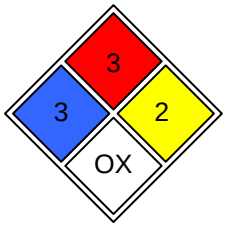


Amherst College

Department of Biology



Basic Regulatory Training

For

The Chemical Hygiene Plan

Biological Safety

Hazard Communication

Emergency Procedures

and

Waste Management


Completed 05/08, Updated, Adapted and Revised 03/14

Table of Contents

Introduction

Welcome! If you are being asked to take this training, you work in a Biology Department laboratory. We hope that your time in the laboratory is very productive and enlightening. We also hope that it is safe. With that in mind, this manual presents the basic safety standards, emergency procedures, waste management protocols and other information that you will need to know before beginning work in the laboratory. Please read it carefully and make sure that you understand everything. You will be asked to take a short, multiple-choice quiz afterward. Please keep this manual as a reference as you may need it in the future. Questions about anything in this manual, or other questions about regulatory requirements for laboratories, can be directed to the Chemical Hygiene Officer (Dr. Kristi Evenson-Ohr, x2736, kohr@amherst.edu, 428 Merrill).

Laboratory Safety

Safe laboratory practices and worker protections are mandated on a federal level and enforced by the Occupational Safety and Health Administration (OSHA). OSHA requires that all companies of 10 or more employees provide training that is commensurate with the duties of each employee's position to ensure their safety. The minimum training and protective equipment that must be provided according to OSHA for laboratory personnel is promulgated through the laboratory standard, hazard communication and right-to-know laws. It is important to stress here that the following is meant to apply to most laboratories. Each research laboratory is different, however, and therefore you should always consult with your P.I. (Principal Investigator, the professor whom you are working with) about specific hazards for equipment, procedures, chemicals and organisms used in individual cases. The Chemical Hygiene Officer can also provide assistance with hazard assessment and training.

Hazard Communication/Right-To-Know

The hazard communication standard (HCS or HAZCOM) and right-to-know laws basically say that all employees have the right to know what hazards they are exposed to, and

employers are obligated to inform their employees of the hazards encountered during the performance of their job functions. The most significant hazards in most of our laboratories are the chemical and biological materials students work with. HAZCOM (29 CFR 1910.1200) requires that all chemical manufacturers follow specific labeling requirements for the products they sell. At a minimum, this must include:

(1) identity of the material (commercial or trade, IUPAC or common name);
(2) associated hazards, including target organ effects;
(3) name, address and phone number of the manufacturer.

Hazard Classification and Definitions

OSHA, and other safety organizations, has defined many hazards for chemicals. These fall into three categories: environmental, physical and health hazards. Environmental hazards must be disposed of properly. Disposal will be discussed in the Waste Management section of this manual. Physical hazards are typically based on physical and/or chemical properties of materials, such as the temperature above which a liquid gives off enough vapor to ignite in the presence of an ignition source (*i.e.*, flashpoint). The specific terms used to describe these hazards are ones that often sound familiar to scientists, but occasionally these terms have a different meaning in a regulatory sense (*eg.*, see "oxidizer" below; this is clearly not the same as an "oxidizing agent"). Physical hazard classifications include:

Flammable (for a liquid; has a flashpoint below 37.8ºC)

(for a solid; likely to cause fire through friction, chemical reaction, or is readily ignited)

(for a gas; forms a mixture with air that can be readily ignited)

Combustible (a liquid with a flashpoint between 37.8-93.3ºC)

Explosive (releases heat, pressure or gas when exposed to elevated temperature, pressure or shock)

Water reactive (reacts with water to release a gas that is either flammable or a health hazard)

Oxidizer (initiates or promotes combustion in other materials)

Pyrophoric (will ignite spontaneously in air at a temperature of 54.4ºC or less)

Unstable (or reactive; vigorously polymerizes, decomposes, or self-reacts under conditions of shock, pressure or temperature)

Physical hazards often create health hazards. However, health hazards are defined based on biological testing and exposure analysis. These include:

Carcinogen (likely to cause cancer)

Lachrymator (an older term no longer used, but you may see it; irritates the eyes)

Irritant (inflames tissue)

Corrosive (destroys tissue)

Sensitizer (likely to cause allergic reactions after repeated exposure)

Allergen (likely to cause allergic reactions)

Toxic (Oral - Kills 50% of rats tested at oral doses of 50-500 mg/kg body weight)

(Contact – Kills 50% of rabbits tested when 200-1000 mg/kg body weight is in continuous skin contact for 24 h.)

(Respiratory – Kills 50% of rabbits breathing the substance for 1 h. at air concentrations between 200-2000 ppm)

Highly toxic (Oral - Kills 50% of rats tested at oral doses of 50 mg or less/kg body weight)

(Contact – Kills 50% of rabbits tested when 200 mg or less/kg body weight is in continuous skin contact for 24 h.)

(Respiratory – Kills 50% of rabbits breathing the substance for 1 h. at air concentrations below 200 ppm)

Target Organ Effects (Likely to specifically damage particular organs, tissues or other structures)

Hepatoxin (Likely to cause liver damage)
Neurotoxin (Likely to cause damage to the nervous system)
Nephrotoxin (Likely to cause kidney damage)
Tetrogen (Likely to negatively affect embryonic or fetal development)
Mutagen (Likely to cause replication errors in DNA)

Hemato-poietic agent (Likely to decrease hemoglobin function and cause oxygen deprivation)

For more thorough definitions of these items, or to find more hazard classifications, please consult the Amherst College Chemical Hygiene Plan (https://www.amherst.edu/offices/enviro_health_safety/) or the OSHA regulations (http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10099).

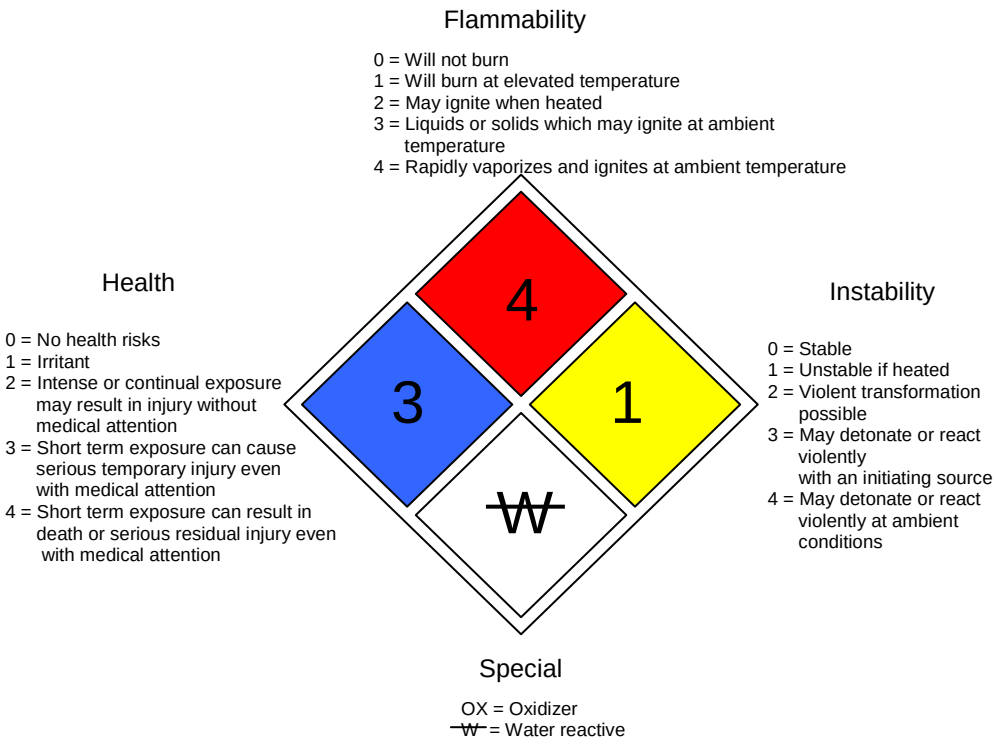
In addition to the hazards associated with chemicals, OSHA also recognizes the term **Biohazard**, a combination of the words "biological" and "hazard". Biohazard refers to organisms, viruses or products of organisms (*eg.*, toxins) that present a health risk to humans. Biohazardous materials will bear the universal biohazard symbol (Figure 1). The background may be either fluorescent orange or orange-red.

Figure 1: The Universal Biohazard Symbol

Common Hazard Classification Systems

You will occasionally come across labels that summarize the physical and health hazards of a material by means of a National Fire Prevention Association (NFPA) diamond, shown in Figure 2.

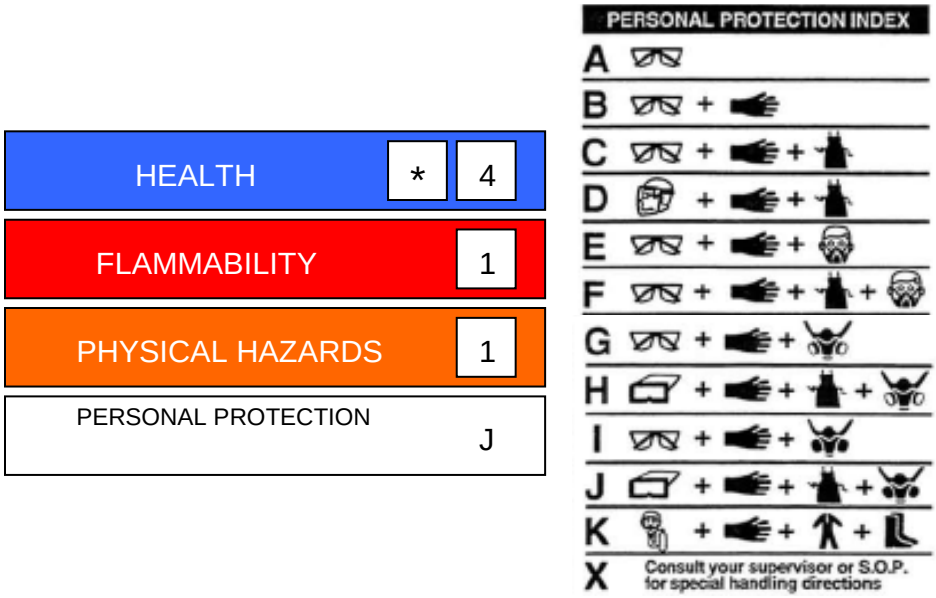
Figure 2: The NFPA Hazard Diamond



The diamond has color coded regions which correspond to specific hazard categories. The white area is used to denote special hazards, like oxidizers (symbol: OX) or water reactive materials (symbol shown in the figure above). Each of the three main areas (health, flammability and instability, or blue, red and yellow, respectively) will contain a number between 0-4. Zero represents only a minimal risk of the specific hazard, while a 4 indicates an extreme risk. Similar to the NFPA diamond is the Hazardous Materials Identification System (HMIS) diamond, though there are some differences between the two. The HMIS system is sometimes used as bars (Figure 3), instead of the diamond format. Additionally, post 2002, the yellow instability section was replaced by an orange physical hazard section. The HMIS system also

includes an asterisk (*) in the health section for materials which pose a chronic (*i.e.*, result from long-term exposure and produce long-term effects) health hazard. The white section of the HMIS is used to convey what kinds of personal protective equipment should be worn while handling the material. HMIS uses letter codes for this, though most manufacturers will actually put cartoons depicting the equipment in this section to prevent confusion. For more information on the diamonds and special symbols, visit the NFPA (http://www.nfpa.org) or HMIS (http://www.paint.org) websites.

Figure 3: A Representative HMIS Bar Label and Code Key for Personal Protective Equipment



The Globally Harmonized System (GHS)

The newest of the wide-spread hazard classification systems (and the one that is the current law of the land) is the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). The purpose of GHS, as the name suggests, is to create a single international system for hazard identification and communication. GHS creates hazard classes and categories (listed below, taken from Appendix A and B of 29 CFR 1910.1200) based on empirical data which are then assigned pictograms, signal words, hazard statements and precautionary

statements to convey hazards and how to minimize them to the users of the materials on the container labels. All of the hazard classes belong to one of three types of main hazard: health, physical or ecological. Only the health and physical hazard classes have been adopted by OSHA, but the chart below includes the ecological hazard classes as well because many manufacturers use these on labels. It is also likely that the EPA will require these classifications in the future, so it's good to be able to recognize them. The categories of each hazard class provide a ranking of the severity of the hazard within a particular class. The categories are usually listed as numbers (with a few exceptions) with the lowest number indicating the most significant risk. Unlike the previous hazard communication law, GHS uses well defined empirical data to classify materials into particular hazard classes and categories. In fact, the definitions of the hazard classes and categories are so precise and lengthy that the exact definitions are prohibitively long to list here. Rather, the chart below gives more qualitative definitions for each hazard class and category. The exact definitions can be obtained from Appendix A for the health hazards and from Appendix B for the physical hazards.

Each hazard class and category require label elements for containers that are prescribed by Appendix C of 29 CFR 1910.1200. The elements of a GHS label include the pictogram, signal word, hazard statements and precautionary statements. The pictograms, shown below, are meant to quickly convey information about hazards in an easy to understand format. In addition to the pictogram, a signal word ("danger" or "warning") is used to indicate the severity of the hazard. Danger indicates the most severe hazards. For example, a label for a flammable liquid in category 1 is required to have the flame pictogram with the signal word "Danger". Hazard statements are assigned to alert users to particular hazards for a given hazard class/category combination, and are also required on the label. For example, for a flammable liquid in category 1, the hazard statement would be "Highly flammable liquid and vapor"; or for an acutely toxic-oral category 1 it would be "Fatal if swallowed". Precautionary statements are also assigned to particular materials based upon that material's hazard class/category classification, and must appear on the label. Precautionary statements alert users to ways to prevent or mitigate hazards associated with improper storage or handling, or respond to emergencies. As such, they can be

Hazard Class	Categories	Qualitative Definitions	Pictograms
Acute Toxicity (Oral)	1	May cause death or immediate ill effects from eating (low dose)	
	2		
	3		
	4	May cause death or immediate ill effects from eating (high dose)	
Acute Toxicity (Dermal)	1	May cause death or immediate ill effects from contact with skin (low dose)	
	2		
	3		
	4	May cause death or immediate ill effects from contact with skin (high dose)	
Acute Toxicity (Inhalation)	1	May cause death or immediate ill effects from inhaling dust, mist, vapors, spray (low dose)	
	2		
	3		
	4	May cause death or immediate ill effects from inhaling dust, mist, vapors, spray (high dose)	
skin corrosion/irritation	1A	Causes irreversible damage to tissue (1A-1C)	
	1B		
	1C		
	2	Causes reversible damage to tissue	
eye damage/irritation	1	Causes lasting tissue damage in the eye	
	2A	Causes tissue damage/changes to the eye that are reversible	
	2B	Causes changes to the eye that are reversible	none

sensitization-respiratory	1A	Leads to hypersensitivity of the airways following inhalation (High frequency)	
	1B	Leads to hypersensitivity of the airways following inhalation (Low to Moderate frequency)	
sensitization-skin	1A	Leads to an allergic response following skin contact (High frequency)	
	1B	Leads to an allergic response following skin contact (Low to Moderate frequency)	
germ cell mutagenicity	1A	Cause mutations in germ cell lines that can be transmitted to progeny	
	1B	Likely to cause mutations in germ cell lines that can be transmitted to progeny	
	2	May cause mutations in germ cell lines that can be transmitted to progeny	
carcinogenicity	1A	Induces cancer or increases its incidence (based on human subject data)	
	1B	Induces cancer or increases its incidence (based on animal subject data)	
	2	suspected of inducing cancer or increasing its incidence	
toxic to reproduction	1A	Causes adverse effects on sexual function and fertility and/or has adverse effects on the development of offspring	
	1B	Presumed to cause adverse effects on sexual function and fertility and/or have adverse effects on the development of offspring	
	2	Suspected of causing adverse effects on sexual function and fertility and/or having adverse effects on the development of offspring	
	lactation	Interfere with lactation and/or are present in breast milk if absorbed	none

Specific target organ toxicity (Single exposure)	1	causes specific, non-lethal, target organ toxicity arising from a single exposure to a chemical at low dose	
	2	causes specific, non-lethal, target organ toxicity arising from a single exposure to a chemical at moderate dose	
	3	Causes narcotic effects or respiratory irritation which is reversible	
Specific target organ toxicity (Repeated exposure)	1	causes specific, non-lethal, target organ toxicity arising from a repeated exposure to a chemical at low dose	
	2	causes specific, non-lethal, target organ toxicity arising from repeated exposure to a chemical at moderate dose	
Aspiration Hazard	1	Results in severe acute effects such as chemical pneumonia, pulmonary injury or death following entry of the substance directly through the oral or nasal cavity, or indirectly through vomiting, into the trachea and lower respiratory system	
Explosives	Unstable explosive	Thermally unstable and too sensitive for normal handling, transport or use	
	1.1	mass explosion hazard-the entire quantity can explode instantaneously	
	1.2	projection hazard, but not mass explosion	
	1.3	fire hazard and minor blast and/or projection hazard	
	1.4	explosion largely confined to package, no appreciable projection hazard	
	1.5	Mass explosion hazard but insensitive, so low probability of explosion	none
	1.6	extremely insensitive, low mass explosion hazard	none
Flammable Gases	1	gases capable of forming extremely flammable mixtures with air under normal conditions	
	2	gases capable of forming flammable mixtures with air under normal conditions	none

Flammable Aerosols	1	aerosols containing extremely flammable components	
	2	aerosols containing flammable components	
Oxidizing Gases	1	gas which can cause or contribute to the combustion of materials more than air does	
Gases under Pressure	Compressed Gas	a gas under pressure that is still a gas	
	Liquefied Gas	a gas under pressure that is partially liquefied	
	Dissolved Gas	a gas under pressure dissolved in a solvent	
	Refrigerated Liquefied gas	a gas under pressure partially liquefied by cooling	
Flammable Liquids	1	vapor above liquid is capable of making a flammable mixture with air at very low temperatures	
	2		
	3		
	4	vapor above liquid is capable of making a flammable mixture with air at higher temperatures (not higher than 93°C)	none
Flammable Solids	1	solids that can be easily ignited by an ignition source or may contribute to fire through friction, fast burning	
	2	solids that can be easily ignited by an ignition source or may contribute to fire through friction, slower burning	
Self-Reactive Substances and Mixtures	Type A	undergoes explosive thermal decomposition with detonation	
	Type B	undergoes explosive thermal decomposition without detonation	
	Type C	possesses self-reactive explosive properties but cannot undergo thermal decomposition or detonation as packaged	
	Type D	partially detonates, or medium response to heat	

Self-Reactive Substances and mixtures cont'd	Type E	no detonation, low heat response	
	Type F	no detonation, low heat response, low explosive power	
	Type G	no detonation, no heat response, no explosive power	none
Pyrophoric liquids	1	Liquids which may ignite in contact with air	
Pyrophoric solids	1	solids which may ignite in contact with air	
Self-Heating Substance and Mixtures	1	readily reacts with air to produce heat, but will not ignite unless in large quantities or after long periods of time	
	2	reacts with air to produce heat, but will not ignite unless in large quantities or after long periods of time	
Substances and Mixtures Which, In Contact with water, emit flammable gases	1	Reacts vigorously with water to produce gases which can ignite	
	2	Reacts readily with water to produce gases which can ignite	
	3	Reacts slowly with water to produce gases which can ignite	
Oxidizing Liquids	1	Liquids which cause the combustion of other materials	
	2	Liquids which greatly contribute to the combustion of other materials	
	3	Liquids which contribute to the combustion of other materials	
Oxidizing Solids	1	Solids which greatly increase the burn rate of a material	
	2	Solids which increase the burn rate of a material	
	3	Solids which somewhat increase the burn rate of a material	
Organic Peroxides	Type A	can detonate rapidly as packaged	
	Type B	does not rapidly detonate, but may undergo a thermal explosion as packaged	

Organic Peroxides Cont'd	Type C	no thermal explosion or rapid detonation, but possesses explosive properties	
	Type D	partially detonates, or medium response to heat	
	Type E	no detonation, low or no effect from heat	
	Type F	no detonation, low heat response, low explosive power	
	Type G	no detonation, no heat response, no explosive power	none
Corrosive to metals	1	Reacts readily with metals	
pyrophoric gas	1	gas which may ignite in contact with air	
simple asphyxiant	1	can displace oxygen, and therefore led to injury or death	none
combustible dust	1	Dusts capable of forming mixtures with air that can be ignited	none
Hazardous to Aquatic Environment, Long-term hazard	1	Very toxic to aquatic life with long lasting effects	
	2	Toxic to aquatic life with long lasting effects	
	3	Harmful to aquatic life with long lasting effects	none
	4	may cause long lasting harmful effects to aquatic life	none
Hazardous to Aquatic Environment, Acute hazard	1	Very toxic to aquatic life	
	2	Toxic to aquatic life	none
	3	Harmful to aquatic life	none
Hazardous to the ozone layer	1	known to deplete the ozone in the upper atmosphere	

allocated to one of four categories: prevention, response, storage or disposal. For example, for an acutely toxic-oral category 1 material, a prevention precautionary statement would be “Wash hands thoroughly after handling.”; a response precautionary statement would be “If swallowed: Immediately call a poison center/doctor.”; a storage precautionary statement would be “Store locked up.”; and a disposal precautionary statement would be “Dispose of contents/container in accordance with all applicable regulations.” Not all hazard class/category combinations will have all four types of precautionary statements, and many combinations will have multiple precautionary statements of one particular type. Appendix C of the GHS final rule (29 CFR 1910.1200) is used to assign the pictograms, signal words and hazard and precautionary statements based on the hazard class and category of a particular material. Because all of the label elements are prescriptive, a GHS label on a container of a given material from Sigma-Aldrich should look the same and contain all of the same information as that from Alfa Aesar, or for the same material in lab somewhere in Europe from a European manufacturer. More information on the GHS system can be found at OSHA’s GHS website (http://www.osha.gov/dsg/hazcom/global.html).

Safety Data Sheets (SDS) and Material Safety Data Sheets (MSDS)

In addition to labeling requirements, the HAZCOM and right-to-know laws also require that chemical manufacturers provide customers with a safety data sheets (SDS) or material safety data sheets (MSDS) for hazardous materials purchased through the manufacturer. SDS/MSDSs provide more extensive and specific information than manufacturer labels in most cases. SDSs are the new documents for these sheets required by the GHS rule. Like all things GHS, SDSs must follow a particular format and contain specific information specified in Appendix D of 29 CFR 1910.1200 so that particular information is always located in the same location on the documents. MSDSs are the documents required under the old HAZCOM standard, and do not necessarily follow a particular format or contain the same information. MSDSs are currently being phased out and replaced by SDSs.

The SDS is a 16 section document which must contain the following information in the following order (unless noted as non-mandatory, in which case that section can be excluded):

(1) Product Identification
(2) Hazard Identification
(3) Composition/Information on Ingredients
(4) First Aid Measures
(5) Fire-fighting Measures
(6) Accidental Release Measures
(7) Handling and Storage
(8) Exposure Controls/Personal Protection
(9) Physical and Chemical Properties
(10) Stability and Reactivity
(11) Toxicological Information
(12) Ecological Information (Non-mandatory)
(13) Disposal Considerations (Non-mandatory)
(14) Transport Information (Non-mandatory)
(15) Regulatory Information (Non-mandatory)
(16) Other information, Including date of preparation or last revision

The specific information that should be included for each heading is listed in Appendix D of the GHS rule. Facilities which use hazardous materials are required to keep copies of the SDSs for these materials. All individuals working with any of these materials must have access to these SDSs during normal working hours. Our department maintains SDS/MSDSs for every hazardous material in all of our laboratories. These are kept on the third floor of McGuire, by the main department offices. This area is always accessible so that students, faculty and staff have around-the-clock access to the SDS/MSDSs. SDS/MSDSs may also be obtained electronically in most cases. Most manufacturers allow downloads of these for their products in pdf format from their websites. Sigma-Aldrich has a particularly extensive collection of thorough SDSs for

each of their products, which may be accessed from their website (www.sigma-aldrich.com). If you need assistance with understanding a SDS/MSDS, please contact the Chemical Hygiene Officer. Your P.I. is also a great source of information about the hazards of materials in the lab. Other excellent resources for determining health hazards of materials include tox-net (http://toxnet.nlm.nih.gov/), the NIOSH/WHO website (http://www.cdc.gov/niosh/ipcs/nicstart.html) and the NIOSH/OSHA website (http://www.osha.gov/SLTC/healthguidelines/index.html).

Labeling Requirements

Just as manufacturers must comply with the HAZCOM standards, there are requirements for how materials must be labeled in laboratories. This includes solutions, reagents, samples and commercial products transferred from original containers. **All containers must be labeled** with the name of the material; abbreviations are not acceptable, except for units. For example, a 6 molar HCl solution should be labeled as "6 M Hydrochloric Acid". Even if a material is not hazardous, abbreviations cannot be used. The main hazard of the material must also appear on the bottle. For the hydrochloric acid solution, this would be "Corrosive". Other good labeling practices include dating all containers and placing your name or initials on the bottle. The type of label and ink used should also be compatible with the contents of the container. Frequently small droplets of liquid materials that run down a bottle during transfer will corrode a label or dissolve the ink. This is especially true for most organic solvents which readily cause many inks to smear. Grease pens are typically a good choice for labeling containers with these items. A clear piece of tape can also be placed over the label to protect it. If a label does smear such that it is no longer legible, please replace it. Containers must also be stored such that the labels are visible without moving the container. Therefore, please make sure that all materials returned to a shelf have the label facing forward.

The Laboratory Standard and Biological Safety

In addition to labeling requirements, OSHA has created a whole series of requirements that are meant to ensure safety in the laboratory. These are detailed in the laboratory standard (29 CFR 1910.1450 and appendices, available on OSHA's website www.osha.gov), which directs all laboratory facilities to develop a chemical hygiene plan and appoint a Chemical Hygiene Officer to implement that plan. The chemical hygiene plan includes standard operating procedures (SOPs), guidelines for chemical storage, fume hood requirements, hazard exposure and control plans, and many other items. Amherst College's chemical hygiene plan in its entirety can be accessed through the environmental health and safety website (https://www.amherst.edu/offices/enviro_health_safety/). It is important to remember that the chemical hygiene plan is meant to be generally inclusive of all laboratories on campus. Therefore, not every part of the plan may apply to your laboratory. Also, there may (and should) be additional SOPs for laboratory specific items, such as the operation of equipment or working with particular hazardous materials, which would not be included in the plan. You should ask your P.I. about these. You should always be trained by your P.I. or trained coworkers before performing any hazardous operations.

Biological Safety

Along with the general OSHA requirements, biological laboratories will need to comply with other sets of standards that are established by the Center for Disease Control (CDC), National Institute of Health (NIH), Massachusetts Department of Public Health (DPH) and the Amherst Board of Health. These requirements are based on hazard assessments of individual laboratories. All of the laboratories at Amherst College that utilize rDNA or biological agents/toxins are assigned a biosafety level (BSL) based on a risk assessment of each laboratory. The risk assessment considers both the hazards of the organisms or agents involved (such as transmission route, infective dose, severity of illness, availability of vaccines), and of the procedures used in the laboratory (such as open centrifuging and sonicating, which can lead to aerosol formation). Risk assessments will be discussed in more detail a bit later. There are currently four general biosafety

levels, numbered 1-4, with 1 presenting the lowest risk. Brief descriptions of these (from the Amherst Board of Health) are given below. Additional information can also be obtained from "Biosafety in Microbiological and Biomedical Laboratories" (BMBL) available from the CDC website at http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm, or from "Guidelines for Research Involving Recombinant DNA Molecules" available from the NIH website at http://oba.od.nih.gov/rdna/nih_guidelines_oba.html.

BSL-1: Suitable for work involving well characterized agents not known to consistently cause disease in immunocompetent adult humans, and presents minimal potential hazard to laboratory personnel and the environment.

BSL-2: Builds upon BSL-1. Suitable for work involving agents that pose moderate hazards to personnel and the environment.

BSL-3: Applicable to clinical, diagnostic, teaching, research or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure.

BSL-4: Required for work with dangerous or exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission.

Most of our laboratories working with rDNA or biological agents/toxins fall into the BSL-1 category, with the exception of one (soon to be two) BSL-2. Your P.I. will inform you what your laboratory's level is. Additionally, this information can be found on the safety information card located on the door to your laboratory. Your laboratory must, at a minimum, follow the requirements listed here for your laboratory's biosafety level classification. Be aware that there may be additional requirements if you are also working with hazardous chemicals, or in a laboratory where these materials are in use. (Those hazards will be covered in the General Standard Operating Procedures section of this manual.) Other hazards (radioactive materials, work with animals, etc.) may also require additional precautions. ***In all cases, the most stringent relevant requirements must be followed.***

General Biosafety Practices

All laboratories are required to use standard microbiological practices and techniques to minimize potential hazards. Appropriate training in these techniques, all laboratory specific hazards and proper operation of equipment shall be provided by the P.I. Safety equipment, such as gloves, lab coats, chemical splash goggles, closed containers and biological safety cabinets (BSC), shall be used as appropriate to minimize all potential exposures.

BSL-1 General Laboratory Practices

1. Access to the laboratory shall be limited. Guests shall be accompanied by laboratory personnel. Doors to the laboratory shall be closed and locked when it is unoccupied.

2. All laboratory personnel must wash their hands with soap and water after working with rDNA, rDNA organisms or biological agents/toxins, and prior to exiting the laboratory.

3. No food or drink is permitted in the laboratory at any time, including gum. Application of cosmetics and handling of contact lenses is also prohibited within the laboratory.

4. Mouth pipetting is strictly prohibited.

5. Broken glassware must not be handled by hand. Dust pans and brushes or tongs may be used. Broken glassware must be placed in the white boxes designated for this purpose. Plasticware should be substituted for glassware when possible. Contaminated glassware shall be disinfected prior to disposal (either chemically or thermally), or placed in a sharps waste container if it is safe to do so.

6. The use of sharps (needles, razor blades, scalpels) is discouraged. If sharps must be used, the following must be followed. Sharps must be disposed of in appropriate, puncture resistant receptacles designed for this purpose. Filled sharps containers should be referred to the Biology Safety Coordinator (Ms. Maureen Manning, x8328). Needles must not be bent, broken, sheared, recapped, removed from disposable syringes, or otherwise manipulated by hand prior to disposal. Reusable sharps must be stored with all sharp edges covered in block of Styrofoam, or similar material. Contaminated reusable

sharps must be decontaminated (chemically or thermally, as appropriate) prior to storage.

7. Gloves must be worn if there is a potential for rDNA, rDNA organisms or biological agents which are hazardous (*i.e.*, infectious) to contact the skin. Gloves shall be removed if contaminated or if the integrity of the protective material has been compromised. Contaminated gloves must be placed in the biohazard bin for autoclaving. Do not reuse gloves. Gloves shall not be worn outside of the laboratory. Hands must be washed thoroughly after gloves are removed. Alternatives to latex gloves must be available to individuals with latex allergies.

8. All procedures must be performed such that the generation of aerosols and/or splashes is minimized. Centrifuge tubes containing hazardous (*i.e.*, infectious) materials must be capped prior to centrifuging. Sonication of such solutions must be conducted in a BSC, chemical fume hood, or the solution must be in a loosely covered container during the sonicating procedure. Chemical splash goggles must be worn while performing procedures likely to generate splashes or aerosols. Lab coats must be worn when splashes are likely.

9. All work surfaces must be decontaminated after the completion of work and after any spills of infectious materials. Solutions of 70% ethanol or 10% bleach may be used, as appropriate. Bleach solutions must be prepared fresh daily, and all stock bottles of bleach must be used within six months of opening. All equipment and instruments must be decontaminated prior to service or removal from the laboratory.

10. All cultures, stocks, plates or other items containing rDNA, rDNA organisms or biological agents must be disinfected prior to disposal, or sent offsite for treatment. Liquid phase materials must be brought to a concentration of 10% bleach and allowed to stand for 20 min. (or until the indicator changes color for media) prior to disposal. Bleach must be used within six months of opening. Material which cannot be chemically disinfected must be disposed of in an appropriate biohazard container. See the Waste Disposal section of this manual for details on disposal of medical/biological waste.

11. Each laboratory P.I. shall provide training to laboratory employees regarding their duties, necessary precautions to prevent exposures and exposure evaluation processes. This shall include risk factors that increase susceptibility to infection. At risk individuals are encouraged to self-identify to health services for counseling and guidance. Training is required at least annually or when procedures change such that additional training is justified.

BSL-2 General Laboratory Practices

1. Access to the laboratory shall be restricted. The doors to the laboratory will remain locked when unoccupied. Only authorized personnel (*i.e.*, those individuals working in the lab) may enter the area.

2. All laboratory personnel must wash their hands with soap and water after working with rDNA, rDNA organisms or biological agents/toxins, and prior to exiting the laboratory.

3. No food or drink is permitted in the laboratory at any time, including gum. Application of cosmetics and handling of contact lenses is also prohibited within the laboratory.

4. Mouth pipetting is strictly prohibited.

5. Broken glassware must not be handled by hand. Dust pans and brushes or tongs may be used. Broken glassware must be placed in the white boxes designated for this purpose. Plasticware should be substituted for glassware when possible. Contaminated glassware shall be disinfected prior to disposal (either chemically or thermally), or placed in a sharps waste container if it is safe to do so.

6. The use of sharps (needles, razor blades, scalpels) is discouraged. If sharps must be used, the follow must be followed. Sharps must be disposed of in appropriate, puncture resistant receptacles designed for this purpose. Filled sharps containers should be referred to the Biology Safety Coordinator (Ms. Maureen Manning, x8328). Needles must not be bent, broken, sheared, recapped, removed from disposable syringes, or otherwise manipulated by hand prior to disposal. Reusable sharps must be stored with all sharp edges covered in block of Styrofoam, or similar material. Contaminated reusable sharps must be decontaminated (chemically or thermally, as appropriate) prior to storage.

7. In addition to item 7 under *BSL-1 General Laboratory Practices* above, gloves, chemical splash goggles (and face masks or shields, if necessary) and lab coats must be worn. Contaminated protective equipment (goggles, face shields and lab coats) must be properly disinfected (either thermally or chemically) prior to donning again or laundering. Contaminated face masks should be disposed of in the biohazard bin. Used protective equipment must not be stored in non-laboratory areas, such as offices.

8. In addition to item 8 under *BSL-1 General Laboratory Practices* above, all work with live cultures of rDNA organisms or biological agents must be performed inside of a Class II BSC.

9. All work surfaces must be decontaminated after the completion of work and after any spills. Solutions of 70% ethanol or freshly prepared10% bleach may be used, as appropriate. Bleach solutions must be prepared fresh daily, and all stock bottles of bleach must be used within six months of opening. All equipment and instruments must be decontaminated prior to service or removal from the laboratory.

10. All cultures, stocks, plates or other items containing rDNA, rDNA organisms or biological agents must be disinfected prior to disposal, or sent offsite for treatment. Liquid phase materials must be brought to a concentration of 10% bleach and allowed to stand for 20 min. (or until the indicator changes color for media) prior to disposal. Bleach must be used within six months of opening. Material which cannot be chemically disinfected must be disposed of in an appropriate biohazard container. See the Waste Disposal section of this manual for details on disposal of medical/biological waste.

11. In addition to item 11 under *BSL-1 General Laboratory Practices* above, the P.I. shall ensure that no laboratory personnel conduct work involving rDNA, rDNA organisms, or biological agents/toxins of a BSL-2 categorization until proficiency in standard and special microbiological practices has been demonstrated.

12. A sign incorporating the universal biohazard symbol and name of the organism or toxin in use must be posted on the laboratory door, along with emergency contact information.

General Standard Operating Procedures (SOPs) - Policies

The chemical hygiene plan provides general SOPs for a variety of common situations. Many of these could also be referred to as policies, because some involve things that you must (or must not) do in a laboratory. Some of these policies overlap the biosafety requirements, however, some do not. Therefore, please make sure that you are familiar with the additional requirements for laboratories which also use hazardous chemicals. Some of the general additional policies which apply to most biology laboratories are:

- Chemical splash goggles must be worn by *everyone* in the laboratory whenever *anyone* in the laboratory is working with hazardous materials (i.e., hazardous chemicals or BSL-2 level work). You must also wear chemical splash goggles whenever you are performing an operation likely to generate splashes, regardless of the chemical or biological hazards.

- Open-toed shoes and other clothing which exposes the body must not be worn. Lab coats should be worn when splashes are likely.

- Long hair should be tied back.

- Working alone in the laboratory is prohibited unless specific permission is obtained from your P.I. If you have a need to work alone, and permission, you must notify the campus police (x2291) of where you will be working and for how long. Make sure you have planned an exit route in the event of an emergency. You should also use a "buddy system". Let someone know where you are and the approximate time you will return or contact them. If they do not hear from you by the expected time, the person should contact the campus police at x2291.

- All exposures to and spills of hazardous materials, and near miss or actual incidents must be reported promptly to the Chemical Hygiene Officer (Dr. Kristi Evenson-Ohr, kohr@amherst.edu, x2736).

- Laboratories should be kept as orderly as possible at all times to minimize hazards and regulatory scrutiny during inspections (*i.e.*, messy labs "look" like they might have violations, and so inspectors will frequently examine these closer than tidy labs).

- Unattended reactions or operations involving hazardous materials must be labeled with the identity of the hazardous materials present, the words "Let Run" and the contact information of the responsible person.

- Any substance which is characterized as a carcinogen, acute toxicant or reproductive toxin (*eg.*, mutagen, teratogen) should be used only in a designated area of the lab that is clearly defined and marked as such. For some labs, this might be the entire lab space. All personnel working in the lab space should be aware of the area, the potential hazards and emergency procedures. Designated areas should be decontaminated in so far as possible upon completion of work with highly hazardous materials. Cleanup can be made easier by use of plastic backed bench paper, Pigmat (chemically absorbent padding), or other types of disposal coverings. Refer these items to the Biology Department Safety Officer for disposal once the work is complete.

- Containers of all liquid or solid materials must be covered (*i.e.*, capped, covered with parafilm or a watchglass, have a reflux condenser, etc.) when not in use. Prepared materials or reactions which evolve gases should have loose fitting covers, or some other form of pressure outlet, and be in a fume hood until gas evolution has ceased. Gas trapping techniques should be used for significant quantities of hazardous gases when possible (*eg.*, bubbling HCl through a sodium hydroxide solution). Venting directions for gas evolving materials (*eg.*, fuming nitric acid) purchased through manufacturers must be strictly obeyed.

- Gloves cannot be worn outside of the laboratory.

- Gloves must be worn when working with corrosive or toxic compounds, and should be worn with sensitizers and allergens. Gloves must also be worn for all BSL-2 operations. It is good practice to *never* have skin contact with laboratory chemicals, regardless of the perceived hazard level.

- The type of gloves worn must afford appropriate protection against the material you are working with. Some types of gloves are permeable to certain materials, or afford adequate protection for only a very short period of time. You should consult the MSDS for specific materials, or the Chemical Hygiene Plan for glove selection. Contaminated gloves should be removed as soon as possible. Alternatives to latex gloves must be available to individuals with allergies to latex.

- Chemical fume hoods (or biological safety cabinets when biological materials are in use) must be used for work with all materials which pose a known or suspected inhalation hazard. When using a chemical fume hood, the sash should not be raised above the green dot. Materials within the hood should be placed at least 6 in. back from the opening. The doors to the laboratory should also be shut to ensure proper hood flow. If a fume hood seems to not be working properly, notify the Chemical Hygiene Officer immediately and do not use the hood. Work which is BSL-2 must be conducted in a Class II biological safety cabinet when possible. Biological safety cabinets are also recommended for procedures in which sterility is important and for all operations in which splashes are likely.

- Secondary containers must be used for the transport of hazardous materials between rooms (*eg.*, bottle carriers, or other secondary containers). Carts must also be used for secondary containers which have lids that could open if the container were dropped.

- Never return unused materials to the stock bottle once dispensed. Trace contamination can lead to potentially disastrous reactions.

- Consumer foodstuffs or equipment (*e.g.*, sugar, aspirin, microwaves, refrigerators) used in a laboratory must be labeled “For laboratory use only, not for human consumption” or “Chemical Use Only”, as appropriate.

- Access to eyewashes, safety showers, exits and fuse boxes must not be obstructed.

- Electrical equipment with frayed cords or exposed wires must not be used.

- Open flames must not be left unattended. All combustible (eg., wood, paper, etc.) and flammable (eg., ethanol, isopropanol, etc.) material must be removed from the area of the flame. Do not use larger flames on burners than is necessary for the work being performed.

- Belt pumps must have a guard covering the belt.

- Each laboratory must have an up to date door card indicating the P.I. and after hours contact information, the hazards and the types of emergency equipment found in the laboratory.

- Gas cylinders must be firmly affixed to walls, or other immovable items, during storage and use.

- Gas cylinders which are not in use (*i.e.*, do not have a regulator attached) must have the cap in place.

- Bleach used for decontamination and waste treatment must be used within six months of opening to prevent lose of potency. Bleach solutions must be prepared fresh daily.

The above list is by no means all inclusive. If you have questions or concerns about specific practices or equipment in your laboratory, consult your P.I. or the Chemical Hygiene Officer.

Unfortunately, even with laboratory specific SOPs, it is not possible to have policies or instructions regarding every possible situation you might encounter in a laboratory. Therefore, you need to proactively assess the hazards of all operations (*i.e.*, conduct a risk assessment). This is discussed in more detail a bit later.

Specific Standard Operating Procedures (SOPs) – Emergencies

In addition to the general SOPs discussed above, the chemical hygiene plan includes specific procedures for emergency situations. It is extremely important that you are familiar with these procedures so that when an emergency does arise, you can respond appropriately. A key factor to successful execution of emergency procedures is knowledge of the location of emergency equipment and nearest exit. Therefore, please make sure you know the location of each of the following for the lab(s) that you will be working in.

(1) Eye wash station(s)

(2) Safety Shower(s)

(3) Fire Extinquisher(s)

(4) First Aid Kit

(5) Telephone (Emergency number is x2111)

(6) Spill containment and/or neutralizing agents (bleach, ethanol, dust pan, sodium bicarbonate, dilute acetic acid, spill pillows, vermiculite, sand, cat litter, etc.)

(7) Exits from the room

(8) Fire alarm activation switch

(9) Nearest exit from the building

The following will detail the procedures for the most common (or reasonably anticipated) emergencies for our labs. Your specific laboratory may have procedures for other occurrences, so please check with your P.I.

(1) BSL-2 Spill Procedure

-Alert everyone in the lab to the spill and evacuate the immediate area.

-Wear the appropriate personal protective equipment to clean up the spill. At a minimum this must include gloves, chemical splash goggles, a face mask or shield, and a lab coat.

- If the spill clean-up requires additional respiratory protection, do not attempt to clean up the spill. Instead, evacuate the lab, closing the door behind you, and report the incident to the campus police at x2111.

-Identify the organism or toxin, the need for respiratory protection and any other hazardous conditions present (eg. hazardous chemicals involved in the spill).

-Remove broken glass, if any, with tongs or some other mechanical device. Do not use your hands. If a dust pan and brush must be used, attempt to minimize the spread of the contaminated liquid.

-The collection apparatus and glass should then be decontaminated by soaking in a freshly prepared 10% bleach (or 70% ethanol, if appropriate) disinfectant solution for 20 min., followed by rinsing with water.

-The glass can then be disposed of in the glass waste box.

-Place an absorbing towel or spill pillow, as appropriate to the spill size, on the liquid.

-Carefully disinfect the area with the disinfectant solution. Ensure that all of the liquid is contained by the absorbing material, and let stand for 20 minutes.

- After the elapsed time, transfer the absorbing material to a biohazard bag.

-Apply more disinfectant solution to the spill area and contain with absorbing material.

-After all of the liquid is absorbed, transfer the absorbing material to the biohazard bag.

-After disinfecting, rinse the spill area with water and paper towels, or mop.

-Contaminated gloves and face masks should be placed in the biohazard bag. Other contaminated personal protective equipment can be disinfected as appropriate. Lab coats should be autoclaved prior to laundering. The Biology Safety Coordinator should be contacted for disposal of the biohazard bag. Do not put these items in the medical/biological waste bin.

-Wash hands thoroughly with soap and water before exiting laboratory.

-If the campus police were not contacted to clean up the spill, report the incident as soon as possible to the Environmental Health and Safety Manager (Mr. Rick Mears, x8189).

- Any spill or accident involving rDNA that leads to personal injury (i.e. needle stick), illness or a breach of containment must be reported to the Office of Biotechnology Activities (OBA) at NIH.

(2) BSL-2 Exposure Procedure

- Small spills to the hands or lower arms should be washed thoroughly with soap and water.

-If a person is splashed in the eyes, assist the person to the eyewash station, and flush the eyes for 15 minutes.

-Wear gloves, lab coat and chemical splash goggles while assisting the victim, and ensure that it is safe to help them.

-Contaminated clothing should be removed for spills to the body, and placed in a biohazard bag or bin.

-Wear gloves, lab coat and chemical splash goggles while assisting the victim.

-Wash the affected area thoroughly with soap and water.

-Contact the campus police at x2111 to report the incident and request medical evaluation.

-Report the identity of the organism or toxin and type of exposure (i.e, splash to the skin or eye, inhalation, etc.)

-Reports of all exposures must be made to the P.I. and Institutional Biosafety Committee (IBC).

Spills of non-infectious materials handled at BSL-1 containment levels do not require special clean-up procedures. Exposures to non-infectious materials handled at BSL-1 containment levels should be treated by washing with soap and water for skin contact, or flushing the eyes with water for 15 minutes for eye contact. If irritation develops, seek medical evaluation.

(3) Small Hazardous Chemical Spill (less than 100 mL)

If a small quantity of hazardous chemical material is spilled (less than 100 mL) the following should be done.

-Alert your coworkers to the spill and evacuate the immediate area (and the room) if necessary

-If it is safe to do so, and you are comfortable doing so, neutralize and/or contain the spill as appropriate

-Use baking soda (i.e., sodium bicarbonate) to neutralize acids, and absorbent material to contain the liquid

-Use dilute acetic acid (vinegar) to neutralize bases, and absorbent material to contain the liquid

-Use a spill pillow, vermiculite, sand, cat litter, or other approved absorbent species to contain other liquids

- Dispose of the cleanup material in a solid (blue) labeled hazardous waste container

-If you do not think it is safe to clean up the spill, contact the campus police at x2111 to report the incident (identify the type and quantity of material spilled if possible)

-If possible, provide the MSDS to the first responder

(4) Large Hazardous Chemical Spill (more than 100 mL)

If a large quantity of hazardous chemical material (more than 100 mL) is spilled the following should be done.

-Alert your coworkers in the immediate area and evacuate the laboratory, closing the door behind you

-Contact the campus police at x2111 to report the incident (identify the quantity and type of material spilled if possible)

-If possible, provide the MSDS to the first responder

(5) *Hazardous Chemical Spills Affecting a Person*

If a hazardous material is spilled on someone in the lab, the following should be done.

-Alert your coworkers in the immediate area and evacuate the lab, if necessary

-Have someone call x2111 to report the incident to the police and request medical assistance

-If possible, provide the MSDS to the first responder

-If it is safe to help the victim, do so

-Always ensure your own safety before helping others

-Wear appropriate personnel protective equipment (i.e., gloves, goggles, lab coat)

-If the spill affected the victim's eyes

-Help the victim position his/her head over the eyewash fountain

-Depress the lever on the fountain and flush the eyes for 15 min.

-If the victim is unable to keep or hold his/her eyes open, assist them

-If the spill is to another part of the body

-Remove all affected clothing from the victim

-Put the victim under the safety shower and pull the handle, or use a sink if the spill is to an easily accessible area of the arm or hand

-Flush the affected area for 15 min.

-Remain with the victim until emergency response personnel arrive

(6) *Fires*

In the event of a fire in the laboratory, the following should be done.

-Alert your coworkers and evacuate the lab, closing the door on the way out

-Alert anyone you see in the hallway

-Pull the nearest fire alarm

-Evacuate the building by the nearest exit and assemble with your coworkers in a pre-defined location at least 50 ft. away from the building

-You should ask your P.I. where the meeting place is for your lab immediately after reading this manual

-Do not use the elevators

-Do not move from the meeting location until a head count has been taken

-The building may be re-entered when the fire alarm has been silenced and the fire marshall has declared it safe to do so

-Fire extinguishers may be used only if the following circumstances apply
-You have been trained to use a fire extinguisher

-The fire alarm has been activated

-It is safe for you to extinguish the fire

Conducting a Risk Assessment

The first thing you should do before starting any new procedure for which there is not a specific SOP is conduct a risk assessment. Risk assessments seek to identify all hazards associated with a planned procedure, and remove or mitigate the risks created by those hazards, or otherwise change the procedure, to simultaneously accomplish the goals of the experiment, and ensure the health and safety of the researchers performing the experiment. Conducting a risk assessment can be daunting, even for individuals with years of laboratory experience, because there can be many types of hazards, not all of which are immediately obvious. There are also many different ways to conduct a risk assessment, and certain approaches are better for some situations than others. Therefore, a prescriptive, “check list” type of approach is not necessarily desirable. The key feature of any good risk assessment is that it uncovers as many sources of potential hazards as possible, thereby effectively eliminating unrecognized hazards. Good risk assessments should also prioritize hazards and risks based on severity and probability of occurrence, and should most definitely eliminate all risks and hazards that are simultaneously high severity and high probability of occurrence. Contingency plans, or what one should do if something does go wrong, should also be in place, and communicated to all relevant parties. A more succinct way of stating this is that a risk assessment should follow the principles of RAMP:

1. *Recognize* all hazards.
2. *Assess* the risks of those hazards.
3. *Minimize* risk by mitigating hazards.
4. *Prepare* for emergencies.

While prescriptive approaches are not desirable on a large scale, RAMP provides a good starting point for all risk assessments. Below is a very broad and general, but by no means all inclusive, series of questions that one might use to apply RAMP.

1. What is the goal of the experiment you wish to perform? This is important to keep in mind as you go through the process of assigning hazards and assessing risk. Obviously, any modifications made to a procedure will still have to achieve the goals of the experiment.

2. Identify all equipment, chemicals, biological organisms and other materials associated with the planned procedure. A list might be helpful.

3. Attempt to identify any hazards associated with the use of any of the items on the list, or the circumstances of the procedure. This is the really difficult part where things can be overlooked. It is a good idea to have multiple people review this area, particularly your PI. Their laboratory experience will enable them to recognize potential hazards that less experienced researchers might not be aware of. What apparatus is to be used, and what are the hazards? For example, glassware containing a vacuum or higher than atmospheric pressure could implode or explode, respectively. Electronic instrumentation might present an electrical hazard if it is dismantled while plugged in or without discharging capacitors, or if used in wet areas. What chemicals are to be used? Look at the MSDSs for these materials, and some of the other sources of information listed previously for chemicals. What are the health hazards of the material (*eg.*, toxic, carcinogen, corrosive, etc.) and what are its routes of entry (*eg.*, inhalation, skin absorption, etc.)? What are the exposure limits to the materials? What are the symptoms of exposure to the material (*eg.*, noticeable odor, headaches or nausea)? Are any of the

chemicals highly reactive (*eg.*, pyrophorics, shock sensitive materials, oxidizers, water-reactives, strongly incompatible with other materials, etc.)? Do the materials degrade in storage to form something more hazardous (*eg.*, peroxide forming materials, etc.)? What biological organisms are to be used? Are any of the organisms considered to be infectious or transgenic? What are the potential routes of exposure? Are radioactive materials involved? Are sharps used? Is there a potential for exposure to harmful levels of electromagnetic radiation (*eg.*, lasers, flash lamps, etc.)? Are there temperature extremes involved (*eg.*, cryogenics or heat)? Are there synergistic hazards, *i.e.*, hazards resulting from the presence and interaction of two or more items? For example, many hazardous items dissolved in DMSO can readily penetrate the skin, usually much more so than the hazardous material itself, or if the material was dissolved in a different solvent.

4. What is the level of severity of each hazard, and the probability that it will create a problem within the given procedure? Any items which create a risk that is high severity and high probability of occurrence must be removed or mitigated in the next step. Ideally, we would like all risk to be low severity and low probability of occurrence.

5. Plan to remove or mitigate the hazards using substitutions of less hazardous items and procedures, engineering controls, administrative controls and personal protective equipment. The list should always be applied in the order above with substitutions of less hazardous items or practices being tried first and personal protective equipment being the last line of defense against hazards. It will not always be possible to use all four options, but it is frequently possible to use more than one option to remove or reduce risk. Substitution might include changing reagents (eg., using SYBR safe instead of ethidium bromide.) Engineering controls eliminate or greatly reduce the hazard through use of mechanical equipment or other technologies. An example is the chemical fume hood or biological safety cabinet. Administrative controls reduce individual exposure to hazards by limiting individual contact with the hazard through work practices. Examples include many general and standard operating procedures, like keeping the lab tidy to minimize hazards, not recapping needles prior to disposal and not eating or drinking in the lab. Use

of personal protective equipment (PPE), like goggles, gloves and lab coats, is the last line of defense, and is generally used in conjunction with other methods.

6. Ensure that your modified procedure still meets the goals of your experiment and eliminates all high risk, high probability situations. For example, if you have changed materials or equipment, ensure that the new materials and equipment do not create new, unrecognized hazards. You should also have plans in place for emergencies, such as equipment failure, loss of power or a chemical spill (see the previous section for common emergencies).

Chemical Storage

In addition to SOPs, the chemical hygiene plan includes guidelines for how materials should be stored. Many chemicals are incompatible with one another, and therefore should not be stored together. Other items, like flammable or combustible materials, present fire risks if they are not stored properly. Chemical segregation can be a very complicated process when laboratories have large quantities of materials and limited space. If you find that you do not have enough room to safely store your materials, contact the chemical hygiene office to discuss potential relocation, reallocation or disposal of materials. The following are some general guidelines for storage. Please consult the Chemical Hygiene Officer for specific items.

(1) Minimize the storage of materials on benches and in fume hoods (unless the fume hood is specifically used only for storage).

(2) Flammable materials not in use must be stored in an approved flammable materials cabinet, safety cans, or an explosion proof refrigerator. Non-explosion proof refrigerators have a sign saying “No Flammables Allowed”.

(3) Acids and bases must be separated

(4) Oxidizers must be stored separately from all other materials

(5) Corrosive and oxidizing materials may not be stored above the benchtop level

(6) Liquid materials should have secondary containment (i.e., a lipped shelf or bin) and only compatible materials may be stored in the same secondary container

(7) Water reactive materials must be stored in dessicators, an inert atmosphere, or be otherwise protected from moisture (*eg.*, sodium in mineral oil)

(8) Stock BSL-2 organisms and toxins must be stored in locked units (*i.e.*, freezers, dewars, etc.) which bear the universal biohazard symbol and the name of the organism or toxin

Please note that these guidelines are for general storage. The extent to which the chemicals in your laboratory need to be segregated depends on the quantity and type of materials in your laboratory. The general rule of thumb here is that if two chemicals react with each other, then they should not be stored together. And you should always ask your P.I. or the Chemical Hygiene Officer if you are not sure.

Waste Management

Laboratories generate a variety of wastes, all of which must be handled appropriately. Most waste can be defined as a solid waste, which includes common household waste, most recyclable refuse and chemical waste, among other discarded items. Municipal solid waste is solid waste which is not regulated for disposal and which is not recyclable. Examples from a laboratory would include paper towels, gloves which are not grossly contaminated, used (and empty) plastic pipets and cuvettes. These items can all be placed in the normal trash receptacles. Broken glassware and used Pasteur pipets are also municipal solid waste, however, these items should be placed in the glass waste boxes available in the laboratories to protect the custodians who remove the trash from the hazard. Paper items which may be recycled should be placed in the appropriately labeled beige bins. Empty boxes and other packaging items from received shipments may be placed in the hallway for collection by the custodian. Apart from municipal solid waste, all other laboratory waste is regulated for disposal as it is considered either regulated solid waste, or biological/medical waste. The following details how this waste must be handled. Questions about classifications of specific waste items should be directed to the Chemical Hygiene Officer.

Regulated Solid Waste

Most of the laboratories in the Biology department generate chemical waste, either directly through chemical use, or through the use of products and devices which contain chemicals. It is crucial for the health and safety of the campus community and the protection of the environment that such waste is managed appropriately. The federal and state governments (through the Environmental Protection Agency (EPA) and Department of Environmental Protection (DEP), respectively) have also mandated by law that this waste is handled, stored and disposed of in a specific manner. Very large fines have been imposed on organizations (including colleges and universities) which have failed to meet the requirements of these laws. At a federal level, these laws fall under the Resource Conservation and Recovery Act (RCRA). Under RCRA each state (through a DEP) is charged with enforcement of the specific federal requirements and can enact more stringent regulations as the legislature deems necessary, as is the case in Massachusetts. The following details the major parts of RCRA and the DEP regulations that pertain to our laboratories, as generators of regulated solid waste.

Universal Waste

Our department generates two types of solid waste that are regulated by the EPA and DEP: universal and hazardous waste. Universal waste consists of specific products and devices of no further use which have hazardous and recyclable constituents. Universal waste items must be handled, labeled and packaged in accordance with federal and state regulations, and cannot simply be tossed in a dumpster or garbage can. Universal waste includes, but is not limited to: batteries, fluorescent lights, sodium and mercury vapor lamps, mercury thermometers and manometers, computer monitors and television tubes. Amherst College is permitted to store universal waste for a period of not more than one year following the start of accumulation in an approved area. We currently store our waste fluorescent lights and batteries in 130 Merrill. There is also a collection canister for used batteries on the third floor by the package delivery area. For disposal of other types of universal waste, please contact the Chemical Hygiene Officer for assistance.

Hazardous Waste

The majority of the regulated waste generated in this department is considered to be hazardous. A hazardous waste is material of no further use to which one or more of the following apply:

(1) possesses at least one of the four characteristics of hazardous waste;

(2) is a significant hazard to human health and safety or the environment if it is improperly stored, treated or disposed;

(3) is an unidentified or abandoned material;

(4) is "waste-like", which would include dusty containers of materials on shelves, materials in rusty cans, seeping containers, etc.

The latter two items, 3 and 4, are criteria used during inspections to impose fines; more on these in a bit.

The four characteristics of a hazardous waste are ignitability, corrosivity, reactivity and toxicity. A waste is classified as ignitable if it:

(1) is a liquid and has a flashpoint of less than 60ºC;
(2) is a flammable or pyrophoric solid;

(3) is a flammable gas;

(4) is an oxidizer.

Note well that ignitability is not the same as flammability (see the definition of flammable under the Hazard Communication section). It is a violation of federal and state law to use "flammable" in place of "ignitable" on a hazardous waste container. Similarly, labeling a useful material as "ignitable" instead of "flammable" implies that the material is waste. Be careful not to confuse these terms.

A waste is classified as corrosive if it:

(1) is aqueous and has a pH less than or equal to 2, or greater than or equal to 12.5;

(2) corrodes steel at a rate greater than 6.35 mm per year at 55ºC.

A waste is classified as reactive if it:

(1) is unstable;

(2) reacts violently or forms toxic byproducts when exposed to water or heat;

(3) is an explosive;

(4) contains cyanide (CN^-) or sulfide (S^{-2}).

Again, please note that the definition of reactive for hazardous waste is different than the definition of unstable (or reactive) for hazardous materials.

A waste is classified as toxic if it:

(1) contains any component (or form of the component, such as salts, complex ions, etc.) listed in 310 CMR 30.125 at or above the specified concentration;

(*eg.*, lead, mercury, barium, arsenic, silver, chromium, cadmium, pyridine, cresols, chloroform, carbon tetrachloride, benzene, 2-butanone)

(2) contains one or more acutely hazardous components listed for toxicity in 310 CMR 30.136 in *any* concentration;
(*eg.*, all cyanide salts, sodium azide, acrolein, benzyl chloride)

(3) is a commercial product or spill residue thereof of a component listed for toxicity in 310 CMR 30.133;

(*eg.*, acetonitrile, acetyl chloride, aniline, dichloromethane, hydrogen fluoride, nitrobenzene)

Please consult the regulatory text for the full list of toxic hazardous waste constituents (http://www.mass.gov/dep/service/regulations/310cmr30.pdf). In Massachusetts, polychlorinated biphenyls (PCBs) and oil (petroleum, vegetable, silicon, etc.) waste are also considered hazardous. Additionally, the Commonwealth has provisions that expand the definition of "toxic" to waste items which are not specifically listed in the regulatory text as such, yet for which there exists sufficient evidence that the material is hazardous to people or the environment (see item 2 of the hazardous waste definition). Under this definition, most chemical waste can be classified as toxic. Ethidium bromide is a classic example of a material which is not specifically regulated,

yet which must be disposed of as hazardous waste because of this expanded definition of toxicity.

In addition to waste generated through normal laboratory processes, both the DEP and EPA treat unknown, abandoned, expired or leaking chemicals as hazardous waste until proven otherwise. The burden of proof is ours, not theirs. Chemical and biological analysis to determine the hazards can be very expensive. Additionally, with the blanket definition of toxicity, most chemicals on the shelf of an average laboratory are considered hazardous. Therefore, if you find expired, unlabeled or leaking materials, please contact the chemical hygiene office immediately for disposal of these items.

It is always your responsibility, as the generator of a waste, to determine whether or not it is hazardous. Questions about specific waste streams should be referred to the Chemical Hygiene Officer.

Hazardous waste is never permitted to be disposed of via drains or normal trash receptacles. Every laboratory in the department which generates hazardous waste has a satellite accumulation area (SAA) where the waste is disposed. A typical SAA is shown in Figure 5.

Figure 5: A Satellite Accumulation Area (SAA) for Hazardous Waste

All waste containers must be capped (except when waste is being added) and kept in an SAA, with adequate secondary containment (*i.e.*, all of the containers must be in a bin which is capable of holding all of the liquid contents should the containers fail). Empty waste containers of various sizes are available from the Biology Safety Coordinator. You are encouraged to use empty reagent bottles as waste containers, if possible, to minimize cost. When selecting a waste container, please keep in mind that the container must be compatible with the contents of the waste. For example, it is generally not a good idea to choose a thin plastic waste container for organic solvents and liquids, as many of these materials can dissolve certain plastics. Similarly, a metal bottle would be a poor choice of container for a corrosive material, like hydrochloric acid or elemental mercury.

All waste in a given container should be compatible, and fully listed on the container. To assist with labeling and waste segregation, Amherst College uses preprinted, color-coded labels (available from the Biology Safety Coordinator) for waste containers. These labels are designed to comply with specific requirements of the EPA (*i.e.*, listing the EPA identification number and having the words "Hazardous Waste" on the bottle, etc.) so it is important that you use these labels and not ones of your own. The colors of the labels correspond to the type of waste as follows:

Orange = Aqueous Waste

Yellow = Non-halogenated Organic Waste

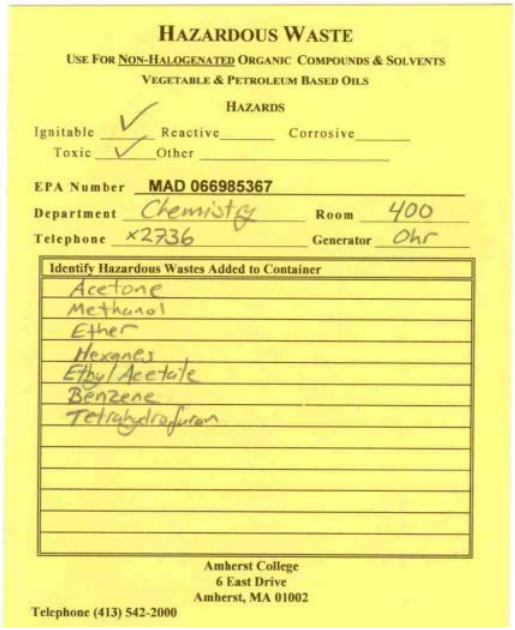
White = Halogenated Organic Waste

Blue = Solid or Elemental Mercury Waste

Please do your best to use the appropriate color label for a given waste container. The waste container label, Figure 6, must be completely filled out. The most significant hazard (ignitable, reactive, corrosive or toxic) ***must*** be specified, however, please do your best to ensure that ***all applicable*** hazards are indicated. The generator should be the P.I. for the laboratory. You must list the contents you are adding to the container each and every time you add something, unless it is already listed on the contents. Names of components must be fully spelled out, in ink or permanent marker, and legible; do not use

molecular formulas or abbreviations like H_2O, EtOH, THF, DMF, TFA, etc. You may use either common or IUPAC names. For multi-component reagents (*e.g.*, Benedict's solution, Lucas reagent, pH = 9 buffer, etc.), please list each component on the label. Do not dispose of incompatible wastes in the same container (*eg.*, use separate aqueous

Figure 6: A Properly Filled Out Hazardous Waste Label


HAZARDOUS WASTE

USE FOR NON-HALOGENATED ORGANIC COMPOUNDS & SOLVENTS
VEGETABLE & PETROLEUM BASED OILS

HAZARDS

Ignitable ✓ Reactive_____ Corrosive_____
Toxic ✓ Other _____

EPA Number MAD 066985367
Department Chemistry Room 400
Telephone x2736 Generator Ohr

Identify Hazardous Wastes Added to Container
Acetone
Methanol
Ether
Hexanes
Ethyl Acetate
Benzene
Tetrahydrofuran

Amherst College
6 East Drive
Amherst, MA 01002
Telephone (413) 542-2000

(orange label) containers for highly concentrated acids and bases). Special attention should be given to strong oxidizing agents (*eg.*, concentrated nitric and sulfuric acids, concentrated hydrogen peroxide); these items should not be disposed of in waste containers which contain other materials. The Biology Safety Coordinator will collect full waste containers during the weekly inspection of the SAAs. If you have a full waste container before then, please contact the Biology Safety Coordinator for immediate disposal. Full containers must be removed from the SAA within three days of being filled. (Note: if you plan on generating more of the same waste, it is very important that you call when a container is full. No SAA may contain more than one container with the exact same contents.)

Biological/Medical Waste

Biological or medical waste is regulated by the Massachusetts Department of Public Health and the Amherst Board of Health. It is defined as "waste that because of its characteristics may cause, or significantly contribute to, an increase in mortality or an increase in serious irreversible or incapacitating reversible illness; or pose a substantial present potential hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed" and includes the following:

1. **Human blood or blood products**: includes blood in the liquid state, body fluids visibly contaminated with blood and materials which are contaminated with blood. Used feminine hygiene products are specifically excluded.

2. **Pathological Waste**: includes human anatomical parts, organs, tissues, fluids and materials contaminated with body fluids other than urine. Specifically excluded are: teeth and bone without visible tissue, nasal secretions, sweat, sputum, vomit, urine or fecal materials that do not contain visible blood or involve confirmed diagnosis of infectious disease.

3. **Cultures and Stocks of Infectious Agents and their Associated Biologicals:** includes all discarded stocks of infectious agents and any material which has come into contact with these stocks. This includes discarded live and attenuated vaccines intended for human use.

4. **Contaminated Animal Waste:** includes contaminated carcasses, body parts, body fluids, blood or bedding from animals known to be infected with a zoonotic disease reportable to the Massachusetts Department of Agricultural Resources under 105 CMR 300.140, infected with diseases that pose a risk to human health (as determined by the State Epidemiologist or Public Health Veterinarian) or which have been inoculated with infectious agents, including those for the purpose of production of biologicals or pharmaceutical testing. This also includes animal carcasses used in research or teaching applications, regardless of whether or not they are infected with any diseases.

5. **Sharps:** includes all unwanted needles, syringes, lancets, scalpel blades, razor blades, suture needles. This also includes pipets and other glass/plastic ware used in connection with a medical procedure.

6. **Biotechnology By-Product Effluents: "**Any discarded preparations, liquids, cultures, contaminated solutions made from microorganisms and their products including genetically altered living microorganisms and their products."

The biology department is a large generator of such waste, particularly of sharps and biotechnology by-product effluents.

Sharps Waste

Most of our labs produce waste needles, razor blades, cannulae and other so-called "sharps" waste. The disposal of sharps is strictly regulated in Massachusetts. All sharps must be disposed of in containers which are puncture resistant, appropriately colored (fluorescent orange, orange-red or red) and which have the word "biohazard" and the biohazard symbol such as those shown in Figure 7. These containers are available from the Biology Safety Coordinator. Full containers should likewise be referred to the Biology Safety Coordinator for collection and disposal.

Figure 7: Sharps Waste Containers

Biotechnology By-Product Effluent Waste

Biotechnology by-product effluent waste includes all cultures, stocks and solutions of microorganisms. This type of waste is considered medical or biological waste in Massachusetts, and as such, is regulated for disposal. That means it cannot simply be dumped down the drain, or discarded with the municipal solid waste stream without proper treatment. Two types of waste are specifically excluded from this regulated category, because the Amherst College Institutional Biosafety Committee (IBC) has declared that these items are safe for disposal prior to treatment. These include aqueous media left over after harvesting *E. coli* or *S. Cerevisiae* which are NIH exempt, in which *only a small amount* of viable organisms remain in the solution. Large amounts of these organisms, such as colonies on plates or in stock solutions, are regulated. All other biotechnology by-product effluent waste is strictly regulated. If you are unsure whether or not your specific waste is regulated, please check with your P.I., or the IBC (contact Professor David Ratner). The following are the approved treatment methods for liquid and solid biotechnology by-product effluent waste.

On-Site Treatment of Approved Liquid Medical or Biological Waste
This applies only to waste which cannot solidify or otherwise impede the flow of material through drains! If the waste can solidify (eg., contains agar), or would otherwise obstruct the flow, do not dispose of it via the drain!

1. Liquid waste materials should be treated as they are created and not stored. If liquid materials must be stored prior to treatment and disposal, they must be placed in a tight sealing container marked with the biohazard symbol and the word “Biohazard”. The container must be stored in an area with secondary containment that is designated for medical or biological waste storage only.
2. Add enough household bleach to the material to bring the final bleach concentration to 10% by volume and mix. Bleach must be used within six months of opening.
3. Allow the mixture to stand for 20 minutes.
4. Rinse the material down the drain with copious amounts of water.

Off-Site Treatment of Approved Solid Medical or Biological Waste

1. All solid medical or biological waste must be placed in a red, fluorescent orange or orange-red plastic biohazard bag displaying the biohazard symbol and the word "Biohazard". The bag must meet the American Society for Testing Materials (ASTM) standard D1922-06a and ASTM D1709-04. The bag should be placed in a secondary container with cover (such as a plastic bin with tight fitting cover) until it is ready for treatment. The bag should have the name of the lab and the date it was placed in the bin written on the side. The bin should be marked to indicate that it is for medical/biological waste storage only.
2. The lid to the secondary container must be tight fitting, and must remain on the secondary container unless waste is actively being added to the container. Do not fill the containers more than 2/3 full.
3. Containers must be referred to Lori Nichols (x2081) or Maureen Manning (x8328) for collection and transport. The material will be sent offsite for treatment.

Medical/Biological Waste Which Must Be Treated Off-Site

All other medical/biological waste, such as anatomical parts or animal carcasses from dissections, must be sent offsite for disposal. All unwanted anatomical parts or carcasses must be disposed of in a red, fluorescent orange or orange-red biohazard bag bearing the universal biohazard symbol and the word "Biohazard". The bag must meet the American Society for Testing Materials (ASTM) standard D1922-06a and ASTM D1709-04. The bag should be placed in a secondary container with cover (such as a plastic bin with tight fitting cover) until it is ready for shipment or transport to another storage area, or it should be placed in the approved freezer in Life Sciences 112 for medical waste storage. The Biology Safety Coordinator will prepare the waste for shipment once the storage area is full.

Chemical Inventory System (Vertere)

As part of the emergency planning and community right to know act (EPCRA), the toxic substance control act (TSCA) and RCRA, Amherst College is required to maintain an accurate inventory of all chemical substances present on campus. The

college is required to report the identity, quantity and location of these materials to various state and local emergency response entities on an annual basis to assist personnel with deciding appropriate courses of action during an emergency situation. Additionally, the EPA and DEP require the college to account for the ultimate fate of materials coming into the campus by comparing the inventory history to the waste stream. In other words, the inventory plays a crucial role in demonstrating proper waste management to regulatory agencies. The inventory also serves a practical purpose for the various research laboratories on campus by providing online access to a list of all materials present on campus. This facilitates "borrowing" of materials required in small quantities, which reduces costs to individual laboratories and promotes safety by minimizing the amount of waste and potentially hazardous materials stored on campus.

Amherst College utilizes the Vertere inventory system, an online database. Online access is available through the Chemistry Department website (https://www.amherst.edu/academiclife/departments/chemistry), the Environmental Health and Safety website (http://www.amherst.edu/~ehs/) or directly at http://chemwatch.amherst.edu/VimWebV2/(S(rv54luerqupc0bfjkbkhbv45))/Login.aspx. You must have an account to gain access. To create an account, contact the chemical hygiene office. Once you have logged in, select the "Chemical" tab, followed by "View/Update" on the left side of the screen. This will bring up the search screen. You may search for chemicals by name, CAS number, department, location, user (*i.e.*, P.I.), tag (barcode) number, or any combination of these.

The barcode (shown in Figure 8) is a unique number assigned to every chemical when it arrives on campus and is used to track the material. When a material arrives in your laboratory, it should be barcoded and the chemical's information should be entered on the inventory sheet for your laboratory (like that shown in Figure 9). The top portion of the sheet is used to add newly received items to the inventory. In the sheet shown in Figure 9, the 200 proof ethanol is being added to the inventory system. Please make sure you include all of the information requested for the chemical. Storage location is especially important. Please be sure to indicate the room number, and any other information (for example, if it will be stored in a refrigerator in the indicated room). Some rooms have multiple refrigerators or freezers, so please make sure you indicate the

Figure 8: The Amherst College Barcode

appropriate unit (usually designated as A, B, C, etc.) You can check the storage locations in Vertere to see a list of possibilities.

When a material is moved from one location to another, or if a container is emptied, it must be transferred or decremented, respectively, and updated in the inventory system. To transfer or decrement a material, simply fill out the appropriate information (chemical name, bar code number, new storage location) on the lower section of the sheet. In the sheet in Figure 9, 2-propanol is being transferred from 130 C to 216. Again, please indicate the exact storage location. When a container is emptied, list only the chemical name and barcode number (or write “empty” in the Transfer column, if you prefer). In Figure 9, toluene is being disposed. The sheets are collected on a weekly basis by the Biology Safety Coordinator and updated in Vertere. Empty containers of hazardous materials should be rinsed appropriately before discarding. You may also place empty containers in a storage bin for collection, or use them as waste containers.

Figure 9: Amherst College Inventory Sheet

AMHERST COLLEGE

Chemical Inventory Log
Department: Biology **Room/Lab: Merrill 130 C** **Responsible Person: Maureen Manning** **Date: February 24, 2009**

Increment Section

Chemical Name	Manufacturer	Size	CAS #	Catalogue #	Storage Location	AC Barcode #
200 Proof Ethanol	Pharmco-AAPER	1 pt.	64-17-5	111000200	130 C	75463

Decrement/Transfer Section

Put the Chemical Name and Barcode # on this side if you want the chemical deleted or the container is empty or you are transferring the chemical.

Add the Room and Building to on this side to indicate where the chemical will be transferred.

Chemical Name	AC Bar Code #	Transfer to Room	Transfer to Building
2-propanol	89023	216	
toluene	96304		

File: Amherst College ChIM Inventory Sheet.wpd

Shipments of Chemicals and Other Materials

After getting this far in the training, it probably will come as no surprise to you that shipments of hazardous materials and recombinant organisms are also regulated. As part of the Department of Transportation (DOT) and the International Air Transportation Association (IATA) regulations, anyone who packages, offers for transport in commerce, or actually transports a hazardous material or recombinant organism by road, rail, ship or air must have appropriate training. The nature of this training is intensive and lengthy, and well beyond the scope of this manual. Therefore, if you ever have a need to ship an item from the department (no matter how "non-hazardous" it may seem) please contact the Biology Safety Coordinator for assistance. Please note that materials considered hazardous for shipment include recombinant organisms, batteries (cell phones, ipods, computers, etc.), most metals that are granular or powdered, most paints, dry ice,

charcoal and perfume. Given that such seemingly benign, everyday items fall under regulatory scrutiny, it is imperative that you ask before shipping anything from the department. Additionally, many laboratory materials are regulated by TSCA, which has additional requirements that must be met for shipping materials (*eg.*, including MSDSs with shipments and declaring items for research purposes only). Violation of any of these laws can endanger the health and welfare of the public, and also incur huge monetary fines and possible prison sentences for the offending party or parties.

Additional Items

This manual includes the basic annual training that is required by various regulatory agencies for most of the laboratories in the biology department. However, you should always receive specific and hands-on training from either your P.I. or trained and experienced coworkers. Additionally, you may require further training, depending on the nature of your project. For example, if you work with human tissue or fluids, animals which could bite you, or certain pathogens, you are required to have bloodborne pathogen training. Additional training is also required for work with radioactive materials and most lasers. Please contact the Biology Safety Coordinator if you need any additional training, or suspect you might. We wish you a productive and safe research experience!