administrative Controls • Complete the Beryllium Use Review Form. • Machining of beryllium prohibited Training: • Beryllium Use at BNL (TQ-BERYLLIUM1) PPE: Nitrile glove Comply with the SBMS Subject Area: “Beryllium”.	**Negligible (0-20)**

C. Environmental Hazards, Tasks and Controls (include on/off site transportation and products/services)

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Any amount of hazardous waste generation** **Default Controls:** Engineering Controls • Waste will be accumulated in chemically compatible containers that appropriately contain/protect the waste. • Waste containers will be closed in a tray (secondary containment) in the Satellite Accumulation Area (SAA). Administrative Controls • All hazardous waste containers will have a (red) “Hazardous Waste Label” that has the generator’s name and the chemical contents (trade name/formula not acceptable). • All waste will be accumulated in closed containers and kept in an established and posted SAA until ready for transfer to the 90-Day Haz Waste Area for pick-up by Waste Management. • For pick-up by Waste management, complete the Nonradioactive Haz Waste Control Form and consult the 90-Day Area Manager to gain access/transfer the waste to the 90Day Area. Training: Hazardous Waste Gen. (HP-RCRIGEN3). PPE: When handling waste materials follow PPE requirements specified for the specific materials. Comply with the SBMS Subject Area: “Hazardous Waste Management”.	**Negligible (0-20)**

D. Radiation Hazards, Tasks and Controls
None

E. Biological Hazards, Tasks and Controls
None

F. Offsite Work Hazards, Tasks and Controls
None

G. Other Issues (Security, Notifications to Other Organizations, Community Involvement, etc.)
None

H. Recommended Exposure Monitoring

- None

Description or comments:

I. EPHA Determination

Chemical Name	Quantity (lbs, gal)	Location (Bldg/Room#)

IV. Perform Work Within Controls

A. Recommended Training and Medical Surveillance Summary

- Cryogen Safety (HP-OSH-025-24)
- NSLS Env Aware for SAA Manager (LS-ENV-SAA)
- Laboratory Standard (HP-IND-220)
- Cryogen Safety (HP-OSH-025)
- Hazardous Waste Generator (HP-RCRIGEN3)
- Beryllium Use at BNL (TQ-BERYLLIUM1)

- Compressed Gas Safety (TQ-COMPGAS1)

B. Personnel Training, Qualification, and Authorization List

Employee/Guest Name	Life/Guest#	Dept	Required Training Course(s)	Signed
Mike Sullivan	S5438	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Compressed Gas Safety (TQ-COMPGAS1) [EXPIRES: 5/17/2015] Laboratory Standard (HP-IND-220) [EXPIRES: 5/31/2013] Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 7/22/2012] Cryogen Safety (HP-OSH-025-24) [EXPIRES: 3/15/2013] NSLS Env Aware for SAA Manager (LS-ENV-SAA) [UNASSIGNED: EXPIRES: 11/15/2013]	6/8/2011 12:00:00 AM
Rhijuta D'Mello	J6432	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Laboratory Standard (HP-IND-220) [EXPIRES: 11/3/2012] Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 11/9/2012] Cryogen Safety (HP-OSH-025-24) [EXPIRES: 11/3/2012]	
Sayan Gupta	X6686	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Laboratory Standard (HP-IND-220) [EXPIRES: 8/24/2013] Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 3/29/2013] Cryogen Safety (HP-OSH-025-24) [EXPIRES: 8/24/2013]	
Jen Bohon	Y7275	LU	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Laboratory Standard (HP-IND-220) [EXPIRES: 8/26/2012] Cryogen Safety (HP-OSH-025-24) [EXPIRES: 8/30/2012]	

C. Emergency Procedures
Follow NSLS Emergency Plan. Spill control supplies are located outside room 1-166 and 1-171.

D. Transportation
None

E. Logistical Interactions
None

F. Termination/Decommissioning

Any chemicals or equipment not used will be disposed or transferred to another group. No decontamination will be required

V. Provide Feedback

After discussions about the process, it was decided to mix the TFA solutions in 1-161 where the corrosive is stored. This will minimize the risk of carrying corrosive liquids in the hallway between labs 5/09 To save the staff time and to help go GREEN, we have installed RealVNC client software on the Mass spectrometer data collection and data processing computers. This enables the control of data acquisition and analysis from any computer that has internet access with the use of a BNL crypto card. We have also installed this software on the X3B beamline computers to allow staff to collect data and also troubleshoot problems that the general users might have during their beamtime. This helps us to be able to solve problems quickly without the need to travel to BNL during the nighttime hours, reducing the chance of accidents, lowering our carbon footprint and saving valuable beamtime.

VI. Attachments

Attached Files:

06-03-10 LEEF 161&163.pdf

Lab 1-163 ODH calculation.docx