NISTIR 7389

MARINE ENVIRONMENTAL SPECIMEN BANK: Clean Room and Specimen Bank Protocols

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January 2007

U.S. Department of Commerce
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DISCLAIMER

Certain commercial equipment or instruments are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the National Institute of Standards and Technology nor does it imply that the equipment or instruments are the best available for the purpose.

THE ROLE OF NIST IN ENVIRONMENTAL SPECIMEN BANKING

The National Institute of Standards and Technology (NIST) has been involved in long-term biological and environmental specimen banking for over 20 years. In 1979, the National Biomonitoring Specimen Bank (NBSB) was established in Gaithersburg, Maryland, at what is now the NIST Center for Neutron Research (NCNR). The NBSB is a direct result of a pilot Environmental Specimen Bank Program that was sponsored by the Environmental Protection Agency (EPA) (Wise and Zeisler, 1985). More recently (2001), NIST completed the construction of the Marine Environmental Specimen Bank (Marine ESB). The Marine ESB is located in the Hollings Marine Laboratory in Charleston, South Carolina, and is devoted to the cryogenic banking of well-documented and preserved environmental specimens collected as part of ongoing research and monitoring programs conducted in the marine and coastal environment. NIST operates both the Gaithersburg and Charleston facilities as a single environmental specimen bank program. NIST personnel from both the NBSB and the Marine ESB collaborate in specimen bank project planning, development of banking protocols, and day-to-day operations of the facilities. Specimens maintained in both banks have been collected as part of other agency research and monitoring programs (Table 1).

Specimen banking provides researchers with the ability to look at environmental trends over long periods of time through retrospective analysis of archived samples. Over the last ten years, the specimen bank inventory has included primarily marine samples, with the majority of the tissues from marine mammals (Becker *et al.,* 1997). Three ongoing environmental monitoring programs that have provided a large percentage of these samples are the Alaska Marine Mammal Tissue Archival Project (AMMTAP), the Marine Mammal Health and Stranding Response Program (MMHSRP), and the Seabird Tissue Archival and Monitoring Project (STAMP).

The AMMTAP was initiated in 1987 with sponsorship from the U.S. Department of the Interior, Minerals Management Service (MMS). The project is now conducted as a collaboration among the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NOAA/NMFS), the U.S. Geological Survey's Biological Resources Division (USGS/BRD) and NIST. The goal of this program is to establish a representative collection of marine mammal tissues taken during Alaska Native subsistence hunts for future contaminant analyses and documentation of long-term trends in environmental quality (Zeisler *et al.,* 1992). Detailed descriptions of the project and protocols have been published (Becker *et al,.* 1991; 1993).

The MMHSRP was initiated in 1990 by NOAA/NMFS to collect marine mammal tissues and bank them for long-term storage. In 1992, the National Marine Mammal Tissue Bank (NMMTB) was formally established by Federal Legislation (Public Law 102-587) and a portion of tissues collected by NMFS's MMHSRP as well as the samples collected by AMMTAP are archived in the NMMTB. The MMHSRP obtains specimens from individual and mass strandings of marine mammals and from animals taken incidentally during commercial fishing operations. This program emphasizes contaminant monitoring and information management. Detailed descriptions of the MMHSRP have been published (Lillestolen *et al.*, 1993 and Becker *et al.*, 1994).

Table 1. Programs providing specimens to the National Biomonitoring Specimen Bank (NBSB) and the Marine Environmental Specimen Bank (Marine ESB)

Bank	Specimens	Program (Sponsor)[1]
NBSB	Human livers	Human Liver Specimen Bank (EPA)
NBSB	Food specimens	Nutrients in Human Diet Program (FDA)
NBSB	Human blood spots	Human Blood Spot Collection (DOD)
NBSB	Human blood serum	Human Blood Serum Collection (NCI)
NBSB & Marine ESB	Mussels	Mussel Watch Program (EPA) National Status and Trends Program (NOAA) EXXON VALDEZ Damage Assessment Program (NOAA)
NBSB	Oysters	National Status and Trends Program (NOAA)
NBSB	Fish tissues[2]	National Status and Trends Program (NOAA) EXXON VALDEZ Damage Assessment Program (NOAA)
NBSB	Marine sediments	National Status & Trends Program (NOAA) EXXON VALDEZ Damage Assessment Program (NOAA)
NBSB & Marine ESB	NMMTB:[3] marine mammal tissues[4]	Marine Mammal Health & Stranding Response Program (NOAA) Alaska Marine Mammal Tissue Archival Project (NOAA/USGS)
Marine ESB	Bird eggs	Seabird Tissue Archival & Monitoring Project (USGS; USFWS; BIA; NPRB) Peregrine Falcon Monitoring Plan (USFWS)
Marine ESB	Bird feathers	Peregrine Falcon Monitoring Plan (USFWS)

[1]U.S. Environmental Protection Agency (EPA)
Food and Drug Administration (FDA)
U.S. Department of Defense (DOD)
National Cancer Institute (NCI)
National Oceanic and Atmospheric Administration (NOAA)
U.S. Geological Survey (USGS)
U.S. Fish and Wildlife Service (USFWS)
Bureau of Indian Affairs (BIA)
North Pacific Research Board (NPRB)
[2]liver and muscle
[3]National Marine Mammal Tissue Bank (NMMTB)
[4]liver, kidney, fat, blubber, muscle, blood

The third major contributor to the Marine ESB is STAMP, which was designed and implemented in 1999 to serve as a systematic, long-term program to identify and track anthropogenic contaminants in Alaskan seabirds over a timeframe of decades. This project is being conducted with financial support from and collaboration with the U.S. Department of the Interior, USGS/BRD, U.S. Fish and Wildlife Service Alaska Maritime National Wildlife Refuge (USFWS/AMNWR), Bureau of Indian Affairs (BIA), and, most recently, the North Pacific Research Board (NPRB). An important part of STAMP is the archival of a representative collection of seabird eggs from Alaskan colonial seabird species for future contaminant analyses and documentation of long-term trends in environmental quality. Currently, seabird eggs from colonies of common murre (*Uria aalge*), thick-billed murre (*U. lomvia*), black-legged kittiwakes (*Rissa tridactyla*), glaucous-winged gulls (*Larus glaucescens*), and glaucous gulls (*L. hyperboreus*) are collected and banked. Additional species and types of biological specimens will be collected in the future. A description of this project with protocols has been published (York *et al.*, 2001).

Other projects with specimens archived at the Marine ESB include the American Peregrine Falcon Project (APFP) and the NOAA National Status and Trends Program, Mussel Watch Project (NS&T/MWP). The APFP was established in 2003 through an agreement between the USFWS and NIST. NIST provides support to the Monitoring Plan for the American Peregrine Falcon (USFWS, 2003) by cryogenically archiving peregrine falcon (*Falco peregrinus*) egg contents and feathers for future chemical analysis.

The NS&T Program was initiated by NOAA in 1984 as a result of concerns about the condition of the Nation's coastal and estuarine ecosystems (Lauenstein *et al*., 1987). One element of the NS&T Program was to archive samples for retrospective analyses. Two monitoring components were initiated by this program: the National Benthic Surveillance in 1984 and the Mussel Watch Project in 1986. The goal of these two components is to provide highly reliable data on concentrations of toxic chemicals in marine fishes, shellfishes, and sediments; to measure biological parameters that accurately reflect anthropogenic stress; and to assess and recommend Federal actions needed to maintain or improve marine environmental quality. NOAA, in collaboration with NIST, developed protocols specifically for the NS&T Specimen Bank for collecting, processing, and archiving samples from both components of the NS&T Program (Lauenstein *et al*., 1987, 1996). For the Mussel Watch Project, mussel tissue, oyster tissue, and sediment were collected from approximately 30 sites per year, three stations within a site, and two batches of approximately 16 to 18 mussels or two batches of 10 oysters per station. Specimens were collected in conjunction with ongoing NS&T sampling efforts for monitoring from 1986 through 1992 from over 250 sites (Lauenstein *et al.*, 1996). The banking component of the NS&T Program was discontinued in 1992. In 2005, NOAA resumed banking mussels and oysters from the Mussel Watch Project at the Marine ESB. Protocols developed in 1984 for collection, processing, and archiving are being followed.

In addition to the protocols developed by NIST for the collection, processing, and banking of environmental samples, NIST personnel have referred to the best practices for repositories guideline (ISBER, 2005) developed by the International Society of Biological and Environmental Repositories (ISBER) members for day-to-day activities. This document provides a wide-range of information and successful strategies for setting up and maintaining a specimen bank. The NBSB and the Marine ESB are members of the ISBER.

MARINE ESB, HOLLINGS MARINE LABORATORY

In 1995, an agreement was signed between NIST and NOAA/NMFS establishing the National Marine Analytical Quality Assurance Program (NMAQAP) to assess and improve the quality of analytical measurements in the marine environment. As part of this agreement, NIST was asked to improve capabilities of assessing trends in marine environmental quality by expanding environmental specimen banking activities. In response, construction of the Marine ESB in association with the Hollings Marine Laboratory, Charleston, South Carolina, was completed in 2001.

The Marine ESB is dedicated to banking marine environmental specimens through many different programs. Because specimen banking must ensure that the sample does not become contaminated or change in chemical composition during collection, processing, storage, and homogenization procedures, it is important that all protocols be followed precisely. Although the NBSB facility in Gaithersburg and the Marine ESB in Charleston maintain identical operating procedures for the handling of the same kind of matrices (tissues, eggs, mussels, etc.), the following information specifically applies to the Marine ESB.

Location

The Marine ESB is located in the Hollings Marine Laboratory (HML) on the Fort Johnson Marine Resources Research Center campus at 331 Fort Johnson Road, Charleston, South Carolina (Fig. 1). The HML is a multi-institutional, multi-disciplinary laboratory operated by NOAA. It provides science and biotechnology applications to sustain, protect, and restore coastal ecosystems, emphasizing linkages between the environment and the health of marine organisms and humans. In addition to NOAA and NIST, the South Carolina Department of Natural Resources, the Medical University of South Carolina, and the College of Charleston are partners in execution of programs of the HML and have employees and contractors located there. The Marine ESB occupies approximately 356 m^2 (3,832 ft^2) of the HML first floor. This includes office space, International Organization for Standardization (ISO) Class 5 clean-air space for processing samples, ISO Class 7 clean-air space for the production of analytical reference materials, and a freezer room with ISO Class 7 clean-air conditions (Fig. 2). Refer to section below, Clean Room Properties, for definition of ISO class clean rooms.

Clean Room Properties

The classification and certification of the Marine ESB clean-air laboratories follow the guidelines provided by the International Organization of Standardization, ISO 14644-1: Clean rooms and associated controlled environments – Part 1: Classification of air cleanliness (ISO 14644-1: 1999(E)). This document replaces the Federal Standard 209 (E) document (U.S. General Services Administration, 1992; Institute of Environmental Sciences and Technology, 1997). An ISO Class 5 clean room is designed to provide clean filtered air, exhaust particulate matter as fast as possible, and maintain ISO Class 5 air quality when there is a minimum of activity in the room. ISO Class 5 air is defined as a measurement of no more than 3,520 particles per m^3 (100 particles per ft^3) that are 0.5 μm in diameter or larger. ISO Class 6 air is defined as a

Figure 1. The Hollings Marine Laboratory: Entrance View

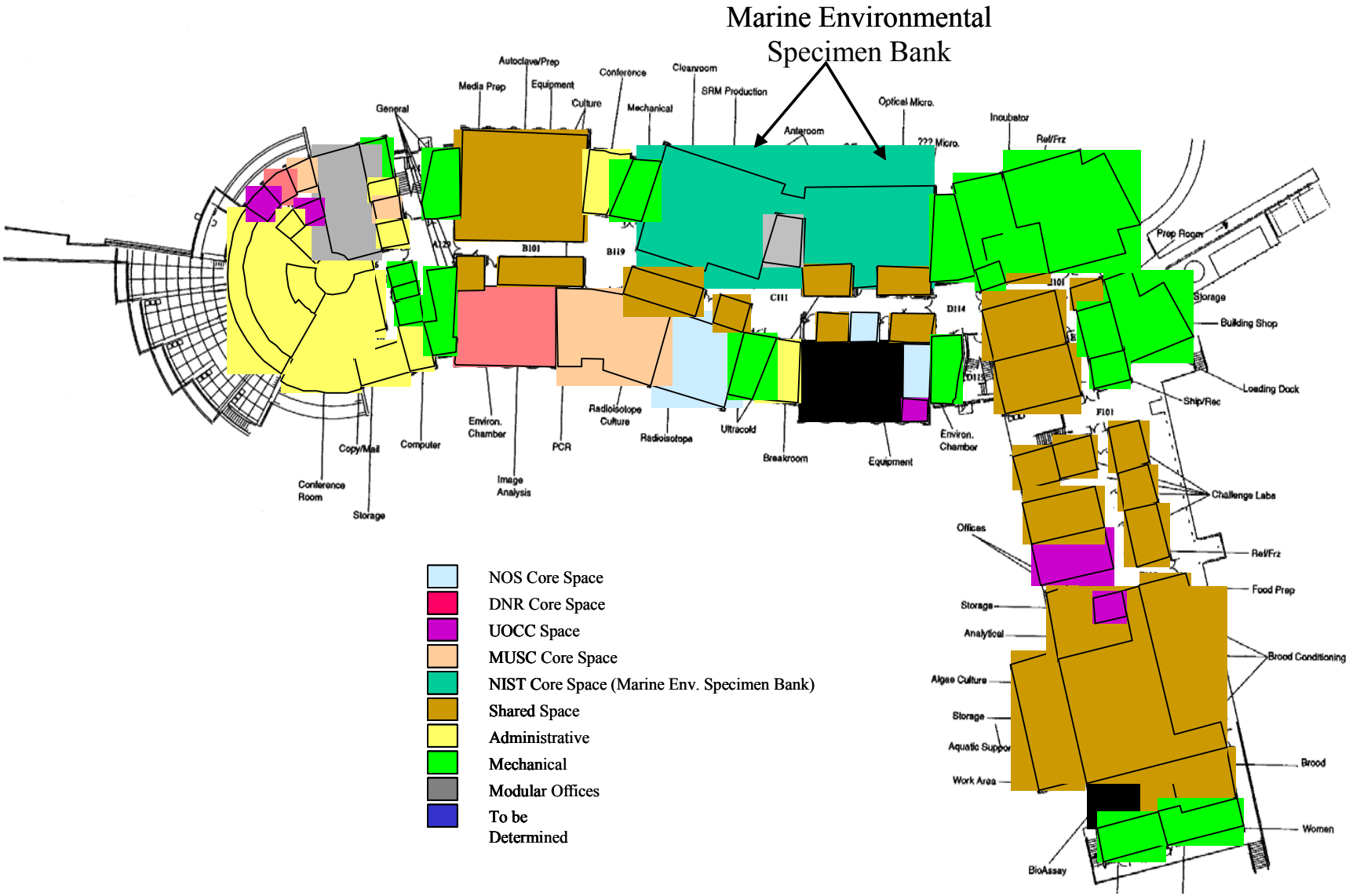


Figure 2. The Marine Environmental Specimen Bank: First Floor of the Hollings Marine Laboratory

measurement of no more than 35,200 particles per m^3 (1,000 particles per ft^3) that are 0.5 μm in diameter or larger. ISO Class 7 air is defined as a measurement of no more than 352,000 particles per m^3 (10,000 particles per ft^3) that are 0.5 μm in diameter or larger

The Marine ESB has been designed to contain an ISO Class 5 Clean Room for sample processing, an ISO Class 7 Freezer Room where specimens are cryogenically stored, an ISO Class 7 Reference Material Production Facility, and two ISO Class 6 ante-rooms. These clean-air laboratories control the concentration of airborne particles to specified limits and are essential in minimizing contamination of samples during processing. The ISO Class 5 Clean Room provides a clean sample preparation work area for materials that will be analyzed for organic and inorganic constituents. At present, organic analyses are performed primarily for chlorinated pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and brominated flame retardants (polybrominated biphenyl ethers and hexabromododecane). The inorganic analyses includes the determination of trace and ultra-trace quantities of elements, with particular attention being given to metals and trace element speciation.

As mentioned above, the purpose of the Marine ESB is the long-term preservation of specimens that are representative of the environment or organism immediately prior to collection. A major concern of specimen banking efforts is that the samples are collected, processed, and stored under conditions that minimize contamination of specimens or any other changes in their chemical composition (Wise and Koster, 1995). Clean technique allows the sample to be processed while minimizing inadvertent contamination and protects the sample's integrity from possible extraneous addition of the chemical analytes of interest. Clean technique is often confused with sterile technique, which is used to prevent the transfer of a bacteriological or viral species to an individual or medium that does not contain this agent.

Since it is impossible to point out every source of contamination that may be encountered, some of the most obvious sources will be described to provide a general awareness of the problem. For example, a 1 μg flake of stainless steel contains approximately 100 ng of nickel (National Bureau of Standards, 1986). The natural occurrence of nickel in human liver is at levels of 1 ng/g to 2 ng/g of sample. Approximately 1 g of sample is used for analysis; therefore, a microgram flake of stainless steel in the sample could produce an analytical result which is 50 to 100 times higher than the true value of nickel in the sample.

In the clean room the air, implements, bench tops, working personnel, and documents providing processing protocols are all potential sources of contamination. The air may contain vapors of contaminants as well as particles of dust, cigarette ash, and wear particles from motors. Cigarette ash contains relatively high amounts of cadmium and organic compounds. Cigarette smoke also contains trace quantities of hundreds of organic compounds. Implements and working surfaces may be contaminated with chemicals used for cleaning and salt and oils from human contact. Also, a common contaminant introduced by human hands is gold from jewelry. The dust-free vinyl gloves that are used should be changed frequently as they are easily contaminated. For example, picking up a pen to record a mass contaminates the gloves. Grasping a Teflon bottle, adjusting eyeglasses, and touching one's face, touching the outside of

the bag that contains the clean Teflon sheets or bags also contaminates the gloves. Therefore, caution should be taken at all times while inside the clean areas.

Each of the rooms that make up the Marine ESB serves a different purpose. It is important to know the function and the types of equipment that are used in each room.

ANTE-ROOMS, ISO CLASS 6

There are two ISO Class 6 ante-rooms (Rooms C102 and D103) (Fig. 3), one that provides access to the Clean Room and the other that provides access to the Freezer Room. These ante-rooms are used by Marine ESB personnel to change into proper 'clean' garments before entering the clean-air laboratories. Disposable laboratory coveralls, hoods, and shoe covers are provided in this room and must be worn at all times while in the clean-air spaces. The material used to make these garments is non-woven Tyvek, a highly effective contamination control fabric that is resistant to penetration by airborne particles. These garments are processed and packaged in a clean environment by the manufacturer and are disposable, but can be worn several times before soiling and tears occur.

Figure 3. ISO Class 6 Ante-Room

Extreme caution must be taken while working in the clean-air laboratories to prevent further contamination of samples; therefore the following precautions should be taken before entering an ante-room and proceeding to the Clean Room or Freezer Room:

- Do not use cosmetic substances such as excessive makeup, alcohol based perfumes, or after shave products.
- Avoid clothing with excessive lint, such as sweaters, turtlenecks, torn and frayed clothing, or any item that has been worn in a dusty environment.
- Do not bring in items that may cause further contamination, such as lead pencils, dirty chemical bottles, paper towels, cardboard boxes, paper products, or other particulate products.
- Do not smoke, chew gum or tobacco, bring in food or drink, or bring personal items to all specimen bank areas.
- Do not use pencils or erasers, only use ballpoint pens in all areas of the Marine ESB.

The following gowning and de-gowning procedures (in numerical order) must be followed in ante-rooms before entering and exiting the Clean Room or Freezer Room.

Gowning Procedure:

1. Wash hands thoroughly before entering an ante-room.
2. Upon entering, step onto the sticky mat to remove loose particles on street shoes.
3. Place the hood over the head, making sure all hair is inside the hood.
4. Choose the closest fit coverall size and put it on, making sure to secure the hood inside of the coverall when zipping up.
5. Place disposable shoe covers carefully over shoes and legs of coverall.
6. If facial hair is present, place a beard cover on, over the hood, again making sure all hairs are inside the cover.
7. Step onto the second sticky mat and enter the Clean Room or Freezer Room.

De-gowning Procedure:

1. From the Freezer Room or Clean Room, step into the ante-room, remove gloves, and dispose of them in the trash can provided.
2. Remove the beard cover, if applicable, and dispose of it in the trash can.
3. Remove the hood and inspect it for heavy soiling or damage. If it is dirty or damaged, discard it in the trash can. If it is clean, hang it on a hook or hanger provided.

4. Remove the shoe covers and again, inspect for damage or excessive amount of dirt. If dirty or damaged, discard the covers in the trash can; if clean, place them near or in the supply cabinet.

5. Remove the coverall and inspect it. If it is dirty or damaged, discard it in the trash can; if not, hang it on the hook or hanger provided.

Some of the cleaning supplies are stored in the ante-rooms. These include a clean-room mop, water bucket, sticky roller, lint-free cloths, aerosol cans of isopropyl alcohol (Deconahol), and a high efficiency particulate air (HEPA) filtered vacuum cleaner. The proper use of these cleaning materials is discussed in detail in the Maintenance Section. All items that are brought into the Marine ESB clean-air laboratories, either through the ante-rooms or the set of double doors in the Reference Materials Production Facility, must be vacuumed before wiping down with the lint-free cloth and Deconahol and then vacuumed a final time.

FREEZER ROOM, ISO CLASS 7

Specimens that are collected for long-term banking are archived in liquid nitrogen vapor-phase freezers (-150 ºC) that are located in an ISO Class 7 Freezer Room (Room D104) (Fig. 4). This Freezer Room is connected with the ISO Class 7 Reference Materials Production Facility. Along with the liquid nitrogen vapor-phase freezers, there are also -80 ºC upright electric freezers and several other pieces of equipment that are either used or stored inside this room (these are listed in Table 2).

Figure 4. ISO Class 7 Freezer Room

Liquid Nitrogen Piping System

All freezers in the Freezer Room are connected to a liquid nitrogen in-line piping system. This system provides vacuum-jacketed insulated piping along with a cryovent device located in the specimen bank to ensure that the entire pipeline is kept at cryogenic temperatures. The system is monitored by a Gordinier Electronics Keep Cold (Model 279) thermocouple sensor controller, located on the wall inside the specimen bank. Liquid nitrogen is brought into the specimen bank through an intricate piping system that distributes liquid nitrogen to multiple freezer stations. Each freezer station consists of a single supply line with either one or two connection valves, totaling forty-one connection valves. These valves provide liquid nitrogen to 30 liquid nitrogen vapor-phase -150 ºC freezers, 10 ultra-cold -80 ºC electric freezers, and one cryogenic vibrating mill (in the Reference Materials Production Facility). The ultra-cold electric freezers are connected to the liquid nitrogen piping system as a part of a back-up system. The liquid nitrogen bulk storage tank for the Freezer Room, Reference Material Production Facility, and Clean Room operations is stored behind the HML building in a 12 m^3 (3,170 gallons) vertical storage tank with a telemetry system to the commercial supplier (Fig. 5). The main distribution lines are equipped with multiple flow valves strategically placed to ensure safety. The Centron System: Monitoring, Alarming, and Data Management System is used to ensure correct temperatures and liquid nitrogen levels are maintained for each freezer. In general, a node is located above each freezer that has three probes/channels to monitor the temperatures at the top and center inside each freezer and one dry contact connected to each individual control panel on each freezer. See the Safety section of this manual for a full description of this system.

Figure 5. Marine ESB Vertical Liquid Nitrogen Storage Tank

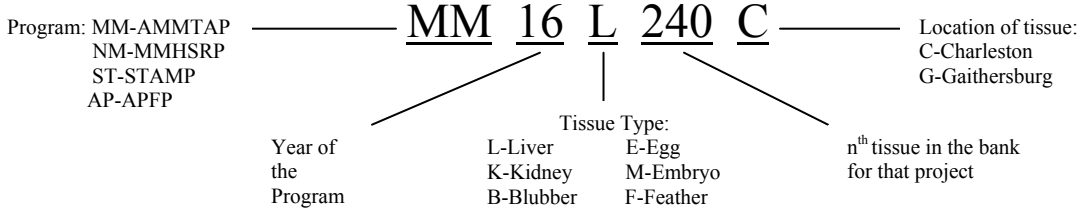
Table 2. Equipment in the Freezer Room and Reference Materials Production Room at the Marine ESB

Equipment Type	Quantity	Purpose
MVE XLC 1841 and 1830HE Liquid Nitrogen (-150 ºC) Vapor-Phase Freezers	13	Permanently store archived environmental samples
Thermo Forma Model 8500 Series (-80° C) Ultra-Cold Electric Freezer	10	Store Standard Reference Materials and control materials
Acid Storage Cabinet	1	Store hydrochloric and nitric acids, used for cleaning Teflon materials
PC for Database Management	1	Database provides specimen management, storage management, and system administration functions for samples archived at the Marine ESB
MVE Cryo Shipper	10 to 12	Shipment of samples to and from the Marine ESB
MVE SC 14/2V, 4/2V	10 to 12	Shipment of homogenized materials to and from the Marine ESB
Centron Monitoring System	3	Probes per liquid nitrogen vapor-phase freezer
	1	Probe per -80 ºC upright freezer
	2 each	Temperature, humidity and oxygen sensors
Gordinier Model 279 Keep Cold Controller	1	Maintains/controls liquid nitrogen in pipeline and vents excess gas
Nilfisk Vacuum Cleaner	1	Four-stage filtration system for best performance in cleanroom environments
Compressed Air Smashing Machine	1	Smashes large tissues into fragments during the homogenization process
Polypropylene Storage Cabinet	2	Store Teflon jars prior to cleaning; store cleaning supplies
Air Compressor	1	Provides air to smashing machine and Teflon heat sealer
Clean Room Depot Teflon Heat Sealer	1	Seals Teflon and fluorinated ethylene-propylene (FEP) bags
Cryo-Cyl 230 LP	1	Stores excess liquid nitrogen for emergency purposes
Vinyl Strip Shield Curtain	1	Minimizes particle exchange between the Storage Room and Reference Material Production Room
Vinyl Strip Shield Curtain	1	Provides an ante-room between double doors and Reference Material Production Room for cleaning large pieces of equipment
Palla S.T.C. Cryogenic Vibrating Mill	1	Cryo-homogenize large quantities of frozen material into fresh frozen powder
Millrock Quanta Series Freeze-Dryer PC\PLC	1	Freeze drying system for large quantities of frozen materials
MVE XLC 1841 Liquid Nitrogen (- 150° C) Vapor Phase Freezer	1	Working freezer for cryogenically homogenizing materials

Archival Protocols and the Specimen Tracking Database

The specimen tracking database that was first used at the Marine ESB was the Archival Specimen Tracking Retrieval Operation (ASTRO) database system. ASTRO is a PC-based graphical program that resembles other Windows-based applications allowing one to point and click to communicate with the software. It was designed for the Centers for Disease Control and Prevention (CDC) to ensure that sample collections are properly maintained and managed. The ASTRO system automated data entry and data query operations to facilitate admission of specimens, cataloging of collections, specimen retrieval, specimen tracking, data sharing, allocation and tracking of available storage space, and overall storage facility management (Ayal, 1999). A user's guide (Ayal, 1999) was produced that introduced the database. Unfortunately this system was unable to store additional data, including analytical data that are generated after a sample is cryogenically homogenized and transferred out of the tissue bank. A system that is able to provide that capability, along with other sample management features, is Freezerworks Unlimited (FUL), a relational database model that was developed by Dataworks Development, Inc. Dataworks Development also provides technical support and scheduled maintenance releases and upgrades. Therefore, the Marine ESB facility is now using this system as the specimen tracking database.

After a sample(s) is collected according to the protocol for that specific project, it is shipped frozen, in a biological dry shipper, to the Marine ESB for archival. For the NMMTB, STAMP, and APFP projects a field datasheet is completed and shipped with each sample (see Appendix A). For other projects, an Excel file with sample collection information that corresponds with the samples arriving at the facility is sent from the field collector to the Marine ESB. Once the shipper is received on site, the samples are checked to ensure that they are still in a frozen condition and that they are properly marked and correspond with the information on the field datasheets. All samples are temporarily placed inside a stainless steel basket in a liquid nitrogen vapor-phase freezer until a permanent archival location is determined. Each sample is assigned a NIST storage identification number according to the diagram shown below:



A NIST Storage Datasheet is completed using the newly assigned storage ID number (see Appendix A), and a permanent location is chosen by determining the next space available inside the freezer according to the FUL database. The NMMTB, STAMP, and APFP samples are placed in pre-labeled cardboard tubes and the NS&T mussels are placed in pre-labeled stainless steel baskets. There is a different data entry screen for each project based on the original

information that is collected in the field. Detailed information from that field data sheet is entered into the FUL database along with the location of the samples.

The tissue samples that are collected for the NMMTB are divided in two 150 g portions each, A and B. Portion A is collected for long-term storage (i.e., decades) and portion B is used for multiple analyses for different analytes. Additionally, these portions are stored in separate freezers to minimize the possibility of sample loss due to equipment failure. Currently, most of the NMMTB 'A' subsamples are archived at the NBSB and all 'B' and the remaining 'A' subsamples are located at the Marine ESB. After the samples have been properly stored, a report is printed from that shipment. A copy of each shipment report is then placed in a binder for permanent storage. For the NMMTB project, the field data sheet and NIST storage data sheet information is also entered into a national database system in Oracle. This is being developed by the primary sponsor of the project, NOAA/NMFS. The data is backed up nightly and a copy of the tape is taken to an off-site location for storage. The STAMP project will be included in this database in the future.

Biological Dry Shippers

The biological dry shippers are designed to transport biological samples at cryogenic temperatures and provide a reasonable holding time, three to ten days depending on the size of the shipper. There are primarily two sizes of shippers that are used, the MVE Cryo Shipper XC, which holds 10 L of liquid nitrogen and the MVE SC14/2V, which holds 8.7 L of liquid nitrogen (Fig. 6). The protocol to properly charge a biological dry shipper or liquid nitrogen dewar at the HML Fill Station is described in Appendix C.

Figure 6. Biological Dry Shippers; Cryo Shipper XC (left) and SC14/2V (right)

REFERENCE MATERIALS PRODUCTION FACILITY, ISO CLASS 7

Within the Marine ESB is the Reference Materials Production Facility (Rooms C104 and C105) with ISO Class 7 clean-air conditions. This area is used to produce large quantities of frozen materials for the preparation of Certified and Standard Reference Materials, as well as control materials for intercomparison exercises. The materials are cryogenically homogenized using a Palla S.T.C. Vibrating Cryomill (Fig. 7). The final product is a fresh frozen powder that is stored in -80 ºC upright freezers. The material can also be freeze dried using the large capacity Millrock Quanta Series Freeze-Dryer PC/PLC (Fig. 7) that is also located in the facility.

Figure 7. Vibrating Cryomill (left) and Freeze-Dryer (right) Located in the Reference Materials Production Facility

Movement of Large Equipment into the Marine ESB

Within the Reference Materials Production Facility is a set of double doors that is used as an emergency exit directly into the hallway. The doors are also used to bring large pieces of equipment into the Specimen Bank. In order to maintain clean-air conditions, a vinyl strip shield curtain was hung from the ceiling to the floor along an open area to create a 3.66 m x 2.74 m (12' x 9') ante-room for cleaning large equipment before bringing it into the clean room area (Fig. 7). In addition to the positive pressure of the clean rooms, the strip shield is a secondary precaution to minimize particle flow from the hallway into the ISO Class 7 clean room. See Maintenance of Clean-Air Laboratories section for details on the proper cleaning procedures of large pieces of equipment.

Figure 8. Double Doors and Vinyl Strip Shield Curtain in the ISO Class 7 Clean Room

CLEAN ROOM, ISO CLASS 5

The Clean Room is an ISO Class 5 (Room C103) clean area that is used for cleaning Teflon supplies (i.e., cryo-mills, jars, smashers, etc.) and for preparation of samples for analyses. The cleanliness level of this room is critical because it is in this room that samples are exposed to airborne particles through the homogenization process. There are many pieces of equipment needed for the preparation before and during homogenization, and these items along with other equipment used in the Clean Room are listed in Table 3.

Table 3. Equipment in the Clean Room

Equipment Type	Quantity	Purpose
MVE XLC 1211 Liquid Nitrogen (-150 °C) Vapor-Phase Freezer	1	Working freezer for cryogenically homogenizing materials
TS-Specialized 250 Disk Mill	2	Grind small particles of tissue into powder, for use with medium and large Teflon disk mills
Solvent Storage Cabinet	1	Store ethyl alcohol and chloroform, used for cleaning Teflon materials
Centron Monitoring System	3 each	Probes per liquid nitrogen vapor-phase freezer
	1 each	Temperature, humidity and oxygen sensors
Polypropylene Laminar Flow Fume Hood	2	Teflon and titanium materials are cleaned under this hood
Vertical Laminar Airflow Hood	1	Cleaned Teflon and titanium materials are placed in the hood to dry
Marble Table and Analytical Balance	1	Weigh homogenized aliquots
Balance	1	Weigh samples >1 kg
Met One Particle Counter	1	Measures airborne particles in a number of size ranges
Desiccator Cabinet	1	Air-tight cabinet to store freeze-dried materials
Polypropylene Storage Cabinet	1	Store clean Teflon materials and other supplies
Diaphragm Liquid Pump	2	Transfer chemicals/water from storage tank to bottles for cleaning supplies
Polypropylene Acid/Solvent Cart	2	Tranfer chemical bottles safely from storage cabinet to fume hood
Milli-Q Synthesis A-10 Filtration System	1	Water filtering system for cleaning and processing samples

Exhaust Hood Usage/Cleaning of Teflon and Titanium

Two polypropylene exhaust hoods are provided in the ISO Class 5 Clean Room for the cleaning of supplies. Cleaning procedures for Teflon and titanium materials are posted near each exhaust hood (see Appendix B). The date that the solvent/acid bottle was opened is documented on the label of the bottle. Water from the Milli-Q Synthesis A-10 filtration system is used for all water rinses. The chemicals can be used for approximately 6 to 8 cleaning sessions before disposal. See the Chemical Hygiene Plan (Appendix C) for proper solvent and acid disposal procedures. Solvent, acid, and strong alkali solutions can not be poured down any sink in the Marine ESB.

Cryogenic Homogenization

Cryogenic homogenization is a process that transforms a solid frozen tissue into a particulate powder and provides identical (i.e., homogenous) sample aliquots. Identical sample aliquots are necessary to allow for valid comparison of data between various researchers and analytical techniques. The Teflon disk mill is recommended as an effective device for size reduction and homogenization of biological tissue. Operation at cryogenic temperatures reduces loss of volatile components and changes in composition during the size reduction step.

Cryogenic homogenization is performed in the Clean Room following strict clean-room procedures. All equipment used during the homogenization process is made of titanium or Teflon. This reduces the sources of unavoidable contamination to two types of materials, titanium from the knives and perfluorinated compounds from the Teflon supplies. Additionally, the titanium and Teflon equipment is precleaned, as mentioned above, to eliminate any surface contamination. The following list of equipment and instructions pertain to the homogenization of a single sample (i.e., liver, kidney, blubber, egg contents).

Equipment

The following pieces of equipment are used for cryogenic homogenization and should be checked to see that everything is in proper working condition the day before homogenizing is conducted:

Liquid Nitrogen (-150 ºC) Vapor-phase Freezer (Fig. 9)
Compressed Air Smashing Machine (Fig. 10)
Air Compressor
TS-250 Disk Mill Shaker (Figs. 9 and 11)
Lint-free Cloths

Figure 9. Liquid Nitrogen Vapor-Phase Freezer and TS-250 Teflon Disc Mill Shaker

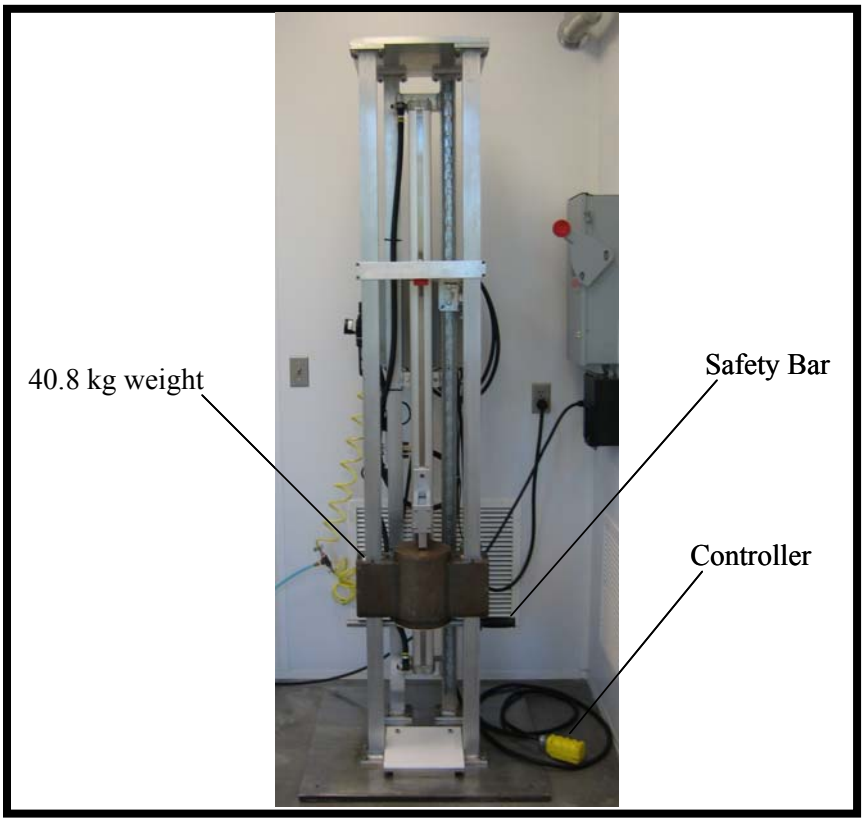


Figure 10. Compressed-Air Smashing Machine

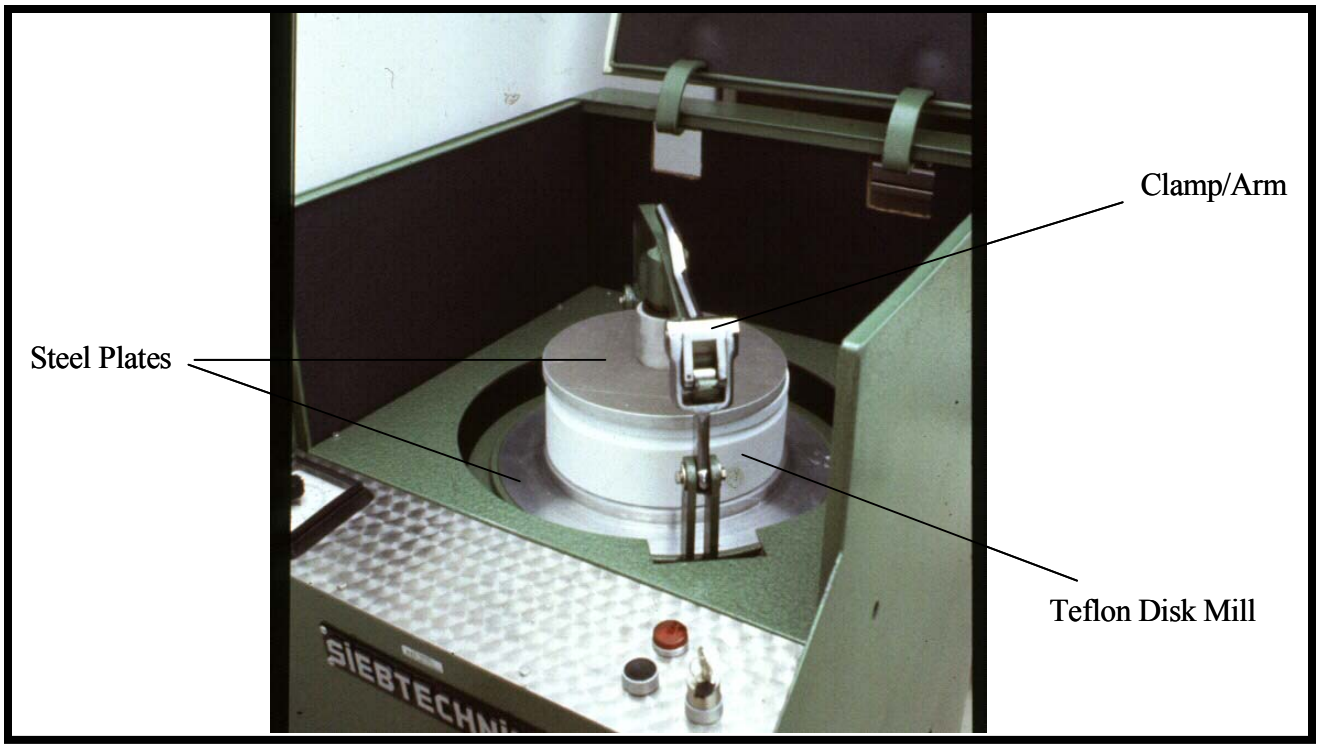


Figure 11. TS-250 Teflon Disc Mill Shaker with Teflon Disc Mill Mounted

The items below require cleaning and cooling to liquid nitrogen vapor-phase temperature before homogenization is conducted:

Teflon disk mill (Figs. 11 and 12)
Teflon smasher (Fig. 12)
Teflon jars (15 mL) or bags
Teflon scraper and scoop (Fig. 12)
Teflon-handled titanium bladed knife
Stainless steel plates for shaker (Fig. 11)

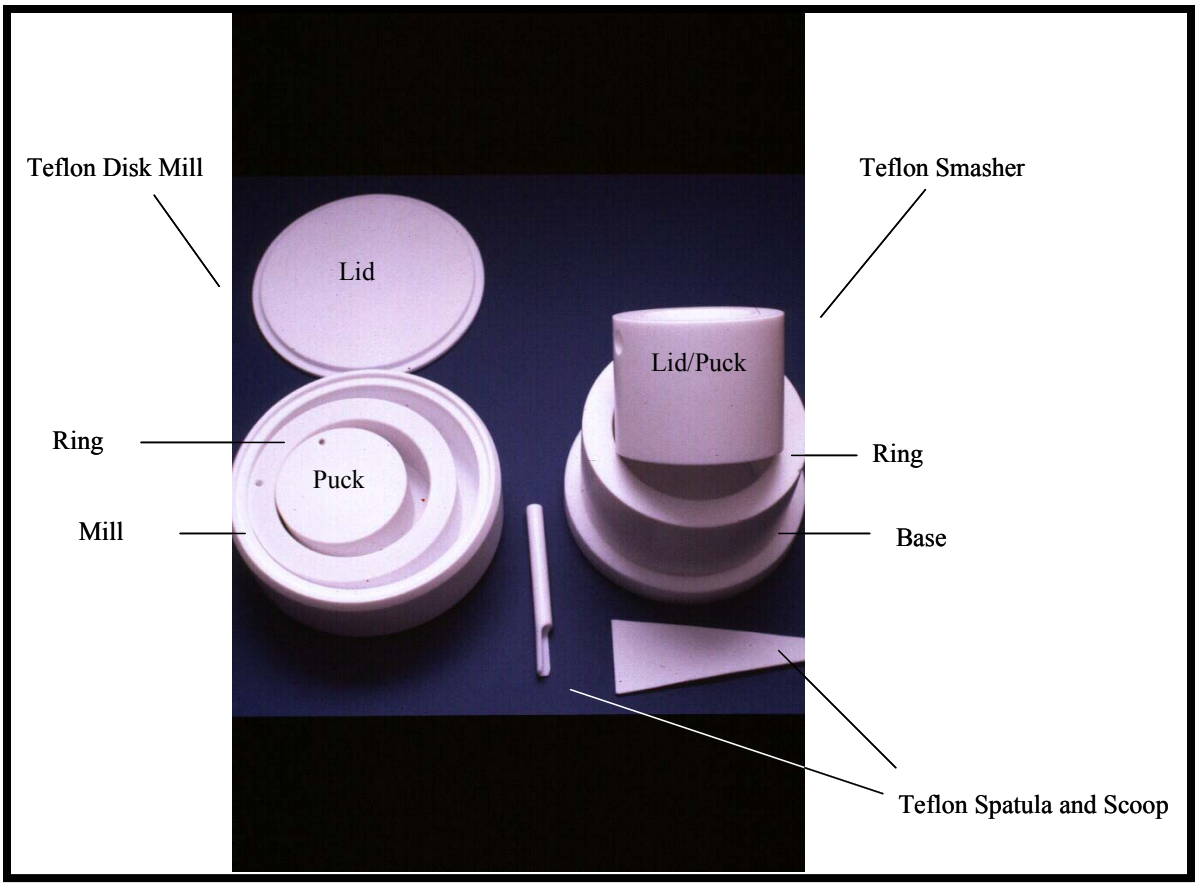


Figure 12. Teflon Materials Needed for Homogenization

Homogenization (Individual Sample)

Locate the sample that is to be homogenized and move it to the working LN_2 vapor-phase freezer. Make sure all equipment to be used during the homogenization has been cleaned properly following the NIST cleaning procedures (See Appendix B). With a pre-cut lid label, properly label 24 15 mL Teflon jars with the NIST Storage ID Number and the Aliquot Number (B001, B002,…, B024). Preweigh all Teflon jars with lids and labels in place on a 4-place analytical balance and record the masses in a laboratory notebook using indelible ink. Put all of the small items (Teflon jars, scraper, scoop, and knife) in a Teflon-lined plastic tray and place the tray in the LN2 freezer to cool overnight. Place the Teflon smasher, disk mill, and shaker plates at the bottom of the same freezer but place them on small boxes so they can be easily lifted out of the freezer.

On the day of homogenization, remove the sample that is to be homogenized from the working LN_2 freezer and follow the steps below:

1. Remove the puck (lid) from the Teflon smasher and place it inside the freezer.

2. Remove sample (tissue) from Teflon jar and place approximately 150 g to 200 g inside the Teflon smasher.

3. Place the puck of the smasher on top of the fragments and place the smasher at the bottom of the compressed air smasher.

4. Remove the safety bar and press the UP button until the 40.8 kg weight is released onto the Teflon smasher. This will fracture the sample into small fragments. (Fig. 13)

5. Press the DOWN button until the claw has clamped onto the 40.8 kg weight.

6. Press the UP button until the safety bar can be replaced.

7. Quickly put the smasher back inside the freezer.

8. Remove the lid and inner puck from the disk mill and place them inside the freezer.

9. Remove the puck from the smasher and with the Teflon spatula, carefully scrape the fragments from the bottom of the puck into the disk mill. Scrape remaining pieces off of the bottom plate of the smasher into the disk mill. Set the smasher aside in the freezer.

10. Scrape all pieces inside the disk mill into the outer ring area. Do not force tissue fragments into this area; if a fragment is too large, place it back in the smasher and repeat Steps 2 through 7. Once all pieces are placed loosely in the outer ring, place the inner puck back in the center of the disk mill and place the lid securely on top.

11. Remove the shaker plates from the freezer and place the bottom plate on the TS-250 disk mill shaker. Quickly place the disk mill onto the shaker plate, place the top shaker plate on top of the disk mill, and fasten the shaker clamp, making sure it is on tight (Fig. 11).

Figure 13. Sample of Tissue in Fragment Form and Frozen Powder Form

12. Close the lid and set the timer for 3 minutes (2 minutes for egg contents). Turn the machine on and wait for it to stop.

13. Once the machine has stopped, place the shaker plates and the Teflon disk mill back in the freezer, remove the mill lid, and scrape any tissue remaining on the lid into the disk mill. Verify that the tissue is sufficiently homogenized and has completely turned in to powder. If the tissue is still in fragments there is probably a piece of tissue that is jammed between the mill and the ring (Fig. 13). Remove the puck from the disk mill and move the fragments back into the inner ring area with the spatula until the inner ring is loose enough to be removed from the disk mill completely. Move all pieces toward the center of the disk mill and replace the inner ring, making sure there are no pieces underneath the ring. Repeat steps 8, 9, 10, and 11. Repeat these steps until the tissue is in powder form (Fig. 13). If there are any hard pieces found after repeating the steps several times, the equipment or material is too warm to work with and you must stop homogenizing and wait several hours for the sample and equipment to cool down.

14. Using the Teflon scoop, fill all jars in numerical sequence, making sure the lids are placed on tightly. Wipe down each jar individually with a lint-free cloth just prior to placing on a 4-place analytical balance. Record the masses in the laboratory notebook. Place the jars in a storage box and return to a LN_2 vapor-phase freezer for long-term storage.

15. Fill out the sample storage form including the homogenization date, the masses of each sample, any comments necessary, and identify the permanent storage locations.

16. Update all databases that contain the sample storage data form with the same new information, including the FUL, Excel, and Word databases.

Note: When removing the Teflon disk mill lid and smasher puck, place them upside-down inside the freezer so that the side of the lid that touches the tissue does not come in contact with any part of the freezer or other items in the freezer. Also, all work should be completed with the equipment inside of the working freezer, except when transferring Teflon equipment to and from the smashing unit and the TS-250 disk mill. Because the cold temperatures of the equipment and the tissue are critical, it is important to work quickly to keep these items inside the freezer as much as possible.

SAFETY

The Marine ESB has been equipped with several safety features throughout each of the rooms to ensure safe laboratory operating procedures (Table 4). And because the Marine ESB is located in a NOAA Facility, all personnel must abide by NOAA safety rules and policies. NIST and NOAA are both federal government agencies under the Department of Commerce therefore many of the safety rules and policies are similar. The rules and policies referred to in this document as a part of the appendix are that of the HML facility. The HML Safety Committee has written a Laboratory Chemical Hygiene Plan and Emergency Preparedness Plan that are

Table 4. Safety Equipment Located in the Marine ESB

Room Name	Room Number	Safety Feature
Ante Rooms	C102 and D103	Chemical Spill Kit First Aid Kit Material Safety Data Sheets Job Hazard Analysis Form Chemical Inventory
Freezer Room	D104	Centron Monitoring System Smoke Detector Fire Alarm Strobe Light Humidity and Oxygen Sensor Liquid Nitrogen In-Line Piping System Telephone Incoming Page Speaker
Reference Material Production Facility	C104 and C105	Centron Monitoring System Smoke Detector Fire Alarm Strobe Light Humidity and Oxygen Sensor Liquid Nitrogen In-Line Piping System Acid Storage Cabinet Incoming Page Speaker Eyewash Station Emergency Exit (set of double doors)
Clean Room	C103	Centron Monitoring System Smoke Detector Fire Alarm Strobe Light Humidity and Oxygen Sensor Liquid Nitrogen In-Line Piping System Eyewash Station Teflon-lined Fume Hoods Solvent Storage Cabinet Incoming Page Speaker Telephone

strictly enforced throughout the entire laboratory (see Appendix D). A Job Hazard Analysis Form has also been designed (see Appendix D). This form must be filled out for each individual laboratory and it must be posted outside of the laboratory. This form includes information on the work activities that are completed in the laboratory and what Personal Protective Equipment (PPE) is required to be worn during those activities. All personnel who work in that laboratory

must sign this form. Also, a list of chemicals that are used in the laboratory must be posted outside the entrance of that laboratory (see Appendix D). In addition to those two lists, Material Safety Data Sheets (MSDS) for all chemicals used in each laboratory must be placed in an easily accessible area near the chemicals. In the Marine ESB, these sheets have been posted inside each of the two ante-rooms on the wall. All forms must be updated every 6 months.

Hurricane Evacuation

The Atlantic hurricane season is from June 1 to November 30. Special actions must be taken in order for the Marine ESB to be prepared for a hurricane evacuation. If a mandatory evacuation is announced, the following steps must be followed before leaving the HML building:

1. The computers in the Freezer Room and in the NIST Offices must have the latest back up of files on the NIST designated server. These include the FUL database inventory and all Paradox files with completed field data sheets for all samples inventoried. This server will be taken out of the building by authorized information technology (IT) personnel.

2. A hard copy of the Marine ESB inventory must be printed from the FUL database for all freezers. This will ensure that if a freezer has a mechanical breakdown and the inside of the freezer does not maintain cryogenic temperatures, those samples can be labeled properly. This hard copy must be taken out of the building.

3. All -150 °C liquid nitrogen freezers must be filled with liquid nitrogen to the 'High Level Fill' mark just before evacuation.

4. All temperatures and liquid nitrogen levels must be recorded in the black NIST Laboratory notebook and taken with personnel out of the building.

5. The binder that contains information on miscellaneous items that are stored in the Marine ESB must be taken out of the building.

6. The Cryo-Cyl 230LP must be filled with liquid nitrogen and brought into the Freezer Room.

7. All computers and other electrical equipment must be covered with plastic in case of water leaks due to roof damage.

Updates on facility status and campus access will be posted on the Fort Johnson campus voice mail system. To access the voice mail system from an off-site telephone, dial (843) 762-8888. Follow the automated instructions by entering your mailbox extension and password followed by # when prompted. In addition to the voice mail system employees can also obtain updates via the NOAA toll free hotline at 1-888-662-2911 or information can be accessed online at www.homelandsecurity.noaa.gov or www.noaa.gov.

Centron Environmental Monitoring and Control System

The Centron System: Environmental Monitoring and Control System, created by Rees Scientific Corporation, has been installed to continuously monitor the temperatures of all freezers located in the Marine ESB as well as monitor the room conditions: temperature, percent humidity, and oxygen levels. Three probes have been placed in each of the LN_2 vapor-phase freezers, 2 probes record the top and center temperatures of each freezer and one probe is a dry contact to the freezer's individual computer monitoring system. Each individual liquid nitrogen freezer has its own computer monitoring system that monitors more than just the temperatures of the freezer, for instance, it also monitors the time it takes to fill a freezer. If the freezer fills for more than a half hour, the computer will alarm, indicating that the freezer may be overfilling. When the freezer's computer goes into alarm, for whatever reason, it will trigger the Centron probe to alarm. One probe has also been placed at the top of each of the -80 °C ultra-cold upright freezers. An alarm will sound if pre-set condition ranges are exceeded. The phone alarm notification system is designed to notify remote users of any alarms that are triggered. The Centron system uses several phone lists that were created by personnel. The Day list is activated Monday through Friday from 0800 to 1700 hours, the Evening List is activated Monday through Friday from 1701-0759 hours and the Weekend List is activated Saturday and Sunday from 0800 to 0759 hours. The Day List includes the office/laboratory phone numbers of Marine ESB personnel, the Evening and Weekend Lists include home and cellular phone numbers of Marine ESB personnel. The system will continue calling the entries until a user has acknowledged the call by entering his/her individual password and inhibiting the probe that is in alarm for a designated time period. Each alarm can be inhibited for 0 to 999 hours. This allows the person time to reach the laboratory and repair the freezer or if necessary, move samples from the damaged freezer into another freezer. The system ensures that trained personnel will be able to correct the problem or error within the Marine ESB before any damage has occurred. The Centron System stores all information that is provided by the trained user (i.e., the date and time the alarm was acknowledged, the probe that went into alarm, and comments from the user that answered the alarm which includes cause of the alarm event).

MAINTENANCE OF CLEAN-AIR LABORATORIES

In general, cleaning should begin in areas that require the most critical level of cleanliness and proceed toward areas of less critical requirements. The following cleaning methods are to be followed to ensure that each room maintains the ISO Class 5, 6, and 7 levels. Basically, the preferred clean room cleaning protocol is to vacuum an area, wipe that area with a cleanser and lint-free cloth and vacuum the area again.

1. Vacuum ceiling panels using a dry vacuum with a soft brush attachment. To protect the HEPA filters, only the grid surrounding the filters should be cleaned, using overlapping strokes following the grid pattern in one direction only. Remove spots with a commercial cleanser, deionized (DI) water, and lint-free cloth and then vacuum again to remove any loosened particles.

2. Vacuum lighting units with a soft brush attachment. Open the unit and wipe the bulbs with a lint-free cloth dampened with DI water, vacuum horizontal surfaces, and close unit. Wipe all trim pieces.

3. Vacuum walls first, starting at the ceiling and working in vertical lines toward the floor. Next, use a sticky roller to wipe the wall, using overlapping movements from the ceiling towards the floor. Vacuum again using a soft brush attachment.

4. Clean doors, frames, and components using a vacuum, followed by wiping with a lint-free cloth that has been moistened with Deconahol and DI water. Doors should be cleaned while they are ajar.

5. Wipe glass surfaces and windows with a lint-free cloth that has been dampened with Deconahol. With a dry wiper or squeegee, dry the window starting at the top and move vertically towards the bottom.

6. Use a lint-free cloth dampened with Deconahol and DI water to clean piping systems. Wipe from top to bottom, using overlapping strokes. Vacuum pipes with a curved pipe attachment and then re-wipe the pipe, if necessary, to remove stubborn grime and spots.

7. Vacuum freezers with a soft brush attachment. Use a lint-free cloth dampened with DI water to clean the top and sides of the freezer, use overlapping strokes to wipe the top of the freezer and then from top to bottom for the sides of the unit. Re-vacuum with a soft brush attachment to pick up any loose particles.

8. Wipe work stations/bench tops with a lint-free cloth moistened with Deconahol and DI water. Start at the rear of the work surface and use overlapping strokes in one direction only. Use a new area of the wiper for each stroke.

9. Vacuum the surface of the floor first, place the sticky mop or vacuum on the floor and pull toward you, lift and move the vacuum so that the next stroke starts adjacent to and slightly overlaps the first stroke. Next, wash the floor with a solution of DI water and floor cleanser using a clean mop head. Rinse with DI water and a new mop head, leaving the rinse water on the floor long enough to completely saturate any film build-up. Mop up the rinse water, making sure to change the water after mopping every 10 to 15 square feet of floor surface. Because the floor is the dirtiest part of the clean room, it is important to keep the wash water and rinse water clean by changing it after 10 to 15 square feet of surface has been mopped. After the floor is dry, vacuum again, using the same movement as described above.

10. All supplies and equipment that are brought into the Marine ESB are to be cleaned using these same protocols. Vacuum all items using a dry vacuum with a soft brush attachment, inside and outside if necessary. Wipe items with a lint-free cloth that has been moistened with Deconahol and vacuum again.

The cleaning schedule for the Marine ESB will depend upon the amount of work being conducted in the rooms and the number of particles that are detected on the particle counter that is stored in the Class 5 Clean Room. A monthly particle count is taken to ensure the rooms maintain the ISO Class 5, 6, and 7 levels. The particle counter is a portable laser counter that is pre-set to count 0.3 μm and 0.5 μm particles over a programmable time period. For the Marine ESB, the instrument is placed in four separate areas of each room for a 5 minute time period and the data are stored inside the particle counter. It can be exported into a separate format if needed. If the particle counts are above the class levels required, a thorough cleaning should be conducted using the above cleaning procedure. Because the Clean Room is two class levels higher than the Freezer Room, entry from one room to the next should be kept to a minimum. A sticky mat with 30 adhesive layers has been adhered to the floor in front of the door leading from the Class 5 to the Class 7 to trap any particles that are on the bottom of the shoe covers. This will help minimize the number of particle exchanges between the two rooms. When the top layer of the sticky mat is dirty, it is removed to expose the next sticky layer. The sticky mat is replaced with a new mat when all layers have been removed.

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APPENDIX A

MARINE ESB DATA SHEETS

NMMTB Field Datasheet
STAMP Field Datasheet
APFP Field Datasheet
NIST Storage Data Sheet

National Marine Mammal Tissue Bank Form

MESB Sample Processing - Page 1

Field ID: _____ Other ID Number: _____

Common Name: _____ Genus species: _____

Stranding Type: (choose all that apply)
- ☐ Single ☐ Incidental Take..... ☐ Fisheries ☐ Other (specify): _____
- ☐ Mass ☐ Live Capture..... ☐ Rescue ☐ Other (specify): _____
- ☐ UME ☐ Subsistence Add'l. Remarks: _____

Condition: (choose one) ◉ Alive ◉ Fresh Dead ◉ Euthanized

If euthanized, with what and how much: _____

Was animal in rehabilitation? (choose one) ◉ Yes ◉ No *If yes:* ____ *Where:* _____ *From:* dd / mm / yy *To:* dd / mm / yy
(please attach clinical/medical records)

Animal Location: State: _____ County: _____ City/Island/Community: _____

Ocean/Bay/Sea: _____

Locality Details: _____

Latitude: _____ N Longitude: _____ W

Time of death (Zulu)..... dd / mm / yy hr Place of Death: _____

Internal body temp. of animal: _____ ◉ C ◉ F *Rigor?* ◉ Yes ◉ No

If transported before tissue removal:
- *Vehicle Type:* _____ *Length of Transport:* _____
- *Ambient weather condition:* _____
- *Remarks:* _____

Time of tissue removal (Zulu).... dd / mm / yy hr Place of tissue removal: _____

If transported before processing:
- *Transportation storage:* ☐ *Dry ice* ☐ *Wet ice* *Other:* _____
- *Ambient weather condition:* _____
- *Interim storage of tissue:* ☐ *Teflon bag* ☐ *Teflon jar* *Other:* _____
- *Remarks:* _____

Time of tissue processing.... dd / mm / yy hr Place of tissue processing: _____

Ambient temperature at processing: _____

Time of interim freezing..... dd / mm / yy hr Freezer type: ☐ LN_2 ☐ -80degC ☐ -30degC Other: _____

Time shipped to MESB..... dd / mm / yy hr

Time received at MESB..... dd / mm / yy hr

Additional comments: _____

Sample weights:	Blubber (g):	Liver (g):	Kidney (g):	Whole Blood (mL):	Plasma (mL)	Serum (mL):	Other:
A							
B							

National Marine Mammal Tissue Bank Form

Field ID: _____ Genus species: _____

Sex: ◉ Female ◉ Male

Total length: _____ ◉ cm ◉ in ◉ Actual ◉ Estimated

Total weight: _____ ◉ kg ◉ lb ◉ Actual ◉ Estimated

Age Class: *(choose one)* ◉ Adult ◉ Subadult ◉ Pup/calf ◉ Yearling ◉ Unknown | ◉ Actual ◉ Estimated

Age: GLG's: _____ Other: _____

Method used: _____ Date aged: dd /mm / yy

By whom: _____

Epiphysis: ◉ Open ◉ Closed fused ◉ Fused invis

Reproductive condition:

☐ Sexually Mature

☐ Pregnant

☐ Lactating

Testis/Ovaries: *(cicle one)*

	Length:	Mid-Width:	Mid-depth:		Weight:	
Left:				◉cm ◉in		◉kg ◉lb
Right:						

Fetus length: _____ ◉ cm ◉ in

Corpora lutea #: _____ Corpora albicantia #: _____ Corpora hemmorghagicum #: _____

Specify Units of Measurement: ◉ cm ◉ in

Cetaceans:

Snout to ant. ins. of flipper: _____

Snout to center of genital aperture: _____

Snout to center of anus: _____

Flipper length: _____

Fluke width: _____

Fluke notch to anus: _____

Total counts: ..... UL/LL: _____ UR/LR: _____

Girth:
- Axillary: _____
- Max: _____ ---- _____ *(Location)*
- Anal: _____

Blubber thickness:
- Thoracic: _____
- Dorsal: _____
- Lateral: _____
- Ventral: _____

Pinnipeds:

Nose to tail length: _____

Ant. length of foreflipper: _____

Axillary girth: _____

Bacculum length: _____

Ant. length of hind flipper: _____

Blubber thickness over post. end of sternum: _____

Other blubber thickness: _____ ---- _____ *(Location)*

Polar Bears:

Girth of neck of axis: _____

Girth of neck at shoulders: _____

Skull length: _____

Sea Otters:

Snout to angle of mouth: _____

Skull length: _____

Axillary girth: _____

Right forepaw width: _____

Skull width: _____

Tooth Wear: ◉ Heavy ◉ Med. ◉ Light ◉ None

Extimate of body fat stores:

	None:	Little:	Average:	Excessive:
Subcutaneous:	◉	◉	◉	◉
Groin: ____ cm	◉	◉	◉	◉
Kidneys:	◉	◉	◉	◉
Mesenteric:	◉	◉	◉	◉

National Marine Mammal Tissue Bank

Field ID Number: _____ Genus species: _____

Was animal necropsied? ◉ Yes ◉ No

Necropsied by: _____ dd / mm / yy

(Please attach necropsy report) Date

Samples collected:

Histological samples:

Individual/Organization: _____ Final destination: _____

Tissues sampled: *(Choose all that apply)*

☐ Liver	☐ Kidney	☐ Blubber	☐ Stomach	☐ Heart	☐ Intestine
☐ Lung	☐ Pancreas	☐ Adrenals	☐ Brain	☐ Muscle	☐ Skin
☐ Trachea	☐ Spleen	☐ Thymus	☐ Colon	☐ Thyroid	☐ Esophagus

Other: _____
(Please list) _____
_____

Lymph Nodes: ☐ Submandibular ☐ Prescapular ☐ Axillary ☐ Hilar ☐ Mesenteric

Other l.n.: _____

Other samples collected:	Type of storage: (Z-frozen, F-formalin, DMSO, ETOH)	Where located (Ind./Org.):
Teeth:		
Genetics (skin):		
Skull:		
Reproductive tract:		
Mammary tissue:		
Ovaries:		
Gonads/testes:		
Parasites:		
■ *List type and location:*		
Stomach:		
■ *List contents if applicable:*		

Other contaminant samples:
(List tissue type, storage type and where located)

Additional samples:
(List tissue type, purpose of collection, storage type and where located)

National Marine Mammal Tissue Bank

Field ID Number: _____ Genus species: _____

Photos taken: ◉ Yes ◉ No ◉ Digital ◉ Film If yes, how many? _____

(send copy with samples for NIST archive)

Video taken: ◉ Yes ◉ No

Disposition: _____

(primary location for photos and/or video)

General comments: _____

(Field notes)

General appearance of individual: _____

General appearance of organs: _____

NMMTB Protocol: ◉ Standard ◉ Modified

Please note any modifications: _____

Form prepared by: _____

Name

_____

Affiliation

A copy of this form and Level A Data Form should be shipped with samples to:

ATTN: Rebecca Pugh
National Institute of Standards and Technology
Hollings Marine Laboratory
331 Fort Johnson Rd
Charleston, SC 29412
(843) 762-8952

NMMTB's Chain of Custody

Field ID Number: _____

Other ID Number: _____

NMMTB Reference/Storage ID Numbers: _____ _____

_____ _____

_____ _____

1.	Collector's signature	Method of transfer to processing stage	dd / mm / yy Date
2.	Processor's signature	Method of transfer to shipping stage	dd / mm / yy Date
3.	Shipper to NMMTB's signature	Method of transfer to MESB	dd / mm / yy Date
4.	Receiver's signature		dd / mm / yy Date

Each person in possession of the tissue must sign and date the form.

SEABIRD TISSUE ARCHIVAL MONITORING PROGRAM
NATIONAL BIOMONITORING SPECIMEN BANK
SEABIRD DATA FORM

I

Sample ID Number:_____ **Species:**_____

Geographic Area:_____ **Lat.:**_____ **Long.:**_____

Colony Name:_____ **Sample Source:**_____

Site ID Number/Name:_____

II

Sample Type: Liver Kidney Muscle Feather Egg Blood Other_____

Date and Time of Death (If Applicable):_____

Date and Time of Collection:_____ **Method of Collection:**_____

Weather Conditions:_____

Field Storage Conditions:_____

Pre-shipment Storage Conditions:_____

Date and Time Shipped from Study Site:_____

Protocol: Standard Modified (Please note modification below)

Comments:

Additional Samples:

Collected by:_____

SEABIRD DATA FORM (con't.)

III **Sample ID Number:**_____

Date and Time of Sample Preparation:_____ **Processor:**_____

Date and Time of Freezing (-80° C or below):_____

Date and Time of Shipping to NBSB:_____ **Shipper:**_____

Date and Time Received at NBSB:_____ **Receiver:**_____

IV **Histological Samples:**

Individual/Organization:_____

Final Destination:_____

Tissues Sampled:_____

V

Sample Weight:	**A**	**B**		
Liver			**Egg (whole)**	
Kidney			**Egg (contents)**	

Egg Measurements: **Length:**_____**cm** **Breadth:**_____**cm**

Eggshell Thickness:_____**mm** **(mean of ten measurements)**

VI

Form Prepared by:_____
(Print Name)

_____
(Signature)

A copy of this form should be shipped with samples to:

Rebecca Pugh
Research Biologist
NIST
219 Fort Johnson Road
Charleston, SC 29412
(843) 762-8647

Peregrine Falcon Egg Contaminants Data Sheet

Collector name and affiliation: _____

Processor name and affiliation: _____

Date Collected: _____ Date Processed: _____

Nest Number or location: _____

Egg Number or description: _____

Nest status at time of collection: _____
(laying, incubating, abandoned, with chicks - how many, post-fledging, etc.)

Egg Length (three measurements, 0.1 mm): _____ , _____ , _____ Average _____

Egg Width (three measurements, mm): _____ , _____ , _____ Average _____

Whole Egg Weight (0.01 g): _____

Weight of displaced H_2O (egg volume) (0.01 g): _____

Contents weight:
a) Tare weight of teflone bag (0.01 g) : _____
b) Weight of bag plus contents (0.01 g): _____
c) Weight of contents (b-a): _____

Conversion factor = $\frac{\text{contents weight}}{\text{displaced } H_2O \text{ weight}}$ = _____

Contents condition (age of embryo, state of decay, etc.) and other comments:

_____

_____

Where are the membranes? Inner: _____ Outer: _____

Eggshell thickness after > 10 days of air drying (note whether either, neither, or both membranes are included in the measurements):

First eggshell half: _____ _____ _____ _____ Avg: _____
Second eggshell half: _____ _____ _____ _____ Avg: _____ Overall Average: _____
Dry shell weight (mg) after > 10 days of air drying: _____

NIST SPECIMEN BANK

Sample Storage

	Storage ID Fz	Rack	Box	Wt (g)	Date In Day	Mo	Yr	Date Out Day	Mo	Yr	Initials

Comments _____

_____

Subsamples	LN2	Other	Storage ID Fz	Rack	Box	XY	Wt (g)	Date In			Date Out			Initials
B001														
B002														
B003														
B004														
B005														
B006														
B007														
B008														
B009														
B010														
B011														
B012														
B013														
B014														
B015														
B016														
B017														
B018														
B019														
B020														

APPENDIX B

INSTRUCTIONS FOR CLEANING TEFLON AND TITANIUM SUPPLIES

Teflon and titanium supplies are cleaned using the following steps. The supplies are placed in a polythylene storage tank located under a fume hood. They are soaked in each chemical or Milli-Q Synthesis 18.2 MΩ·cm resistivity water (H_2O) rinse for a designated time period. Hydrochloric acid (HCl) and nitric acid (HNO_3) are both diluted with Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O at the ratios listed below. The chemicals are transferred back into the bottles by using a chemical-resistant electric pump. Old chemical bottles are used to store hoses from the pump after each use, one bottle for acids and one bottle for solvents.

TEFLON

Chemical/Water	Time in Liquid
Chloroform ($CHCl_3$)	1 h
Ethyl Alcohol (EtOH)	1 h (older bottle)
Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse	
HCl/H_2O (1:2)	4 h
Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse	
HNO_3/H_2O (1:2)	4 h

Above soaks, excluding water rinses, should completely cover the items in the vat.

Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse
Ethyl Alcohol Rinse (can be used approximately 4 times before disposal)
Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse - First Rinse
Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse - Final Rinse

Above soaks, excluding H_2O rinses, use one bottle of liquid.

TITANIUM

Chemical/Water	Time in Liquid
EtOH	1 h to 2 h
Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse	Overnight
HC)/ H_2O (1:10)	½ h
HNO_3/ H_2O (1:10)	½ h
Milli-Q Synthesis 18.2 MΩ·cm resistivity H_2O rinse	

APPENDIX C

HOLLINGS MARINE LABORATORY POLICY ON USE OF THE LIQUID NITROGEN FILL STATION

Hollings Marine Laboratory Policy on Use of the Liquid Nitrogen (LN_2) Fill Station

The liquid nitrogen (LN_2) fill station, located at the back of the Hollings Marine Laboratory (HML), is available for use to all HML researchers. The fill station is connected to the NIST-owned 3,000 gallon LN_2 bulk tank. This tank is set up with a telemetry system that is linked directly to the LN_2 supplier so that when the liquid level falls below a certain point, the supplier delivers more LN_2 automatically. This is important because the bulk tank provides LN_2 to all of the freezers located in the Marine Environmental Specimen Bank, ten -80 ºC upright freezers, and fifteen LN_2 vapor-phase freezers. In order to use the fill station, the researcher must obtain a code number for the lock on the gate from the HML front desk and follow the procedures below:

- Use only containers that have been designed and manufactured according to all applicable codes and specifications for liquid-nitrogen temperatures and pressures (i.e., dewar, shipper, cryogenic tank, cylinder)
- Wear proper personal protective equipment (PPE) when filling a container. Safety glasses or goggles and cryogenic gloves (thermal insulated gloves) shall be worn at all times when handling cryogenic liquids or when in the proximity of someone handling cryogenic liquids. There should always be a pair of cryo-gloves and a face shield located inside of the cage; if that PPE is missing, please contact Rick Meitzler, x8842 or Raluca Semeniuc, x8870.
- Other PPE that must be worn when handling liquid nitrogen includes a long-sleeved garment (i.e., shirt or lab coat), long pants, and closed-toed shoes.
- Individual sets of ear plugs are also provided on the wall next to the fill station. These plugs are not required but are provided for your comfort to block the excessive noise that comes from the fill hose when filling a container or to protect your ears if there is a valve leak.
- To fill an open container, remove the fill line (hose) from the left polyvinyl chloride (PVC) pipe that is attached to the wall. The fill lines should always have a diffuser on the end of it, if it does not, contact Rick Meitzler, x8842 and/or Cleve Robertson, x8934. Place the fill line in the container and open the top valve. It will take several minutes for the nitrogen gas to be vented from the fill line. The fill line will dispense liquid after the gas has been pushed through and the piping and fill line are at the proper temperatures. Someone must be in attendance at the fill station at all times during the transfer operation.
- To fill a closed container, remove the fill line (hose) from the right PVC pipe that is attached to the wall, this fill line does not have a diffuser on the end of it. Then follow the instructions that were provided by the manufacturer for your closed container of choice.

For problems with the fill station immediately contact:
Rebecca Pugh (x8952) or
Cleve Robertson (x8934)

HML Liquid Nitrogen Fill Station

APPENDIX D

HOLLINGS MARINE LABORATORY SAFETY MANUALS AND FORMS

Chemical Hygiene Plan
Emergency Preparedness Plan
Job Hazard Analysis Form
Chemical Inventory for Rooms C103 and C104

CHEMICAL HYGIENE PLAN

HOLLINGS MARINE LABORATORY

CHARLESTON, SC

National Ocean Service
National Oceanic and Atmospheric Administration
Department of Commerce

South Carolina Department of Natural Resources

Medical University of South Carolina

National Institute of Standards and Technology

University of Charleston

November 2006, Revision F

TABLE OF CONTENTS

INTRODUCTION

The purpose of the HML Chemical Hygiene Plan is to establish uniform, safe, and efficient practices in the laboratories and to assist in the safety instruction of new laboratory employees. The contents of the manual are general in nature. Specific problems should be referred to the Area Safety Representative or laboratory supervisor. Additional specifics are included in Section VIII.

It is the policy of the HML to do all that is reasonable to prevent injury to persons, and damage to property, and to protect the employees, facility, the environment, and the public from injury, fire or other damage. In order to achieve these goals the HML has instituted a comprehensive safety program. The Director urges the active cooperation and commitment of all branches and employees. Ongoing dialogue and feedback is encouraged. HML's management supports this program in its promotion of employee safety and health. Aspects of the overall safety program include:

- Safety policies and procedures
- Incident reporting and investigation
- Emergency preparedness
- Hazardous materials and hazardous waste management programs
- Fire protection
- Safety education and training

These facets of the facility safety program are also incorporated by reference into the Chemical Hygiene Plan.

The employees who work with chemicals bear the primary responsibility for safety on the job, not only for themselves but also for fellow employees. They must obey all safety rules; they must report unsafe conditions to the Safety Committee, and if they are not certain about proper safety procedures, they must consult their supervisors.

The attitude of the employee is the key to employee and environmental safety. If he/she is interested and willing to follow the simple safety rules outlined in this manual, there will be little chance of injury or damage from material being handled in the laboratory.

The Safety Committee and management expect that all laboratory staff from all of the Joint Partnership Institutions (National Oceanic and Atmospheric Administration (NOAA), National Institute of Standards and Technology (NIST), South Carolina Department of Natural Resources (SCDNR), Medical University of South Carolina (MUSC) and College of Charleston (CofC)) will live up to the spirit and intent of this manual and make their laboratory a safer and better place to work.

CHEMICAL HYGIENE PLAN

The following written chemical hygiene plan has been established for the:

HOLLINGS MARINE LABORATORY

to comply with the U.S. Department of Labor, Occupational Safety and Health Administration Standard 29 CFR 1910.1450, Occupational Exposure to Hazardous Chemicals in Laboratories and other applicable regulations issued by DOC.

The senior official at this site has primary responsibility for this location's chemical hygiene program. Functional responsibility has been delegated to:

To be determined and assigned by each lab user group's Area Safety Representative (ASR)

All tenant components at this site are covered by the program as such: NOAA employees, MUSC employees, SCDNR employees, NIST employees, and College of Charleston employees and all of the above designated representative.

Upon request, the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) shall have access to this written plan at the following location:

http://hmlintranet/safety/ (the latest version of the plan) and/or in each HML laboratory.

Familiarize yourself with the plan's location nearest your lab and list same on your Job Hazard Analysis (JHA) posted form.

PART ONE

PERSONNEL RESPONSIBILITIES

HML has established a chain of command to handle specific safety responsibilities within the facility. The HML Safety Committee, in conjunction with individual laboratory supervisors, holds primary responsibility for developing and maintaining a safe working environment for laboratory workers.

The responsibilities of various staff positions are described below.

A. ALL LABORATORY STAFF

All laboratory staff is expected to obey the safety rules and to report all unsafe conditions and all accidents. Each person working with or around chemicals must be properly trained and is responsible for remaining aware of the hazards associated with these chemicals and handling them in a safe manner. If there is any doubt as to the specific hazards associated with a material, or to the proper method of handling, the employee is expected to ask his or her supervisor or the Environmental Health and Safety (EH&S) Officer for the appropriate information.

B. SAFETY COMMITTEE

The Safety Committee is responsible for overall coordination of safety policies within HML including routine inspections. The Safety Committee is chaired by the HML EH&S Officer, with member representation from each of the Joint Partnership Institutions (NOAA/National Ocean Service (NOS), NIST, SCDNR, MUSC, and CofC).

C. LABORATORY SUPERVISOR

1. Provide assistance, information, or instruction to employees regarding safety issues identification of hazards or potential hazards, and ensure that adequate supervision is provided.

2. Provide laboratory employees with information, as applicable, to allow an effective review of existing procedures and policies in regard to safety issues.

3. Ensure that workers comply with the Occupational Health and Safety Act and that they carry out all prescribed safety measures and procedures.

4. In addition to posting a copy of the Occupational Health and Safety Act poster in a conspicuous place in the HML copier/fax room # A-108, post a copy of any OSHA inspection reports and responses to orders issued.

5. Ensure that all equipment, whether leased, rented, or owned outright is maintained in good condition.

6. Ensure that proper facilities, equipment, protective devices, or services are provided and maintained in good order for safe handling, storage, and disposal of chemicals and biological materials or wastes.

7. Maintain a list of all hazardous materials used in the lab and keep the Chemical Inventory List up to date.

8. Ensure compliance with legislative requirements regarding dangerous drugs, radioactive isotopes, hazardous chemicals or reagents, biological materials, or wastes in conjunction with the EH&S Officer.

9. Ensure that workers are familiar with the Chemical Hygiene Plan and comply with all its requirements.

10. Enforce proper procedures in chemical handling, storage, dispensing, and transportation within the lab and any related bulk chemical storage rooms, as appropriate.

11. Ensure that all new employees are properly trained, adequately supervised, and familiarized with all aspects of lab safety to ensure adherence to Job Hazard Analysis (JHA) protocols for their lab areas.

12. Be involved in clean-up of significant lab chemical spills and ensure that proper procedures and precautions are carried out. Carry out all required reporting of spills or releases.

13. Ensure that waste is disposed of in an appropriate manner.

D. LABORATORY DIRECTOR

Under the OSHA Laboratory Standard, the Laboratory Director of the facility has ultimate responsibility for the implementation of this plan and must, with other administrators, provide continuing support for institutional chemical hygiene (CFR 1910.1045 OSHA law).

PART TWO

RULES FOR SAFE PRACTICE

A. GENERAL SAFETY

1. Fundamental Rules

a. Do not eat, drink, smoke, or apply cosmetics in the laboratory, as per OSHA mandates.
b. Do not put any objects, i.e., pencils, fingers, swabs, etc. in the mouth, ears, or nose.
c. Do not bring food into the laboratory. Food and beverages are prohibited from lab entry.
d. Mouth pipetting is forbidden.
e. Keep your lab coat buttoned while working in the laboratory. When leaving the laboratory remove your coat and wash your hands. Lab coats should not be worn outside of the lab or work area and must be changed at least once a week (more frequently if soiled).
f. Cover all cuts, abrasions, open sores, and bruises with waterproof band aids or disposable gloves and report all injuries to your supervisor.
g. Read all labels and warning signs. Adhere to strict product labeling for all laboratory products.
h. Hair should be tied back, if shoulder length or longer. Jewelry may need to be removed if working with machinery or instruments.
i. Keep the work area tidy and free of unnecessary equipment and materials.
j. Shoes with open toes or open heels are prohibited in all areas. Full shoe covering required.
k. Conduct only authorized work; no horseplay is permitted to take place in the laboratory.
l. Clean up all spills and leakages immediately. See instructions for clean-up of specific spills.
m. All electrical equipment should be grounded and kept in good condition.
n. Keep all corridors, doorways, and emergency exits accessible and free from hazards.
o. Acquaint yourself with local procedures in case of fire, accident, explosion, or other emergency by learning the layout of your building and the location of the emergency exits, telephones, fire fighting equipment (and how it works), and first aid equipment.
p. Eye protection must be worn inside of the laboratories at all times. JHA's should indicate when and what type of eye protection is appropriate for activities within each lab.

> ***WARNING:*** *Employees who wear contact lenses should be aware that fumes from concentrated acids and solvents can cause eye irritation and damage to lenses. Should eye irritation occur, remove lenses immediately and rinse eyes with clean water.*

q. Appropriate gloves are recommended when handling any chemicals. Be sure that gloves are resistant to the particular material being handled. Such guidance is often available on the MSDS for that particular chemical. For questions please contact the EH&S Officer at extension 8842.
r. When it is not practical to wear gloves, extra care should be taken to avoid exposure.
s. Report all injuries, spills, and other releases of hazardous materials to the Lab Supervisor and or the Safety Committee Representative. Additionally, notify the safety and/or facility offices at extension's 8808, 8934 & 8842.

NOTE: *Never let reporting interfere with seeking of emergency medical care in case of an actual medical emergency.*

2. **Safety Equipment**

Safety and emergency equipment is an integral and important part of each laboratory. This equipment includes fire extinguishers, fire blankets, eyewash fountains, safety showers, laboratory hoods, laboratory sinks, and first-aid and spill kits.

Safety Equipment required for a general laboratory includes:

1. Eye Wash Fountains

An eyewash fountain should be capable of providing a gentle stream or spray of aerated water for an extended period of time, usually 15 minutes, although 30 minutes may be required. The minimum flow rate should be at least 1.5 liters per minute for 15 minutes.
The eyewash should be located as close to the safety shower as possible, so that the eyes may be rinsed while the body is being showered. Plumbed eyewash units must be activated weekly to flush the line and to verify proper operation.

2. Safety Showers

Safety showers are for immediate first-aid treatment of personnel contaminated with hazardous materials and for extinguishing clothing fires. Every laboratory worker should be familiar with the location and proper operation of safety showers. Each shower must be activated weekly (where drains are installed) to flush the line and to verify proper operation. The certification card should be inspected to ensure showers have been tested in the last 30 days.
The shower should be equipped with a quick-opening valve that can remain open without being held and requires manual closing. The minimum recommended time of operation is 15 minutes.

3. Laboratory Fume Hoods

When properly used, laboratory hoods can act as a barrier between the laboratory worker and potential hazards, such as chemical splashes, fires, and minor explosions.

4. Laboratory Sinks

The laboratory sink is essential for safety in the laboratory. Employees must wash

their hands with soap and water after removal of gloves, before leaving the laboratory.

5. First Aid Kits

A first aid kit should be clearly marked and available to all laboratory workers. The kit should be inspected periodically and the contents replenished as needed. An attached tag or sticker can serve as documentation of inspection.

6. Spill Clean-Up Kit (kept in the HML hallways for shared use)

Suggested Items for Small-Spill Kit:

- Safety goggles
- Lab coat
- Heavy gloves appropriate for the material (see MSDS sheets for information)
- 5 gallon plastic bucket
- Small bag of absorbent (kitty litter)
- Acid/Base neutralization materials:
  Acid spill - sodium bicarbonate
  Base spill - monosodium phosphate
- Small broom and dustpan

7. Fire Extinguishers (See #3 below).

3. Fire Regulations & Extinguishers

a. Fire extinguishers - 5 lbs. (2.5 kg) dry-chemical type (at least 10BC rating) suitable for class ABC fires except in areas containing machinery with integrated circuits. These areas should have a CO_2-type extinguisher. Placement should be at the exit of each room, or as required, permanently mounted to a wall, cupboard, or similar. There should be approximately one extinguisher located every 30 feet (9.144 m) with discretion used in sharing between one or more rooms. The above outline is provided only as a general guide. Please refer to 29 CFR 1910.157 and/or Fire Prevention Code regulations to verify compliance with requirements. To report a fire incident in HML dial 9 - 911.

NOTE: *HML is a fully automated fire sprinkler facility.*

b. Fire blankets - Should be available in areas containing open flames, flammable liquids, flammable gases, and corrosive chemicals rated as fire hazards. Blankets should be located at exits or adjacent to fire extinguishers. Travel routes should be free from obstruction and travel distance no greater than 70 feet (21.34 m). Learn the location of your nearest fire blanket in HML.

c. Safety cans - flammable chemicals > 4 gallons (15.1 L) should be kept in UL-approved safety containers having a spring-loaded cap and a flame arrester.

d. Safety pumps - must be used when dispensing from containers larger than 5 gallons (18.9 L).

NOTE: *Safety pumps with flame arresters are required when dispensing flammable materials from a metal container to a metal container.*

e. Flammable storage cabinets - for storage of flammable or combustible liquids only. Do not store corrosive materials in these cabinets.

4. **Lighting and Noise Levels**

a. Lighting - it is essential that each work area have sufficient lighting.
b. Noise levels - should not exceed those recommended by OSHA, generally 85 dB.

If the noise level is in excess of the standard, efforts must be made to reduce the level. (Possible solutions are: enclosing noisy equipment, acoustical treatment of walls or ceiling, vibration damping of noisy machines, replacing metal-metal contact with synthetic material-material contact). For a noise determination survey contact the EH&S Officer at extension # 8842.

A. Electrical Equipment

a. Always read the instructions before attempting to assemble apparatus or to operate it.
b. All equipment must be UL approved and have three-prong plugs.
c. Do not use cords with worn insulation. Replace connections immediately when there is any sign of thinning insulation.
d. Make sure the prongs are dry before plugging into any circuit.
e. Electrical units which are to be operated in an area where flammable vapors may be present should be explosion proof.
f. Disconnect all electrical equipment before servicing. Electrical service supply should be well grounded with adequate circuit protection.
g. Bench tops made of conducting material (e.g. stainless steel) should be grounded.
h. No connections to the main service lines should be made by anyone but a licensed electrician.
i. Multiple adapters that can lead to overloading and bad connections should never be used.
j. Fuses or circuit breakers of the correct rating should be used on all equipment at all times but "ground" connections must never be fused.
k. Labs should have sufficient outlets, suitably spaced to allow for convenient connection of each item of electrical equipment likely to be used at one time.

The following signals are indicative of electrical hazards and should be corrected if found:

- Shock received when touching any part of electrical equipment.
- Power receptacles that are the non-grounded type (two-wire instead of three-wire) or are cracked or do not hold the plug securely.

- Power plugs having only two prongs that are connected to a receptacle through a "cheater" (grounding plug to non-grounded receptacle adapter) or have bent or broken prongs.
- Power cords that are frayed, burned, nicked, cracked, or otherwise damaged or are so short that they require an extension cord. Power cords having lengths in excess of the distance between the equipment and the electrical outlet must be neatly coiled. Power cords running across the floor where personnel must walk are a hazard and are not permitted.
- Equipment that is dirty or shows evidence of fluid spillages or has been obviously damaged.
- Multiple electrical equipment attached to an adaptor.
- Electrical noise shown on meter readings, scope patterns, and strip chart recorder traces making them difficult or impossible to read.
- Wet or moist surfaces on electrical equipment. Ground fault circuit interrupter (GFCI) circuits that buzz or trip regularly.

B. Thermal Equipment

a. Heating baths - be sure the thermoregulator works properly. Water baths must be checked daily for temperature and water level.

b. Autoclaves/ovens - avoid steam and heat burns by being familiar with good operating techniques.

c. Outlets

1) Should be checked for grounding using a circuit tester every three (3) months by the Safety Team as part of routine laboratory inspection. Call ext. 8808 if questions or need testing sooner.

NOTE: *Electrical Shock - Turn off electricity first. If the patient is not breathing, phone for emergency assistance dial 9-911 and, if trained, begin CPR immediately.*

6. **Centrifuges**

Centrifuges should be securely anchored either by strong suction cups or wheel brakes and should be located where vibration will not cause items to fall off nearby shelves.

Any centrifuge that does not have an interlocking device to prevent:

- The lid from being opened while the centrifuge is still in motion and;
- The head from spinning while the lid is open.

must have a sign affixed to it or near it stating:

"DO NOT OPEN LID WHILE CENTRIFUGE HEAD IS IN MOTION."

Centrifuges are dangerous unless operated correctly; therefore, the following procedures should be strictly followed:

a. Balancing

Accessories and contents should be carefully balanced and the load distributed symmetrically around the head before starting. Containers should be the right size for swing out heads; buckets must always be properly seated in the heads.

b. Starting and Stopping

The lid must be closed before use and remain closed until the head has stopped rotating. Changes in speed should be made gradually so as not to destroy the motor and the maximum speed recommended must not be exceeded. The centrifuge must be stopped by turning the speed control knob (rheostat) to zero.

c. Breakage

When a breakage occurs, at least 10 minutes (preferably 30) should be allowed for aerosols and droplets to settle before clean-up is attempted. If practical, the buckets, broken tube(s) and contents, trunnions, and head should be autoclaved or immersed in 100% Lysol® for 10 minutes. Decontaminate the inside of the bowl with Lysol, allowing 10 minutes for disinfection, rinse with water and allow to dry, then reassemble for use. Disposable gloves and respirator should be worn for clean-up procedure. Daily cleaning of the inside of the centrifuge bucket with Lysol is recommended. Immediate clean up of any blood splattered inside the centrifuge is a must. The unit should be unplugged before cleaning to avoid electrical shock.

7. **Pipetting**

Mouth pipetting is prohibited. The use of safety bulbs and other mechanical pipettors is mandatory. A disposable wipe should be used to remove excess fluids from the tip of a pipette. Fluid should not be drawn up to the top of the pipette. Contents should be expelled gently down the wall of the receptacle to avoid splashing and aerosol formation. Soiled pipettes should never be placed on benches but placed gently into hypochlorite solution with tips down and cotton plugs removed, the pipette being completely immersed.

B. CHEMICAL PROPERTIES AND DEFINITIONS

1**. Flammable Liquids**

Since flammable liquids can be found in most laboratories, knowledge of the properties of flammable liquids is important for all laboratory personnel. Flammable liquids are volatile, and it is the vapor of these flammable chemicals, not the liquid, which ignites and burns. The vapors are often heavier than air and tend to settle on the floor and to flow down stairways, air ducts, elevator shafts, etc. Frequently, ignition of this vapor trail with its resultant flashback can occur at some distance from the source of the vapor. Common sources of ignition are electrical equipment, open flames, hot surfaces, cigarettes, and static electricity, etc. Since flammable liquids such as carbon disulfide are immiscible in and denser than water, they can settle in the bottom of drains, e.g. the U section of a sink drain, and be ignited not only by the above sources of ignition, but also by certain chemicals such as perchloric and nitric acid.

NOTE: *To report a fire, call 9-911 or pull an alarm box located at the Emergency Exit egress in the HML corridors.*

Certain flammable solvents such as ethyl ether, isopropyl ether, dioxane, and tetrahydorfuran will form peroxides, which explode if allowed to concentrate by evaporation or by distillation. Improper handling of most flammable liquids can lead to health hazards - skin reactions and inhalation illnesses.

The meaning of certain words should be understood by everyone who works with flammable chemicals:

a. Flammable liquid is a liquid that has a flash point of less than 37.8 ^{o}C, e.g. acetone, ethyl alcohol, and xylene.
b. Combustible liquid has a flash point equal to or greater than 37.8 ^{o}C but not exceeding 93.3 ^{o}C, e.g., fuel oil, kerosene and varsol.
c. Flash point is the temperature at which a liquid gives off vapors sufficient to form an ignitable mixture with the air near the surface of the liquid. For example, the flash points of acetone, diethyl ether, and xylene are approximately -15 ^{o}C, -45 ^{o}C and 24 ^{o}C respectively. A good source for flash point information is NFPA 325M (National Fire Protection Association, Batterymarch Park, Quincy, MA). Each MSDS also has data.
d. Ignition temperature is the temperature to which a mixture must be raised to initiate combustion. Only a small part of a flammable vapor-air mixture need be heated to the ignition temperature to result in self-sustained combustion. A static electric spark lasting only a fraction of a second is sufficient. Some organic solvents have dangerously low ignition temperatures, e.g., diethyl ether 185 ^{o}C; carbon disulfide 100 ^{o}C, etc.

A type-B portable fire extinguisher is the extinguisher of choice for putting out fires involving flammable solvents. The discharge should be directed at the base of the fire, but care must be exercised not to spread the burning flammable liquid. ABC-rated extinguishers are also used for such fires universally. All extinguishers at HML are ABC rated and hands-on training is available through the Safety Office on a regular basis. (Contact ext. # 8842).

2. **Corrosive Chemicals**

Concentrated acids and bases must be added to water to minimize the possibility that the heat of reaction will cause eruption of the corrosive. Never add water to a concentrated acid or base as the water will layer on the top of the more densely concentrated acid or base. The extreme heat produced may boil and project the upper layer. Since the fumes of concentrated corrosives can cause severe external and internal burns, these solutions should be handled in a fumehood with the employee wearing rubber gloves, rubber apron, and safety glasses. If a spill occurs, neutralize spills of concentrated acid with dry sodium carbonate or bicarbonate, and neutralize spills of concentrated alkali with citric or boric acid. Keep a supply on hand.

Drips of acids or alkalis on the sides of containers are best cleaned off with paper towels. Plastic stoppers are better than glass stoppers for glass bottles holding an alkaline

solution. Alkalis tend to bind glass to glass making it sometimes impossible to remove a glass stopper. For safe transportation of corrosives, protective packaging should be used. When a corrosive chemical is to be disposed of, it should first be neutralized before being flushed down the drain with large volumes of water. Disposal must be in compliance with waste disposal policies as well as state and local regulations.

3. **Explosive Chemicals**

Explosive chemicals are those chemicals which have a higher propensity to explode under a given set of circumstances (extreme heat, pressure, mixture with an incompatible chemical, etc.) than other chemicals. MSDSs are a good resource for this determination.

4. **Toxic Chemicals**

Several toxic chemicals are commonly used in the laboratory at HML. It is best to review the Material Safety Data Sheet prior to handling new chemicals introduced to the laboratory. For additional information and special details concerning materials suspected of being toxic or otherwise having potential harm consult the EH&S Officer or the Laboratory Supervisor.

5. **Compressed Gases**

A compressed gas is defined as a gas having pressure in the container of 40 psi (275,790.3 pascal) or greater at 70 ºC. Also, any flammable liquid having a Reid vapor pressure exceeding 40 psi (275,790.3 Pa) at 38 ºC is classified as a compressed gas. (The regulations define the minimum pressure but not the maximum pressure in a cylinder that can be above 6000 psi (41,368,543.68 Pa) for non-condensable gases.)

The gas pressure within a cylinder depends on its physical state. For example, "permanent" gases exert a pressure proportional to the amount of gas in the cylinder; while gases that are liquefied in the cylinder, e.g., carbon dioxide, propane, ammonia, etc., exert vapor pressure as long as liquid remains.

1. **Flammable Chemicals**

A) NO SMOKING

B) Know the location of and the proper use of each type of fire extinguisher in your area.

Flammable liquids must not be stored in domestic-type refrigerators; an explosion may result. To report a fire call 9-911 when in HML.

a. Flammable liquids in glass are best stored in approved storage cabinets. They should never be stored with oxidizing agents, e.g., nitric, perchloric, and sulfuric acids. Safety cans should be used whenever possible but should not be subjected to extreme changes in pressure or temperature. If there is any sign of a vapor or liquid leak, transfer the liquid to another approved container.
b. After opening of container, flammable liquids in excess of 4 liters must be stored in safety cans or safety cabinets. One liter glass bottles or smaller should be used when contamination from the safety can may interfere with analytical or clinical results. Safety cans with a spring-action cover have five important functions:

a. Pressure relief valve;
b. To prevent leakage or spillage if the can is dropped;
c. To minimize vapor escape;
d. To prevent a fire from entering a safety can; and
e. To smother a fire inside the safety can.

Normally, this cover should prevent leakage of a liquid when the safety can is inverted. Safety cans should not be stored in closed compartments that are subject to extreme changes in pressure or in temperature. The pressure release feature of a safety can may cause flammable vapors to leak into the closed compartment. The purpose of the flame arrester is to prevent propagation of a flame into a safety can and ignition of the vapors inside the can.

c. The glass bottles should never be more than 75% full; leaving a vapor space of at least 25% for expansion.
d. When dispensing flammable liquids (metal-metal), the dispensing container and safety can must be well grounded and bonded.
e. Static electricity is generated when liquids, especially flammable liquids, move in contact with other materials, e.g., pouring, pumping, etc. If the static electric charge becomes sufficiently great, a spark can occur from one metal container to the other container and ignite the vapor-air mixture. Good wire-metal electrical contacts must be made. The purpose of the bonding is to minimize the potential differences between the dispensing drum and the safety can; grounding is to minimize the potential differences between the containers and the ground.
f. 500 mL is the maximum recommended volume of any type of flammable liquid that should be stored on a laboratory shelf.
g. All containers must be well labeled. No abbreviations are permitted; use the common name of the chemical product as listed on the MSDS sheet.
h. Flammable liquids should never be heated with an open flame, hot plate, or uninsulated resistance heater. The preferred sources of heating are a heating mantle, steam bath, or hot water bath. Know your pre-planned emergency actions just in case.
i. When shaking flammable liquids in closed containers, e.g., separatory funnels, release the pressure frequently or the stopper may be forced out and the worker will be sprayed with the chemical.
j. All spills must be cleaned up immediately.
k. Flammable liquids must not be exposed to potential sources of ignition, e.g., electric motors, Bunsen burner flames, bacticinerators, etc.
l. OSHA-approved fume hoods with explosion-proof fans should be used where possible when handling volatile substances, e.g., for organic solvent extractions.
m. Keep inventory control of all flammable chemicals in the laboratory and storage areas.
n. The maximum amount of flammable liquid that may be stored at any one time in a location is 235 liters. Liquids may be stored in vented UL-approved safety cabinets or in sealed containers of no more than 23 liters capacity each. (Sealed means closed by a lid or other device from which no liquid will escape at normal room temperature and which has not been opened since it has been filled and sealed by the supplier).
o. Flammable or combustible liquids should never be stored in a basement or below ground.

2. **Corrosive Chemicals**

a. Before transporting a carboy of acid or base, check to make certain the neck of the bottle is not broken. Use proper transport containers or Department of Transportation (DOT) legal secondary transport containers.
b. Never store strong acids with bases or either of the two with flammable liquids or oxidizing chemicals. Perchloric acid should be stored by itself. Keep sealed when not in use. Corrosive chemicals are best stored in special ventilated cabinets.
c. On-site storage of corrosives should be limited in quantity.
d. All acid or alkali solutions must be clearly labeled. Glass containers storing alkaline solutions should have plastic rather than glass stoppers as alkali tends to bind glass.
e. Strong acids or bases should be handled in fumehoods with glass partitions lowered to provide protection to hands and face. Bottles should be placed in a sink with absorbent cloth or towel covering the neck when opening.
f. Safety glasses, rubber aprons, rubber gloves, and lab coats are necessary when handling concentrated acids or alkalis.
g. Never add water to acids or alkalis; always add a concentrated acid or base to water, a small portion at a time. Using moist paper towels clean off any acid or alkali drips remaining on the outside of a container. Pour with container below eye level to avoid eye injury and with label up to avoid drips contaminating the label.
h. Neutralize spills of concentrated acid with sodium carbonate or sodium bicarbonate and neutralize spills of concentrated alkali with monosodium phosphate.
i. Ensure all glassware used to hold corrosive chemicals is well rinsed with water before sending to wash-up.

NOTE: ACID AND ALKALI BURNS *- The burned areas must be washed with large volumes of water, for a period of five times longer than is necessary to stop the burning sensation. The area must then be covered with sterile dressing and then aluminum foil or plastic wrap to prevent exposure to air.*
No ointments, creams, baking soda, or other substances should be applied. Severe burns should be examined by a physician. An incident report shall be filed using the HML facility's incident report form.

3. **Explosive Chemicals**

a) Ethers
Ethyl ether is a highly volatile and flammable solvent requiring special storage and disposal procedures. With exposure to air, peroxides will form. When the peroxides are concentrated by evaporation of the ether, an explosion will occur.
Isopropyl ether and other ethers also form peroxides readily. The following precautions must be adhered to:

- Ethyl Ether is preferably obtained in metal cans, and stored in safety containers as opposed to glass bottles.
- All opened bottles must be dated when opened and expiration date sealed.
- "Inhibited" grades, i.e., containing small amounts of water or alcohol, can be used longer (no more than 6 months) than the pure "non-inhibited" grade which must be disposed of within a few weeks following exposure to air. The latter often contains butylated hydroxytoluene.

- It is not advisable to leave a container around with very little ether left in it. Such containers must be promptly disposed of to minimize the risk of explosion. Empty containers will be picked up by HML's hazardous waste contractor.
- Store all ether cans in a cool place and away from direct heat and sunlight. Explosion-proof refrigerators only may be used.
- Stabilization of peroxided ether:
    - To the container add approximately 100 mL of a 5% ferrous sulfate solution for each liter of ether. For smaller volumes adjust the amount accordingly, i.e., 1 mL 5% ferrous sulfate solution for each 10 mL ether.
    - Mix (do not shake)

4. **Toxic Chemicals and Unknown Toxicity**

If there is any uncertainty about the hazards of a chemical, contact the Area Safety Representative or Laboratory Supervisor.

Chemicals of special interest are shown below:

a. **Azide Solutions** - Sodium azide is a preservative commonly used in many in vitro diagnostic products. Continual discharge of wastes into drains can bathe the drain pipeline with solutions of sodium azide and therefore is not permitted. Over a period of time, the azide reacts with copper, lead, brass, or solder in the plumbing system to form an accumulation of lead and/or copper azide. Both of these compounds are extremely explosive. Solutions containing sodium azide should not be discharged down drains.

b. **Cyanide** - Avoid contact of cyanide solutions with acids. Acids react with cyanides to produce hydrocyanic acid (prussic acid) vapor which is potentially lethal.

c. **Mercury** - Mercury is extremely toxic. Every laboratory should do a mercury assessment and include it as part of the Chemical Hygiene Plan. Mercury should be stored in plastic, air-tight containers, away from direct heat or sunlight, and at as low an ambient temperature as possible. Contact the HML EH&S Officer if you have or use mercury containing products at HML. (We are eliminating mercury use in labs, contact ext 8842).

d. **Pesticides** - Pesticides are neurotoxic agents and are highly toxic. Any laboratory using pesticides should conduct a pesticide assessment and include it as part of the Chemical Hygiene Plan. This plan should address appropriate storage conditions for pesticide stocks and disposal of spent wastes.

NOTE: *Contact the Lab Supervisor and/or the EH&S Officer for proper clea- up procedures if a spill occurs.* (If in doubt, always contact for clarification and proper disposal verification).

5. **Carcinogenic Chemicals**

Currently regulated by OSHA as carcinogenic:

2-Acetylaminofluorene	Acrylonitrile
4-Aminodiphenyl	Asbestos
Benzene	Benzidine
Bis-chloromethyl ether	1,2-Dibromo-3-chloropropane
3,3'-Dichlorobenzidine (+ salts)	4-Dimethylaminoazobenzene
Ethylene oxide	

Inorganic arsenic a- Naphthylamine 4-Nitrobiphenyl B-Propiolactone	Ethyleneimine Methyl chloromethyl ether b- Naphthylamine N-Nitrosodimethylamine Vinyl chloride

Other recognized carcinogens:

Analgesics with Phenacetin Myleran Chromium and certain compounds Cyclophosphamide Melphalan Mustard gas	Azathioprine Chlorambucil Conjugated estrogens Diethylstilbestrol PUVA (Methoxsalen with Ultraviolet A Therapy) Thorium dioxide

Suspected carcinogens:

Adriamycin 2-Aminoanthraquinone 1-Amino-2-methylanthraquinone o-Anisidine hydrochloride Beryllium and some compounds 1,3 Butadiene Carbon tetrachloride Chlorinated paraffins 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea 4-Chloro-o-phenylenediamine p-Cresidine Dacarbazine 2,4-Diaminoanisole sulfate 1,2-Dibromo-3-chloropropane 1,4-Dichlorobenzene 3,3'-dichlorobenzidine HCL Methylene chloride Diepoxybutane Diethyl sulfate 3,3'-Dimethoxybenzidine Dimethylcarbamoyl chloride Dimethyl sulfate 1,4-Dioxane Direct blue 6 Estrogens (not conjugated) Ethylene oxide Formaldehyde gas	Aflatoxins o-Aminoazotoluene Amitrole Benzotrichloride Bischloroethyl nitrosourea Cadmium and some compounds Chlorendic acid Chloroform 3-Chloro-2-methylpropene C.I. Basic red 9 mono HCL Cupferron 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) 2,4 Diaminotoluene 1,2 Dibromoethane 3,3'-dichlorobenzidine 1,2-Dichloroethane 1,3-Dichloropropene Di(2-ethylhexyl)phthalate Diglycidyl resorcinol ether 3,3'-Dimethylbenzidine 1,1-Dimethylhydrazine Dimethylvinyl chloride Direct black 38 Epichlorohydrin Ethyl acrylate Ethylene thiourea Hexachlorobenzene

Carcinogenic materials should always be handled with care. Use protective equipment such as gloves when handling, and avoid breathing vapors.

6. **Compressed Gases (e thru u)**

e. The cylinder contents must be clearly identifiable.
f. Cylinders must be handled carefully and not rolled, slid, or dropped; large cylinders should be transported on a wheeled cart; do not lift a cylinder by its cap.
g. Cylinders must always be securely fastened whether in storage, transit or use.
h. The cylinder valves must not be tampered with. Never force connections or use homemade adapters. Use only approved equipment. Never repair or alter cylinders, valves, or safety relief devices.
i. Compressed gas cylinders should only be used with a regulator. Close the cylinder valve when the compressed gas is not being used.
j. When a compressed gas cylinder is "empty", turn off the cylinder valve and label the cylinder as "empty". Store separately from full cylinders. Current cylinder status tags must be attached to the tanks at all times.
k. Compressed gas cylinders should be stored in well-ventilated areas away from ignition sources, heat, flame, and flammable chemicals. Cylinders should never be artificially cooled. Cylinders must never be placed where they can become part of an electrical circuit.
l. Never completely empty a compressed gas cylinder.
m. Empty gas cylinders must never be refilled on laboratory premises. Never attempt to mix gases in cylinders.
n. If a gas cylinder leaks, close the valve and clearly identify the cylinder as unusable and hazardous. Remove the cylinder outdoors to a well-ventilated location away from possible sources of ignition if the gas is flammable. Contact the supplier.
o. The protective caps should be kept on the cylinders at all times except when the cylinders are in active use.
p. Avoid using a wrench on valves equipped with hand wheels. Never hammer a valve to open or to close it.
q. Use only soapy water to check for gas leaks. Extended lines must be marked every 8 feet.
r. It is best not to store cylinders containing flammable gases with oxygen cylinders. They should be separated by a minimum of 20 feet (609.6 cm).
s. Hydrogen should be handled with care as it ignites easily.
t. Compressed air tanks should have an air pressure of less than 30 pounds per square inch (206,842.7184 Pa).
u. Small propane fuel tanks when exhausted are not to be refilled and should be disposed of according to the facility hazardous waste disposal plan.

D. GENERAL HANDLING OF SPILLS

All spills should be promptly evaluated to determine the following:

1. What is the material, and what are the hazards?
2. Does the employee have the ability to handle the problem?
3. Is there a risk to other employees?

For any spill, regardless of its size or type of chemical involved, first alert fellow employees and visitors that a spill has occurred. If extreme danger is present due to flammability and/or noxious fumes, evacuate all personnel from the affected area. Secure the area if necessary.

Before attempting to contain or clean up a chemical spill, determine the appropriate level of protective equipment required and put it on! Appropriate protection might include safety goggles, apron, gloves, and/or respiratory protection. Next, determine the appropriate method of handling by reviewing the Material Safety Data Sheet, label, or other source of information.

Spillages of organic solvents, acids, or alkali are effectively cleaned up with "Spill Control Pillows". These pillows contain highly absorbent silica which is capable of retaining up to 1 liter of liquid. Be aware that these pillows do not reduce the toxicity or flammability hazard of the material - they are to absorb the material and prevent further spreading. Strong oxidizing acids will destroy the pillow fabric. Some chemicals, e.g., strong acids, will have to be neutralized or diluted before they can be absorbed onto the pillow.

Clean Up Procedure

i. Initiate any appropriate action to prevent a larger problem. If the liquid is flammable, all sources of ignition (electrical, open flame) must be shut off. To reduce irritant vapors the area of the spill should be well ventilated.
ii. Alert all staff to avoid area of spill.
iii. Wear protective clothing when cleaning up spill (chemical resistant gloves, eye protection, respirator, etc. based on the specific hazards of the material).
iv. Determine the volume of the spill. For each liter of liquid spilled use one Spill Control Pillow. Large spills with flowing liquid should be diked to prevent further spread. Small spills (under 100 mL) can be easily handled with absorbent paper towels and copious rinsing with water.
v. Clean-up. Press the pillow into the spill and allow absorbent action of the silica to absorb the spill. Do not use a "wiping" action to clean-up the spill.
vi. Disposal. Spill Control Pillows cannot be re-used or used to clean up spills of different solvent as violent chemical reactions can occur. Used pillows should be containerized, placed in a appropriate container, labeled, and held for disposal.
vii. Decontamination. Rinse the area with water and dry. Check yourself to determine whether or not you have spilled any chemical on yourself or your clothing. If so, discard clothing or clean as appropriate. After the area is clean and fumes have dissipated, evacuated personnel may return to the room and normal operations may resume.

Note the following special precautions:

a) **Acid and Alkalis** - Spill Control Pillows do not neutralize acid or alkali. It is important to first neutralize acid spills with dry sodium carbonate (or bicarbonate).Neutralize alkali spills with boric acid. Add until bubbling ceases. Flush with copious amounts of water.

b) **Organic Solvents**

As the Spill Control Pillow does not eliminate the fire hazard associated with organic solvents it is recommended that the Pillows be placed in a fume hood until they can be properly containerized prior to disposal.

c) **Mercury**

Spills of mercury must be cleaned up immediately to prevent toxic vapors from entering the air and to prevent contamination of surface or water systems by large or microscopic droplets. If traces of mercury are left in laboratories as the result of mercury spills, worker health can be affected over a period of time. In areas of spills, mercury vapor in the air can exceed 20 mg/m^3 (threshold limit values (T.L.V.) is 0.05 mg/m^3) depending on the conditions in the lab and the amount spilled.

It is recommended that each branch with equipment containing mercury stock a Mercury Spill Control Kit. In addition, all other locations should stock Mercury Absorbent Sponges to handle small spills.

The Mercury Spill Control Kit contains:

- A hand operated suction pump to pick up large quantities of mercury.
- Mercury absorbent sponges for swabbing up small particles from benches, floors, and other smooth surfaces.
- An absorbent powder that reacts with mercury to form a harmless amalgam. This powder is suited for collecting mercury from rugs, crevices and other poorly accessible areas. If additional supply is needed, it can be made by mixing equal parts of zinc powder and sulfur powder.
- Protective eye glasses.
- Plastic, disposal bag.

Large Spills (>1 lb. (0.4535924 kg)) - Wear protective plastic gloves, respirator, and chemical-resistant clothing. Use the suction pump to pick up puddles and droplets of mercury. For efficient operation of the pump, keep the reservoir at about the same level as the spill. The stainless steel adapter tube is useful for picking up mercury droplets in crevices. Transfer all mercury in the reservoir to a plastic container with a screw cap. Seal tightly and label "Waste Mercury". After removal of large mercury droplets with the suction pump, use either the sponges or the powder to complete clean-up (see below).

Small Spills - On flat surfaces use the mercury absorbent sponges. Dampen the sponge and slowly wipe the contaminated area. Allow for complete absorption of all free mercury. Return the used sponge to its plastic bag, seal tightly, and label "Mercury Waste". Waste material should be held for disposal according to the

facility Waste Disposal Plan. For spills that are difficult to access, sprinkle the contaminated area with a fine layer of the mercury absorbent powder. Overlay the powder with wet paper towels to moisten the powder. Allow 10 to 15 minutes for the mercury to react with the powder and form an amalgam. As the amalgam lowers the vapor pressure of mercury, it can then be cleaned up by conventional means (e.g., a vacuum cleaner) and disposed with the routine waste.

E. CHEMICAL EXPOSURES

CHEMICAL BURNS

If you witness or are made aware of an accident where an individual receives a chemical burn:

1. Remove the individual from chemical contact as promptly and completely as possible. Contaminated clothing should be removed immediately.

2. The affected area should be flushed with water by emergency shower, face flush, or whatever means is quickly available. If the individual is wearing safety goggles, do not remove them until the face area has been flushed. When chemical is splashed in the eyes, the lids should be forcibly held apart so that the entire surface of the eye is flushed.

3. Do not use neutralizing agents, buffering agents, or chemical antidotes.

4. When the affected area has been flushed extensively, the employee should be taken to the emergency unit. If a stretcher is necessary to transport the employee, call 9-911 for an ambulance.

5. The Supervisor, if not present, should be contacted immediately.

CHEMICAL INHALATION

When an individual inhales chemical gases, vapors, fumes, or mists and exhibits any symptoms of adverse exposure:

a. Remove the victim from exposure immediately. If located in a confined space or the chemical is irritating, a gas mask or Scott Air Pack should be used in the rescue and is located in room E112. DO NOT ATTEMPT TO USE THIS EQUIPMENT UNLESS YOU HAVE BEEN TRAINED IN ITS PROPER USE. Inform a supervisor immediately if assistance is required. Dial 9-911 for emergency assistance.

b. Dilute the concentration of the material in air by opening windows and nearby laboratory hoods.

c. Keep the victim warm and lying down.

d. If the victim has stopped breathing, begin artificial respiration. CPR (CARDIOPULMONARY RESUSCITATION) SHOULD ONLY BE ADMINISTERED BY CERTIFIED PERSONNEL). Select someone to dial 9-911 for an EMS ambulance.

e. If the victim has not stopped breathing, they should be taken to the Emergency Unit for additional medical treatment. If a stretcher is needed, call 9-911.

f. The source of the chemical gas or vapor should be cut off. Depending on the type of chemical, respiratory protection equipment may be required.

g. Contact the Supervisor immediately.

F. WASTE DISPOSAL PLAN

It is the policy of HML to comply with all federal, state, and local regulations regarding the proper storage, handling, labeling, transportation, and disposal of all regulated wastes, including hazardous (chemical), infectious, and radiological materials. The HML Hazardous Waste Management Plan is administered by the Area Safety Representative. The plan addresses the management of chemical and residual wastes, and is incorporated by reference into this Chemical Hygiene Plan. Specific aspects of the plan as they affect waste management generated by the laboratory are summarized in this section.

PERSONNEL RESPONSIBILITIES

In the laboratory, specific responsibilities apply to all employees and supervisors from each Joint Partnership Institution (NOS, NIST, SCDNR, MUSC and CofC). These responsibilities are described below.

ALL LABORATORY WORKERS

The individual laboratory worker bears the primary responsibility for proper waste management practices, as control over the proper labeling, storage, and minimization of wastes is best implemented at the point of generation. Workers are responsible for proper labeling and identification of chemical, infectious, and radiological wastes, and should be aware that improper disposal presents potential liability to the individual as well as the facility. Federal, state, and local controls prohibit the disposal of regulated hazardous wastes without specific permitting. No hazardous wastes should be combined with municipal trash or poured down the sink.

All hazardous wastes should be properly containerized, labeled, and stored prior to transfer to the storage, under the direction of the EH&S Officer.

There are a few specific exemptions to this policy and they are detailed below:

i. **Acids or alkalis** should be neutralized and diluted with water before being poured down the drain.

ii. **Non-regulated, non-liquid chemical wastes, ion-exchange resins and non-regulated powdered dyes and stains** may be disposed of in the normal trash. Any questions on waste determination should be directed to the Safety Committee.

Chemical wastes should be considered hazardous if they have the characteristics of ignitability, corrosivity, reactivity, or if they are specifically listed.

iii. **Non-regulated, non-volatile, water-soluble liquids** may be poured down the drain with copious amounts of water. Any questions about the proper identification of these materials or procedures for proper dilution should be directed to the Area Safety Representative.

iv. **Flammable liquids** must be collected in safety cans for off-site disposal. Do not pour these materials down the drain.

v. **All wastes** should undergo a Resource Conservation and Recovery Act (RCRA) hazard determination prior to any disposal or mixing with other wastes. Hazardous wastes should be stored properly according to hazard class, with an accumulation date being placed on each container once accumulation begins. Hazardous wastes may not be stored on site for more than 90 days. A qualified waste management company must handle all hazardous wastes generated by the laboratory. All wastes must be labeled immediately upon placement in a storage container.

vi. Each employee should attempt to minimize wastes generated by utilizing good housekeeping practices and by buying only what supplies are needed. Avoid buying excessive quantities of any chemical, particularly those with expiration dates.

vii. Chemicals should not be evaporated in fume hoods for the purpose of disposal. Particular care should be taken when evaporating solvents in the hood for any purpose; certain materials, such as ethers, may form explosive peroxides when evaporated to dryness.

LABORATORY SUPERVISOR

The Laboratory Supervisor is responsible for overall direction of the hazardous waste disposal plan within the laboratory.

AREA SAFETY REPRESENTATIVE

The Area Safety Representative assists in making waste determinations, reviews requests for on-site disposal approval, advises laboratory personnel on regulatory compliance, and is responsible for the overall hazardous waste management program, in conjunction with the EH&S Officer.

TO BE FOLLOWED IN ALL LAB ACTIVITIES
Hollings Marine Laboratory
Safety and Health Rules

Authorized persons only are permitted in the laboratory. All visitors must register and must be accompanied by a responsible employee. No visitors under age 18 without advance coordination as limited by law.

1. Approved eye protection must be worn by all persons in laboratories at all times. Visitors' spectacles will be available for use by those not having suitable glasses.

2. Persons handling corrosive or highly toxic substances must wear gloves impervious to the material.

3. Laboratory coats are furnished to employees and are to be worn whenever performing laboratory work.

4. Good personal hygiene shall be practiced at all times. Samples, chemicals, solvents, biologicals or other hazardous materials contacted by the hands or other parts of the body must be washed off immediately. Safety showers shall be used in case of a major chemical contact.

5. Smoking is not permitted in the laboratories or within any federal building, structure, or vehicle by law.

6. No eating or drinking can be permitted in laboratories. Never use laboratory glassware for food or beverage.

7. Long hair must be tied up or worn under a cap, especially around machinery, heat sources, or aerosols.

8. Wearing of jewelry is discouraged. Rings can be damaged and can lead to irritation from chemicals retained under them. Jewelry may be required to be constrained and/or removed.

9. Employees shall do no laboratory work while alone in the building, except with specific approval of their supervisors.

10. Horseplay and practical joking are not permitted.

11. Full shoes are required inside all labs. No open toe or heels permitted.

12. All driving on government business must be within legal limits and in conformance with all applicable regulations. Defensive driving should be practiced at all times.

13. All injuries, no matter how slight, must be reported to the laboratory supervisor.

14. Know your nearest escape route for use in an emergency. Be familiar with fire and evacuation alarms.

15. Know where your nearest fire extinguisher and fire blanket is located. Be sure you know how to use it.

16. Know where your nearest safety shower and eye wash fountains are located. Be sure you know how to use them.

17. Wipe up spills on bench or floor promptly. Correct other hazards or report them to your supervisor.

18. Label all containers for positive identification. Add hazard warnings when contents are flammable, corrosive, toxic, radioactive, etc. This includes proper chemical identification and hazard information date, name of researcher, and other appropriate information.

19. Maintain complete, valid records of all samples, reagents, analyses, and results. Protect records from damage or loss.

20. Handle glassware carefully to avoid injury. Discard broken glassware into the designated container.

21. Avoid pressure or vacuum in glassware unless it is specifically designed for the conditions.

22. Use autoclaves, steam sterilizers, and other pressurized equipment strictly according to written procedure. Safety relief devices and controls must function properly.

23. Gas cylinders must be secured with a strap or chain when being stored, used, or transported. Adapters between cylinder valves and regulators may not be used. Caps must protect valves when cylinders are not in use.

24. Use the hood for all operations involving toxic or flammable materials. Verify hoods are tested for flow rate at least once a year. The face velocity should range between 100 and 200 feet per minute (0.508 and 1.016 meter per second).

25. Flammable materials (flashpoint 100 ^{o}F ore less) are best kept in safety cans. Glass bottles containing flammable and when preferred for purity, must be kept in a flammable liquids cabinet; OSHA regulations limit the size of glass bottles for flammables. Secondary containers are always a plus toward preventing breakage.

26. Use syringes in accordance with proper procedures. Do not use excessive force on a syringe. Dispose of syringes and needles in sharps container.

27. Dispose of all samples and test materials in accordance with the facility waste disposal plan. Avoid putting hazardous materials into the sewer unless instructed to do so. Avoid purchasing excess materials that will have to be disposed of. Always verify any questions with the EH&S Officer and your Lab Supervisor.

28. All electrical equipment must be grounded by a ground fault circuit interrupter. Equipment or cords with poor insulation or base terminals must be repaired before reuse. Extension cords are prohibited. All wiring is to be done by a qualified electrician.

29. Learn how to lift properly to avoid back injury. **Get help for heavy loads!**

PART THREE

INFORMATION AND TRAINING

Introduction

Safe operation of the laboratory is largely contingent upon personnel awareness and knowledge. These skills apply to the ability of employees to obtain information and use it in the performance of their duties. It applies to familiarity with procedures as well as the hazards of chemicals and equipment in the laboratory.

The OSHA Lab Standard requires the laboratory to provide information and training to verify employees are aware of the hazards of chemicals present in their work areas. Information is to be provided at the time of an employee's initial assignment to a work area and prior to assignments involving exposures to chemicals they have not worked with before. Refresher training is to be provided on a schedule determined by the EH&S Officer.

A. INFORMATION PROGRAM OUTLINE/EXPOSURE LIMITS

The following information is provided on exposure to hazardous chemicals in the laboratory:

- Signs and symptoms associated with exposures to hazardous chemicals used in the laboratory
- Permissible exposure limits or other recommended exposure limits
- Location of material safety data sheets (MSDSs) and other available reference material on hazards, safe handling, storage and disposal of the hazardous chemicals used in the laboratory. At present, MSDSs are available online at www.msdsonline.com (ID:noaamsds; Password: msds).

B. TRAINING PROGRAM PARAMETERS

The following information is included in the required training program:

- Physical and health hazards of chemicals in the work area
  - Measures that employees can take to protect themselves from these hazards, including specific procedures such as work practices, personal protective equipment to be used, and emergency procedures
  - Methods and observations that may be used to detect the presence or release of hazardous chemicals
- Updated training requirements.

All new employees will receive this training prior to working unsupervised in the laboratory. Any employees transferred from other work areas to the laboratory will also receive training. Annual refresher training will also be provided. Training will be implemented through a combination lecture/audio-visual presentation format.

The HML Chemical Hygiene Training Program is administered through the Area EH&S Officer. Laboratory Supervisors are expected to inform the Area Safety Representative when new chemicals are introduced to the laboratory and when job changes are instituted requiring additional training for employees.

***NOTE**: Maintain a record of training*

PART FOUR

CRITERIA FOR CONTROL MEASURES

Criteria for determining what control measures are needed to assure safe handling of hazardous materials in the laboratory include the following:

- Description of operations that must be conducted within laboratory hoods or with an enclosure or exhaust ventilation
- Description of specific handling practices
- Description of personal protective equipment to be used
- Air monitoring to determine exposure levels and establish respiratory protection requirements.

In particular, control measures for the handling of extremely hazardous chemicals must be described. For example, highly toxic materials may require special protective measures. Several sources are used for making these determinations. Following is a list of sources that are available to all laboratory employees:

- "Threshold Limit Values and Biological Exposure Indices", American Conference of Governmental Industrial Hygienists, Cincinnati, OH. (1990 or later edition).
- "Right to Know Pocket Guide for Laboratory Employees", Genium Publishing, Schenectady, NY, 1991.
- "Condensed Chemical Dictionary", Van Nostrand & Reinhold, NY, NY. (11th or later edition).

A. SPECIFIC HANDLING PRACTICES

All employees from each Joint Partnership Institution (NOS, NIST, SCDNR, MUSC, and CofC) are urged to follow the general safety rules regarding the handling of chemicals and chemical containers. These rules are referenced in PART TWO of the Chemical Hygiene Plan.

B. PERSONAL PROTECTIVE EQUIPMENT

Most personal protective clothing and equipment is provided by the laboratory to employees and visitors when and where this is necessary. It is the responsibility of each employee to be certain that the appropriate clothing is worn as necessary. The most fundamental piece of personal protective clothing is provided by each employee for his/her own use. This is the normal clothing worn in the laboratory. Clothing should be worn to minimize skin surfaces available for direct contact through splashing. Therefore, all employees should wear long sleeve and long legged clothing or long lab coats and oxford style shoes or sneakers. Avoid short sleeve shirts, short trousers or skirts, and

open-toed shoes or sandals. Additional personal protective gear available includes: (such as, but not limited to)

- Eye Wear
- Gloves
- Aprons
- Respirators
- Face Shields
- Full foot coverings
- Radiation monitors

C. RECORDS

Accident records, including recommendations to prevent a recurrence, will be retained in the Administrative Office (Nancy Davey) and by the EH&S Officer (Rick Meitzler). Employees incurring an accident or illness believed to be work related will complete and file a Form CD-137 (Report of Accident/Illness within 24 hours). OSHA 300 forms are required by law by each user agency and postings as by law annually.
Additionally, these agencies will continue to adhere to their internal reporting policies.

1. Chemical Hygiene Plan records will document that facilities and precautions are compatible with current knowledge and regulations.

2. An inventory of Room 266, volatile solvents storage, will be recorded each year and purchase restrictions applied to specific items, if needed.

3. Records of hazardous chemicals stored in individual work areas will be posted inside the entrance to each room and periodically updated. Once a year workers will be instructed by Division Chiefs to update their lab inventories. Any chemical with one or more NFPA (diamond) Codes rated at 2 or above may be considered hazardous. MSDS data is a separate listing from a chemical lab inventory.

4. Records of high-risk substances (e.g., toxins or HCN) will be maintained in the laboratories by Project Leaders, and will include amounts on hand, amounts used, and names of workers involved.

5. Medical records will be retained by the Laboratory in accordance with the requirements of state and federal regulations and who may elect to track such records.

D. SIGNS AND LABELS

1. All containers shall be labeled as to contents and date prepared, generated, or purchased. This includes reagent bottles, chemical waste containers, and receptacles. The labels on purchased chemical containers, including hazard and first-aid information, shall not be removed. **If you add it or transfer it to another container you must label it, even if it is water.**

2. Emergency telephone numbers will be posted for supervisors, emergency personnel, and workers responsible for equipment and facilities.

3. There will be prominent signs for locating fire extinguishers, exits safety showers, eyewash fountains, and other safety and first aid equipment.

4. There will be warnings at areas or equipment where special or unusual hazards exist.

E. HOUSEKEEPING

General housekeeping is an integral part of chemical hygiene and good safety practice. A clean work area is much safer than a cluttered or dirty one. Some appropriate housekeeping measures include:

- Keep all aisles, hallways, and stairs clear of all chemicals.
- Keep all work areas, especially work benches, clear of all clutter and obstructions.
- All working surfaces and floors should be cleaned regularly.
- Access to emergency equipment, showers, eyewashes, and exits should never be blocked.
- Wastes should be kept in the appropriate containers and labeled promptly and properly.
- Laboratory staff should be considerate and aware of housekeeping staff. The typical housekeeping staff is not properly trained in the handling of chemicals and should not face situations where they must make decisions regarding the proper handling, storage, or disposal of chemicals. Therefore, all chemicals should be placed in proper storage areas by the end of each workday; all spills should be promptly cleaned up with arrangements made for waste disposal; and all chemicals should be properly labeled.

F. AIR MONITORING

HML has established an Air Quality Monitoring Program to ensure a safe work environment for all employees. This program is summarized below:

Personnel Responsibilities

All *laboratory supervisors* are responsible for the following:

- Knowledge of air quality regulations applicable to their department
- Informing employees of the hazards associated with chemicals used in the department, providing information on known air quality, and advising employees of air monitoring performed
- Reporting suspected air quality problems to the Health & Safety Manager
- Maintain air quality standards as required and implement corrective action as necessary.

All *laboratory personnel* are responsible for the following:

- Report suspected air quality problems

- Follow policies and procedures designed to maintain air quality, such as replacing lids on containers and working in fume hoods

- Correctly use and maintain personal protective equipment.

The *Area Safety Representative* is responsible for the following:

- Assist laboratory supervisors in determining air monitoring requirements in each workplace

- Arrange for air monitoring via the HML safety office for appropriate devices in each testing area

- Ensure that air monitoring meets regulatory requirements and implement corrective action as required

- Review results with laboratory supervisors and any impacted laboratory personnel.

PART FIVE

LABORATORY VENTILATION

Laboratory ventilation is a key factor in controlling employees' exposure to hazardous substances. Ventilation is provided in two ways: through the facility's heating and air conditioning system, and through fume hoods utilized in the laboratory. OSHA defines a fume hood as a "device located in the laboratory which is enclosed on five sides with a moveable sash or fixed particle enclosure on the remaining side. It is constructed and maintained to draw air from the laboratory and to prevent or minimize the escape of air contaminants into the laboratory. It allows chemical manipulation to be conducted in the enclosure without insertion of any part of the body other than the hand and arm. Walk-in hoods with adjustable sashes meet the above requirements if the sashes are adjusted during use so that the airflow and the exhaust of air contaminants are not compromised and employees do not work inside the enclosure during the release of airborne toxic substances."

Following are additional requirements applying to fume hoods in the laboratory:

Ventilation will not be obstructed or modified except by qualified mechanical engineers. Ventilation in areas where noxious fumes or flammable liquids are handled should provide a minimum of six air changes per hour. Ventilation in areas where fungal, mycobacterial, or viral specimens are handled should provide a negative air pressure with respect to the rest of the laboratory.

Fume hoods are used for the safe handling of noxious, corrosive, or volatile chemicals. Fume hoods are not be used as a substitute for Biological Safety Cabinets (laminar flow hoods). The following policies concerning fume hoods in the laboratory will apply:

Construction: No fume hoods constructed of flammable materials will be permitted in the laboratory. Although specialty hoods for select agents are of lab-grade composite materials, open flame sources are not permitted in these hoods.

Toxic fumes: Whenever toxic substances, corrosive aerosols, carcinogens, mutagens, or teratogens are handled in a fume hood, the minimum face velocity must be 100 cubic feet per minute (fpm). For hoods not meeting this requirement, the velocity may be increased by lowering the sash. If the velocity cannot be increased, the hood may not be used for the aforementioned materials. For effective use, materials should be handled at least six inches away from the hood opening.

Inspection: All hoods will be inspected at least annually by a qualified, contracted engineer. Anytime a fume hood's air handling system is altered or serviced, the hood must be inspected before being placed in service. Any new fume hoods installed must be inspected by the contracted engineer before being placed in service. Inspected hoods shall have a sign affixed to them stating the inspection interval, last inspection date, average face velocity, location of the fan that serves the hood, and the inspector's name and dated initials.

PART SIX

PRIOR APPROVAL FOR USE OF SPECIAL HANDLING of CHEMICALS with Legal Restrictions of Various Laws in Health, Safety, Environmental and Terrorism Mandates.

The OSHA Laboratory Standard requires that if a particular laboratory operation, procedure, or activity requires prior approval from the employer or any supervisor, the circumstances and the approval procedure must be described in the plan.

The following ***require written prior approval from the Laboratory Supervisor, Center Director and EH&S Officer:***

- Radio-isotopes
- PCBs
- Pesticides
- Highly Infectious Agents
- Extremely Hazardous Specialty Gases

PART SEVEN

MEDICAL PROGRAM

A. EXPOSURE MONITORING

The OSHA Laboratory Standard requires the Chemical Hygiene Plan to describe the conditions under which the employer is required to provide laboratory employees who work with hazardous chemicals the opportunity to receive medical attention and any follow-up examinations that the examining physician determines to be necessary. The three conditions under which medical consultation and medical examinations must be provided without cost, without loss of pay, and at a reasonable time and place are as follows:

- Whenever an employee develops signs or symptoms associated with a hazardous chemical to which the employee may have been exposed in the laboratory

- Whenever an event takes place in the work area such as a spill, leak, explosion, or other occurrence resulting in the likelihood of a hazardous exposure

- Whenever exposure monitoring reveals an exposure level routinely above the permissible exposure limit (PEL) or action level for an OSHA-regulated substance for which there are exposure monitoring and medical surveillance requirements.

B. SIGNS AND SYMPTOMS OF EXPOSURE

Material Safety Data Sheets, labels, and various reference materials describe potential signs and symptoms of exposure to chemicals. Following is a partial summary of symptoms or signs that are indicative of overexposure to hazardous materials:

Abdominal cramps
Alopecia (loss of hair)

Amenorrhea (stoppage of menstruation)
Amnesia
Analgesia (loss of sensitivity to pain)
Anesthesia (loss of feeling)
Angina pectoris (chest pain)
Anorexia (loss of appetite)
Anosmia (loss of sense of smell)
Anuria (lack of urination)
Anxiety
Aphasia (inability to talk coherently)
Apnea (breathing temporarily stopped)
Areflexia (loss of reflexes)
Argyria (blue colored tissue from silver)
Arrhythmia (irregular heartbeat)
Arthralgia (joint pain)
Asphyxia (suffocation)
Asthenia (loss of strength or energy)
Asthma (difficulty in breathing)
Ataxia (incoordination, unable to walk straight)
Athetosis (slow writhing movements of fingers)
Atrophy (reduction in size or function of body parts)
Blindness
Blurred vision
Bradycardia (slow heart beat)
Bronchitis
Burn (tissue damage)
Cancer (abnormal tissue growth)
Cataracts
Changes in body/breath odor
Cheilitis (inflammation of the lips)
Chemical pneumonitis (inflammation of the lungs)
Chills
Chloracne (reddish skin rash)
Chorea (jerky uncontrolled movements of limbs)
Colic (abdominal pain due to intestinal gas)
Collapse
Coma
Confusion
Conjunctivitis (inflamed and reddened eyes)
Constipation
Convulsions
Coughing
Coughing blood
Cyanosis (blue to purple skin color)
Dark urine
Dehydration (excessive loss of body water)
Delirium (mental confusion)
Dental erosion

Depression, mental
Dermatitis (inflamed and reddened skin)
Diaphoresis (profuse perspiration)
Diarrhea
Disequilibrium (inability to maintain balance)
Disordered gait (change in walking pattern)
Dizziness
Drooling
Drowsiness
Dysarthria (difficulty in speaking clearly)
Dysosmia (impaired sense of smell)
Sysphagia (difficulty in swallowing)
Dyspnea (difficulty in breathing)
Dysuria (painful or difficult urination)
Eczema (itching and burning skin)
Edema (fluid retention, swelling)
Emaciation (extreme low weight)
Embolism (obstruction of a blood vessel)
Emphysema (difficulty breathing)
Epistaxis (nosebleed)
Erythema (reddened skin)
Euphoria (exaggerated feeling of well-being)
Fasciculation (muscle twitching under skin)
Fainting
Fatigue
Fever
Fibrillation (rapid muscle contractions)
Fluorosis (darkening of the teeth)
Footdrop (dragging of the foot while walking)
Frostbite
Gangrene (tissue death)
Gasping (difficulty catching breath)
Gastroenteritis (inflammation of the stomach and intestine)
Giddiness (dizziness, silliness)
Glossitis (tongue swelling)
Halitosis (foul-smelling breath)
Hallucination
Headache
Hematuria (blood in the urine)
Hemiparesis (paralysis of one side of the body)
Hemorrhage (bleeding)
Hyperemia (congestion of blood in a body part)
Hyperkinesis (excess activity or motion)
Hyperpigmentation (excessive coloring of the skin)
Hyperthermia (elevated body temperature)
Hyperventilation (sudden rapid breathing)
Hypocalcemia (calcium deficiency of the blood)
Hypothermia (lowered body temperature)

Hypoxia (insufficient oxygen)
Icterus (tissue discoloration)
Impotence (loss of sexual ability)
Incoordination
Inflammation (swelling, redness)
Inflexibility (rigidity, inability to move)
Insomnia (inability to obtain normal sleep)
Interstitial fibrosis (scarring of the lungs)
Involuntary defecation
Involuntary urination
Iridocyclitis (inflammation of the iris)
Irritability
Itch
Jaundice (yellow discoloration of skin or eyes)
Keratosis (horny growths on skin)
Lacrimation (excessive eye tearing)
Lassitude (sense of weariness)
Lesion (injury to tissue)
Lethargy (sluggish feeling)
Lightheadedness (dizziness)
Lipid granuloma (inflamed lung tissue)
Lipid pneumonia (from aspiration of oily materials)
Malnutrition
Melena (black tarry vomit or stools)
Menstrual changes
Metallic taste
Miosis (pupil contraction)
Miscarriage
Myotonia (temporary muscle rigidity and spasm)
Narcosis (stupor or uncontrolled sleeping)
Nasal ulceration (perforation of nasal tissue)
Nausea
Necrosis (localized death of tissue)
Neoplasm (abnormal tissue growth)
Nephrotoxin (poisonous to the kidney)
Nervousness
Neuritis (inflammation of the nerves)
Nocturia (excessive urination at nighttime)
Numbness
Ochronosis (dark spots on skin)
Oliguria (decreased urination)
Opisthotonos (spasms with body arched from head to heels)
Oxide pox (dermatitis from oxide contact)
Pallor
Palpitations (forceful heartbeat)
Paralysis
Paresthesia (abnormal tingling)
Paroxysm (sudden recurrence of disease)

Perforation (opening through a tissue)
Pharyngitis (sore throat)
Phlebitis (swollen, painful vein)
Photophobia (inability to tolerate light)
Photosensitization (allergic reaction to light)
Phototoxicity (irritant reaction to light)
Pneumoconiosis (material particles in the respiratory track)
Prostration (marked loss of strength)
Proteinuria (presence of protein in the urine)
Ptosis (drooping of upper eyelid)
Pulmonary edema (fluid in the lungs)
Pyorrhea (swollen, bleeding gums)
Pyuria (pus in urine)
Respiratory distress
Rhinorrhea (excessive nasal discharge)
Salivation (discharge of saliva)
Scotoma (blind spot in field of sight)
Seizure
Sensitization (allergic reaction)
Shock (depression of all bodily functions)
Siderosis (lung and tissue damage from iron particles)
Silicosis (lung condition from silica dusts)
Spasms
Stomatitis (swelling of the mouth lining)
Strabismus (lack of coordinated eye movement, crossed eyes)
Sweating (excessive moisture on skin)
Swelling (of tissues)
Tachycardia (abnormal rapid heartbeat)
Tachypnea (increased respiratory rate)
Tetany (intermittent muscle spasms)
Tick (skin twitch)
Tinnitus (ringing in the ears)
Tracheobronchitis (coughing, difficulty breathing)
Tremors (shaking, trembling)
Tumor (swelling or growth)
Ulceration (tissue destruction)
Urticaria (skin eruption)
Vertigo (feeling of whirling motion)
Vesiculation (blisters)
Vomiting
Wheezing
Wrist drop (inability to extend hand at wrist)

C. MEDICAL SURVEILLANCE PROGRAM (NOAA has no programs for line offices. Each must establish its own, if necessary)

PART EIGHT (Federal OSHA Mandatory Action)

CHEMICAL INVENTORIES / LABORATORY SPECIFIC PRACTICES

CHEMICAL INVENTORIES

Chemical Inventories are maintained by each laboratory supervisor and the overall yearly inventory is posted by the lab supervisor in an area designated by the NOAA Facility EH&S Officer. Each of the Joint Partnership Institutions (NOS, NIST, SCDNR, MUSC and CofC) will be responsible for updating and maintaining current chemical inventories for their researchers within the HML. The posting of such inventories will be placed at the laboratory primary entrance in addition to posting the Job Hazard Analysis (JHA) for each lab room (such data must be kept current at a minimum of annually). Contact the HML EH&S Officer for assistance (Ext. #8842).

LABORATORY - SPECIFIC PRACTICES

Detailed manuals specific to the lab and its activities will be prepared prior to the action of such activities. In full compliance with CDC guidelines, OSHA and NIOSH standards, Toxic or Extremely Hazardous Substances Registration Laws, and directions of policy as promulgated by the HML Executive or Science Boards and in conjunction with the Principal Investigator's a written request for authorization to conduct any such regulated activities within the HML facility (Blank forms are on the HML intranet) must be completed.
Written investigation requests must be submitted and approved in writing starting with the HML director for authority to conduct any regulated activities at HML. The director will review the application request and pass the application to all parties of interest prior to the final activities being approved / disapproved for start up at the HML facility. This coordination may involve and follow collaborative agencies' internal review processes when seeking approval, in addition to the HML considerations.

Questions that affect the approval process will be coordinated for the best resolution through the director and any final discrepancies may be set with the director for consideration to the HML Science Board or Executive Committee. The director will be the responsible and coordinating authority at the HML.

NOTE: *No work of any kind may start prior to the final approval of such written request by all parties of interest and fully signed by those parties.*

Operations of Biological Safety Level Three (BSL_3) Laboratories

The HML has two primary BSL_3 Suite pairs sharing anterooms that are special built containment facilities. BSL_3 laboratories may be operated and used for research involving highly toxic chemicals (natural and anthropogenic) and/or highly infectious agents (bacteria, viruses, biotoxins). Research conducted in BSL_3 will follow procedures and protocols outlined by the U.S. Centers for Disease Control and Prevention (CDC) as specified in Appendix D, ***via written approval prior to any agents being introduced***: Each user/applicant protocol request will be reviewed by the HML Biosafety Committee on an individual basis with replies forwarded to the Center Director and Science Board as well as to the applicant user for review and concurrence (To be completed by the BioSafety Committee and EH&S Officer, verifying CDC guidelines).

All actions must conform to the requested guidance of the BioSafety Committee prior to any activities being permitted.

APPENDICES

APPENDIX A

CONVERSION CHARTS

METRIC/ENGLISH

TEMPERATURE

From ºC to ºF ºF = 1.8 X ºC + 32
From ºF to ºC ºC = 0.556 X ºF - 17.8

VOLUME

1 mL	=	0.0338 fluid ounces	1 oz	=	29.573 mL
1 liter	=	2.1134 pints	1 pint	=	473.166 mL
1 liter	=	1.0567 quarts	1 quart	=	946.332 ml
1 cc	=	0.06102 cubic inches	1 ci	=	16.3872 cc

WEIGHT/MASS

1 gram	=	0.03527 ounces
1 oz	=	28.3495 g
1 kilogram	=	35.274 ounces
1 oz	=	0.0283 kg

APPENDIX B

HML HAZARDOUS CHEMICAL SPILL/DISCHARGE REPORT

PURPOSE

This form should be completed following hazardous chemical spills. The objectives of this form include: 1) an effective review of applicable regulatory reporting requirements, 2) investigation of ways to prevent a reoccurrence, and 3) to verify that all resultant hazardous wastes are properly handled.

INSTRUCTIONS FOR COMPLETION

This form should be completed for all spills meeting any of the following conditions:

a. An employee was injured as a result of the spill or release
b. The release resulted in evacuation of a work area
c. The release is believed to have resulted in an employee's exposure to an OSHA 1910.1000 air contaminant above the allowable STEL (short-term exposure limit) or PEL (permissible exposure limit) level
d. Staff members other than employees stationed in the immediate area were involved in spill cleanup

Only spills of regulated hazardous or infectious substances need to be reported. Contact the Safety Committee if there is any question on the regulatory status of a material.

NOTE: *A hazardous substance becomes a hazardous waste when spilled, under normal circumstances.*

APPENDIX C

GLOVE SELECTION FOR COMMON LABORATORY CHEMICALS

SUBSTANCE	PREFERRED GLOVE	ACCEPTABLE ALTERNATIVE
Acetaldehyde	NT	NP, NH
Acetic acid	NR, NT, NP	
Acetone	BR	TF
Acrylonitrile	NP	
Ammonium hydroxide	NP,NT, PVC	
Aniline	NT	NP, PVC
Benzaldehyde	NT	PVC
Benzene	PVA, VT	
Benzyl chloride	TF	
Bromine	TF	
Butane	NP	
Butyraldehyde	BR, TF	NP, PVC
Cadmium oxide	NP, NT	
Calcium hypochlorite	NP, NT	
Carbon disulfide	PVA	
Carbon tetrachloride	PVA, VTR	NT
Chlorine	NP, SX	BR, NT
Chloroacetone	NP	
Chloroform	PVA, VT	NT
Chromic acid	PVC, BR	
Cyclohexane	NP, NT, VT	
Dibenzyl ether	NP	
Dibutyl phthalate	BR, NT, PVA, VT	
Diethanolamine	NP, PVC, BR, PVA	TF
Diethyl ether	NT	NP
Dimethyl sulfoxide	BR, NP	
Ethanol	BR	NP, TF
Ethyl acetate	BR, PVA, TF	
Ethylene dichloride	TF, VT	PVA
Ethylene glycol	NT, PVC	NR, NP
Fluorine	NR, NP	
Formaldehyde	BR, NT, PE, VT	
Formic acid	NP,NT, PVC	
Freon 113	NT, TF	VT
Furfural	BR	PVA
Glutaraldehyde	BR, NP, VT	
Glycerol	NT, PVC	
Hexane	NT, PCA, TF, VT	
Hydrobromic acid	NP, PVC	
Hydrochloric acid	BR, SX	NR, NP, NT, PVC
Hydrogen peroxide	PVC	NR, NP, NT

Iodine	SX	PE
Isopropyl alcohol	NT	NP, TF
Maleic acid	NR, NP, NT, PVC	
Methylamine	BR, NT, VT	NP
Methyl cellosolve	BR	
Methyl chloride	PVA, NP	
Methyl ethyl ketone	BR, TF	NP, NT
Methylene chloride	PVA	NT
Monoethanolamine	BR, NP, NT, VT	PVC
Morpholine	BR	PVA
Naphthalene	TF	NT
Nitric acid	BR, SX	NP
Oxalic acid	BR, NR, NP, NT, PVC, VT	
Perchloric acid	NR, NP, NT, PVC	
Peroxyacetic acid	BR	BT
Phenol	BR, VT	NP
Phosphoric acid	NR, NP, NT, PE, PVC	
Potassium hydroxide	BR, NP, NT, PVC	NR
Propylene dichloride	None appropriate	
Sodium hydroxide	BR, NR, NP, NT, PVC	
Sodium hypochlorite	NR, NP,NT, PVC	
Sulfuric acid	BR, NR, NP, PE, PVC, TF	
Toluene	PVA, TF, VT	
1,1,1-Trichloroethane	PVA, VT	
Trichloroethylene	PVA, VT	
Tricresyl phosphate	BR, PVA, PVC	VT
Triethanolamine	BR, NP, NT, PVA, PVC	
Trinitrotoluene	NP	

KEY

PE = polyethylene
PVA = polyvinyl alcohol
PVC = polyvinyl chloride
SX = Saranex
TF = Teflon
VT = Viton

APPENDIX D

PROCEDURES AND PROTOCOLS FOR OPERATION OF BIOLOGICAL SAFETY LEVEL 3 LABORATORIES (CDC)

Those procedures and protocols can be found at: http://bmbl.od.nih.gov/

as well as any and all required/documented approvals/registrations as per CDC mandates.

Other useful links are: http://www.absa.org/
http://www.phac-aspc.gc.ca/msds-ftss/index.html#menu?
http://www.absa.org/resriskgroup.html

EMERGENCY PREPAREDNESS PLAN

HOLLINGS MARINE LABORATORY

U.S. DEPARTMENT of COMMERCE
NOAA, NATIONAL OCEAN SERVICE
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
MEDICAL UNIVERSITY OF SOUTH CAROLINA
SOUTH CAROLINA DEPARTMENT OF NATURAL RESOURCES
COLLEGE OF CHARLESTON

May 2006 (Rev. E)

TABLE OF CONTENTS

GENERAL EVACUATION INFORMATION

Persons Authorized to Order Evacuation

Center Director - Fred Holland (Director)

Office of Administration – Susan Smith/Pat Luck/Tammie Herrin

General Supervisory Engineer - Martin Burnett/Cleve Robertson

Notification for Emergency Evacuation

Fire - Fire Horns and Strobe Lights

Explosion or Gas Leak - Fire Horns and/or Public Address by Speaker Phone Ext # 74

Suspicious Object - Phone Address Announcement by Speaker Phone Ext # 74

Bomb Threat - Phone Address Announcement by Speaker Phone Ext # 74

Major Chemical Spill - Phone Address by Speaker Phone Ext # 74 or Fire Horns

Reporting Site

Fire / Explosion / Suspicious Object / Major Chemical Spill:

All employees should report to the parking lot in front of the building near the HML Building main parking lot. If a fire alarm is set off during a thunderstorm and no fire is apparent, employees should report to the front lobby and await further instruction.

Bomb Threat: All employees report to the guard shack just inside the main gate where facility personnel will conduct a head count and verification.

Building Re-entry

Re-entry to the building will be announced by the Center Director or designated official.

EMERGENCY PERSONNEL

Designated Official

Fred Holland, Center Director

Occupant Emergency Coordinator

Donna Owens / Pat Luck / Susan Smith/ Tammie Herrin

Team Coordinator

Martin Burnett, Facility Security Officer- # 762-8808
Richard Meitzler, Safety Officer -#762-8842 / 7842 or Raluca Semeniuc- IH- #762-8870
NIST Coordinator - Paul Becker -#762-8861 / 8916 NIST lab
MUSC Coordinator - Darlene Middleton -#762-8851
UofC Coordinator - Jack DiTullio -#762-8858 / 8945 lab
SCDNR Coordinator Bob Chapman -#762-8860, 8882 lab; Brandon Eleby #762-8886

Area Monitors	**Area**
Donna Owens / Susan Smith/Tammie Herrin	Administration (Rm.A100 - 135)
Mike Sellers	Open Office Area (Rm. A200 - 231)
Mats Lundqvist	Section B 1st Floor (Rm. B101 -122)
Ed Wirth	Section B 2nd Floor (Rm B201 - 214)
Rebecca Pugh / Michael Ellisor	Section C 1st Floor (Rm C101 - 114)
Steven Christopher	Section C 2nd Floor (Rm C201 - 213)
Steve Morton	Section D 1st Floor (Rm D101 - 119)
Peter Moeller	Section D 2nd Floor (Rm D201 - 221)
Cleve Robertson	Section E 1st Floor (Rm E101 - 124)
Cleve Robertson/ Kevin Corcoran	Section E 2nd Floor (Rm E201 - 204)
Lou Burnett / Erin Burge	Section F (Rm F101 - 138)
Colden Battey / John Siegling	Aquatic Production
Safety Officer - R.Meitzler / Raluca Semeniuc	BSL_3

Disabled Staff Monitor Pat Luck / Susan Smith

Damage Control Team Martin Burnett, Cleve Robertson, Kevin Corcoran and Safety Officer- R. Meitzler / Raluca Semeniuc- IH

FIRE EVACUATION PROCEDURES

1. **Be prepared for a fire emergency** by making note of the nearest fire extinguisher and the route to nearest emergency exit from your work area. Our fire alarm consists of loud horns and strobe lights. Most alarms will be activated by one of the smoke detectors or heat detectors located throughout the building.

2. **Upon detection of a fire**, regardless of size, do the following immediately and in sequence, unless there are others available to permit simultaneous execution.

   - **Attempt to extinguish the fire immediately** using the closest available fire extinguisher **only if you judge the fire small enough to be controllable and you have been trained to use a portable extinguisher and you have a safe way out and anticipate no undue harm to you. (Always keep a means of escape)**

   - If a fire is too large, or the fire extinguisher is inadequate, **leave the area immediately.**

- **Activate the fire alarm** (if it has not sounded**)** by pulling one of the alarm pull stations which are located by each exit door and in the main hallways in the building. Leave the building immediately. The James Island Fire Department will be notified directly once the fire alarm is activated.

3. **Upon hearing a fire alarm**, personnel will:

   - **Stop work immediately**. Terminate all meetings or telephone calls and if time permits, close doors, turn off lights, and turn off equipment except those marked "Do Not Turn Off". Do not attempt to remove personal belongings or records.

   - **Evacuate the building**. Proceed without delay to the closest emergency exit, or an alternate exit if necessary to avoid the fire location. There is a fire exit door at the following locations, A101 Lobby, A130 Stair Well, D119 Stair Well, rear of E101 Hallway, E114 Hallway, F101 Entrance, F115 Entrance and F 133 Entrance. Additionally there are three exits in the Brood Conditioning Rooms F118, F119 and F120.

     Area Monitors should assist in evacuating personnel. Check to see that lights are off, doors are closed, and people are out. If the alarm sounds when you are far from your area, leave the building by the nearest exit.

     Upon exiting the building, go to the paved parking lot in front of the building by the HML Building and or the rear of the building depending on location relative to the nearest exit and join the other employees. All driveways and roadways must be kept clear for emergency vehicles and fire equipment. (Be prepared for a roll call of all occupants)

   - **Visitors and Handicapped Employees**. Area Monitors and/or employees nearest visitors will ensure that visitors are aware of alarms and evacuate the building with employees. Area Monitors will make sure that handicapped employees are assisted in leaving the building safely in the event of a fire alarm. (Ensure that the roll call accountability includes each visitor)

4. **Return to the building** only after an official all clear is given by management authority.

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OTHER EVACUATIONS

1. **Chemical Spill**. In the event of a chemical spill, notify others in the area of the problem. Do not walk through the spill and do not attempt to clean it up without proper protective equipment. Notify the Safety Officer and / or the Facilities Manager. If it is a minor liquid spill, use the appropriate absorbent from your lab or from one of the spill cleanup kits in the hallways. Seal the contaminated absorbent material in a plastic bag and label it for disposal. If it is a major spill that is immediately hazardous, close the door, notify others, and if there is a major fire hazard pull the fire alarm as you exit the building. Air handlers shut off automatically with the fire alarm, and fire personnel will have adequate personal protective equipment to clean up the spill. Fire personnel with self-contained breathing respirators can also handle toxic spills if it is decided that they are needed.

2. **Bomb Threat**. If a bomb threat against HML is received by an employee, it shall be reported immediately to the front desk (8811). The receptionist will notify the Center Director or designee (Director or Administrative Officer) for appropriate action. There will always be someone at the front desk to answer the phone. If a decision is made to evacuate the building, the Center Director or his representative will make the following announcement over the public phone address system ext #74, (NOTE: The fire alarm system will not be used for a bomb threat):

   Attention Staff. The Laboratory has received a bomb threat. Please turn off all non-essential equipment and utilities, close your door and leave the building by the nearest exit. Go to the guard shack just inside the main gate and remain there until further notice. This is not a test. Do not stay in the building. Repeat.

   The Center Director or designee will call 911 for the police and fire authority. **No evacuation order will be given without the approval of the Center Director or Acting Director**. *However*, **when there is an immediate danger**, such as an actual fire or explosion, the premises shall be evacuated at once by sounding the fire alarm system (at any pull station if not already sounding), leaving by the nearest exit, and proceeding to the assembly point near the main gate.

   If you receive a call from a person stating there is a bomb in the building, **keep the caller on the line** as long as possible unless you have reason to believe a detonation is imminent (use judgement). Be alert to the telephone number shown on the phone screen you are using & copy the displayed number for authorities.

   - Ask the caller to repeat the message.
   - Record (write), if possible, every word spoken by the person making the call.
   - Attempt to inform a coworker of the situation through hand signals and notes.
   - Try to get word of the threat to the receptionist at the front desk.

   ✓ If the caller does not indicate the **location of the bomb or the time of possible detonation**, you should ask the caller to provide the information.

   ✓ Pay particular attention to **any strange or unusual noises** such as motors running,

background music, or any other noises which might indicate where the call is being made. Listen closely to the voice (male - female), voice quality, accents or speech impediments.

- ✓ Immediately after the caller hangs up, the person receiving the call should **report this information to the Center Director and the Administrative Officer.**

- ✓ In the event you see a suspicious looking object during or before an evacuation, do not disturb the object. Notify the Center Director (ext.8813) or the Receptionist (ext.8811) and inform them of its location.

INTRUDER ON PREMISES

In the event an intruder(s) is/are observed on the premises during working hours,

- Call the reception desk (ext.8811) stating nature of intrusion (demonstration, vandalism, theft, bomb, arson etc.) and whether intruder has a weapon.
- The receptionist will call the County Police (911) or MRRI Security (762-5044). Do not attempt to apprehend an intruder.
- If possible record appearance of individual(s), sex, race, clothes, hair, behavior etc., and means of escape (foot, car, bike etc.)
- Record license plate number if possible.

TRAPPED IN FREEZER ALARM

There are four locations of alarms in the building to alert others of a person trapped in one of the walk-in freezers. Flashing red lights and high pitched audible alarms are located in Section B outside Room's B118 and B208, Section D outside Room D108, Section E outside Room E104 and Section F outside Room F106. When one of these alarms sounds, check walk-in freezers in the following order to determine if anyone is trapped inside.

1. Check the main walk-in freezers, Rooms E104, F106 and B208.
2. Check Environmental Chambers Rooms B118, D108 and F106.

If you should find yourself trapped in a freezer (unable to open the door), press in the large red button beside the exit door. This will set off the audible / visible alarms described above. As a general rule, do not enter a freezer alone unless someone else is aware of it. If it is after hours or on a weekend you should definitely not enter a freezer unless someone else is keeping a close watch. If you push the alarm button accidentally, pull it back out and let someone know. If after hours, page the on-call maintenance person (numbers are posted on doors to Room=s E105, E201 and F116 hallway door.) and let them know that an emergency trip to the lab is not needed.

NATURAL DISASTER RESPONSE

Hurricanes are our most common natural disaster, but we can expect to have adequate warning so that we can make preparations (as described below) and be elsewhere when it arrives. Tornadoes are most likely to occur during a hurricane, but there is a chance of a life threatening tornado during a thunderstorm and a remote but real chance of a severe earthquake.

- The Center Director (or designee) shall assess the situation and advise the Administrative

Officer and/or Receptionist of any specific information or instructions to be issued to Area Monitors and employees in general.

- The Receptionist will make the following announcement:

May I have your attention. A tornado (or high winds, earthquake aftershocks etc.) is expected momentarily. Please turn off all non-essential equipment and utilities and go to the nearest interior shelter area until further notice. Repeat.@

- Preferred shelter areas in the main lab building are located in the main hallways. Avoid the more hazardous labs, or use hallways in Section A - Interior windowless rooms and maintenance areas. Close all doors and proceed to a shelter area. Find a place along a wall and wait for further instructions from Area Monitors. The first floor laboratory hallway offers the better protection area when the end doors are closed.

HURRICANE PREPAREDNESS

Responsibilities

- **Center Director** - is responsible for decisions related to implementation of this plan during an emergency.

- **HML Science Board** - is responsible for overseeing the emergency planning process and ensuring their respective branches are secure.

- **Chief, Office of Administration** - is responsible for operational elements of the plan prior to, during, and following an emergency situation, will notify major local television and radio stations of employee work dismissal.

- **NOAA Supervisors and Supervisory Scientists from NIST, SCDNR, MUSC and UofC** - are responsible for ensuring that employees are familiar with this plan and comply with its policies and procedures.

- **Employees -** are responsible for securing their areas such as covering items with plastic cover, removing critical equipment from low/floor areas, and securing all related personal items.

- **HML Safety Committee** - is responsible for updating the plan, along with Rick Meitzler (ext. 8842).

Hurricane Preparations

- Hurricanes will be monitored via television and the Internet to provide advance warning of any potential threat to the Charleston area. When a hurricane watch is issued, preparations

will be made to secure the laboratory. Prior to a weekend or holiday, preparations will be initiated at an earlier stage of the hurricane threat. The weather conditions may be clear during early readiness.

- Normal supervisory channels will be maintained and special instructions transmitted through supervisors. Each employee will be responsible for securing his/her work area and adjacent unoccupied areas. Computers will be backed up and plastic sheeting will be distributed to cover critical instruments and records. Records and computers will be raised off the floor if possible and protected with plastic sheeting.

- Assist as needed to remove and/or secure loose objects outside the building such as empty drums or materials on the loading dock. Ensure that trash dumpster is closed.

- Government vehicles will be moved to high ground, away from trees. Laboratory boats will be filled with water and/or tied down if they have not been moved to a location for safe haven.

- Check to insure that all gas and water faucets, including the main distilled water faucet in the mezzanine, are shut off. Shut off unnecessary electrical equipment and ensure that all equipment that must remain operable is supplied by emergency power.

- All employees will leave the building and the Fort Johnson premises when dismissed by their supervisors and return only after the hurricane threat has passed. Inside doors will be closed but not locked. Outside doors will be locked.

- If a hurricane threat develops rapidly over a weekend, supervisors or their designees will request that employees come in to the Laboratory and secure their areas. Normally, preparations will have been made in advance, allowing all employees to secure their homes and to evacuate the area if they so choose. No personnel or tenants shall remain on site to ride out any hurricane threat.

Communication Procedures

If employees have been dismissed, or are off duty as a hurricane approaches, they will be expected to check the HML/CCEHBR telephone voice mail system for instructions. Information will be distributed through the **voice mail message center (762-8888)**. Should our phone system fail, there is a **backup system** (via SC Public Information Phone System) PIPS at **1-866-246-0133**. Follow the menu selections.

Official NOAA Weather Service Hurricane Watch/Warnings for the Greater Charleston Area will be used for the decision making process. Charleston County Emergency Preparedness Advisories for the Greater Charleston Area will be adhered to, especially those advisories affecting coastal islands.

Dismissing Employees

During Work Hours - During a Hurricane Watch and after Center facilities are secured, there will be a liberal leave policy, allowing employees to secure their homes and make travel plans. After Center facilities are secured and a Hurricane Warning is imminent, Administrative Leave will be
granted.

During Non-Work Hours - All employees will utilize the HML voice mail system to obtain

instructions from the Center Director. If Laboratory facilities have not already been secured, employees will be expected to report to duty to secure facilities under a Hurricane Watch unless excused by their supervisor (weather conditions permitting). If a Hurricane Warning is issued, employees will be on Administrative Leave and will not be expected to report to work.

Resumption of Operations

All employees should check the HML voice mail regularly (or the 800 number if necessary) for instructions. Employees will be expected to report back to duty upon the lifting of Hurricane Warnings, with the following exceptions:

1. If severe damage to the area occurs, instructions will be given by voice mail.
2. If less than four hours remain in the standard day (8:00 - 4:30) employees may choose to take advantage of the liberal leave policy to meet personal obligations.

Supervisors will assess the damage in their respective areas and submit a written report to the Chief, Office of Administration as soon as possible after the emergency. The HML will inspect for structural damage and/or mechanical failure and advise the Facilities Manager/General Supervisor Engineer and/or Administrative Officer and supervisors as soon as possible.

Information on the Web

The attached information on hurricane preparedness (modified), and additional information on various types of storms, can be found at: http://www.nhc.noaa.gov/

For useful information on local conditions, forecasts, warnings, and facilities for evacuation, try the Charleston National Weather Service and add the appropriate zip code for any specific area:
http://www.erh.noaa.gov/ifps/MapClick.php?CityName=Charleston&state=SC&site=CHS

For the South Carolina Emergency Management Division office of the SC Adjutant General:
Contact: http://www.scemd.org (803) 737-8700
South Carolina DOT: http://www.scdot.org
SC American Red Cross: http://www.redcross.org/sc/lowcountry
SC State web site: http://www.myscgov.com
FEMA: http://www.fema.gov
Weather-NWS at Charleston: http://weather.noaa.gov/chs

Recommendation: Add these sites to your Bookmarks or Favorites list.

Are You Ready for a Hurricane?

Know What Hurricane WATCH and WARNING Mean

- **Watch**: Hurricane conditions are possible in the specified area of the Watch, usually within 36 hours.
- **Warning**: Hurricane conditions are expected in the specified area of the Warning, usually within 24 hours.

Prepare a Personal Evacuation Plan

- Identify ahead of time where you could go if you are told to evacuate. Choose several places:

a friend's home in another town, a motel, or a shelter.
- Keep handy the telephone numbers of these places, as well as a road map of your locality. You may need to take alternative or unfamiliar routes if roads are closed or clogged.
- Listen to NOAA Weather Radio or local radio or TV stations for evacuation instructions. If you are advised to evacuate, do so immediately.

Assemble a Disaster Supplies Kit

- First-aid kit and essential medications
- Canned food and can opener
- At least three gallons of water per person (one gallon per day per person for three days)
- Protective clothing, rainwear, and bedding or sleeping bags
- Battery -powered radio, flashlight, and extra batteries
- Special items for infants, elderly, or disabled family members
- Written instructions for how to turn off gas and water if authorities advise you to do so. (Remember, you'll need a professional to turn them back on)

Prepare for High Winds

- Install hurricane shutters or precut 3/4 inch marine plywood for each window. Install anchors and pre-drill holes in the plywood so you can put it up quickly.
- Make trees more wind resistant by removing diseased or damaged limbs, then strategically removing branches so that wind can blow through.

Know What to Do When a Hurricane WATCH is Issued

- Listen to the advice of local officials, and leave if they tell you to do so.
- Complete preparation activities.
- If you are not advised to evacuate, stay indoors away from windows.
- Be aware that the calm 'eye' is deceptive; the storm is not over. High winds resume from the opposite direction once the eye passes over. Trees, shrubs, buildings, and other objects damaged by the first winds can be broken or destroyed by the second winds whose force is opposite the first winds.
- Be alert for tornadoes. Tornadoes can happen during and after a hurricane passes over. Remain indoors, in the center of your home, in a closet or bathroom without windows.
- Stay away from flood waters. If you come upon a flooded road, turn around and go another way. Cars are easily swept away by flood waters.

Know What to Do After a Hurricane Is Over

- Listen to NOAA Weather Radio or local radio or TV stations for instructions. Check the NOAA Voicemail Message Center (843-762-8888) or the Emergency Message Center at 1-866-246-0133 – PIPS (public information phone system via SC EMD (803-797-8300).

HOMELAND SECURITY INCIDENTS

During the occurrence of bioterrorism acts or homeland security incidents that may pose a threat to the safety and security of Hollings Marine Laboratory (HML), precautions will be taken to secure the safety of HML including:

- Activation of the Ft. Johnson 5 (NOAA, NIST, SC DNR, MUSC and UofC) to secure the Ft. Johnson Campus including securing and possible closure of the Front Gate.

- Activation of HML Continuity of Operations Plan (COOP) which includes: (1) Notification of the NCCOS Director to Activate the COOP; (2) The NCCOS Director will then notify the NOAA Deputy Acting Administrator to inform NOS Management; (3) The NCCOS Director will then have the NCCOS Deputy Director to notify other Centers.

- The HML COOP Team will include the Center Director (Fred Holland), Deputy Director (Paul Sandifer), Building Supervisor (Martin Burnett), Human Resources (Nancy Davey), Safety Officer (Rick Meitzler), and Branch Chiefs along with appropriate representatives from SC DNR, MUSC, U of C, NIST with NOAA. As may be warranted and threat influenced other appropriate fire and law enforcement departments within the area who may confer and decide on the appropriate levels of response to take to secure the facility and safety /security of HML staff .

- Employees will be dismissed and granted Administrative Leave according to the level of threat posed by the particular incident or act. A liberal leave policy will be granted during such events to ensure that employees are able to address security concerns of their family members. All threat alert phases will coincide with the Homeland Security Advisory System to manage the risk incident or threats.

- Shelter in place procedures also utilize the above guidance and will be based upon direction from Headquarters and / or Local Emergency Management Authorities. All such directives will be authenticated by on-site management and coordinated with HQ and local jurisdictional authorities for status confirmation.

JOB HAZARD ANALYSIS

Hollings Marine Laboratory

Program: **National Institute of Standards and Technology**

Area Supervisor: **Paul Becker**

Room number: **C102-C105**

I. Work Activities: *(list below)*

1) Samples will be homogenized inside of liquid nitrogen (LN_2) vapor phase freezers
2) Cleanup of Teflon and titanium materials using chemicals (acids and solvents)
3) Processing and sub-sampling of marine animal tissues

II. Listing of protective equipment that may be required for these tasks:

Equipment	Applicable Work Activity Number
a. Closed Toe Shoes	1, 2 and 3
b. Lab Coat	
c. Safety Goggles	2 and 3
d. Gloves	1, 2 (cryogenic or laboratory gloves), and 3
e. Fume Hood	2
f. Apron	
g. Dust Mask	
h. Radiation Badge	
i. Face Shield	
j. Hearing Protection	
k. Other: Coverall w/ hood, booties	1, 2 and 3
l. Other: Oxygen Monitor*	1, 2 and 3

* May be required

III. Location of the nearest emergency equipment for this area:

1. Fire: Fire pull alarm is near the exterior door in hallway, near room **inside Room C103** .
   Fire extinguisher mounted on the wall near door to room **C108.**
2. Eyewash and safety shower: Eyewash is in the sink in room **C103** .
   Shower is in room **outside Room C106**.
3. MSDS sheets are on the lab bench and mounted in hallway area near **B121.**
4. Spill Kit and First Aid Kit is in Room **C102**.

This analysis has been identified for the activities in the area by:

Supervisor: _____Date_____

Area Safety Representative: _____Date_____

I have been informed of the possible hazards in this work area and of the Personal Protective Equipment that is required. **Before undertaking any new task which does not appear to be specifically addressed by this hazard analysis, I will seek guidance from my immediate supervisor.**

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

For questions concerning completion of this form, call Martin Burnett @ 762-8808.

JOB HAZARD ANALYSIS

Hollings Marine Laboratory

Program: **National Institute of Standards and Technology**

Area Supervisor: **Paul Becker**

Room number: **D104**

I. Work Activities: *(list below)*

1) Samples will be placed inside liquid nitrogen (LN_2) vapor phase freezers and -80° C upright freezers for archival.
2) Marine animal tissues will be freeze dried
3) Samples will be homogenized inside of liquid nitrogen (LN_2) vapor phase freezers

II. Listing of protective equipment that may be required for these tasks:

Equipment	Applicable Work Activity Number
a. Closed Toe Shoes	1, 2 and 3
b. Lab Coat	
c. Safety Goggles	1, 2 and 3
d. Gloves	1, 2 and 3 (Cryogenic gloves)
e. Fume Hood	
f. Apron	
g. Dust Mask	
h. Radiation Badge	
i. Face Shield	
j. Hearing Protection	
k. Other: Coverall w/ hood, booties	1, 2 and 3
l. Other: Oxygen Monitor*	1, 2 and 3

* May be required

III. Location of the nearest emergency equipment for this area:

1. Fire: Fire pull alarm is near the exterior door in hallway, near room **inside Room D104**.
   Fire extinguisher mounted on the wall near door to room **D109.**
2. Eyewash and safety shower: Eyewash is in the sink in room **C105**.
   Shower is in room **outside Room C106**.
3. MSDS sheets are on the lab bench and mounted in hallway area near **D120.**
4. Spill Kit and First Aid Kit is in Room **C102**

This analysis has been identified for the activities in the area by:

Supervisor: _____Date_____

Area Safety Representative: _____Date_____

I have been informed of the possible hazards in this work area and of the Personal Protective Equipment that is required. **Before undertaking any new task which does not appear to be specifically addressed by this hazard analysis, I will seek guidance from my immediate supervisor.**

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

Signed: _____Date_____
(*Person Working in the Laboratory*)

For questions concerning completion of this form, call Martin Burnett @ 762-8808.

NIST - Chemical Inventory 1/13/07

Room No.	Cas No.	Chemical	Size	# Units	Contact
C103/C104	7647-01-0	Hydrochloric Acid	2.5 L	11	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103/C104	7697-37-2	Nitric Acid	2.5 L	10	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103/C104	7647-01-0	Hydrochloric Acid	4 L	5	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103/C104	7697-37-2	Nitric Acid	4 L	10	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103	7647-01-0	Hydrochloric Acid	5 gal.	1	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103/C104	7647-01-0	Hydrochloric Acid	2.5 gal.	1	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103/C104	7697-37-2	Nitric Acid	4 L	1	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103	67-66-3	Chloroform	4 L	16	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103	64-17-5	Ethyl Alchohol - 200 proof	4 L	25	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
C103/C104/D104		Liquid nitrogen			Rebecca Pugh (x8952)/Michael Ellisor (x8951)
		Water with trace amounts of Hydryochloric acid	4 L	6	Rebecca Pugh (x8952)/Michael Ellisor (x8951)
		Water with trace amounts of Nitric acid	4 L	9	Rebecca Pugh (x8952)/Michael Ellisor (x8951)