NISTIR 6752

NATIONAL BIOMONITORING SPECIMEN BANK NIST-CHARLESTON LABORATORY: Clean Room and Specimen Bank Protocols

Rebecca S. Pugh
Barbara J. Porter
Paul R. Becker
and Stephen A. Wise

NIST
National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

NISTIR 6752

NATIONAL BIOMONITORING SPECIMEN BANK NIST-CHARLESTON LABORATORY: Clean Room and Specimen Bank Protocols

Rebecca S. Pugh[1]
Barbara J. Porter[2]
Paul R. Becker[1]
and Stephen A. Wise[2]

[1]National Institute of Standards and Technology
Chemical Science and Technology Laboratory
Charleston, SC 29412

[2]National Institute of Standards and Technology
Chemical Science and Technology Laboratory
Gaithersburg, MD 20899

January 2002

U.S. Department of Commerce
Donald L. Evans

National Institute of Standards and Technology
Arden L. Bement, Jr., Director

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.

INTRODUCTION

NATIONAL BIOMONITORING SPECIMEN BANK

The National Institute of Standards and Technology (NIST) has been involved in the long term banking of biological specimens for over 20 years. The U.S. National Biomonitoring Specimen Bank (NBSB) was initiated in 1979 from the pilot Environmental Specimen Bank Program under the sponsorship of the Environmental Protection Agency (EPA) (Wise and Zeisler, 1985). It is maintained at the NIST Research Reactor Building in Gaithersburg, Maryland. The NBSB is an archive that provides for the long-term storage of well documented and preserved environmental specimens. Details of the NBSB have been described elsewhere (Wise and Koster, 1995; Wise et al., 1993). Specimens have been collected through numerous research projects that have been developed in collaboration with several different national and international agencies. These projects are summarized in Table 1.

Table 1. Projects within the National Biomonitoring Specimen Bank at NIST

Project	Sponsoring Organization/Agency	Specimen Type
Environmental Specimen Bank	Environmental Protection Agency (EPA)	Human liver, mussels
National Status and Trends Specimen Bank Project	National Oceanic and Atmospheric Administration (NOAA)	Mussels/oysters, sediment, fish muscle and fish liver
Alaska Marine Mammal Tissue Archival Project	NOAA, U.S. Geological Survey/Biological Resources Division (USGS/BRD), Minerals Management Service (MMS)	Marine mammal tissues (kidney, liver, blubber)
National Marine Mammal Tissue Bank	NMFS/Office of Protected Resources (NOAA)	Marine mammal tissues (kidney, liver, blubber)
Trace Nutrients in Human Diet Project	International Atomic Energy Agency (IAEA) US Department of Agriculture (USDA) Food and Drug Administration (FDA)	Total human diet
Seabird Tissue Archival and Monitoring Project	USGS/BRD, USFWS/NWR	Seabird eggs

Specimen banking provides researchers 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). Two ongoing environmental monitoring programs that have provided a large percentage of these samples are the Alaska Marine Mammal Tissue Archival Project (AMMTAP) and the Marine Mammal Health and Stranding Response Program (MMHSRP).

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 between the U.S. Geological Survey/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). A detailed description of the project and protocols have been published (Becker et al., 1991; 1993).

The National Marine Mammal Tissue Bank (NMMTB) was initiated in 1987 by the National Marine Fisheries Service (NMFS) to collect marine mammal tissues and bank them for long-term storage at the NBSB. In 1992, the NMMTB was formally established by Federal Legislation (Public Law 102-587) and a portion of tissues collected by NMFS's MMHSRP are archived in the NMMTB. The MMHSRP obtains specimens from marine mammal strandings and from animals taken incidentally during commercial fishing operations and emphasizes contaminant monitoring and information management. Detailed descriptions of this project have been published (Lillestolen et al. 1993 and Becker et al. 1994).

A third program that currently contributes to the NBSB is the Seabird Tissue Archival and Monitoring Program (STAMP). This project was initiated in 1998 with financial support from the U.S. Department of the Interior, USGS/BRD and is conducted as a collaboration among USGS/BRD, U.S. Fish and Wildlife Service Alaska Maritime National Wildlife Refuge (USFWS/NWR), and NIST. This program was established to archive a representative collection of seabird tissues from representative Alaskan colonial seabird species for future contaminant analyses and documentation of long-term trends in environmental quality. Currently, seabird eggs from common murre (*Uria aalge*) and thick-billed murre (*Uria lomvia*) colonies are collected and banked but additional species and types of specimens will be collected in the future. A description of this project and protocols have been published (York et al. 2001).

NBSB - CHARLESTON LABORATORY

In 1995, an agreement was signed between NIST and NMFS to establish the National Marine Analytical Quality Assurance Program (NMAQAP). Through interlaboratory comparisons and reference material development, the NMAQAP is able to assess and improve the quality of analytical measurements in the marine environment as well as allow for the improvement in the capabilities to assess trends in marine environmental quality by expanding environmental specimen banking activities. As part of this agreement, NIST established a satellite facility of the NBSB in Charleston, South Carolina.

The NBSB-Charleston Laboratory is dedicated to banking only marine environmental specimens, currently through the AMMTAP, MMHSRP and STAMP 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. The NBSB-Gaithersburg facility (hereafter, NBSB-Gaithersburg) and the satellite specimen bank in Charleston (hereafter, NBSB-Charleston), will maintain identical operating procedures for all aspects of the NBSB, but the following information will specifically describe those of NBSB-Charleston.

LOCATION

NBSB-Charleston is located at 219 Fort Johnson Road, Charleston, South Carolina, on the Fort Johnson Marine Resources Center complex in NOAA's National Ocean Service (NOS), Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) building (Fig. 1). The NIST space is approximately 185.81 m^2 (2,000 ft^2) which includes office space, associated storage space, analytical laboratories and NBSB-Charleston. The NBSB-Charleston consists of three rooms, the Ante Room, 8.55 m^2 (92 ft^2), the Specimen Bank, Room 227B, 39.02 m^2 (420 ft^2) and the Clean Room, Room 227A, 33.45 m^2 (360 ft^2)(Fig. 2).

The Hollings Marine Laboratory (HML) will be completed in the fall of 2001. This facility is located on an approximately 8-acre site within the Fort Johnson Marine Resources Center. The HML will promote collaborative and interdisciplinary scientific research through a multi-disciplinary team of scientists. This team includes NIST, NOAA/National Ocean Service, South Carolina Department of Natural Resources, University of Charleston, and Medical University of South Carolina. The NIST space will be approximately 743.22 m^2 (8,000 ft^2) and will include office space, analytical laboratories, a Standard Reference Material production facility and the NBSB. (Fig. 3).

CLASS 100/10,000 FACILITY

The NBSB-Charleston has been designed to contain a Class 100 sample processing area (Clean Room) and a Class 10,000 cryogenic storage room (Specimen

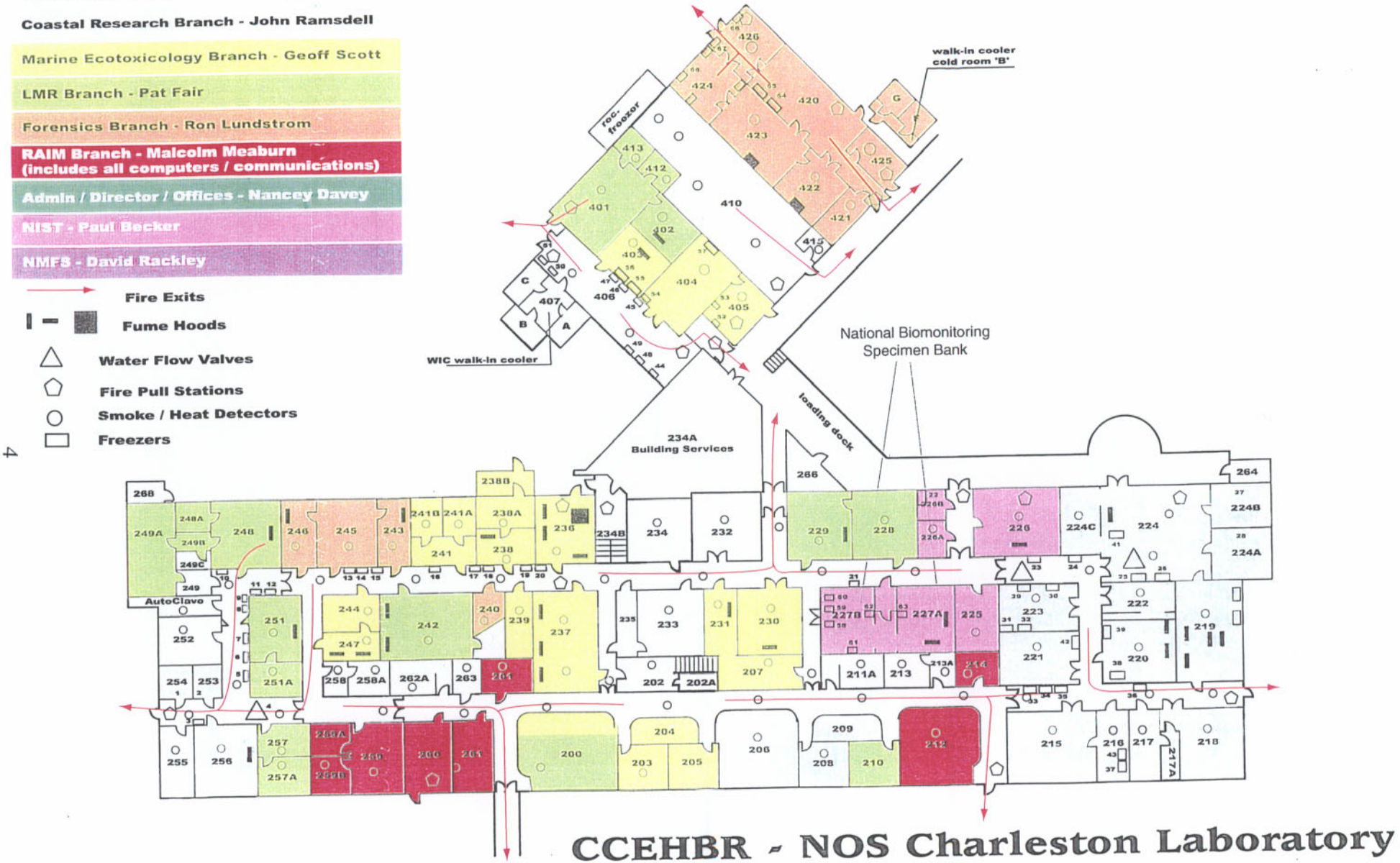


Figure 1. The NBSB-Charleston located within the NOS/CCEHBR Laboratory

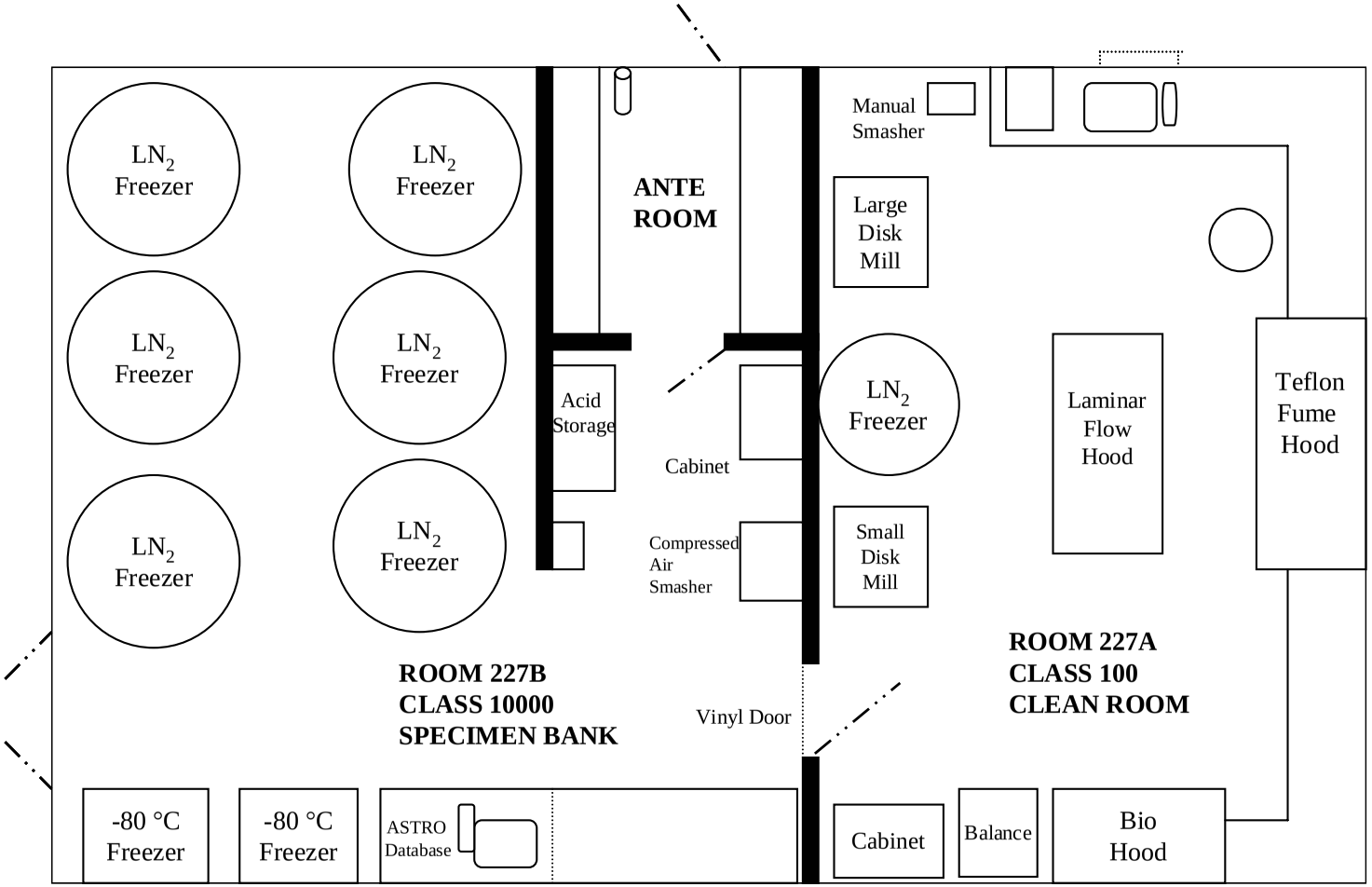


Figure 2. NBSB-Charleston Floor Plan

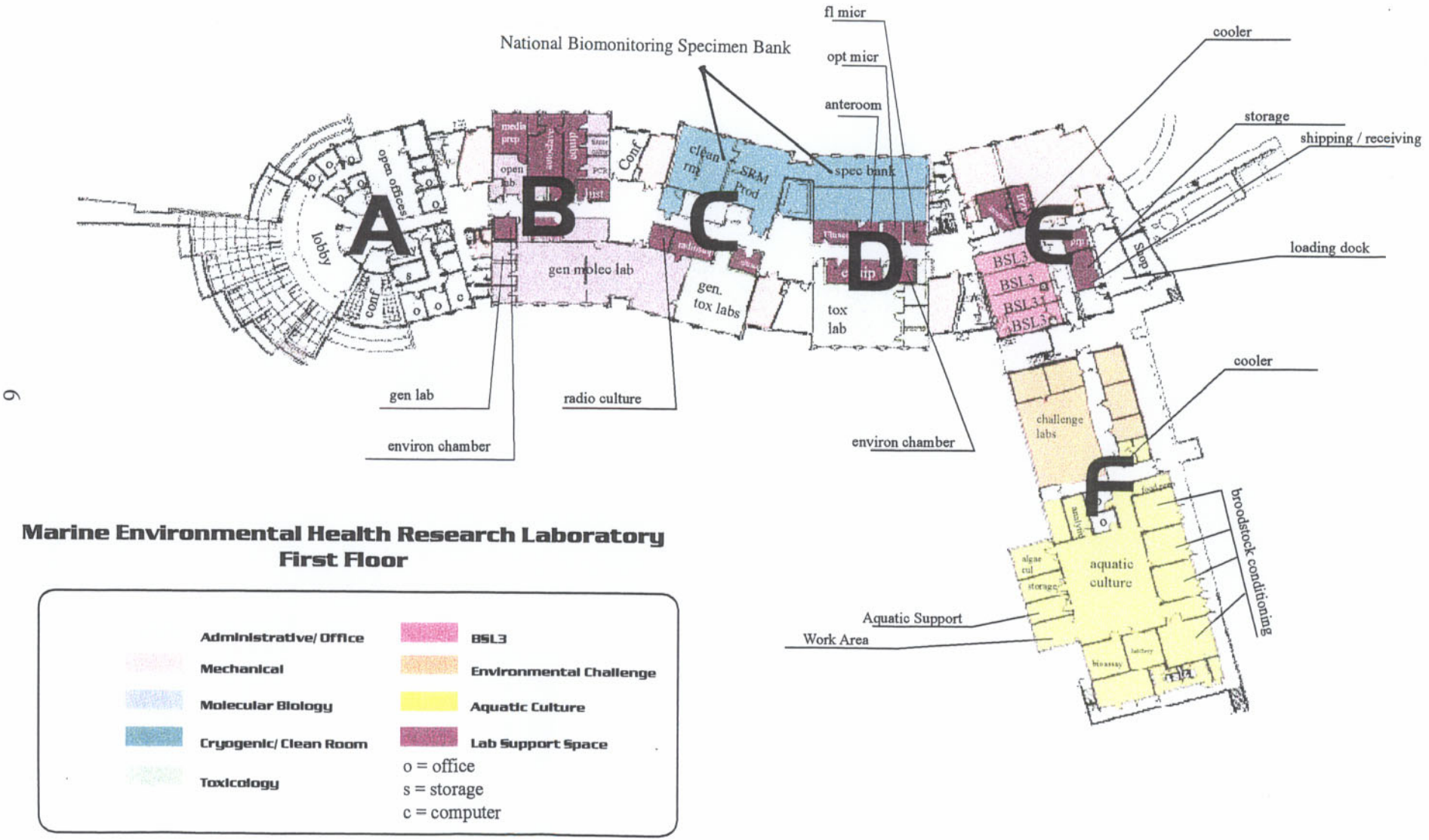


Figure 3. The Hollings Marine Laboratory (HML), First Floor

Bank). The clean laboratories control the concentration of airborne particles to specified limits and are essential in minimizing contamination of samples during processing. The clean room provides a clean sample preparation work area for materials to be analyzed for organic and inorganic constituents. At present, organic analyses are performed primarily for chlorinated pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls; the inorganic analyses center on the measurement of trace and ultratrace quantities of elements, with particular attention being given to metals.

The normal Class 100 clean room is designed to provide clean filtered air, exhaust particulate matter as fast as possible and maintain Class 100 air quality when there is a minimum of activity in the room. Class 100 air is defined as a measurement of no more than 3,530 particles per m^3 (100 particles per ft^3) which are 0.5 µm in diameter or larger. Class 10,000 air is then defined as a measurement of no more than 353,000 particles per m^3 (10,000 particles per ft^3) which are 0.5 µm in diameter or larger. These standard classes of air cleanliness for airborne particulate levels in clean rooms are established by the Federal Standard 209E document (U.S. General Services Administration, 1992; Institute of Environmental Sciences and Technology, 1997). The NBSB Class 100 room is achieved by driving air vertically downward through high efficiency particulate air (HEPA) filters and capturing return air in two of the side walls at ground level. The air is then recirculated back through the HEPA filters (Fig. 4) and is exchanged several times per hour. The airflow in NBSB-Charleston is non-unidirectional. Federal Standard 209E defines this as 'airflow which does not meet the definition of unidirectional airflow'. In other words, it allows the vertical downflow of air to change directions to horizontal at working height. This provides an efficient shielding against any cross-contamination.

As mentioned above, the purpose of the NBSB 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 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 which 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 µg flake of stainless steel in the sample could produce an analytical result which is 100 times higher than the true value of nickel in the sample.

In the clean room the air, implements, bench tops, working personnel, and 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

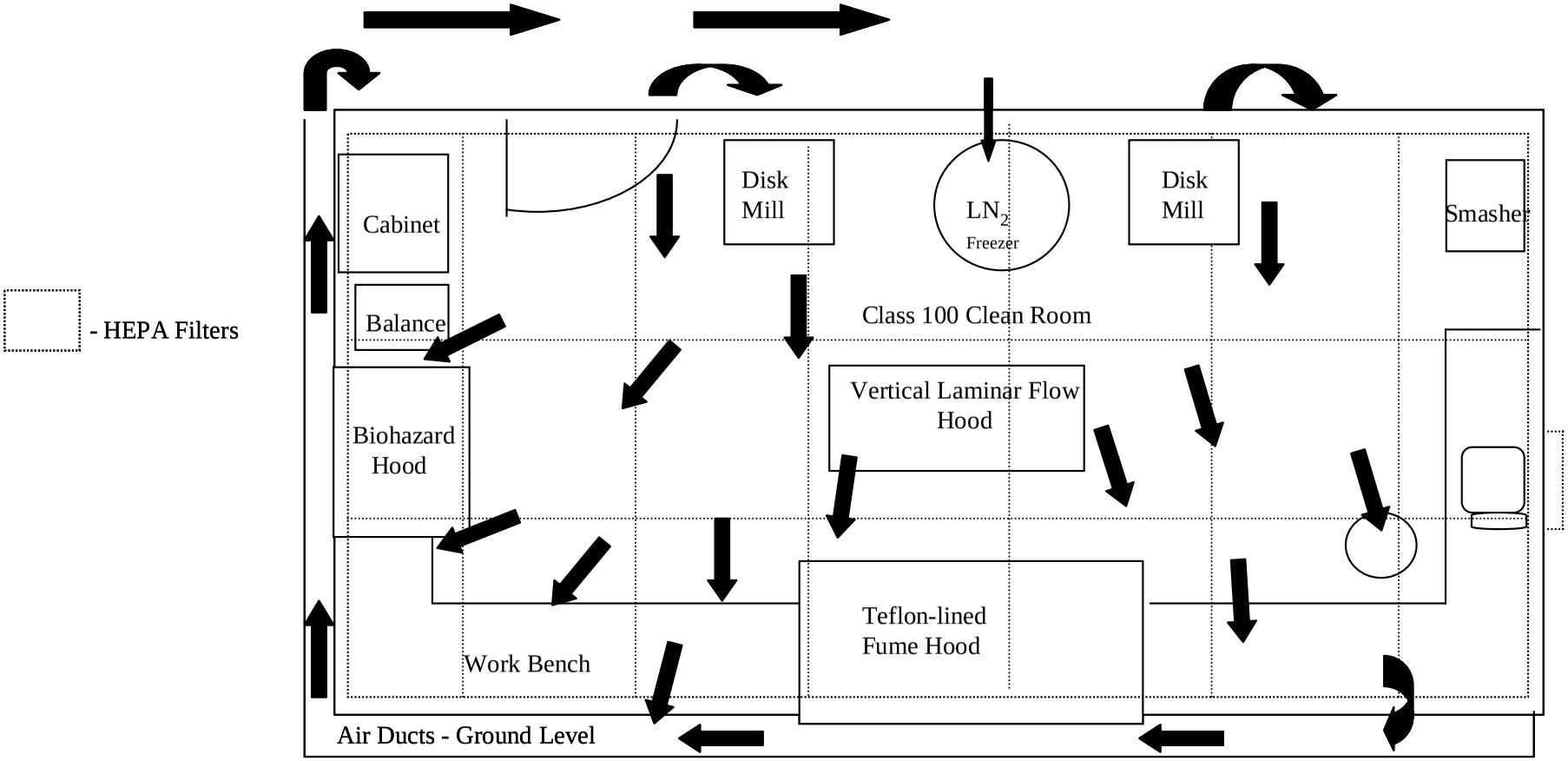


Figure 4. Clean Room Air Flow

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, 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 three rooms that make up the NBSB-Charleston serves a different purpose. It is important to know the function and the types of equipment that are used in each room.

ANTE-ROOM

The Ante-Room, a Class 10,000 room, is used to change into proper 'clean' garments before entering the Specimen Bank and Clean Room. Disposable laboratory frocks, hoods, and shoe covers are provided in this room and must be worn at all times while in the NBSB. 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.

Extreme caution must be taken while working in NBSB-Charleston to prevent further contamination of samples; therefore the following precautions should be taken before entering the Ante-Room:

1. Do not use cosmetic substances such as excessive makeup, alcohol based perfumes, or after shave products;
2. Avoid clothing with excessive lint, such as, sweaters, turtlenecks, torn and frayed clothing, or any item that has been worn in a dusty environment;
3. Do not bring in items that may cause further contamination, such as a lead pencil, dirty chemical bottles, paper towels, cardboard boxes, paper products, or other particulate products;
4. Do not smoke, chew gum or tobacco, bring in food or drink, or bring personal items into the NBSB; and
5. Do not use pencils or erasers, only use ballpoint pens in the NBSB.

The following gowning and de-gowning procedures must be followed in the Ante-Room before entering and exiting NBSB-Charleston:

Gowning Procedure:

1. Wash hands thoroughly before entering the Ante-Room;
2. Upon entering, step onto the sticky mat to remove loose particles on street shoes;
3. Place disposable shoe covers carefully over shoes;
4. Place the hood over the head, making sure all hair is inside the hood;
5. Choose the closest fit frock size and put it on, making sure to close all snaps and secure the hood inside of the frock;
6. If facial hair is present, place a beard cover on, over the hood, again making sure all hairs are inside the cover; and
7. Step onto the second sticky mat and enter the Specimen Bank.

De-gowning Procedure:

1. From the Specimen Bank, 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 the hook next to the Specimen Bank door;
4. Remove the frock and inspect it. If it is dirty or damaged, discard it in the trash can; if not, hang it on the hook underneath the top shelf; and
5. 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 underneath the frock on the floor.

Some of the cleaning supplies are stored in the Ante-Room. These include a clean-room mop, water bucket, sticky roller, lint-free cloths, and an aerosol can of isopropyl alcohol, Deconahol. The proper use of these cleaning materials is discussed in detail in the Maintenance Section. All items that are brought into NBSB-Charleston must be wiped down with Deconahol and a lint free cloth. If an item is extremely dirty, the vacuum cleaner located in the Specimen Bank must first be used before wiping down with the lint-free cloth.

SPECIMEN BANK

The Specimen Bank is a Class 10,000 cryogenic storage facility. Samples that are collected for the AMMTAP, MMHSRP, and STAMP projects are archived in liquid nitrogen vapor-phase freezers (-150 °C) that are located in this room. Along with the liquid nitrogen freezers, there are several other pieces of equipment that are either used or stored inside of the Specimen Bank (these are listed in Table 2).

LIQUID NITROGEN PIPING SYSTEM

All freezers in the specimen bank are connected to a liquid nitrogen in-line piping system. This system provides vacuum insulated piping along with a cryovent device located on the exterior of the building to ensure that only liquid is maintained in the lines, not nitrogen gas. The system is monitored by a Watlow-manufactured series 965 microprocessor-based controller. The controller is located on the wall inside the specimen bank and is set to vent only enough gas to allow the piping to remain full of liquid. There are four liquid nitrogen pipes that enter from the ceiling into the specimen bank with two valves on each pipe. These valves provide the liquid nitrogen to all eight freezers: six -150 °C vapor phase and two -80 °C ultra-cold electric freezers. The ultra-cold electric freezers are connected to the piping system for the liquid nitrogen back-up system. The liquid nitrogen for the Specimen Bank and Clean Room operations is stored behind the CCEHBR building in a 5.7 m^3 (1,500 gallon) vertical storage tank. The Datatron System 3001: 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 top, middle, and bottom temperatures inside the freezer. See the 'Safety' section of this manual for a full description of this system.

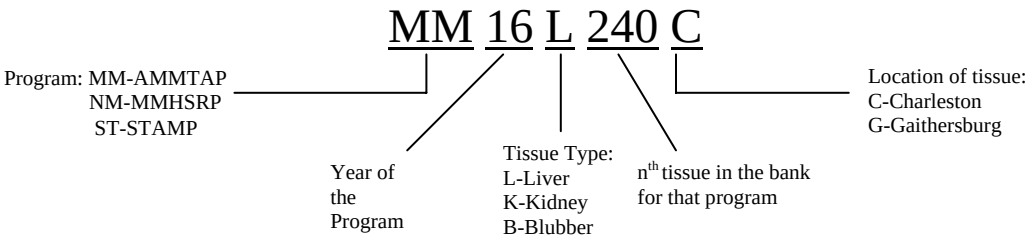
ARCHIVING SAMPLES: THE ASTRO SYSTEM

The operating procedures for storage of specimens at the NBSB-Charleston facility are identical to those used at the Gaithersburg facility except for the use of the Archival Specimen Tracking Retrieval Operation (ASTRO) database system at Charleston. ASTRO is a PC-based graphical program that resembles other Windows-based applications allowing one to point and mouse 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 automates 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) has been produced that introduces the database. The following information describes the use of ASTRO specifically for NBSB-Charleston and the system used to assign the NIST Storage Number which is needed for the ASTRO database.

Table 2. Equipment located in the Specimen Bank at NBSB-Charleston.

Equipment Type	Quantity	Purpose
MVE XLC 1841 Liquid Nitrogen (- 150° C) Vapor Phase Freezer	6	Permanently store archived environmental samples
Forma Model 8517 (-80° C) Ultra-cold Electric Freezer	2	Store Standard Reference Materials and control materials for Quality Assurance Program
Acid Storage Cabinet	1	Store hydrochloric and nitric acids, used for cleaning Teflon materials
PC, Label Printer, and Scanner for ASTRO Database	1	Database provides specimen management, storage management, and system administration functions for samples archived at the NBSB
MVE Cryo Shipper	7 to 10	Shipment of samples to and from the NBSB
MVE SC 14/2V, Cryo Shipper	7 to 10	Shipment of homogenized materials to and from the NBSB
Nodes for the Datatron System 3001: Monitoring, Alarming, and Data Management System	8	One node for each freezer has 3 temperature probes to monitor temperatures at the top, middle, and bottom of each LN_2 freezer and upright freezer
Watlow 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 environements
Compressed Air Smashing Machine	1	Smashes large tissues into fragments during the homogenization process
Polypropylene Storage Cabinet	1	Store Teflon jars prior to cleaning; store cleaning supplies
Air Compressor	1	Provides air to smashing machine, stored in the hallway next to Room 226
Cryo-Cyl 230 LP	1	Stores excess liquid nitrogen for emergency purposes, stored in hallway next to Room 226
Vinyl Strip Shield Curtain	1	Minimizes particle exchange between the Specimen Bank and Clean Room

Once marine mammal tissues have been collected using the NBSB protocols, the 180 mL Teflon jars that the tissues are stored in are placed in one of the -150 °C biological dry shippers and transported to NBSB-Charleston. Individual NBSB field data sheets (see Appendix A) are completed for each animal sampled and are shipped with the samples. Once the shipper is received, 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 data sheets. A NIST Storage Number is then assigned to each tissue that is archived in the specimen bank (See Appendix A).



After all storage numbers have been assigned, a 'New Shipment' is created in ASTRO. This screen contains the following information:

Who shipped the samples,
Date shipped,
Date received at NBSB-Charleston,
Contents of the shipment,
Location where the samples will be stored (Charleston),
Project type (NMMTB),
Collection type (AMMTAP, MMHSRP, STAMP, etc.).

The Teflon jars are then placed in pre-labeled cardboard tubes (racks). Five Teflon jars will fit in each cardboard tube and each individual tube contains only one tissue type (i.e.; liver, kidney, etc.). An inventory of the current available space for each tube is printed from ASTRO so that the samples can be placed in the appropriate tubes. After the new shipment is created the 'Enter New Specimens and Aliquots' screen appears (Fig. 5). This screen allows you to enter the following detailed information about each sample:

Local ID (Animal ID Number),
Specimen ID (NIST Storage Number),
Specimen Source (condition of the animal–Code, 1, 2 or 3),
Collection Date,
Aliquot ID (subsample, A and B),
Aliquot Container Type (Teflon jar, Teflon bag, etc.),
Aliquot Type (tissue type),

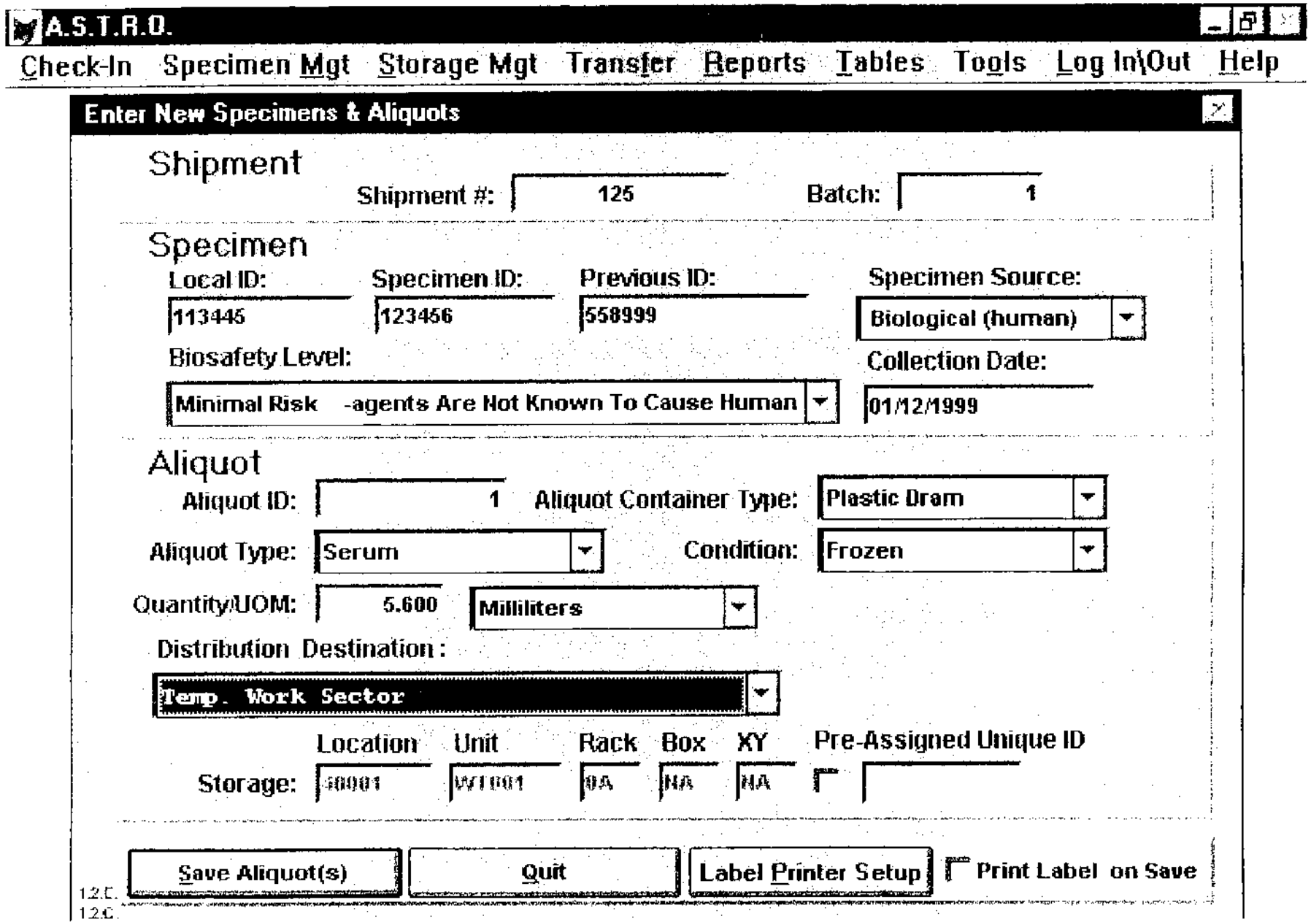


Figure 5. ASTRO 'Enter New Specimens and Aliquots' Screen

Condition (condition of the sample),
Quantity/Unit of Measurement (mass of tissue),
Distribution Destination (Charleston, Gaithersburg, Temporary space), and
Storage Location (Freezer/Rack/Box/XY Location)

Once those data are entered, the 'Standard EPI (epidemiological) Information' screen appears (Fig. 6). This screen allows entering of values for:

Age (neonate, sub-adult, adult, etc.),
Race (species of animal),
Sex (Male, Female, Unknown), and
Geographic Location (where the animal was sampled)

The tissue samples that are collected for the NBSB are divided in two portions, 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, a plan is being implemented to store all 'A' portions at NBSB-Gaithersburg and all 'B' portions at NBSB-Charleston. After the samples have been properly stored, the shipment is posted in ASTRO and a 'Posted Shipment Detail Report' is printed (Fig. 7). This report provides the following information:

Shipping information,
Project,
Collection,
NIST storage number,
Aliquot number,
Animal ID number,
Collection date,
Storage location information,
Tissue type,
Tissue weight,
Condition of the sample,
Unique ID number assigned by ASTRO, and
User ID

A copy of each shipment report is then placed in a binder for permanent storage. The field data sheet and NIST Storage data sheet information is entered into a Paradox database. This database is a computerized duplicate of the hardcopy sheets.

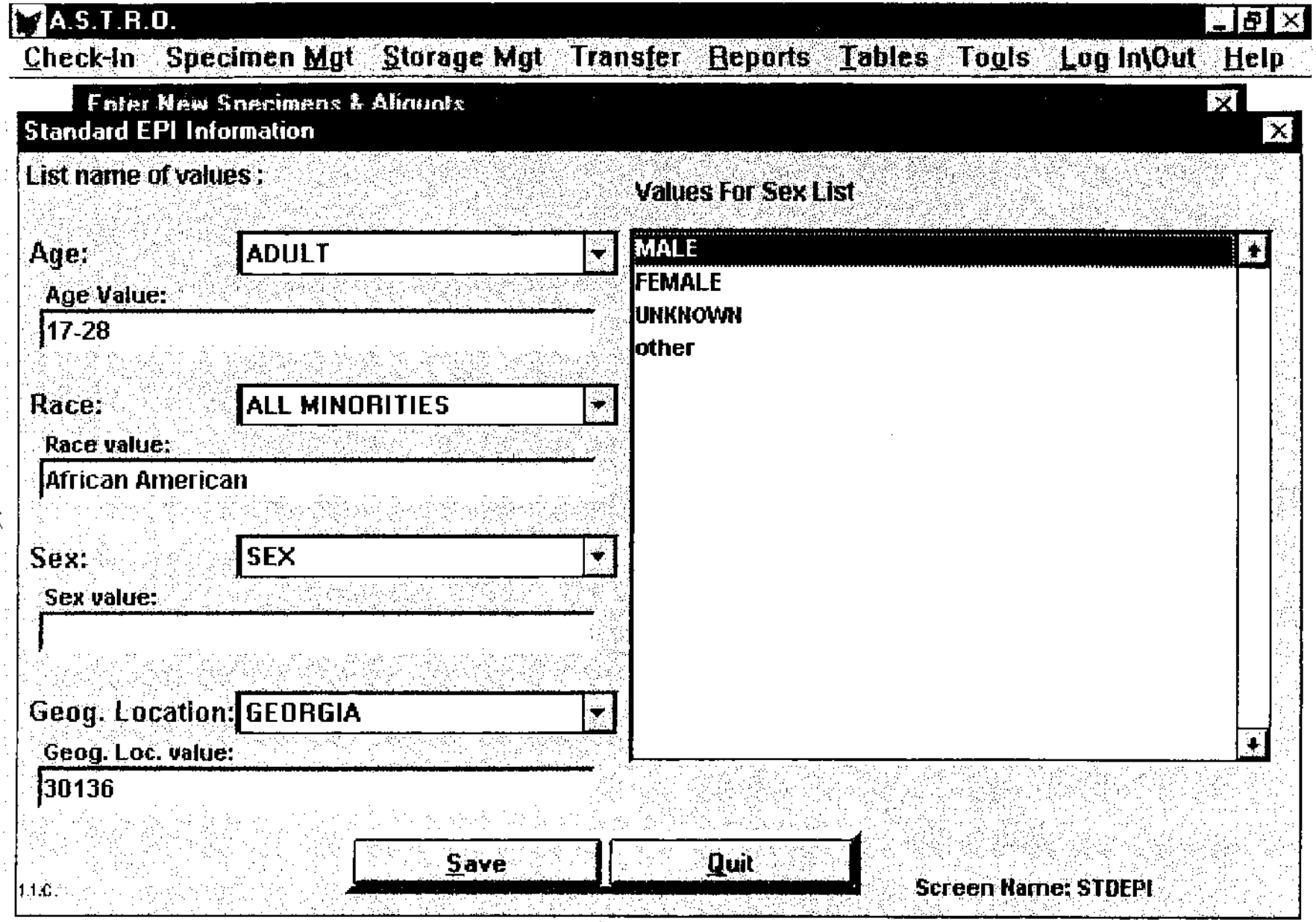


Figure 6. ASTRO 'Standard EPI Information' Screen

SHIPMENT / BATCH 27 / 1 ORGANIZATION **UNC-Wilmington** COURIER **Federal Express** COST $ SHIPPED **04/10/2000**
SPECIMENS 8 # ALIQUOTS 16 RECEIVED **04/11/2000 10:31**
LOCATION **10000** PROJECT **NMMTB NATIONAL MARINE MAMMAL** COLLECT **MM MMHSRP** CHECKED **04/11/2000 10:51**

PROJ. / COLLECT. / SPEC.	ALIQ.	LOCAL ID	COLL. DATE	Location UNIT	RACK	BOX	XY	VOLUME	UOM	TYPE	CONDITION	THAWS	UNIQUE ID	USER
NMMTB MM NM11B121C	1	NEFC5451	02/23/2000	LNAA	CA	AA	AC	127.000	g	BLUBBER	FROZEN -	0	000CI4A5	RSP
NMMTB MM NM11B121C	2	NEFC5451	02/23/2000	LNAB	CI	AA	AB	121.000	g	BLUBBER	FROZEN -	0	000CI4A6	RSP
NMMTB MM NM11B125C	1	NEFSC03701	02/10/2000	LNAA	CA	AA	AD	100.000	g	BLUBBER	FROZEN -	0	000CI4AD	RSP
NMMTB MM NM11B125C	2	NEFSC03701	02/10/2000	LNAB	CI	AA	AC	95.000	g	BLUBBER	FROZEN -	0	000CI4AE	RSP
NMMTB MM NM11K120C	1	NEFC5451	02/23/2000	LNAA	BZ	AA	AE	140.000	g	KIDNEY	FROZEN -	0	000CI4A3	RSP
NMMTB MM NM11K120C	2	NEFC5451	02/23/2000	LNAB	CJ	AA	AC	152.000	g	KIDNEY	FROZEN -	0	000CI4A4	RSP
NMMTB MM NM11K124C	1	NEFSC03701	02/10/2000	LNAA	CD	AA	AA	167.000	g	KIDNEY	FROZEN -	0	000CI4AB	RSP
NMMTB MM NM11K124C	2	NEFSC03701	02/10/2000	LNAB	CJ	AA	AD	102.000	g	KIDNEY	FROZEN -	0	000CI4AC	RSP
NMMTB MM NM11L119C	1	NEFC5451	02/23/2000	LNAA	CC	AA	AB	134.000	g	LIVER	FROZEN -	0	000CI4A1	RSP
NMMTB MM NM11L119C	2	NEFC5451	02/23/2000	LNAB	CL	AA	AA	154.000	g	LIVER	FROZEN -	0	000CI4A2	RSP
NMMTB MM NM11L123C	1	NEFSC03701	02/10/2000	LNAA	CC	AA	AC	120.000	g	LIVER	FROZEN -	0	000CI4A9	RSP
NMMTB MM NM11L123C	2	NEFSC03701	02/10/2000	LNAB	CL	AA	AB	174.000	g	LIVER	FROZEN -	0	000CI4AA	RSP
NMMTB MM NM11M122C	1	NEFC5451	02/23/2000	LNAA	CB	AA	AC	138.000	g	MUSCLE	FROZEN -	0	000CI4A7	RSP
NMMTB MM NM11M122C	2	NEFC5451	02/23/2000	LNAB	CK	AA	AC	137.000	g	MUSCLE	FROZEN -	0	000CI4A8	RSP
NMMTB MM NM11M126C	1	NEFSC03701	02/10/2000	LNAA	CB	AA	AD	181.000	g	MUSCLE	FROZEN -	0	000CI4AF	RSP
NMMTB MM NM11M126C	2	NEFSC03701	02/10/2000	LNAB	CK	AA	AD	180.000	g	MUSCLE	FROZEN -	0	000CI4AG	RSP

BATCH COMMENTS:.

Two bottlenose dolphin samples from Bill McLellan

Figure 7. ASTRO Posted Shipment Detail Report

BIOLOGICAL DRY SHIPPERS

The biological dry shippers are designed to transport biological samples at cryogenic temperatures and provide a reasonable holding time. 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. 8). The static holding time of these shippers are estimated at 14 d and 25 d, respectively (Fig. 9). To properly charge a shipper, the following instructions must be followed:

1. Place a lab coat or nonabsorbent apron, a pair of safety goggles and a pair of loose insulating cryogenic gloves on,
2. Remove the lid of the dry shipper and fill ¾ of the way with liquid nitrogen,
3. Replace the lid and let the shipper stand overnight (24 h),
4. Remove the lid again and fill the shipper ½ of the way with liquid nitrogen,
5. Let stand for 3 h to 4 h and pour off excess liquid nitrogen.

All excess liquid nitrogen must be removed by pouring it out of the shipper in order to transport the shipper as a non-hazardous item. In addition, Teflon jars and bags and glass jars may crack if there is direct contact with the liquid, therefore, it is important to make sure all liquid nitrogen is removed.

Figure 8. MVE Biological Dry Shippers; Cryo Shipper XC (left) and SC14/2V (right)

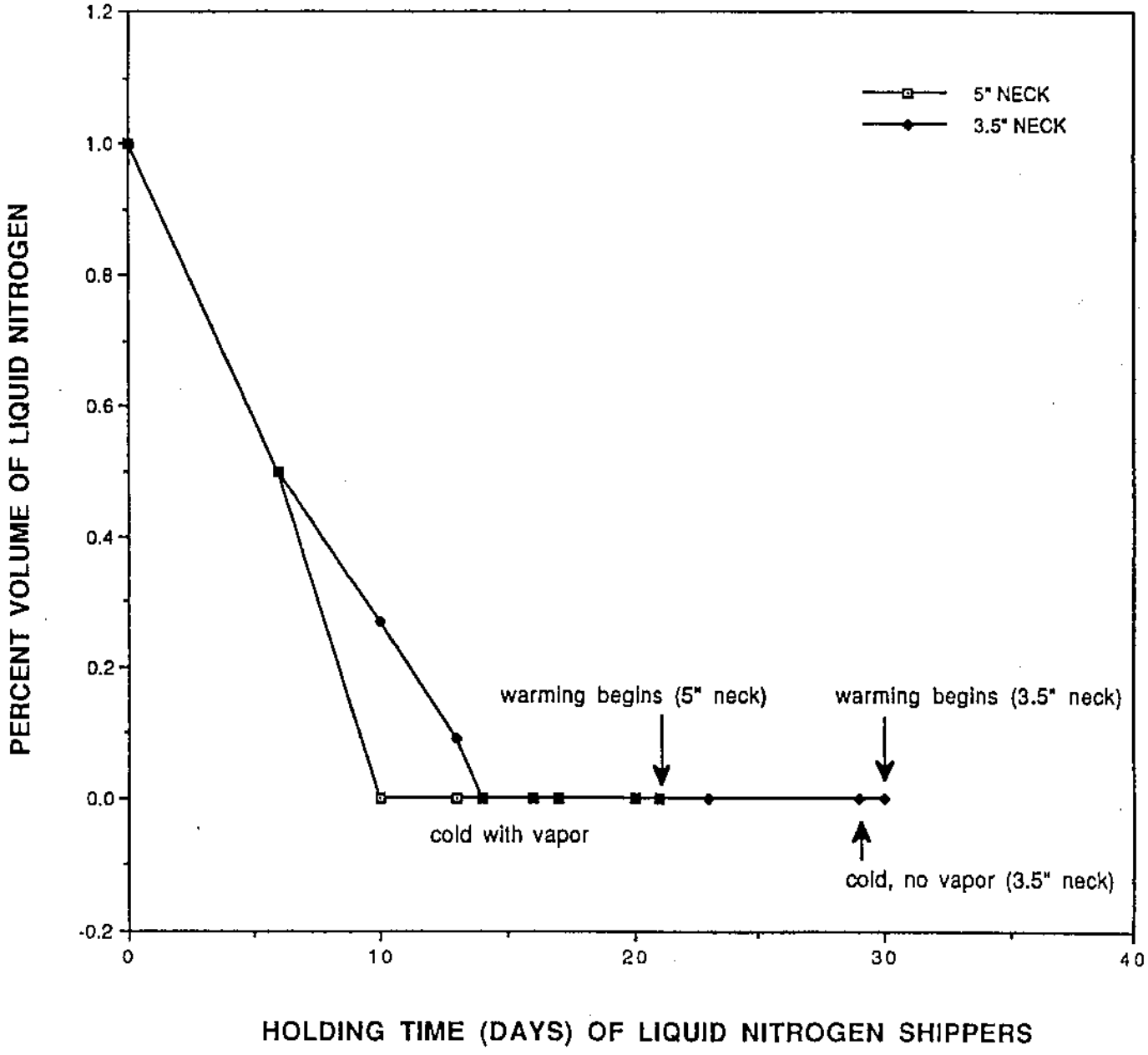


Figure 9. Holding Time of Biological Dry Shippers

CLEAN ROOM

The Clean Room is a Class 100 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.

HOOD USAGE/CLEANING OF TEFLON AND TITANIUM

A special Teflon-lined exhaust hood is provided for the cleaning of supplies. Cleaning procedures for Teflon and titanium materials are posted near the hood (see Appendix A). Always write the date that the solvent/acid bottle was opened on the label of the bottle. HPLC-grade water 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. Do not pour any solvent, acid, or strong alkali solutions down the sink in the Clean Room; it is not constructed to withstand such solutions.

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 obtained by various researchers and analytical techniques. The Teflon disk mill is recommended as an effective device for size reduction and homogenization of biological tissue. Further, 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 contamination that are unavoidable to two types of materials. Additionally, the titanium and Teflon equipment is precleaned, as mentioned above, to eliminated any surface contamination. The following list of equipment and instructions pertain to the homogenization of a single sample (i.e., liver, kidney, blubber).

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 °C) Vapor Phase Freezer
Compressed Air Smasher
Air Compressor

Table 3. Equipment located in the Clean Room at NBSB-Charleston.

Equipment Type	Quantity	Purpose
MVE XLC 1211 Liquid Nitrogen (- 150° C) Vapor Phase Freezer	1	Working freezer for cryogenically homogenizing materials
TS-250 Disk Mill	1	Grind small particles of tissue into powder, for use with small Teflon disk mill
TS-Specialized 250 Disk Mill	1	Grind small particles of tissue into powder, for use with medium and large Teflon disk mills
Manual Smashing Machine	1	Smash large tissues into particles during the homogenization process
Solvent Storage Cabinet	1	Store ethyl alcohol and chloroform, used for cleaning Teflon materials
Acid Storage Cabinet	1	Store hydrochloric and nitric acids, used for cleaning Teflon materials
Nodes for the Datatron System 3001: Monitoring, Alarming, and Data Management System	3	One node each to monitor temperatures in working freezer, temperatures in upright freezer located in the hallway and % humidity in the room
Teflon Heat Sealer	1	Seal Teflon bags
Teflon-lined Fume Hood	1	Teflon and titanium materials are cleaned under this hood
Nuaire Biological Safety Hood	1	
Vertical Laminar Airflow Hood	1	Cleaned Teflon and titanium materials are placed in the hood to dry
Marble Table and Analytical Balance	1	Measure weights of homogenized aliquots
Balance	1	Measure samples >1 kg
Met One Particle Counter	1	Measures airborne particles in a number of size ranges
Desiccator Cabinet	1	
Polypropylene Storage Cabinet	1	Store clean Teflon materials
Diaphragm Liquid Pump	1	Transfer chemicals/water from storage tank to bottles for cleaning supplies

TS-250 Disk Mill Shaker (Fig. 10)
Lint Free Cloths

Items which require cleaning and cooling to liquid nitrogen temperature before homogenizing is conducted:

Teflon disk mill (Fig. 11)
Teflon smasher (Fig. 11)
Teflon jars (15mL) or bags
Teflon scraper and scoop (Fig. 11)
Teflon handled titanium bladed knife
Stainless steel plates for shaker (Fig. 10)

Homogenizing (Individual Sample)

Locate the sample that is to be homogenized and collect all items in the second list. Make sure all equipment 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 and record the masses in a laboratory notebook. Put all of the small items (Teflon jars, scraper, scoop, and knife) in a Teflon-lined plastic tray and place the tray in the LN_2 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 homogenizing, remove the sample that is to be homogenized from storage 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 90 lb weight is released onto the Teflon smasher. This will fracture the sample into small fragments. (Fig. 12)
5. Press the DOWN button until the claw has clamped onto the 90 lb 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

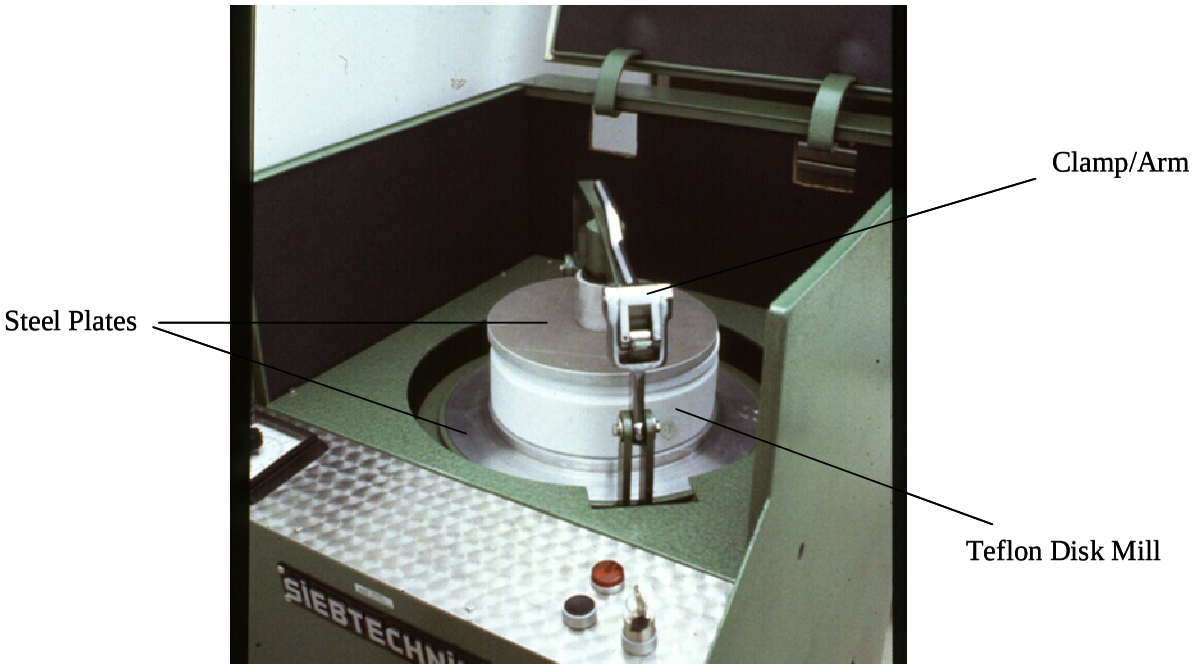


Figure 10. TS-250 Teflon Disk Mill

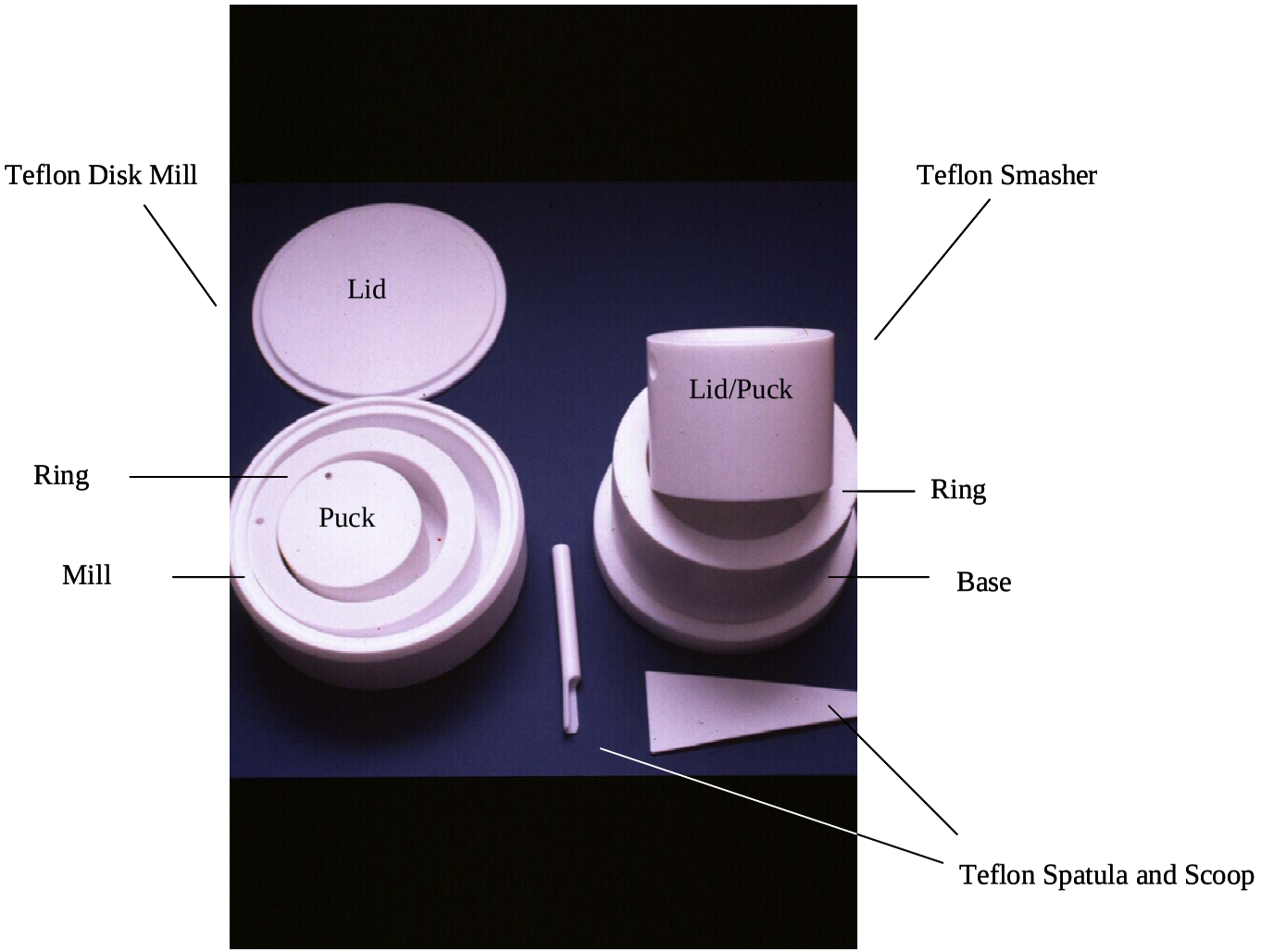


Figure 11. Teflon Materials Needed for Homogenization

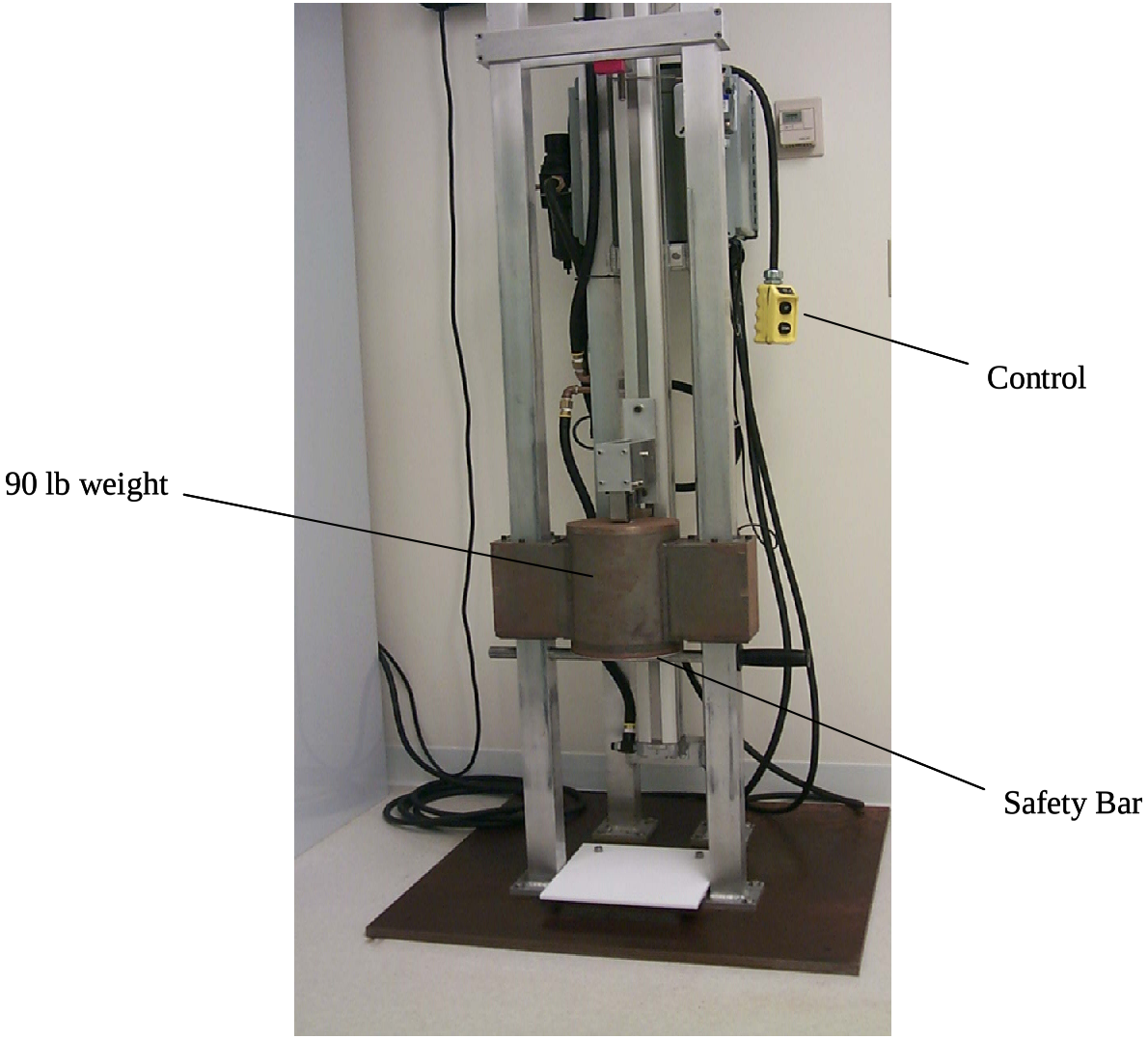


Figure 12. Compressed-Air Smashing Machine

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. 10).
12. Close the lid and set the timer for 3 minutes. 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 into 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 disc mill completely. Move all pieces towards 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 all materials to cool down.
14. Using the Teflon scoop, fill all jars in numerical sequence, making sure the lids are placed on tightly. Weigh the jars and record the masses in the laboratory notebook. Place the jars in a storage box and return to the LN_2 permanent 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 the Paradox computer file that contains the sample storage data form with the same new information and the ASTRO database.

Note: When removing the lids (puck) to the Teflon disk mill and smasher, 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 smahing 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.

Tissue in Fragment Form

Tissue in Powder Form

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

SAFETY

NBSB-Charleston has been equipped with several safety features throughout each of the three rooms to ensure safe laboratory operating procedures (Table 4, Figure 14). In addition, the CCEHBR Laboratory Safety Committee has written a Laboratory Chemical Hygiene Plan and Emergency Preparedness Plan that are strictly enforced throughout the entire laboratory. (See Appendix C). A Job Hazard Analysis Form has also been designed (See Appendix C). 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 C). 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 NBSB-Charleston, these sheets have been posted just inside the Clean Room on the wall.

HURRICANE EVACUATION

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

1. The computer in the Specimen Bank and in the NIST Office must have the latest back up of files on zip disk. These include the ASTRO database inventory and all Paradox files with completed field data sheets for all samples inventoried. These disks must be taken out of the building by authorized personnel.
2. A hard copy of the NBSB inventory must be printed from the ASTRO 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 -180 °C liquid nitrogen freezers must be filled with liquid nitrogen 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. All binders with AMMTAP, MMHSRP, and STAMP Field and NBSB Storage Data Sheets must be taken with personnel out of the building. Also, binders that contain information on miscellaneous items that are stored in the NBSB must also be taken.
6. The Cryo-Cyl 230LP must be filled with liquid nitrogen and brought into the Specimen Bank.
7. All computers and other electrical equipment must be covered with plastic in case of water leaks due to roof damage.

Table 4. Safety Equipment located in NBSB-Charleston.

Safety Feature	Comments
Ante Room	
Emergency Exit	Also used as primary entrance into and out of the NBSB
Fire Extinguisher	
Specimen Bank	
Smoke Detector	
Fire Alarm Strobe Light	
Humidity and Oxygen Sensor	
Emergency Exit	Set of double doors with an emergency break bar.
Liquid Nitrogen In-Line Piping System	Contains Cryo-vent device on the exterior of the building to ensure nitrogen gas is not maintained in the line
Phone Access	
Incoming Page Speaker	
Acid Storage Cabinet	
Clean Room	
Smoke Detector (2)	
Fire Alarm Strobe Light	
Humidity and Oxygen Sensor	
Liquid Nitrogen In-Line Piping System	Contains Cryo-vent device on the exterior of the building to ensure nitrogen gas is not maintained in the line
Eyewash Station	
Emergency Shower	
Teflon-lined Fume Hood	
Window	With no emergency exit in the Clean Room, a window has been added for safety and security reasons
Solvent Storage Cabinet	
Acid Storage Cabinet (2-small)	Located under the Fume Hood
Incoming Page Speaker	
Cryo-Cyl 230LP	Liquid nitrogen cylinder stored in hallway near Room 226

1. Entrance/Emergency Exit
2. Fire Extinguisher
3. Smoke Detector
4. Fire Alarm Strobe Light
5. Emergency Break Bar Exit
6. LN_2 In-Line Piping System
7. Phone Access
8. Incoming Page Speaker
9. Acid Storage Cabinet
10. Humidity & Oxygen Sensor
11. Solvent Storage Cabinet
12. Window
13. Eyewash Station
14. Emergency Shower
15. Teflon Fume Hood

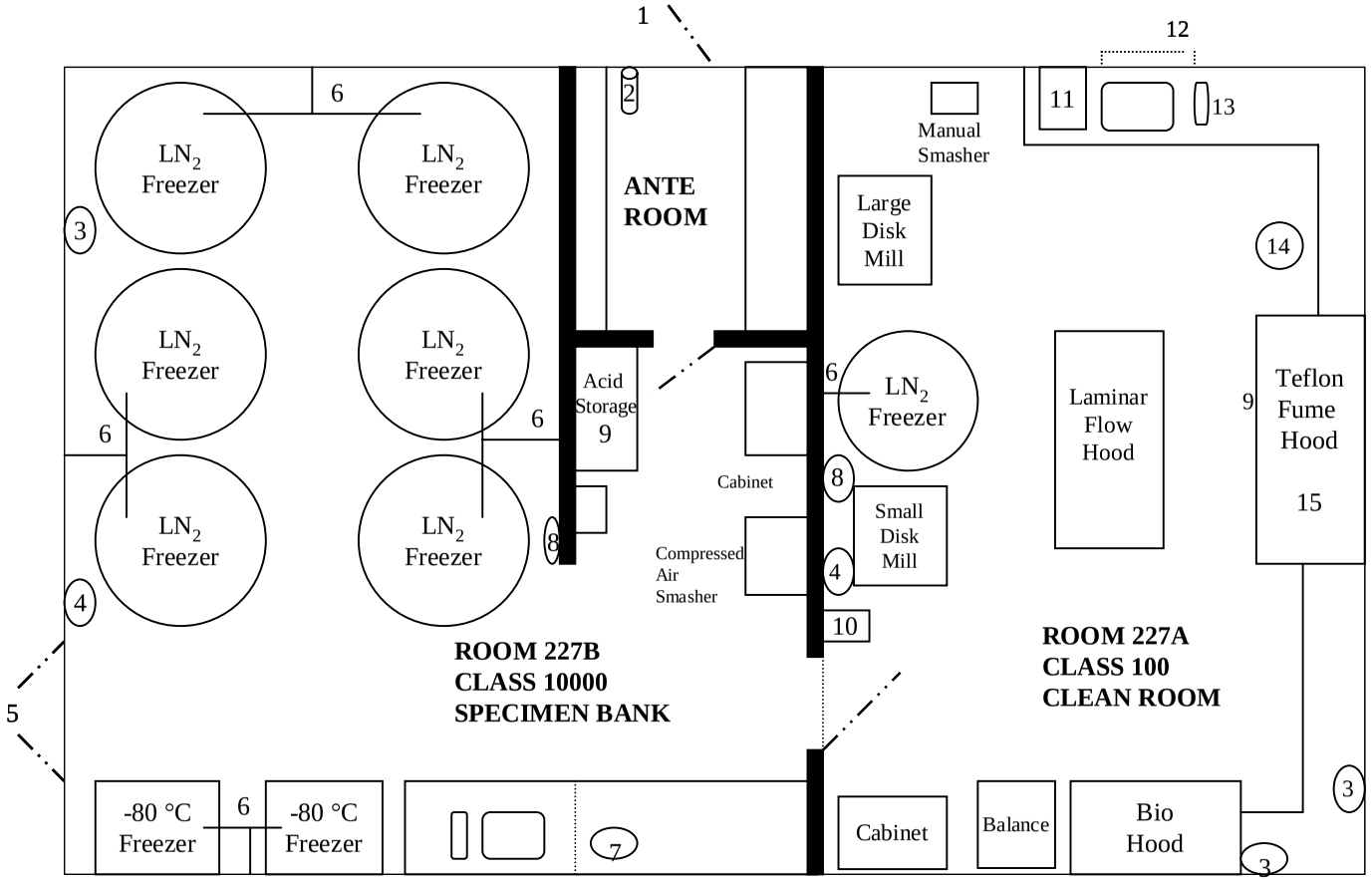


Figure 14. NBSB-Charleston Safety Features

DATATRON

The Datatron System 3001: Monitoring, Alarming, and Data Management System, created by R & D Scientific Corporation, has been installed to continuously monitor the temperatures of all freezers located in NBSB-Charleston as well as monitor room conditions (i.e., temperature and humidity). Three probes (channels) have been placed in each of the liquid nitrogen vapor freezers to record temperatures at the top, center and bottom of each freezer and one probe has 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 and a message providing the alarm number and location of the alarm will display across the LCD display message board, located in the NIST Office Space, Room 225. Also, the phone alarm notification system is designed to notify remote users of any alarms that are triggered either by channels out of their alarm limits or rebooting of the computer system (e.g. due to a power failure). The system uses one of two lists of phone numbers and users, List 1 (0800-1659) and List 2 (1700-0759), and begins calling the entries on the list. The system will continue calling the entries in order until an acknowledgment has been made by one of the users. The system ensures that trained personnel will be able to correct the problem or error within the NBSB before any damage has occurred. The Datatron System will also store all information that is provided by the trained user (i.e., the cause of the alarm event). The computer, LCD message board, and printer for the Datatron 3001 system are located in the NIST Office space in Room 225. A manual has been designed by R & D Scientific Corporation for this system and is stored next to the computer as a reference.

MAINTENANCE

In general, cleaning should begin in areas that require the most critical level of cleanliness and proceed toward areas of less critical requirements (Fig. 15). The following cleaning methods are to be followed to ensure that each room maintains the Class 100 and 10,000 levels. Basically, the preferred clean room cleaning protocol is to vacuum an area, wipe that area with a cleanser and lint-free cloths and vacuum the area again.

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.

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.

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.

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.

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.

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 rewipe the pipe, if necessary, to remove stubborn grime and spots.

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. Revacuum with a soft brush attachment to pick up any loose particles.

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.

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.

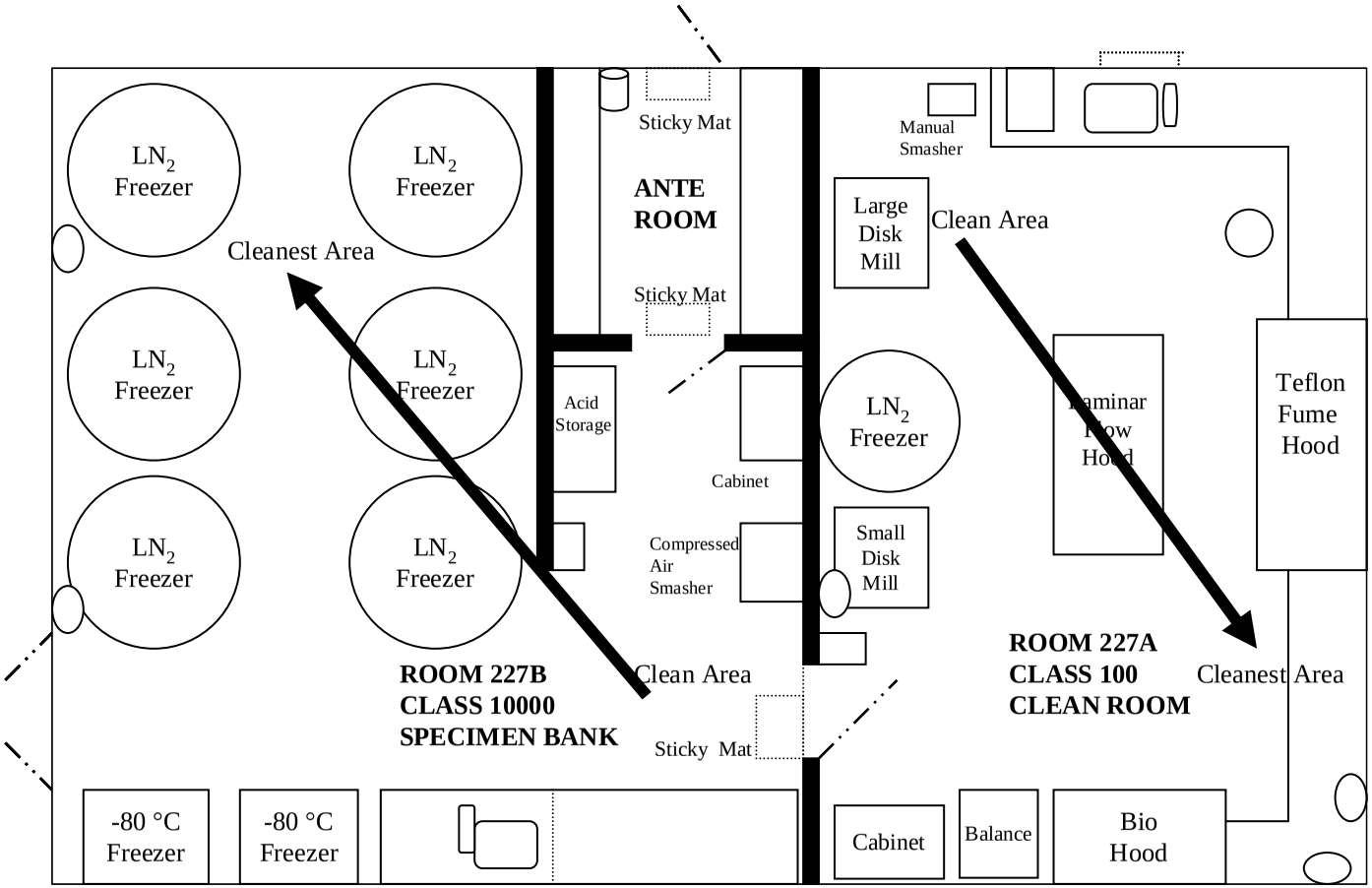


Figure 15. NBSB-Charleston Cleanliness Levels

The cleaning schedule for the NBSB will depend upon the amount of work being conducted in the rooms and the number of particles that are detected on the particle counter. After using the clean room for cleaning supplies or homogenizing samples, the above procedure should be followed precisely, starting with the ceiling and working down to the floors. A weekly particle count will be taken to ensure the rooms are staying at Class 100 and 10,000 levels. If the particle counts are above the class levels required, a thorough cleaning will be conducted. Because the Clean Room is two class levels higher than the Specimen Bank, entry from one room to the next should be kept to a minimum. To help prevent particle exchange between rooms, a 1.4 m x 2.5 m vinyl curtain made of 0.2 m wide strips has been mounted above the door between the Specimen Bank and the Clean Room. A sticky mat has also been adhered to the floor in front of that door to trap any particles that are on the bottom of the shoe covers. These measures will help to minimize the number of particle exchanges between the two rooms.

REFERENCES

Ayal, N. 1998, Archival Specimen Tracking Retrieval Operation User's Guide. Center for Disease Control and Prevention, Atlanta, GA.

Becker, P.R., S.A. Wise, B.J. Koster, and R. Zeisler, 1991, Alaska Marine Mammal Tissue Archival Project: Revised Collection Protocol, **NISTIR 4529,** USDOC, National Institute of Standards and Technology, Gaithersburg, MD.

Becker, P.R., B.J. Koster, S.A. Wise, and R. Zeisler, 1993, Biological specimen banking in Arctic research: an Alaska perspective. *Sci. Total Environ.* 139/140: 69-95.

Becker, P.R., D. Wilkinson, and T.I. Lillestolen, 1994, Marine Mammal Health and Stranding Response Program: Program Development Plan. USDOC, NOAA Tech. Memo. **NMFS-OPR-94-2**, Silver Spring, MD.

Becker, P.R., S.A. Wise, L. Thorsteinson, B.J. Koster, and T. Rowles, 1997, Specimen banking of marine organisms in the United States: current status and long-term prospective. *Chemosphere,* 34(9/10): 1889-1906.

Institute of Environmental Sciences and Technology, 1997, IEST-RP-CC006.2: Testing Cleanrooms. Contamination Control Division, Institute of Environmental Sciences and Technology, 35 pp.

Lillestolen, T.I., P.R. Becker, and D. Wilkinson, 1993, The National Marine Mammal Tissue Bank and Stranding Network Program. *Coastal Zone 93: Proceedings of the 8th Symposium on Coastal and Ocean Management (New Orleans, LA)*, O.T. Magoon, W.S. Wilson, H. Converse, and L.T. Tobin, eds., ASCE, New York, NY, 3: 2494-2504.

National Bureau of Standards, 1986, Certificate of Analysis, Standard Reference Material 101g, 18 Cr-10 Ni Steel (AISI 304L). Analytical Chemistry Division, National Bureau of Standards, Gaithersburg, MD, 2 pp.

U.S. General Services Administration, 1992, Federal Standard 209E: Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones. Institute of Environmental Sciences and Technology.

Wise, S.A., and R. Zeisler, 1985, The U.S. Pilot Environmental Specimen Bank Program. In: International Review of Environmental Specimen Banking. **National Bureau of Standards Spec. Pub. 706**, Wise, S.A., and R. Zeisler, eds., Gaithersburg, MD. 34-45.

Wise, S.A., B.J. Koster, J.L. Langland, and R. Zeisler, 1993, Current activities within the National Biomonitoring Specimen Bank. *Sci. Total Environ.* 139/140:1-12.

Wise, S. A. and B.J. Koster, 1995, Considerations in the Design of an Environmental Specimen Bank: Experiences of the National Biomonitoring Specimen Bank Program. *Environ. Health Perspect.* 103 (Suppl. 3): 61-67.

York, G.W., B.J. Porter, R.S. Pugh, D.G. Roseneau, K. Simac, P.R. Becker, and S.A. Wise. 2001, Seabird Tissue Archival and Monitoring Program: Protocols for Collecting and Banking Seabird Eggs, **NISTIR 6735**, National Institute of Standards and Technology, Gaithersburg, MD.

Zeisler, R., B.J. Koster, and S.A. Wise, 1992, Specimen Banking at the National Institute of Standards and Technology. In: *Analytical Approaches as Related to Specimen Banking*. Rossbach, M., and J.D. Schladot, eds., Stuttgart: Springer Verlag, 37-49.

NISTR 6752
Part 2

APPENDIX A

NATIONAL BIOMONITORING SPECIMEN BANK DATA SHEETS

NBSB Cetacean Field Data Sheet
NBSB Pinniped Field Data Sheet
NBSB Storage Data Sheet

NATIONAL BIOMONITORING SPECIMEN BANK

Animal Field ID Number _____ Species _____

Stranding ☐ Incidental ☐ Subsistence ☐

Geographic Collection Site _____ Lat. _____ Long. _____

Weather Condition: Temp. _____ Precip. _____ Wind _____

Time of Death: day mo yr ☐☐☐ hr ☐☐ Collection Method: Natural ☐ Gun Shot ☐ Euthanized ☐ Other _____

Was animal moved from collection site before necropsy? Yes ☐ No ☐

Method of Transportation (vehicle type and storage temp): _____

Necropsy Location: _____

Sample Type: Liver ☐ Kidney ☐ Blubber ☐ Other _____

Time of Collection: day mo yr ☐☐☐ hr ☐☐ Collected by: _____

Storage of tissue prior to processing: Bulk ☐ Bag ☐ Teflon Jar ☐ Other _____

Type of Storage Unit and Temperature:
Freezer ☐ Temp. _____
Refrigerator/Frz ☐ Temp. _____
Dry Ice ☐
Other ☐ Temp. _____

	day mo yr	hr	
Time of Processing:	☐☐☐	☐☐	Processor _____
Time of LN2 Freezing:	☐☐☐	☐☐	
Time Shipped From Site:	☐☐☐	☐☐	Shipper _____
Time Received at Bank:	☐☐☐	☐☐	Receiver _____

Protocol: Standard ☐ Modified ☐ (Please note modifications below)

Remarks:

CETACEAN SAMPLING FORM

Animal Field ID Number _____

Condition: Alive ☐ Freshly Dead ☐ Other _____

Sex: Female ☐ Male ☐ Total Length ☐ Total Weight ☐

Baleen/Tooth Counts (erupted or total): UL/LL ☐ UR/LR ☐

Age: Estimated _____ Method Used: Tooth ☐ Length ☐ Other _____

If tooth, who/where analyzed? _____

Specify Units of Measurement _____

Snout to melon	☐	Snout to caudal end of ventral groove	☐
Snout to angle of mouth	☐	Snout to center of genital aperture	☐
Snout to blowhole	☐	Snout to center of anus	☐
Snout to center of eye	☐	Flipper length	☐
Snout to ant. ins. of dorsal fin	☐	Flipper width	☐
Snout to tip of dorsal fin	☐	Fluke width	☐
Snout to fluke notch	☐	Dorsal fin height	☐
Snout to ant. ins. of flipper	☐		

Girth: Axillary ☐ Max (location) ☐ Anal ☐

Blubber thickness: Dorsal ☐ Lateral ☐ Ventral ☐

Parasites: Were they collected? Yes ☐ No ☐ (If yes, list type of parasites and where they occurred)

_____

_____

Stomach: Was stomach sampled? Yes ☐ No ☐

If sent - where? _____

Contents: _____

Reproductive condition: Pregnant ☐ Lactating ☐ Fetus length _____

Gonad weight: Left ☐ Right ☐

Animal Field ID Number _____

General Comments:

General Appearance of Individual:

General Appearance of Organs:

Histological Samples:

Individual/Organization: _____

Final Destination: _____

Tissues Sampled:

Liver	☐	Stomach	☐	Lympn Nodes	☐
Kidney	☐	Heart	☐	Type: _____	
Blubber	☐	Intestine	☐		
Lung	☐	Pancreas	☐	Other	☐
Adrenals	☐	Brain Stem	☐	Type: _____	
Muscle	☐	Skin	☐		

Sample Weights: (g)	Liver	Kidney	Blubber	Other
A				
B				

Form Prepared by: _____
Name (print)

_____
Signature

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

National Institute of Standards and Technology
Charleston Laboratory
219 Fort Johsnon Road
Charleston, SC 29412

Attn: Rebecca Papa
(843) 762-8647

NATIONAL BIOMONITORING SPECIMEN BANK

Animal Field ID Number _____ Species _____

Stranding ☐ Incidental ☐ Subsistence ☐

Geographic Collection Site _____ Lat. _____ Long. _____

Weather Condition: Temp. _____ Precip. _____ Wind _____

Time of Death: day mo yr ☐☐☐ hr ☐☐ Collection Method: Natural ☐ Gun Shot ☐ Euthanized ☐ Other _____

Was animal moved from collection site before necropsy? Yes ☐ No ☐

Method of Transportation (vehicle type and storage temp): _____

Necropsy Location: _____

Sample Type: Liver ☐ Kidney ☐ Blubber ☐ Other _____

Time of Collection: day mo yr ☐☐☐ hr ☐☐ Collected by: _____

Storage of tissue prior to processing: Bulk ☐ Bag ☐ Teflon Jar ☐ Other _____

Type of Storage Unit and Temperature:
Freezer ☐ Temp. _____
Refrigerator/Frz ☐ Temp. _____
Dry Ice ☐
Other ☐ Temp. _____

	day mo yr	hr	
Time of Processing:	☐☐☐	☐☐	Processor _____
Time of LN2 Freezing:	☐☐☐	☐☐	
Time Shipped From Site:	☐☐☐	☐☐	Shipper _____
Time Received at Bank:	☐☐☐	☐☐	Receiver _____

Protocol: Standard ☐ Modified ☐ (Please note modifications below)

Remarks:

PINNIPED SAMPLING FORM

Animal Field ID Number _____

Condition: Alive ☐ Freshly Dead ☐ Other _____

Sex: Female ☐ Male ☐ Total Length (cm) ☐ Total Weight (kg) ☐

Tooth Counts (erupted or total): UL/LL ☐ UR/LR ☐

Age: Estimated _____ Method Used: Tooth ☐ Length ☐ Other _____

If tooth, who/where analyzed? _____

Specify Units of Measurement _____

Tip of snout to tip of tail ☐ Ant. length of hind flipper ☐

Ant. length of foreflipper ☐ Blubber thickness over posterior end of sternum ☐

Axillary girth ☐

Blubber thickness: Dorsal ☐ Lateral ☐ Ventral ☐

Parasites: Were they collected? Yes ☐ No ☐ (If yes, list type of parasites and where they occurred)

_____

_____

Stomach: Was stomach sampled? Yes ☐ No ☐

If sent - where? _____

Contents: _____

Reproductive condition: Pregnant ☐ Lactating ☐ Fetus length _____

Gonad weight: Left ☐ Right ☐

Animal Field ID Number _____

General Comments:

General Appearance of Individual:

General Appearance of Organs:

Histological Samples:

Individual/Organization: _____

Final Destination: _____

Tissues Sampled:

Liver	☐	Stomach	☐	Lympn Nodes	☐
Kidney	☐	Heart	☐	Type:	_____
Blubber	☐	Intestine	☐		
Lung	☐	Pancreas	☐	Other	☐
Adrenals	☐	Brain Stem	☐	Type:	_____
Muscle	☐	Skin	☐		

Sample Weights: (g)	Liver	Kidney	Blubber	Other
A				
B				

Form Prepared by: _____
Name (print)

_____
Signature

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

National Institute of Standards and Technology
Charleston Laboratory
219 Fort Johsnon Road
Charleston, SC 29412

Attn: Rebecca Papa
(843) 762-8647

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	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 for the designated time period. 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)
HPLC Grade Water Rinse	
Hydrochloric Acid (HCl) 2:1	4 h
HPLC Grade Water Rinse	
Nitric Acid (HNO_3) 2:1	4 h

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

HPLC Grade Water Rinse
Ethyl Alcohol Rinse (can be used approximately 4 times before disposal)
HPLC Grade Water Rinse-First Rinse (use water from previous final rinse)
HPLC Grade Water Rinse-Final Rinse (new bottle)

Above soaks use one bottle of liquid.

TITANIUM

Chemical/Water	Time in Liquid
Ethyl Alcohol (ETOH)	1h to 2 h
HPLC Grade Water Rinse	Overnight
Hydrochloric Acid (HCl) 10:1	½ h
Nitric Acid (HNO_3) 10:1	½ h
HPLC Grade Water Rinse	

APPENDIX C

NOS/CCEHBR LABORATORY SAFETY MANUALS AND FORMS

Chemical Hygiene Plan
Emergency Preparedness Plan
Job Hazard Analysis Form
Chemical Inventory for Room 227

CHEMICAL HYGIENE PLAN

FOR
CHARLESTON LABORATORY

CENTER FOR COASTAL ENVIRONMENTAL HEALTH
AND BIOMOLECULAR RESEARCH
AT CHARLESTON

NATIONAL OCEAN SERVICE

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

DEPARTMENT OF COMMERCE

JUNE 1998

CONTENTS

CHEMICAL HYGIENE PLAN

The following written chemical hygiene plan has been established for:

CHARLESTON LABORATORY
CENTER FOR COASTAL ENVIRONMENTAL HEALTH
AND BIOMOLECULAR RESEARCH

NATIONAL OCEAN SERVICE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
DEPARTMENT OF COMMERCE

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:

Malcolm B. Hale, Area Safety Representative (ASR)

All NOAA components at this site are covered by the program. As such, NOAA employees, their designated representatives, and, upon request, the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health shall have access to this written plan at the following location:

On the SAFETY/MSDS Shelf in the bookcase in the central hallway outside Room 235 (vending machine room).

CHEMICAL HYGIENE PLAN

FOR

CHARLESTON LABORATORY
CENTER FOR COASTAL ENVIRONMENTAL HEALTH
AND BIOMOLECULAR RESEARCH

NATIONAL OCEAN SERVICE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
DEPARTMENT OF COMMERCE

REGULATORY REQUIREMENT/INTRODUCTION

The Department of Labor, Occupational Safety and Health Administration (OSHA) Standard 29 CFR 1910.1450, Occupational Exposures to Hazardous Chemicals in Laboratories, became effective on May 1, 1990. The Federal Register announcement states that "employers shall have completed an appropriate Chemical Hygiene Plan and commenced carrying out its provisions by January 31, 1991". The new standard recognizes that laboratories typically differ from industrial operations in their use and handling of hazardous chemicals and that a different approach than that found in OSHA's substance specific health standards is warranted to protect workers.

The standard applies to laboratories that use hazardous chemicals in accordance with the definition of laboratory use and laboratory scale as provided in the standard. Laboratories are obligated to maintain employee exposures to hazardous chemicals at or below the permissible exposure limits (PELs) specified in 29 CFR 1910, subpart Z. The manner in which this obligation is achieved will be determined by each employer through the formulation and implementation of a Chemical Hygiene Plan (CHP). The CHP must include the necessary work practices, procedures and policies to ensure that employees are protected from all potentially hazardous chemicals in use in their work area.

The OSHA Standard defines a Chemical Hygiene Plan as a written program developed and implemented by the employer which sets forth procedures, equipment, personal protective equipment and work practices that (i) are capable of protecting employees from the health hazards presented by hazardous chemicals used in that particular work place and (ii) meets the requirements of paragraph (e) of this section (of the standard). Paragraph (e) states that the CHP shall be available to all employees and shall include the following elements: (i) Standard operating procedures for the use of hazardous chemicals, (ii) Control measures to be implemented to reduce employee exposure to hazardous chemicals, (iii) Specific measures to be taken to assure that fume hoods and other protective equipment are functioning properly, (iv) Provisions for employee information and training, (v) The circumstances under which a laboratory activity shall require prior approval, (vi) Provisions for medical consultation and medical examinations, (vii) Designation of personnel responsible for implementation of the Chemical Hygiene Plan, (viii) Provisions for additional employee protection for work with particularly hazardous chemicals. Eleven components of a CHP as recommended by the National

Research Council are included in this plan and are listed in the Table of Contents (page i).

This Chemical Hygiene Plan as well as more specific references relating to chemical safety, and safety in general at this facility, are available on the safety shelf of the bookcase outside Room 235 in the central hallway. Included are:

(1) Charleston Laboratory Emergency Preparedness Plan - Instructions for evacuation of the building for fire alarms or other emergencies and preparation/response for hurricanes and other natural disasters.

(2) A copy of the Hazard Communication Program for the Charleston Laboratory. It includes an inventory list of hazardous chemicals used in this laboratory.

(3) Radiation Safety Manual - Rules and guidelines for those who work with radioactive materials.

(4) Working procedures for the control and containment of biotoxins.

(5) Additional, selected publications on chemical safety and first aid.

BASIC RULES AND PROCEDURES

1. Minimize all chemical exposures. Do not taste laboratory chemicals and avoid any contact with your skin. Smell chemicals only briefly and sparingly if necessary to identify them.

2. Don't underestimate risks. Use general precautions for handling all lab chemicals and use special precautions for chemicals that present special hazards.

3. Provide adequate ventilation. The best way to prevent exposure to airborne chemicals is to use hoods and other ventilation devices.

4. Use the hood for operations which might result in the release of toxic chemical vapors or dust.

5. Use only those chemicals for which the quality of the available ventilation system is appropriate. If hood operation is questionable, arrange for use of a more efficient hood for more hazardous chemicals.

6. Vent apparatus which may discharge toxic chemicals (vacuum pumps, distillation columns, etc.) into local exhaust devices (hoods or direct discharge from building).

7. Do not allow release of toxic substances in cold rooms, clean rooms, incubators, or any rooms which have closed, recirculated atmospheres.

8. Smoking is not allowed in the laboratory building. Do not eat, drink, or apply cosmetics in rooms where lab chemicals are present. Wash hands before conducting these activities.

9. Do not store food or beverages in refrigerators or other areas where chemicals are stored. Glassware or utensils which are used for laboratory operations should not be used to store or consume food.

10. Do not use mouth suction for pipeting or for starting a suction.

11. Do not engage in horseplay or other behavior which might startle or distract another worker or cause an accident.

12. Keep long hair and loose clothing confined. Wear shoes at all times, but sandals or perforated shoes should not be worn where chemicals are being used or mechanical work is being done.

13. Wear safety glasses or goggles when hazardous chemicals are being handled in the laboratory.

14. Wear appropriate gloves when the potential for contact with toxic materials exists.

15. Avoid the use of contact lenses in the laboratory. If they are necessary inform the supervisor and take precautions.

16. Wear lab coat when working with chemicals. Remove lab coat if it becomes significantly contaminated and decontaminate it if it would be hazardous to laundry personnel.

17. Wash areas of exposed skin before leaving the laboratory.

18. Avoid working alone in the building. Do not work alone in the laboratory if procedures could be hazardous.

19. Seek information about hazards, and plan appropriate equipment layout and protective procedures prior to performing work.

20. Be alert to unsafe conditions and see that they are corrected. Warn others of unsafe practices when they are detected. Those working regularly in an area are most aware of possibly unsafe conditions. Formal reports of unsafe conditions may be made on Form CD-351, "Report of Possible Safety/Health Hazard".

21. Pets and unsupervised children are not allowed in the Laboratory.

CHEMICAL PROCUREMENT, DISTRIBUTION AND STORAGE

1. Before a chemical is ordered, investigate the potential for special hazards. Request Material Safety Data Sheet (MSDS) from the supply source. If special hazards exist, less hazardous substitutes should be considered.

2. New solvents will not be ordered when an excess amount that is suitable for use is already in storage.

3. Before a substance is received, information on proper handling, storage and disposal should be determined by those involved.

4. No container may be accepted without an adequate label. Labels must identify the contents and hazard class, and should contain first aid information.

5. All incoming orders will be received and noted by Debbie Braddock (Karen Bauersfeld, backup), prior to distribution.

6. Material Safety Data Sheets (MSDS) are normally mailed by the supplier to the Area Safety Representative (ASR, Malcolm Hale) for each new shipment of chemicals received. The ASR will check to see if the sheets are needed for our central MSDS file or if special precautions are needed. Copies of the MSDS will then be passed on to the person who ordered the chemical. If you are missing a MSDS, first check our central MSDS file outside Room 235, then the Internet or request one from the supplier of the chemical.

7. Chemicals which generate acute hazardous waste (e.g. carbon disulfide) or unstable chemicals (e.g. diethyl ether) will be identified and monitored from purchase to disposal. The ASR will keep a log on future purchases of such chemicals. Wastes will be stored in appropriate, labeled containers and not mixed with incompatible wastes. Smaller containers shall be purchased, unless there is a short-term need for larger volumes.

8. Chemicals such as diethyl ether or dioxane, which may form dangerous peroxides, will be protected from light and heat and kept for no more than 2 years. Small quantities of ether that are being used in the lab will be stored in an explosion-proof refrigerator.

9. Diethyl ether bottles will be dated when opened and disposed

of within 6 months of opening. The waste or outdated ether will be added to solvent wastes in a steel drum for approved commercial disposal.

10. All samples of marine biological toxins shall be stored in the padlocked, explosion-proof refrigerator in Room 216.

11. Environmental samples which appear edible will be marked as unsafe for human consumption during refrigerated storage.

ENVIRONMENTAL MONITORING

1. Regular instrumental monitoring of airborne concentrations is not usually justified or practical in laboratories but may be appropriate when testing or redesigning hoods or other ventilation devices, or when a highly toxic volatile substance is stored or used regularly (e.g. 3 times/week).

2. Chemical monitoring badges are purchased periodically and made available to the staff. If there are complaints of chemical fumes, badges are worn and/or placed in the area to determine time-weighted concentrations of chemicals. Other potential problem areas are periodically monitored with the badges and the analytical results are filed and maintained.

3. Monitoring procedures and requirements for employees working with radioactive materials are described in the Laboratory Radiation Safety Manual, available on the library safety shelf, or see John Bemiss, the Radiation Safety Officer.

HOUSEKEEPING, MAINTENANCE AND INSPECTIONS

1. Laboratory inspections for safety, housekeeping and chemical controls will be performed at least once each six months. Informal inspections of specific areas may be carried out at any time.

2. Areas should be kept orderly and floors cleaned regularly.

3. Eyewash fountains will be inspected every three months. They should be operated at least weekly by the occupant of the laboratory to prevent rust buildup in the water.

4. Safety showers will be inspected and tested every six months.

5. Access to exits, emergency equipment and utility controls shall not be blocked.

6. Hoods will be monitored, and modified if inadequate. They will be tested and certified at least annually. If you suspect that a hood is malfunctioning, notify your supervisor or the ASR.

7. The Area Safety Representative is primarily responsible for scheduled inspections. Informal inspections and safety surveillance are primarily the responsibility of supervisors.

8. The results of scheduled inspections will be given to the appropriate supervisors along with suggestions for correcting any and all violations.

MEDICAL PROGRAM

1. For medical emergencies dial 911 and take appropriate first aid measures.

2. For less critical injuries that require medical attention the employee will be transported to a medical facility. Form CA-16, authorization for medical treatment, should be obtained from the Administrative Officer (Karen Bauersfeld) and signed by the supervisor prior to leaving if possible.

3. When an employee develops symptoms associated with a hazardous chemical exposure, the employee shall have the right to an appropriate medical examination free of charge.

4. In the event of an accident in the laboratory resulting in the likelihood of hazardous exposure, an affected employee has the right to a medical consultation to determine the need for a medical examination. The employee must first notify his/her supervisor and complete a Form CA-16.

5. All medical examinations and consultations shall be performed by a licensed physician without cost to the employee, without loss of pay and at a reasonable time and place. The employee may choose his/her personal physician, but the form (CA-16) authorizing medical procedures must be completed first.

6. Any medical conditions resulting from hazardous chemical exposure which requires treatment will be documented with details of the exposure and detailed statements from the attending physician.

7. Records pertaining to chemical exposures of employees, the resulting medical evaluations, as well as results of periodic monitoring of areas or employees for concentrations of ambient chemicals will be maintained by the Area Safety Representative.

PERSONAL PROTECTIVE APPAREL AND EQUIPMENT

1. Protective apparel (gloves, safety goggles, aprons, etc.) shall be provided from operating funds for the degree of protection required for substances being handled.

2. An easily accessible drench-type safety shower shall be available in areas of higher risk.

3. An eyewash fountain shall be provided in high risk areas.

4. Fire extinguishers shall be readily accessible to all work areas.

5. Respiratory protection, a fire alarm and a telephone for emergency use shall be available nearby to all work areas.

RECORDS

1. Accident records, including recommendations to prevent a recurrence, will be retained in the Administrative Office (Nancy Davey) and by the Area Safety Representative. 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.

hours.

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

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

4. 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.

5. 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.

6. Medical records will be retained by the Laboratory in accordance with the requirements of state and federal regulations.

SIGNS AND LABELS

1. All containers shall be labeled as to contents and date made 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.

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.

SPILLS AND ACCIDENTS

1. Procedures in case of fire, disaster or incidents that require evacuation are described in the Charleston Laboratory Emergency Preparedness Plan, of which each employee should have a copy. A reference copy is on the Safety/MSDS shelf in the Charleston Laboratory Library.

2. General rules for accidents and spills involving hazardous chemicals:

Eye Contact: Promptly flush eyes with water (15 minutes are recommended) and get medical attention.

Ingestion: Check container label and MSDS for first aid recommendations. Get medical attention as soon as possible.

Inhalation: Remove to fresh air and get medical attention.

Skin Contact: Flush thoroughly with water and immediately remove contaminated clothing.

3. In the event of a spill of hazardous material, take steps to contain and absorb it, if personal safety permits. Inform others in the area who might be affected and/or could assist.

4. In the event of a large spill of a hazardous material such that cleanup requirements exceed the in-house capability, sound the fire alarm and have the building evacuated. Fire department personnel having self contained breathing apparatus (SCBA) and protective equipment should be informed of spill conditions upon their arrival.

5. In the event of a serious accident, do not move a worker with a possible serious injury unless the immediate area is not safe. Call 911 for medical assistance, alert others in the laboratory and provide first aid.

6. Use or transfer of hazardous chemicals should take place in a properly operating hood if possible. Have absorbent or neutralizing materials available in case of spills.

7. Contaminated absorbent materials will be placed in a plastic bag or other suitable container and disposed of according to waste regulations.

8. All accidents or near accidents will be investigated and analyzed and the results distributed to all who might benefit. The Administrative Officer (KB) and the Area Safety Representative (MH) are required to maintain records of any accidents that involve injury or property damage.

TRAINING AND INFORMATION

1. Aim: To assure that all individuals at risk are adequately informed about the work in the laboratory, its risks, and what to do if an accident occurs.

2. Supervisors will provide employees with information and training on hazardous chemicals in their work area at the time of their initial assignment, and whenever a new hazard is introduced.

3. Employees will be informed of the location and availability of the written hazard communication program for the laboratory.

4. The Hazard Communication Program, including the required inventory list of hazardous chemicals, will be available on the shelf just outside Room 235 in the section with the Material Safety Data Sheets.

5. Employees shall receive initial Hazard Communication training and annual refresher training in accordance with the requirements of the Hazard Communication Program (HCP). Initial training shall include at least:

(i) Method and observations that may be used to detect the

presence or release of a hazardous chemical in the work area.

(ii) The physical and health hazards of the chemicals used in the work area.

(iii) The measures employees can take to protect themselves from these hazards, including work practices, emergency procedures and personal protective equipment.

(iv) Details of the Hazard Communication Program, including an explanation of labeling systems and material safety data sheets.

6. The objectives of the HCP training are to enable employees to recognize and respond to chemical hazards, become familiar with material safety data sheets and personal protective equipment, and safely handle, use and store chemicals.

WASTE DISPOSAL

1. Aim: To assure that minimal harm to people, other organisms, and the environment will result from the disposal of laboratory chemicals.

2. Room 266 is designated for organic solvent storage, and not for acids, alkalies or waste chemicals. Moderate amounts (1 to 4 gallons) of waste organic solvents may be stored temporarily (on floor to the right inside door) if they are properly labeled as to contents and if the ASR is notified.

3. Waste solvents will be transferred from temporary storage or directly from labs to storage drums in HazMat Storage Building #1 behind the Laboratory building until pickup by a commercial waste disposal company can be arranged. Transfers will be made by the ASR or by Laboratory personnel in consultation with the ASR.

4. The ASR, with assistance from project personnel if needed, will assure that wastes will only be added to drums containing other wastes that are chemically compatible. The volumes and compositions of wastes added will be recorded as the drums are filled. Each staff member is responsible for keeping records of contents of temporary waste containers used in each laboratory.

5. Wastes will be removed from the premises only by EPA certified waste disposal companies.

6. Most waste acids or bases can be disposed of by neutralization in a sink and flushing down the drain with excess water. Special care and sink within a fume hood should be used.

7. Radioactive waste materials will be handled in accordance with the Laboratory Radiation Safety Manual and will be stored in the restricted area in Room 410 until they can be disposed of.

8. All supplies used in toxin bioassays and extracted cell debris are collected in approved biohazard disposal bags. All animal cages are to be treated with a strong sodium hypochlorite solution and steam cleaned following toxin bioassays. When toxic

wastes are neutralized by autoclaving or bleach treatment, they will be placed inside a plain plastic bag without the biohazard warning before they are placed in the dumpster for disposal.

9. Environmental samples that appear edible will be made unpalatable and marked "unsafe for human consumption" prior to disposal.

EMERGENCY PREPAREDNESS PLAN

CENTER FOR COASTAL ENVIRONMENTAL HEALTH
AND
BIOMOLECULAR RESEARCH AT CHARLESTON

U.S. DEPARTMENT of COMMERCE
NOAA, NATIONAL OCEAN SERVICE
NATIONAL CENTERS FOR COASTAL OCEAN SCIENCE

JULY 1999

TABLE OF CONTENTS

GENERAL EVACUATION INFORMATION

Persons Authorized to Order Evacuation

Center Director - Sylvia Galloway (or Acting Director)

Office of Administration - Karen Bauersfeld / Nancy Davey / Martin Burnett

Building Maintenance Supervisor - Robbie Meyer

Notification for Emergency Evacuation

Fire - Fire Horns and Strobe Lights

Explosion or Gas Leak - Fire Horns and/or Public Address

Suspicious Object - Public Address Announcement

Bomb Threat - Public Address Announcement

Major Chemical Spill - Public Address 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 flagpole. 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.

Building Re-entry

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

EMERGENCY PERSONNEL

Designated Official

Sylvia Galloway, Center Director

Occupant Emergency Coordinator

Nancy Davey, Martin Burnett

Team Coordinator

Safety Officer

Area Monitors	Area
Debbie Braddock / Bill Dzienis	Administration (Rm. 100-119)
Paul Bauersfeld / Pete Key	Open Office Area (Rm. 200-214)
Tod Leighfield / Bennie Haynes	Biotoxins (Rm. 215-224)
Rebecca Pugh / Paul Becker	NIST / Back Hall (Rm 225-229)
Laura Webster / Tom Brown	Back Hallway (Rm. 230-247)
John Bemiss / Kathy Moore	Biotech / ADP (Rm. 248-263)
Wayne McFee / A.K. Leight	Bldg. 400 Areas (Rm. 401-426)
Mike Fulton / Paul Pennington	Bldg. 500, Greenhouse & Trailer

Disabled Staff Monitor

Karen Bauersfeld

Damage Control Team

Robbie Meyer, Charles Burnsed, Martin Burnett, Safety Officer

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.**

   - 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 from the building. Leave the building immediately

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 rear of Room 236 and Rooms 243 and 246 have emergency fire exit windows that could be pushed out with a heavy object.

     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 parking lot in front of the building by the flagpole and join the other employees. All driveways and roadways must be kept clear for emergency vehicles and fire equipment.

   * **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 to leave the building safely in the event of a fire alarm.

4. **Return to the building** only after an official "all clear" is given.

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 absorbant from your lab or from one of the spill cleanup kits in the hallways. Seal the contaminated absorbant 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 CCEHBR Charleston Laboratory is received by an employee, it shall be reported immediately to the front desk (8511). The receptionist will notify the Center Director or her designee (Acting Director or Administrative Officer) for appropriate action. If a decision is made to evacuate the building, the Center Director or her representative will make the following announcement over the public address system (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 department. 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 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).

   - 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 / Acting 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.8525) or the Receptionist (ext.8511) 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.8511) 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 three 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 (1) in the back hallway between Rooms 235 and 237, (2) on the back loading dock outside Room 405, and (3) in the Room 406 corridor. 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 407A, B and C.
2. Check Marine Mammals receiving freezer, Room 401.
3. Check walk-in freezers outside Room 420 (Forensics).
4. Check Environmental Rooms (224 A&B, 253, 254).

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 (number is posted on door to Room 234) 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. Tornados 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 shelter area until further notice. Repeat."

- Preferred shelter areas in the main lab building are located in the crossing halls between the back hall and the front hall / bullpen. The main back hallway can also be used, avoiding the more hazardous labs, or use hallways in the administrative and 400 areas. Close all doors, proceed to a shelter area, find a place along a wall and wait for further instructions from Area Monitors.

HURRICANE PREPAREDNESS

Responsibilities

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

- **Center Management Team** - is responsible for assisting in 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.

- **Supervisors** - 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.

- **Laboratory Safety Committee** - is responsible for updating the plan.

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.

- 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. Get records and computers off the floor if possible and protect 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 safe location.

- Check to insure that all gas and water faucets, including the main distilled water faucet in the mezzanine, are shut off. Shut off electrical equipment that need not be running and ensure that all equipment that must remain operable is supplied by emergency outlets.

- All employees will leave the building and the Fort Johnson premises when dismissed by their supervisors and return only after the hurricane threat is 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.

Communication Procedures

If employees have been dismissed, or are off duty as a hurricane approaches, they will be expected to check the 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 CASU) at **1-800-343-0639**. 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 CCEHBR 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. 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 CCEHBR 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:

a) If severe damage to the area occurs, instructions will be given by voice mail.
b) 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 State Maintenance Staff will inspect for structural damage and/or mechanical failure and advise the Facilities Manager and/or Administrative Officer and supervisors as soon as possible.

Information on the Web

The attached information on hurricane preparedness (modified), and much additional information on various types of storms, can be found at: **http://www.kate.net/storms/**

For much useful information on local conditions, forecasts, warnings and facilities for evacuation, try the Charleston Net Home Page at **http://www.charleston.net/** and click on the Storm Center icon.

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 predrill 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 CCEHBR Voicemail Message Center (843-762-8888) or the Emergency Message Center at 1-800-343-0639.

JOB HAZARD ANALYSIS

National Ocean Service CCEHBR at Charleston

ROOM NO 227 PROGRAM(S) NIST

WORK ACTIVITIES: 1. Sample storage in LN_2

2. Sample homogenization 3. Laboratory cleanup

4. Shipper/Dewar LN_2 fill 5.

6.

Personel Protective Equipment (PPE) Requirements

PPE	Applicable Work Activitiy Numbers
Lab Coat	1,2,3,4
Safety Glasses	1,2,3,4
Safety Goggles	
Gloves, Chem Resist	2,3
Gloves, Insulating	1,2,4
Fume Hood*	3
Apron	
Face Shield	
Ear Plugs	
Dust Mask	
Radiation Badge	
Other PPE	
Lab basics	1,2,3
Lab hood	1,2,3
O_2 Monitor*	1

* Not classified as PPE, but a common essential requirement.

These PPE requirements have been identified for this activity by:

Supervisor: [signature] Date 3/15/99

Certified: Malcolm Hale (Area Safety Rep.) Date 3/24/99

I have been informed of the possible hazards in this work area and of the Personal Protective Equipment that is required.

Signed: [signature] (Employee) Date 6/20/99

CHEMICALS USED IN ROOM 227A & B

LIQUID NITROGEN

HYDROCHLORIC ACID

NITRIC ACID

ETHYL ALCOHOL

CHLOROFORM