TEXAS TECH UNIVERSITY
Department *of*
Biological Sciences

BIOLOGICAL SCIENCES
LABORATORY CHEMICAL SAFETY PLAN

POSITION STATEMENT: The DBS regards Chemical Safety issues, and any issues pertaining to the safety and well being of researchers, staff, and students to be of the upmost importance. Our ultimate goal is to provide a safe working environment that complies with federal, state, and university guidelines, regulations, and laws. We strive to achieve this goal while allowing researchers to efficiently pursue their academic endeavors and responsibilities without the burdens of undue and cumbersome regulation.

A. PRINCIPLES FOR WORKING WITH LABORATORY CHEMICALS AND BIOLOGICAL MATERIALS.

This laboratory Chemical Hygiene Plan is mandatory. The procedures, personal protective equipment and other elements of this Chemical Hygiene Plan are designed to minimize exposures. All investigators, fellows, technical staff, students, or any other individuals working in the lab must comply with the precautions and rules outlined below at all times.

The following general principles apply to the use of chemicals and biological materials in this laboratory, _____ Building, Room(s)_____.

1. It is prudent to **minimize all chemical exposures**. Inhalation, ingestion and skin contact with chemical or biological materials should be avoided.
2. **Avoid underestimation of risk.** Exposures should be minimized even for substances of no known significant hazard. Special precautions should be used for work with substances that present special hazards. Assume that any mixture will be more toxic than its most toxic component and that all substances of unknown toxicity should be considered toxic.
3. **Provide adequate ventilation.** Fume hoods and other exhaust ventilation devices should be used properly to prevent exposure to airborne substances.
4. **Use available references to assess the risk of hazardous chemicals.** The Occupational Safety and Health Administration (OSHA) have provided a list of permissible exposure limits (PELs) for a number of chemicals. The American Conference of Governmental Industrial Hygienists (ACGIH) has provided threshold limit values (TLVs) for many chemicals. The Texas Tech University Chemical Hygiene Plan includes a list of Hazardous Chemicals and Select Carcinogens, which should be handled with additional precautions. Carcinogens are listed separately because special handling is required for these chemicals.

B. CHEMICAL HYGIENE RESPONSIBILITIES. Responsibility for this departmental chemical hygiene plan is assigned to the following individuals:

1. **Dr. _____, Chairman of the Department/Division of _____ is responsible for chemical hygiene in this Department/Division.**
2. **Dr. _____, as principal investigator and laboratory chemical hygiene officer, in addition to the general principles listed above, is also responsible for the following:**
   a) Develop, implement and revise this chemical hygiene plan, policies and practices as is needed for the individual lab. Maintain an awareness of current requirements concerning regulated substances.
   b) New incoming graduate students will undergo a 1-day mandatory training session as part of their DBS orientation. During this training all students will be taught general lab safety procedures as outlined in this operating policy.
   c) All undergraduate students engaged in research in DBS' labs are required to have read and understood this operating policy before engaging in research activities.
   d) Ensure that all individuals within a lab have undergone required yearly training as mandated by EHS. These include but are not restricted to: Lab Safety, Safety Awareness, Hazard Communication and Biological Safety. These safety courses are to be taken online at: http://www.depts.ttu.edu/ehs/Web/TrainModDesc.aspx and training certificates are to be kept with the lab as to be easily accessible.
   e) Monitor purchasing, use, and disposal of chemicals and biological materials used in these laboratories.
   f) Ensure appropriate records are maintained (training, audit reports, injury reports, etc.).
   g) Ensure that faculty, staff, and students understand and follow the Chemical Hygiene Plan.
   h) Provide regular formal laboratory hygiene and housekeeping inspections, including routine inspections of any emergency equipment required by the Chemical Hygiene Plan for this laboratory.
   i) Determine the required or appropriate levels of protective apparel and equipment. Make sure protective equipment is available, in working order, and used properly. Provide adequate training for the use of protective equipment.
   j) Ensure that facilities and training for use of any new materials being ordered are adequate.
   k) Seek ways to improve laboratory safety.

3. **Fellows, students, and laboratory staff are responsible for:**
   a) Planning and conducting each experiment in accordance with the laboratory Chemical Hygiene Plan and all applicable University policies and procedures.
   b) Developing and practicing good personal hygiene and chemical and biological safety practices.
   c) Review procedures for substituting a less or non-hazardous procedure which will produce the same results.
   d) Consider procedures to implement a smaller scale operation or experiment when

possible.

e) Implement inventory control by evaluating if a smaller quantity of chemical(s) may be purchased.
f) Educate others in the same working area on the safe and proper purchase, use, storage, and disposal of hazardous materials within the Department of Biological Sciences and Texas Tech University.
g) Accurately label all containers in the work area.
h) Conduct periodic inspections of potential waste generating operations under your direct control.
i) Contact Environmental Health and Safety when hazardous materials need to be collected. Do not accumulate large quantities of unnecessary hazardous materials. See Material Handling and Disposal below for details on preparing materials for collection

4. **Environmental Health and Safety (EHS):**
   a) Provide a timely collection service for hazardous materials that are no longer needed.
   b) Maintain a Hazardous Waste Management Program that emphasizes source reduction.
   c) Arrange for final disposal of hazardous wastes.
   d) Complete all required regulatory reporting.
   e) Act as the University representative for contract agreement, shipment record keeping, and regulatory inspections involving hazardous materials.
   f) Provide information and technical assistance regarding proper purchasing, storage, labeling, and disposal of hazardous materials.
   g) Act as a collection point for individuals who have ideas for improving the Hazardous Waste Management Program.

C. THE LABORATORY FACILITY

1. **Design:** All laboratories are designed in accordance with accepted laboratory standards. No room shall be converted into a laboratory for the use of chemical or biological materials until it is reviewed by Environmental Health & Safety.
2. **Chemical Storage:** All Chemicals are to be stored according to OSHA regulations:
   a) All Chemicals are to be stored with labels intact.
   b) General use chemicals are to be stored separately from chemicals determined to be a health hazard. In addition, within categories, solid chemicals should be stored separately from liquids.
   c) General usage chemicals should be labeled with an easily visible orange sticker.
   d) Health Hazard chemicals should be labeled with and easily visible blue sticker.
   e) MSDS sheets are to be kept electronically or within a binder for chemicals with an NFPA number of 2 or less for any category. Any chemical with a 3 or above for any category will be addressed in a separate Biological Sciences Operating Plan for hazardous chemicals.
   f) A chemical inventory will be maintained for all chemicals within a lab. Chemicals will be designated by chemical name, ACS number, NFPA number and location with the lab (Appendix A).

g) Commonly used buffers and solutions that are made and stored within the lab will be labeled with the common name and if non-hazardous labeled with a visible orange dot.
h) An inventory of commonly used buffers will be maintained (ie PBS: Phosphate Buffered Saline) and will be designated by NFPA number and location within the lab (Appendix B).
i) According to EHS regulations, acids, bases and flammable liquids will be stored in specified, regulated and clearly labeled containers.

D. CHEMICAL INVENTORY GUIDELINES FOR LABORATORIES

1. **Purpose:** In order to comply with numerous regulatory requirements, the University must compile an annual inventory identifying the location (building and room) and quantity of all hazardous materials on campus. One of the major regulatory elements the inventory is used to fulfill is the OSHA Hazard Communication Regulation - 1910.1200 (HazCom). The purpose of HazCom is to ensure that the hazards of chemicals produced or imported are evaluated, and that information concerning their hazards is transmitted to employers and employees. In addition to the OSHA regulations for hazard communication, there are other regulations and guidelines, which require an inventory system.
   a) Environmental Protection Agency (EPA) – Emergency Planning and Community Right-to-Know Act (EPCRA) hazardous chemical storage reporting

b) Department of Homeland Security (DHS) – Chemicals of Interest (COI)
c) Centers for Disease Control and Prevention (CDC) – Select agents and toxins
d) Drug Enforcement Agency (DEA) and Bureau of Narcotics and Dangerous Drugs (BNDD) – Controlled Substances and List I & II regulated chemicals
e) International Building Code - Flammable material and other storage limits
f) Local Fire Department Requirements – Flammable material storage limits

2. **Compliance**: In order to achieve and maintain compliance, the Department of Biological Sciences at Texas Tech has committed to inventorying chemical containers on site as described below in "Items REQUIRED to be Inventoried," except those exempted below under "Items NOT REQUIRED to be Inventoried".
   Initially, all labs will have to comply by manually inventorying all required material items mentioned in the "Items REQUIRED to be Inventoried" below
   On an annual basis, each lab will be responsible for verifying that the items in the inventory match what is currently found in the lab and other storage areas, such as common areas, refrigerators/cold rooms, and freezers. However, there are other materials, which are highly regulated and may require more frequent inventory updates. These materials are items of interest to federal and local agencies, such as Department of Homeland Security, Centers for Disease Control and Prevention, Local Fire Department, etc. and are more controlled to prevent the following:
   a) Release: quantities of toxic, flammable, or explosive chemicals that have the potential to create significant adverse consequences for human life or health if intentionally or unintentionally released, detonated, or involved in a fire.
   b) Theft or Diversion: materials that have the potential, if stolen or diverted, to be abused or used as weapons, which can ultimately lead to significant adverse consequences for human life or health.
   c) Sabotage or Contamination: chemicals that, if mixed with other readily available materials, have the potential to create significant adverse consequences for human health or life.
   Since most of these materials have well established threshold limits which are defined by each agency, EH&S will be assisting with the compliance requirements by performing monthly database queries for labs located within designated control areas, floors, or buildings. Keeping in mind that these are aggregate amounts, if a lab, control area, floor, or building is over the threshold limit, then EH&S will work with all labs within the area to resolve any issues with database information or actual materials stocks

3. **Items REQUIRED to be Inventoried:** Any, but not limited to, chemical containers that have a manufacture's label which denotes physical or health hazards, or whose MSDS denotes hazards, are to be included in the inventory. In general, laboratory chemicals and reagents are inventoried even if the hazard is considered low. Almost all chemicals received from chemical manufacturers such as Sigma-Aldrich, Fluka, Alfa Aesar, Fisher Scientific, Mallinckrodt Baker, Acros, Bio-Rad, Qiagen, Invitrogen, etc., will be included in the lab inventory. The list below provides some examples of common materials that need to be inventoried.
   a) Environmental Health and Safety Listed Chemicals http://www.depts.ttu.edu/ehs/Web/Docs/Chem_Hygiene_Plan.pdf as well as chemicals

listed as hazardous or carcinogenic by OSHA (Appendix C)

b) DEA scheduled materials, to include those materials acquired from the Division of Comparative Medicine (DCM) http://www.justice.gov/dea/pubs/scheduling.html
c) Select agents that are classified as biological toxins (Appendix C)
d) All flammable solvents, to include primary & secondary chemical containers that are brought into the lab from another location (e.g.) 10 gallon carboy of ethanol that is filled from a primary 55 gallon drum at the loading dock and brought into the lab
e) Materials that are transferred or inherited from another lab
f) All organic solvents, including liquid scintillation counting cocktail
g) Other research drugs and therapeutics
h) All chemicals/reagents regardless of hazard
i) Shock sensitive and potentially explosive mixtures produced by the lab must be inventoried (e.g. Bouin's stain made from saturated picric acid solution or serial dilution of ether mixtures). For further guidance in peroxide forming materials and shock sensitive materials see Appendix D.
    a. Reactive or **explosive** materials requiring special attention
    b. Guidelines for Safe Handling and Disposal of Peroxide Forming
j) Gas cylinders, small compressed gas cylinders or small propane cylinders
k) Corrosive cleaning agents (e.g. strong base/acid solutions, RNASE away, Chromerge, etc.)
l) Photographic Chemicals
m) Activated charcoal
n) Chemical kits**
o) Dyes and stains

** Chemicals contained in a kit are usually not individually inventoried. They can be inventoried under the kit name.

4. **Items NOT REQUIRED to be Inventoried:** Even though some items may not be entered into the inventory, the user is still responsible to obtain a current MSDS for the product. The list below provides some examples of common materials that do not need to be inventoried.
    a) Any secondary chemical container that is produced in the lab from a primary chemical container(s) that is already inventoried (e.g.)
        i. 1N NaOH that is made from a commercially available 10N NaOH solution or solid NaOH.
    b) Squirt bottles and spray bottle
    c) Conical and "Falcon" tubes with chemicals or samples in them
    d) Biological material (e.g.) ○ plant or animal tissue, blood or blood products
    e) reproducing biological organisms, bacteria, viruses, fungi or yeast
    f) Enzymes, antibodies, proteins, peptides, nucleic acids
    g) Conjugated antibodies and proteins
    h) Tissue culture media or other growth media
    i) Buffer solutions for pH probes
    j) Non-chemical diagnostic materials that contain a film on any surface (e.g. 96-well plate)
    k) Chemical spill kits
    l) First aid kit components
    m) Food or food additives (unless it will be used for R&D or operational purposes)

n) Office Supplies (appropriate quantities for office administrative purposes)
o) Non-Hazardous metals such as foils, bars, and rods
p) Test strips (pH, peroxide, water hardness, iron, phosphate, etc.)

Note: Each PI or designated person(s) will be responsible for the proper hazard determination (see below) for all mixtures that are commonly made and used in the research lab. For hazard classification guidance concerning mixtures and solutions, the Hazard Communication Standard (29 CFR 1910.1200) states that a mixture (or solution) will be considered as having the same health hazards as the components that comprise ≥10% of the mixture (≥0.1% for known carcinogens in the mixture). If the PI or designated person(s) is not comfortable with making hazard determinations or is unsure about the hazard classification of a particular solution, they should consult with EH&S.

E. COMPONENTS OF THE LABORATORY CHEMICAL HYGIENE PLAN

Basic rules and procedures. All labs must post on the inside of any laboratory door a list of contacts at Texas Tech University (Appendix E) as well as General Laboratory Rules (Appendix F)

1. The following general principles should be used for all laboratory work with chemicals:
   a) Accidents and spills

      Eye contact: Promptly flush eyes with water for 15 min and call:
      a. Tech Police (742-3931)
      b. EHS (742-3328)

      Ingestion: Follow directions for accidental ingestion found on the chemical's Material Safety Data Sheet (MSDS) and call (806):
      a. Tech Police (742-3931)
      b. EHS (742-3328)
      c. TTHSC UMC (775-8200)
      d. Poison Control (296-8900)

      Skin contact: Promptly flush the affected area with water and remove any contaminated clothing. Notify:
      a. Tech Police (742-3931)
      b. EHS (742-3328)

      Spills: Call for emergency spill response:
      a. Tech Police (742-3931)
      b. EHS (742-3328)

      Cuts or Breakage of Skin: Promptly flush the affected area with water and call:
      a. Tech Police (742-3931)
      b. EHS (742-3328)

      All issues: Notify your supervisor.
      If required: Call the employer's occupational medicine provider to arrange a post-exposure evaluation.

b) Avoidance of routine exposure: Develop and practice safe habits that avoid unnecessary exposure to chemicals by any route.
    a. Do not deliberately smell or taste chemicals.
    b. Vent any apparatus that may discharge toxic chemicals (e.g. vacuum pumps, distillation columns, etc.) into local exhaust devices.
    c. Inspect gloves and test glove boxes before use.
    d. Do not allow volatile hazardous substances or asphyxiants to be released in cold rooms or warm rooms since these have contained, recirculated atmospheres.

c) Choice of chemicals
    a. Use only those chemicals for which appropriate ventilation systems are available.
    b. Whenever possible, choose the least hazardous chemical for a given experiment.
    c. Order the smallest amount of chemical necessary.

d) Eating, drinking, smoking, etc.:
    a. Eating, drinking, smoking, handling contact lenses and applying cosmetics is strictly prohibited in areas where hazardous laboratory chemical, biological, or radioactive materials are used or stored.
    b. Storage of food and beverage in containers or in areas that are designated or are used for storage of hazardous laboratory materials is prohibited.
    c. Such activities are permitted in an area (defined as a room with floor to ceiling walls and a closed door) separated from the laboratory space. If a separate area can only be accessed by going through the laboratory, then only covered food or beverages may be carried through the laboratory.
    d. For more details on eating, drinking, etc. in the laboratory, please see the "Eating, Drinking, and Related Activities" policy on the EHS website: http://www.depts.ttu.edu/ehs/Web/

e) Equipment and glassware
    a. Handle and store laboratory glassware with care to avoid damage.
    b. Inspect glassware before each use and do not use damaged glassware.
    c. Use extra care with dewar flasks and other evacuated glass apparatus. If plastic or plastic-coated flasks are not available, shield or wrap glass flasks to contain chemicals and fragments should implosion occur.

f) Exiting
    a. Thoroughly wash hands and any areas of exposed skin before leaving the laboratory.

g) Horseplay
    a. Avoid practical jokes or other behavior that might confuse, startle or distract another worker thus possibly causing an accident.

h) Mouth suction
    a. Never use mouth suction for pipetting or starting a siphon.

i) Personal apparel
    a. Confine long hair and loose clothing. Wear appropriate shoes at all times in the laboratory. Clogs, sandals, perforated or cloth shoes are not appropriate.
    b. Clothing must provide adequate coverage so that there is no skin exposed to hazardous materials.

j) Personal housekeeping
    a. Keep the work area clean and uncluttered, with chemicals and equipment properly labeled and stored.
    b. Clean up the work area on completion of an operation or at the end of each day.

k) Personal protection
    a. Ensure that all persons, including visitors, wear appropriate personal protective equipment (PPE) in areas where chemicals and biological agents are handled.
    b. Wear appropriate gloves when the potential for contact with hazardous materials exists. Inspect the gloves before each use and replace them periodically or when damaged.
    c. Gloves should be removed before existing the lab and should not be worn in public areas of the lab such as computer work-stations or telephone areas.
    d. Do not reuse gloves after removal.
    e. Use low protein, non-powdered latex gloves to help reduce the risk of latex allergies. Contact Employee Health if any lab personnel have an allergic reaction to latex gloves (742-3876).
    f. Always use appropriate containment equipment (e.g. fume hood) when working with toxic or volatile hazardous materials.
    g. Use of respiratory protection requires enrollment in the respiratory protection program, including a medical evaluation and fit test. Contact Environmental Health & Safety if you need to wear respiratory protection (742-3876)
    h. Use appropriate eye protection when handling chemicals ie weighing and pouring of stock chemicals.
    i. Avoid use of contact lenses in the laboratory unless necessary. If contact lenses are worn, chemical splash goggles are required when handling chemicals. Supervisors should be aware of any personnel who wear contact lenses.
    j. Remove laboratory coats immediately upon significant contamination and before exiting the laboratory.
    k. Employees must not take lab coats or other protective clothing home for cleaning.
    l. Use other specialized containment or personal protective equipment as appropriate.

l) Planning
    a. Seek information and advice about hazards before starting an experiment.
    b. Plan appropriate protective procedures and positioning of equipment before beginning and new operation.

m) Unattended operations
    a. Leave lights on, place an appropriate sign on the door and provide for containment of toxic substances in the event of utility service failure (e.g. cooling water) during an unattended operation.

n) Use of a fume hood
    a. Use the fume hood for operations that might result in release of toxic chemical vapors or dusts.
    b. Use a fume hood or other local ventilation device when working with any appreciably volatile substance with a TLV or PEL of less than 50 ppm.

c. Confirm that the fume hood is functioning properly before use. Keep the fume hood sash closed at all times except when operations within the fume hood are ongoing.
d. Keep storage within the fume hood to a minimum. Do not allow items to block vents airflow within the fume hood.
e. Work at least six inches back from the face of the fume hood.
f. If the fume hood is equipped with an on/off switch it should be left on at all times, even when not in use.

o) Vigilance
   a. Be alert to unsafe conditions and see that they are corrected.
   b. Notify your supervisor and TTUPD (742-3931) of any suspicious activities in or around research labs.

p) Waste disposal
   a. Ensure that the plan for each laboratory operation includes training and procedures for disposal of waste in accordance with all federal, state, and local regulations and all applicable Environmental Health & Safety policies. These will be discussed below.
   b. Do not dispose of any hazardous chemicals into the general trash or in the sanitary sewer.

q) Working alone
   a. Avoid working alone in a building.
   b. Do not work alone in a laboratory when working with hazardous materials.

F. HAZARDOUS CHEMICAL USAGE

1. **Introduction:** This guide outlines procedures established for the safe and proper management of hazardous chemical materials located within the Department of Biological Sciences at Texas Tech University. These procedures are intended to promote compliance with federal, state, and local regulations pertaining to hazardous materials. In addition, this guide provides instructions for handling materials that may not be considered hazardous, but have special processing or waste management requirements (Appendix G).

2. **Definition:** Any chemical that poses a hazard to health, property or to the environment. The Chemical Operating Policy of the Department of Biological Sciences at Texas Tech University categorizes any chemical to be hazardous that is designated with a rating of 3 or above related to the NFPA 704 standard for Heath, Flammability, Reactivity or Special.

3. Transportation of Hazardous Chemicals

a) All hazardous material shall be transported within secondary containment of sufficient size to hold the entire contents in the event of a spill or leak.
b) Large volumes or numerous bottles should be transported on carts. Carts used for transporting hazardous materials should have sides of sufficient height to restrain containers on the cart. The wheels of the cart should be of adequate diameter to assure smooth travel throughout the route of transport.
c) At no time shall hazardous materials be left unattended during transport.
d) Hazardous materials must not be left unattended outside any hazardous material collection location.
e) Routes of transport for hazardous materials shall be planned to minimize exposure to

personnel and visitors in the event of a spill.

f) Those transporting hazardous materials should use the freight elevators. If passenger elevators must be used to transport hazardous materials, only empty elevators are to be boarded. Passengers attempting to use the elevator shall be requested to wait for another elevator.

g) In the event of a spill of hazardous material, The Texas Tech procedure for spills found in this policy is to be followed.

4. Storage of Hazardous Chemicals

a) All chemicals should be dated when received and again when opened.

b) Flammable materials in containers larger than one gallon shall be stored in approved flammable material storage cabinets. See EHS Policy on Use and Storage of Flammable Materials.

c) Peroxide forming compounds (e.g, ether, dioxane, THF) shall be disposed of six months after opening, before the manufacturer's expiration date, or, given the absence of a date, one year after receipt.

d) Acids and bases shall not be stored with flammable materials in flammable cabinets. Acids should be segregated from bases.

e) Incompatible chemicals shall not be stored together. For further information on chemical incompatibilities call EHS 5-5918.

f) Stored chemicals should be evaluated annually to determine suitability and integrity for continued use.

g) Chemicals that have been stored for 10 years should be sent for disposal via Texas Tech EHS.

h) A sufficient supply of absorbents and neutralizers should be available at all chemical storage location for use in the event of a spill.

5. Use of Hazardous Chemical Materials

a) When working with a hazardous Chemical material, work practices as directed by the MSDS should be followed.

b) Volatile chemicals with an OSHA Permissible Exposure Level (PEL) of 50 ppm or less should be handled within a chemical fume hood.

c) A sufficient supply of absorbents and neutralizers should be available for use in the event of a spill.

d) For any chemical rated as hazardous by the above definition will require individual training by the PI prior to usage by any member of the laboratory.

   a. The Principal Investigator is responsible for establishing written protocols that utilize hazardous chemicals.
   b. A written protocol must be made available by the Principal Investigator to any lab member who will be using the hazardous chemical during the procedure/assay.
   c. The protocol must be read and understood by any user of the hazardous chemical.
   d. The user of the hazardous chemical must sign and date that he/she has been specifically trained in the usage of this hazardous chemical and understand the hazards of its usage.
   e. A MSDS sheet for each hazardous chemical used within any given protocol must also be included with the protocol.
   f. See Appendix H for examples.

6. Working with Allergens and Embryotoxins/Reproductive Hazard

a) Allergens (examples: diazomethane, isocyanates, bichromates, methyl methacrylate)
   a. Wear suitable gloves to prevent hand contact with allergens or substances of unknown allergenicity.
   b. Women who are pregnant should avoid working with these chemicals if possible.

b) Embryotoxins/Reproductive Hazards (examples: organomercurials, lead compounds, formamide).
   a. If you are a woman of child-bearing age, handle these substances only in a properly functioning fume hood while wearing appropriate personal protective equipment (especially gloves) to prevent skin contact.
   b. Review each use of these materials with the principal investigator or research supervisor and review continuing uses annually or whenever a procedural change is made.
   c. Store these substances, properly labeled, in an adequately ventilated area in a break-resistant secondary container.
   d. Notify supervisors of all incidents, exposures, or spills and follow the posted Injury/Illness procedures

7. **Working with Chemicals of Moderate Chronic or High Acute Toxicity** (examples: diisopropylflurophosphate, hydrofluoric acid and hydrogen cyanide) Supplemental rules to be followed in addition to those listed above:

a) Aim: to minimize exposure to these toxic substances by any route using all reasonable precautions.

b) Applicability: These precautions are appropriate for substances with moderate chronic or high acute toxicity when used in significant quantities.

c) Location: Store and use these substances only in areas of restricted access with special warning signs.

d) Containment equipment: Always use a properly functioning fume hood or other containment device for procedures that may result in the generation of aerosols or vapors containing the substance. Trap any released vapors to prevent their discharge with the hood exhaust.

e) Personal protection: Always avoid skin contact by using gloves and long sleeves (and other protective apparel as appropriate). Always wash hands and any exposed skin immediately after working with these materials.

f) Records: Maintain records of the amounts of these materials on hand, amounts used, and the names of the workers involved.

g) Prevention of spills and accidents: a. Plan in advance how to avoid and handle spills and accidents.
   a. Ensure that at least two people are present at all times if a compound in use is highly toxic or of unknown toxicity.
   b. Store breakable containers of these substances in chemically resistant trays. Work with and mount apparatus above such trays or cover work and storage surfaces with removable, absorbent plastic-backed paper.

h) Waste: Thoroughly decontaminate or dispose of contaminated clothing or shoes. Store contaminated waste in closed, suitably labeled, impervious containers.

8. **Work with Chemicals of High Chronic Toxicity** (examples: dimethymercury and nickel carbonyl, benzo-a-pyrene, N-nitrosodiethylamine, known human carcinogens). Further supplemental rules to be followed in addition to all those mentioned above for work with substances of known high chronic toxicity (in quantities above a few milligrams, depending on the substance):
   a) Access: Conduct all transfers and work with these substances in a controlled area such as a restricted access fume hood, glove box, or portion of the lab designated for use of highly toxic substances. All people with access to this area must be aware of the substances being used and understand and follow all necessary precautions.
   b) Approvals: Prepare a plan for use and disposal of these materials and obtain the approval of the PI (or designee) and EH&S (if necessary) prior to ordering the material.
   c) Non-contamination/Decontamination: Protect vacuum pumps against contamination with scrubbers or HEPA filters and vent them into a hood. Decontaminate vacuum pumps or other contaminated equipment, including glassware, in the controlled area before normal work is resumed there.
   d) Exiting: On leaving a controlled area, remove any protective apparel (placing it in an appropriate labeled container) and thoroughly wash hands and any other areas of exposed skin.
   e) Housekeeping: Use a wet mop or a vacuum cleaner equipped with a HEPA filter instead of dry sweeping if the toxic substance is a powder.
   f) Medical surveillance: If using toxicologically significant amounts of these substances on a regular basis (e.g. three times per week), consult the chemical hygiene officer, EH&S, and a qualified physician concerning advisability of regular medical surveillance.
   g) Records: Keep accurate records of the amounts of these substances stored and used along with the dates and names of users.
   h) Signs and labels: Ensure that the controlled area is conspicuously marked with warning and restricted access signs and that all containers of these substances are appropriately labeled with the chemical identity and warning labels.
   i) Spills: Ensure that contingency plans are in place and that equipment and materials are readily available to minimize exposures of people and property in case of an accident.
   j) Storage: Store containers of these chemicals only in a ventilated, limited access area in appropriately labeled, break-resistant, chemically resistant secondary containers.
   k) Gloves boxes: For a negative pressure glove box, the ventilation rate must be at least two volume changes per hour and the pressure must be at least 0.5 inches of water. For a positive pressure glove box, thoroughly check for leaks before each use. In either case, trap the exit gases or filter them through an appropriate filter before releasing them into the hood.
   l) Waste: Use chemical decontamination whenever allowable. Ensure that containers of contaminated waste (including rinsates from contaminated flasks) are transferred from the controlled area in a secondary container under the supervision of authorized personnel.

9. Excess Hazardous Chemicals
   a) The contents of all containers must be clearly identified.
   b) Toxic, highly reactive or corrosive chemicals should be collected in compatible containers and labeled with all contents clearly identified.

c) All chemicals should be collected for disposal in a container of the same material as that in which they were delivered, or in an approved safety can for flammable liquids.
d) Individual chemicals (or reagents containing hazardous chemicals) should not be mixed for disposal.
e) Whenever possible, different types of excess flammable liquid should be collected separately.
f) Corrosives (or solutions containing corrosives) should be collected in glass or chemical resistant plastic containers.
g) Mixtures of flammable liquids and corrosive chemicals should be collected separately and should not be collected in metal containers.

10. Disposal of Hazardous Materials

a) Excess hazardous material must be disposed of in accordance with Federal and State guidelines. Unwanted chemicals must be disposed through the Texas Tech Hazardous Material Disposal Program (Appendix H)
b) Materials in any of the following categories must be disposed of as hazardous materials:
    a. Ignitable - any substance with a flash point below 60°C (140° F).
    b. Corrosive - any substance with pH of less than or equal to 2.0 or greater than or equal to 12.5.
    c. Reactive - any substance which is unstable, reacts violently with water, forms potentially explosive mixtures with water, generates toxic gases, vapors or fumes when mixed with water or exposed to a pH between 2.0 and 12.5, or capable of detonation or explosive decomposition or reaction.
    d. Toxic - any substance which contains any of the compounds listed by the EPA under the Resource Conservation and Recovery Act at or greater than the listed concentration.
    e. Specific chemicals - any substance containing an EPA listed compound.
c) All containers of excess chemicals must contain the following information.
    a. Specific chemical name (in English). If a mixture, list the components and percentage composition. Non- specific identifiers (i.e mixed solvents, mixed aqueous solutions) will be treated as Unknowns (See Section F below)

   b. Contact name (Principal Investigator or their designee).
   c. Location of lab (Building/room number)
   d. Lab phone number

d) All unwanted/excess materials must be inventoried and submitted to EHS prior to disposal via the EHS On-line Chemical Waste Form. http://www.depts.ttu.edu/ehs/Web/WPRequestForms.aspx
e) Infectious waste shall be placed in a biohazard box and disposed of through EH&S. Departments may also choose to autoclave their infectious waste prior to disposal in the regular trash. If departments choose to autoclave their biological waste, autoclaves must be validated weekly using biological indicators and records of validation must be kept and available for inspection
b) Sharps must be placed in an approved sharps container and disposed of by EH&S.
c) Radioisotopes must be disposed of in accordance with the policies of the Radiation Safety Office (http://www.depts.ttu.edu/ehs/Web/RWDRequestForm.aspx).
d) Non hazardous trash must be removed by custodial services.
a) White paper, letterhead, photocopy paper, computer paper, etc. should be placed in recycling containers.

11. Employee Protection

a) When working with a hazardous material the minimum personal protective equipment shall be:
   a. lab coat or other protective clothing
   b. safety glasses or splash goggles
   c. gloves
b) Personal protective equipment shall not be worn outside the lab or work area.
c) EHS may require modifications to the work area or personal protective equipment to assure protection of personnel. Personal protective equipment may include gloves, eye protection, protective clothing, and/or respiratory protection. All personnel requiring respiratory protection will be enrolled in the Texas Tech Respiratory Protection Program managed by EHS.

12. Procedures

a) Chemical Spill Procedure
b) EVALUATE THE SPILL
   a. Are the materials Innocuous, Corrosive, Flammable, Toxic or Explosive?
   b. Identify all materials by common or chemical name.
   c. Estimate how much is spilled.
   d. Evaluate the degree of danger to patients, staff or visitors.
   e. Evaluate the degree of danger to equipment or property.
c) CONTAIN THE SPILL. Utilize any action designed to prevent the spilled material from spreading and causing increased damage.
d) EVACUATE the area if the spill cannot be contained, OR if the spilled material produces irritating odors, flammable vapors or explosive vapors. (extinguish all spark or ignition sources).
e) CLEAN up the spilled material.
   a. Spills of innocuous material can be cleaned up by laboratory personnel or

equipped staff.

b. Spills of acids, bases and flammables and mercury can be cleaned up by laboratory personnel using appropriate neutralizers/absorbents and proper personal protective equipment.
c. Spills of toxic or explosive material, and large spills of corrosive or flammable materials shall be handled by EHS. Immediately call the Emergency Telephone Number for your campus: EHS 742 -3328, Tech Police (742-3931)
    i. Your name and phone number.
    ii. Precise location of spill.
    iii. Exact description of what was spilled (make sure you state any compounds which may form toxic compounds).
    iv. Any steps you have taken to control the spill.
    v. Any injuries that have occurred.

f) DISPOSE of all contaminated materials in accordance with this Policy.
g) Employees who have been exposed to hazardous chemicals due to a spill or other uncontrolled situation shall promptly report to the Occupational Injury Clinic for their campus or to an Adult Emergency Room when the Clinic is not operating. A Report of Incident shall be completed by the individual's supervisor.
h) Consult EHS at 742-3876 with any question regarding chemical spills and spill clean up.

13. Institutional Biological Safety Committee Protocol

a) If your lab works with recombinant DNA, potentially infectious microorganisms, replication defective viral vectors, or human or animal cells or tissues you must have an approved Recombinant DNA and Hazardous Research Materials Protocol. For more information, see http://www.depts.ttu.edu/ehs/Web/BioISafety.aspx
b) Please keep a copy of your approved protocol in the lab Blue Book for reference.

14. Information and Training Program for use of Hazardous Chemicals

a) All Biological Sciences laboratory faculty and staff are required to have annual documented training covering OSHA and EPA topics. EH&S conducts laboratory safety training free of charge to all Texas tech Employees. See http://www.depts.ttu.edu/ehs/Web/TrainModDesc.aspx to register for a training session or to take training online.
b) A certified statement from the employee stating that the laboratory chemical hygiene plan, the training outline (Appendix I), and standard operating procedures for working with chemical carcinogens/toxins have been explained satisfactorily will be included in the laboratory chemical hygiene plan (Appendix I).
c) The principal investigator shall regularly and continuously review the techniques of laboratory staff. The principal investigator shall also assist them in developing better techniques with an emphasis on safety.

15. List of Hazardous Chemicals and Select Carcinogens

See Appendix J for a list of hazardous chemicals and select carcinogens currently used in _____ laboratory, Building _____.

APPENDIX A

Butler Lab Chemical Log

Updated March 2012

National Fire Protection Association:		Health Hazard	Flammability Hazard		Instability Hazard		Special Hazard	
NFPA Rating	Name	Vendor	Catalog #	Weight	Expiration Date	Date Opened/ Received	Location	Notes
	178 Methyltestosterone C-IIIN	Sigma	M7252-25G	25 g			4 Degree	
	19 - Nortestosterone 17 - Decanoate	Sigma	N-6633	1 g		4/18/90	213A	
	A286982	TOCRIS	4228	10 mg			213A	
	AccuGel 29:1 Acrylamide/Bis-Acrylamide Solution	National Diagnostics	EC-852	1L			4 Degree	
2	Adenosine 5' - triphosphate disodium salt	Sigma	A7699-5G	5 g			-20 Chest	
	Agar	Sigma	A1296	1 kg			213A	
1	Agar	Fisher	BP26411	1 kg			213A	
2	1 OX Ammonium Persulfate for electrophoresis	Sigma	A-3678	100 g			213A	
	Ammonium Sulfate anhydrous	Sigma	A-5132	500 g			213A	
	Anti - Goat IgG (whole molecule - peroxidase antibody produced in rabbit	Sigma	A5420-1ML	1 mL			-20 Chest	
	Anti - Mouse IgG (whole molecule) peroxidase, produced in goat	Sigma	A4416-1ML	1 mL			-20 Chest	
3	Antibiotic Antimycotic Solution Stabilized bio Reagent suitable for cell culture sterile filtered	Sigma	A5955-100ML	100 mL			-20 Freezer	
3	Antibiotic Antimycotic Solution Stabilized bio Reagent suitable for cell culture sterile filtered	Sigma	A5955-100ML	100 mL			-20 Freezer	
3	Antibiotic Antimycotic Solution Stabilized bio Reagent suitable for cell culture sterile filtered	Sigma	A5955-100ML	100 mL			-20 Freezer	
3	Antibiotic Antimycotic Solution Stabilized bio Reagent suitable for cell culture sterile filtered	Sigma	A5955-100ML	100 mL			-20 Freezer	
	Antibody produced in rabbit	Sigma	A9044-2ML	2 mL			-20 Chest	
	Aphidicolin	Sigma	A-0781	1 mg			4 Degree	
	B-estradiol `	Sigma	E2758-1G	1 g			4 Degree	
	Bacto - Agar	BD	214010	454 g		7/23/02	213A	
	Bacto - Yeast Extract	BD	212750	500 g			213A	
2	Benzamidine Hydrochloride Hydrate	Sigma	B6506-5G	5 g			4 Degree	
	Benzyl Benzoate	Sigma	B9550-500mL	500 mL			213A	
1	Calcium Chloride Anhydrous	EM	CX0160-1	500 g			213A	
1	Calcium Chloride Anhydrous	EM	CX0160-1	500 g			213A	
2 1	Calcium Chloride Dihydrate	Fisher		500 g			213A	
	Calphostin C	Sigma	C-6303	0.1 mg			4 Degree	
3	Cantharidin	Sigma	C7632-100MG	100 mg			4 Degree	
3	Carbenicillin Disodium	Sigma	C9231-10G	10 g			4 Degree	
3	Carbenicillin Disodium	Sigma	C9231-10G	10 g			4 Degree	
	Catalase C-9322	Sigma	C-9322	1 g			4 Degree	
	Ciprofloxacin	Fluka/Sigma	17850-25G-F	25 g			213A	

APPENDIX B

Standard Buffers and Acronyms

Updated April 2012

National Fire Protection Association:		Health Hazard	Flammability Hazard	Instability Hazard	Special Hazard	
NFPA Rating	Acronym	Name	Concentration	pH		Location
	$CaCl_2$	Calcium Chloride	1M			Butler Bench
	EDTA	Ethylenediaminetetraacetic Acid	0.5M			Butler Bench
		HI-Tris	2M	8.00		Butler Bench
3	HCl	Hydrocloric Acid	1M			Butler Bench
	LB	Luria Broth	1X, 5X			Butler Bench
	MgCl	Magnesium Chloride	1M			Butler Bench
		Manganese (II) Chloride Solution	1M			Butler Bench
	Hepes	N-2-Hydroxyethlpiperazine-N'-2-Ethanesulfonic Acid		7.20		Butler Bench
	PBS	Phosphate Buffered Saline				Butler Bench
	PBST	Phosphate Buffered Tween-20				Butler Bench
	KCl	Potassium Chloride	1M			Butler Bench
	SSC	Saline Sodium Citrate Buffer				Butler Bench
	$NaHCO_3$	Sodium Bicarbonate	7.50%			Butler Bench
		Sodium Borate				Butler Bench
	NaCl	Sodium Chloride	1M, 2M			Butler Bench
2	SDS	Sodium Dodecyl Sulfate	10%			Butler Bench
3	NaOH	Sodium Hydroxide	1M			Butler Bench
3	S.O.C.	Super Optimal Broth	1X			Butler Bench
		Transfer Buffer	1X, 10X			Butler Bench
		Tris	0.5M, 1M, 1.5M	4.20, 6.80, 7.40, 7.80, 8.00, 8.80		Butler Bench
	TBS	Tris Buffered Saline				Butler Bench
	TE	Tris EDTA				Butler Bench
	Tris-HCl	Tris HCl	1M	7.60		Butler Bench
	TBST	Tris Buffered Saline Tween-20	1X			Butler Bench
	TRIS Buffer	Tris(hydroxymethyl)aminomethane				Butler Bench
	TAE	Tris/Acetate/EDTA	1X, 10X			
	TBE	Tris/Borate/EDTA	1X, 10X			

APPENDIX C
Hazardous Chemical List

This list is based on OSHA's z-list but is not all-inclusive. For complete hazard information, refer to the MSDS for each chemical or contact EH&S.

Acetaldehyde
Acetic anhydride Acetone
2-Acetylaminofluorine
Acetylene tetrabromide
Acrylamide
Aldrin
Allyl chloride
Allyl propyl disulfide
Aluminum
2-Aminoethanol
Ammonia
n-Amyl acetate
Aniline and homologs
Antimony and compounds ANTU
Asbestos
Barium sulfate
Benzene
p-Benzoquinone
Benzoyl peroxide
Beryllium and beryllium compounds
Bismuth telluride
Boron trifluoride
Butadiene (1,3-Butadiene)
2-Butanone (Methyl ethyl ketone)
n-Butyl-acetate
tert-Butyl acetate
sec-Butyl alcohol
Butylamine
n-Butyl glycidyl ether (BGE)
p-tert-Butyltoluene
Calcium oxide
Calcium sulfate
Carbaryl (Sevin)
Carbon dioxide
Carbon monoxide
Chlordane
Chlorinated diphenyl oxide
Chlorine dioxide
Chloroacetaldehyde
Phenacyl chloride
2-Chloro-6-(trichloromethyl) pyridine
Chromium (II)
Chromium metal and insol. Salts
Clopidol

Acetic acid
Acetonitrile
Acetylene dichloride
Acrolein
Acrylonitrile
Allyl alcohol
Allyl glycidyl ether
alpha-Alumina
4-Aminodiphenyl
2-Aminopyridine
Ammonium sulfamate
sec-Amyl acetate
Anisidine (o-, p-isomers)
Arsenic compounds
Azinphos-methylBarium
Benomyl
Benzidine
Benzo(a)pyrene
Benzyl chloride
Biphenyl
Boron oxide
Bromine
Butanethiol
2-Butoxyethanol
sec-Butyl acetate
n-Butyl alcohol
tert-Butyl alcohol
tert-Butyl chromate
Butyl mercaptan
Cadmium
Calcium silicate
Camphor, synthetic
Carbon black
Carbon disulfide
Carbon tetrachloride
Chlorinated camphene
Chlorine
Chlorine trifluoride
a-Chloroacetophenone
Chlorobenzene
Chromic acid
Chromium (III)
Chrysene
Coal dust

Cobalt
Copper
Cotton dust
Crag herbicide (Sesone)
Cresol
Crotonaldehyde
Cumene
Cyanides
Cyclohexane
Cyclohexanol
Cyclohexanone
Cyclohexene
Cyclopentadiene
2,4-D(Dichlorophenoxyacetic acid)
Decaborane
Demeton (Systox)
Diacetone alcohol (4-Hydroxy-4-methyl-2-pentanone)
1,2-Diaminoethane
Diazomethane
Diborane
1,2-Dibromo-3-chloropropane (DBCP)
1,2-Dibromoethane
Dibutyl phosphate
o-Chlorobenzylidene malononitrile
Chlorobromomethane
2-Chloro-1,3-butadiene
Chlorodiphenyl
1-Chloro-2,3-epoxypropane
2-Chloroethanol
Chloroethylene
Chloroform (Trichloromethane)
bis(Chloromethyl) ether
Chloromethyl methyl ether
1-Chloro-1-nitropropane
Dicyclopentadienyl iron
Dieldrin
Diethylamine
2-Diethylaminoethanol
Diethyl ether
Difluorodibromomethane
Diglycidyl ether (DGE)
Dihydroxybenzene
Diisobutyl ketone
Diisopropylamine
4-Dimethylaminoazobenzene
Dimethoxymethane
Dimethyl acetamide
Dimethylamine
Dimethylaminobenzene (N,N-Dimethylaniline)
Dimethylaniline
Dimethylbenzene
Dimethyl-1,2-dibromo-2,2-dichloroethyl phosphate
Dimethylformamide
2,6-Dimethyl-4-heptanone ketone
1,1-Dimethylhydrazine
Dimethylphthalate
Dimethyl sulfate
Dinitrobenzene (all isomers)
Dinitro-o-cresol
Dinitrotoluene
Dioxane (Diethylene dioxide)
Diphenyl (Biphenyl)
Diphenylmethane diisocyanate
Dipropylene glycol methyl ether
Di-sec octyl phthalate
(Di-(2-ethylhexyl)phthalate)
Dibutyl phthalate
o-Dichlorobenzene
p-Dichlorobenzene
3,'-Dichlorobenzidine
Dichlorodifluoromethane
1,3-Dichloro-5,5-dimethyl hydantoin
Dichlorodiphenyltrichloroethane (DDT)
1,1-Dichloroethane
1,2-Dichloroethane
1,2-Dichloroethylene
Dichloroethyl ether
Dichloromethane
Dichloromonofluoromethane
1,1-Dichloro-1-nitroethane
1,2-Dichloropropane

Dichlorotetrafluoroethane
Dichlorvos (DDVP)
Ethyl chloride
Ethyl ether
Ethyl formate
Ethyl mercaptan
Ethyl silicate
Ethylene chlorohydrin
Ethylenediamine
Ethylene dibromide
Ethylene dichloride
(1,2-Dichloroethane)
Ethylene glycol dinitrate
Ethylene glycol methyl acetate
Ethyleneimine
Ethylene oxide
Ethylidene chloride
N-Ethylmorpholine
Ferbam
Ferrovanadium dust
Fluorides (as F)
Fluorine
Fluorotrichloromethane(Trichlorofluoromethane)
Formaldehyde
Formic acid
Furfural
Furfuryl alcohol
Glycerin (mist)
Glycidol
Endrin
Epichlorohydrin
EPN
1,2-Epoxypropane
2,3-Epoxy-1-propanol
Ethanethiol
Ethanolamine
2-Ethoxyethanol (Cellosolve)
2-Ethoxyethyl acetate
(Cellosolve acetate)
Ethyl acetate
Ethyl acrylate
Ethyl alcohol (Ethanol)
Ethylamine
Ethyl amyl ketone (5-Methyl-3-heptanone)
Ethyl benzene
Ethyl bromide
Ethyl butyl ketone (3-Heptanone)
Iron oxide fume
Isoamyl acetate
Isoamyl alcohol (primary and secondary)
Isobutyl acetate
Isobutyl alcohol
Isophorone
Isopropyl acetate
Isopropyl alcohol
Isopropylamine
Isopropyl ether
Isopropyl glycidyl ether (IGE)
Kaolin
Ketene
Lead, inorganic (as Pb)
Limestone
Lindane
Lithium hydride
L.P.G. (Liquefied petroleum gas)
Magnesite
Magnesium oxide fume
Malathion
Maleic anhydride
Manganese compounds (as Mn)
Mercury
Mesityl oxide
Methanethiol
Glycol monoethyl ether
Graphite
Gypsum
Hafnium
Heptane (n-Heptane)
Hexachloroethane
Hexachloronaphthalene
n-Hexane
2-Hexanone (Methyl n-butyl ketone)
Hexone (Methyl isobutyl
ketone)
Methoxychlor
2-Methoxyethanol (Methyl cellosolve)
2-Methoxyethyl acetate (Methyl cellosolve acetate)
Methyl acetate
Methyl acetylene (Propyne)

Methyl acetylene-propadiene mixture
Methyl acrylate
Methylal (Dimethoxy-methane)
Methyl alcohol
Methylamine
Methyl amyl alcohol
Methyl n-amyl ketone
Methyl bromide
Methyl butyl ketone
Methyl cellosolve
Methyl cellosolve acetate
Methyl chloride
Methyl chloroform
Methylcyclohexane
Methylcyclohexanol
o-Methylcyclohexanone
Methylene chloride
Methyl ethyl ketone (MEK)
PCB
Pentaborane
Pentachloronaphthalene
Pentachlorophenol
Pentaerythritol
Pentane
2-Pentanone (Methyl propyl ketone)
Perchloroethylene (Tetrachloroethylene)
Perchloromethyl mercaptan
Perchloryl fluoride
Phenol
p-Phenylene diamine
Phenyl ether, vapor
Phenyl ether-biphenyl mixture
Phenylethylene
Phenyl glycidyl ether (PGE)
Phenylhydrazine
Phosdrin (Mevinphos)
Phosgene (Carbonyl chloride)
Phosphine
sec-Hexyl Hydrazine
Hydrogen bromide
Hydrogen chloride
Hydrogen cyanide
Hydrogen fluoride (as F)
Hydrogen peroxide
Hydrogen selenide
Hydrogen suldide
Hydroquinone Iodine
Methyl formate
Methyl hydrazine
Methyl iodide
Methyl isoamyl ketone
Methyl isobutyl carbinol
Methyl isobutyl ketone
Methyl isocyanate
Methyl mercaptan
Methyl methacrylate
Methyl propyl ketone
alpha-Methyl styrene
Methylene bisphenyl isocyanate (MDI)
Molybdenum (as Mo)
Monomethyl aniline
Monomethyl hydrazine
Morpholine
Naphtha (Coal tar)
Naphthalene
alpha-Naphthylamine
beta-Naphthylamine
Nickel carbonyl
Nickel
Nicotine
Nitric acid
Nitric oxide
p-Nitroaniline
Nitrobenzene
p-Nitrochlorobenzene
4-Nitrodiphenyl
Nitroethane
Nitrogen dioxide
Nitrogen trifluoride
Nitroglycerin
Nitromethane
1-Nitropropane
2-Nitropropane
N-Nitrosodimethylamine
Nitrotoluene (all isomers)
Nitrotrichloromethane
Octachloronaphthalene
Octane
Oil mist, mineral
Osmium tetroxide (as Os)
Oxalic acid

Oxygen difluoride
Ozone
Paraquat
Parathion
Silicon
Silicon carbide
Silver
Sodium fluoroacetate
Sodium hydroxide
Stibine
Stoddard solvent
Strychnine
Styrene
Sulfur dioxide
Sulfur hexafluoride
Sulfuric acid
Sulfur monochloride
Sulfur pentafluoride
Phosphoric acid
Phosphorus (yellow)
Phosphorus pentachloride
Phosphorus pentasulfide
Phosphorus trichloride
Phthalic anhydride
Picloram
Picric acid
Pindone (2-Pivalyl-1,3-indandione)
Platinum (as Pt)
Propane
beta-Propriolactone
n-Propyl acetate
n-Propyl alcohol
n-Propyl nitrate
Propylene dichloride
Propylene imine
Propylene oxide
Propyne
Pyrethrum
Pyridine
Quinone
RDX
Rhodium
Ronnel
Rotenone
Selenium compounds
Selenium hexafluoride
Silica, amorphous
Trichloroethylene
Trichloromethane
Trichloronaphthalene
1,2,3-Trichloropropane
1,1,2-Trichloro-1,2,2-trifluoroethane
Triethylamine
Trifluorobromomethane
2,4,6-Trinitrophenol
2,4,6-Trinitrophenylmethylnitramine
2,4,6-Trinitrotoluene (TNT)
Triorthocresyl phosphate
Triphenyl phosphate
Turpentine
Uranium
Sulfuryl fluoride Systox;
2,4,5-T Tantalum
TEDP (Sulfotep)
Tellurium and compounds
Tellurium hexafluoride
Temephos
TEPP (Tetraethyl pyrophosphate)
Terphenyls
1,1,1,2-Tetrachloro-2,2-difluoroethane
1,1,2,2-Tetrachloro-1,2-difluoroethane
1,1,2,2-Tetrachloroethane
Tetrachloroethylene
Tetrachloromethane
Tetrachloronaphthalene
Tetraethyl lead
Tetrahydrofuran
Tetramethyl lead
Tetramethyl succinonitrile
Tetranitromethane
Tetryl (2,4,6-Trinitrophenylmethylnitramine)
Thallium
4,4'-Thiobis
Thiram
Tin
Titanium dioxide
Toluene Toluene-2,4-diisocyanate (TDI)
o-Toluidine Toxaphene
Tremolite
Tributyl phosphate
1,1,1-Trichloroethane
1,1,2-Trichloroethane

Vanadium	Vinyl benzene
Vinyl chloride	Vinyl cyanide
Vinyl toluene	Warfarin
Xylenes (o-, m-, p-isomers)	Xylidine
Yttrium	Zinc chloride fume
Zinc oxide fume	Zinc oxide
Zinc stearate	Zirconium compound

Select Carcinogens List

Chemical	CAS Number
A-alpha-C (2-Amino-9H-pyrido[2,3-b]indole)	26148-68-5
Acetaldehyde	75-07-0
Acetamide	60-35-5
Acetochlor	34256-82-1
2-Acetylaminofluorene	53-96-3
Acifluorfen	62476-59-9
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Actinomycin D	50-7-60
Adriamycin (Doxorubicin hydrochloride)	23214-92-8
AF-2;[2-(2-furyl)-3-(5-nitro-2-furyl)]acrylamide	3688-53-7
Aflatoxins	1402-68-2
Alachlor	15972-60-8
Alcoholic beverages, when associated with alcohol abuse	n/a
Aldrin	309-00-2
Allyl chloride	107-05-1
Aluminum products	n/a
2-Aminoanthraquinone	117-79-3
p-Aminoazobenzene	60-09-3
ortho-Aminoazotoluene	97-56-3
4-Aminobiphenyl (4-aminodiphenyl)	92-67-1
3-Amino-9-ethylcarbazole hydrochloride	6109-97-3
1-Amino-2-methylanthraquinone	82-28-0
2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole	712-68-5
Amitrole	61-82-5
Analgesic mixtures containing phenacetin	n/a
Androgenic (anabolic) steroids	n/a
Aniline	62-53-3
ortho-Anisidine	90-04-0
ortho-Anisidine hydrochloride	134-29-2
Antimony oxide (antimony trioxide)	1309-64-4
Aramite	140-57-8
Arsenic (inorganic arsenic compounds)	various
Asbestos	1332-21-4
Auramine	492-80-8
Azaserine	115-02-6

Azathioprine	446-86-6
Azacitidine	320-67-2
Azobenzene	103-33-3
Benz[a]anthracene	56-55-3
Benzene	71-43-2
Benzidine [and its salts]	92-87-5
Benzidine-based dyes	various
Benzo[b]fluoranthene	205-99-2
Benzo[j]fluoranthene	205-82-3
Benzo[k]fluoranthene	207-08-9
Benzofuran	271-89-6
Benzo[a]pyrene	50-32
Benzotrichloride	98-07-7
Benzyl chloride	100-44-7
Benzyl violet 4B	1694-09-3
Beryllium and beryllium compounds	various
Betel quid with tobacco	n/a
Bis(2-chloroethyl)ether	111-44-4
N,N-Bis(2-chloroethyl)-2- naphthylamine (Chlornapazine)	494-03-1
Bischloroethyl nitrosourea (BCNU) (Carmustine)	154-93-8
Bis(chloromethyl)ether	542-88-1
Bitumens, extracts of steam-refined and air refined	various
Bleomycins	various
Bracken fern	n/a
Bromodichloromethane	75-27-4
Bromoform	75-25-2
1,3-Butadiene	106-99-0
1,4-Butanediol dimethanesulfonate (Busulfan)	55-98-1
Butylated hydroxyanisole (BHA)	25013-16-5
beta-Butyrolactone	3068-88-0
Cadmium and cadmium compounds	various
Caffeic acid	331-39-5
Captafol	2425-06-1
Captan	133-06-2
Carbon tetrachloride	56-23-5
Carbon-black extracts	n/a
Carrageenan, degraded	n/a
Ceramic fibers (airborne particles of respirable size)	n/a
Certain combined chemotherapy drugs for lymphomas	n/a
Chlorambucil	305-03-3
Chloramphenicol	56-75-7
Chlordane	57-74-9
Chlordecone (Kepone)	143-50-0
Chlordimeform	6164-98-3
Chlorendic acid (approximately 60 percent chlorine by weight)	115-28-6
Chlorinated Parrafins (C12, 60% chlorine)	108171-26-2
alpha-Chlorinated toluenes	various

p-Chloroaniline	106-47-8
Chlorodibromomethane	124-48-1
Chloroethane (ethyl chloride)	75-00-3
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (Lomustine)	13010-47-4
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)	13909-09-6
Chloroform	67-66-3
Chloromethyl methyl ether (technical grade)	107-30-2
3-Chloro-2-methylpropene	563-47-3
4-Chloro-ortho-phenylenediamine	95-83-0
p-Chloro-o-toluidine	95-69-2
Chlorophenols	various
Chlorophenoxy herbicides	various
Chlorothalonil	1897-45-6
Chlorozotocin	54749-90-5
Chromium (hexavalent compounds)	various
Chrysene	218-01-9
C. I. Acid Red 114	6459-94-5
C. I. Basic Red 9 monohydrochloride	569-61-9
Ciclosporin (Cyclosporin A; Cyclosporine)	59865-13-3
Cinnamyl anthranilate	87-29-6
Cisplatin	15663-27-1
Citrus Red No. 2	6358-53-8
Coal gasification	n/a
Coal-tar pitches	n/a
Coal-tars	n/a
Cobalt metal powder	7440-48-4
Cobalt [II] oxide	1307-96-6
Coke Production oven emissions	n/a
Conjugated estrogens	n/a
Creosotes	n/a
para-Cresidine	120-71-8
Cupferron	135-20-6
Cycasin	14901-08-7
Cyclophosphamide (anhydrous)	50-18-0
Cyclophosphamide (hydrated)	6055-19-2
D&C Orange No. 17	3468-63-1
D&C Red No. 8	2092-56-0
D&C Red No. 9	5160-02-1
D&C Red No. 19	81-88-9
Dacarbazine	4342-03-4
Daminozide	1596-84-5
Dantron (Chrysazin; 1,8-Dihydroxyanthraquinone)	117-10-2
Daunomycin	20830-8-13
DDD (Dichlorodiphenyldichloroethane)	72-5-48
DDE (Dichlorodiphenyldichloroethylene)	72-55-9
DDT (Dichlorodiphenyltrichloroethane)	50-29-3
DDVP (Dichlorvos)	62-73-7
N,N'-Diacetylbenzidine	613-35-4

2,4-Diaminoanisole	615-05-4
2,4-Diaminoanisole sulfate	39156-41-7
4,4'-Diaminodiphenyl ether (4,4'-Oxydianiline)	101-80-4
2,4-Diaminotoluene	95-80-7
Diaminotoluene (mixed)	n/a
Dibenz[a,h]acridine	226-36-8
Dibenz[a,j]acridine	224-42-0
Dibenz[a,h]anthracene	53-70-3
7H-Dibenzo[c,g]carbazole	194-59-2
Dibenzo[a,e]pyrene	192-65-4
Dibenzo[a,h]pyrene	189-64-0
Dibenzo[a,i]pyrene	189-55-9
Dibenzo[a,l]pyrene	191-30-0
1,2-Dibromo-3-chloropropane (DBCP)	96-12-8
1,2-Dibromoethane	106-93-4
2,3-Dibromo-1-propanol	96-13-9
p-Dichlorobenzene	106-46-7
3,3'-Dichlorobenzidine	91-9-41
3,3'-Dichlorobenzidine 2HCl	612-83-9
1,4-Dichloro-2-butene	764-41-0
3,3'-Dichloro-4,4'-diaminodiphenyl ether	28434-86-8
1,1-Dichloroethane	75-34-3
1,2-Dichloroethane	107-06-2
Dichloromethane (Methylene chloride)	75-09-2
1,2-Dichloropropane	78-87-5
1,3-Dichloropropene (technical grade)	542-75-6
Dieldrin	60-57-1
Dienestrol	84-17-3
Diepoxybutane	1464-53-5
Diesel engine exhaust	n/a
Di(2-ethylhexyl)phthalate	117-81-7
1,2-Diethylhydrazine	1615-80-1
Diethyl sulfate	64-67-5
Diethylstilbestrol	56-53-1
Diglycidyl resorcinol ether (DGRE)	101-90-6
Dihydrosafrole	94-58-6
Diisopropyl sulfate	2973-10-6
3,3'-Dimethoxybenzidine (ortho-Dianisidine)	119-90-4
3,3'-Dimethoxybenzidine dihydrochloride (ortho-dianisidine dihydrochloride)	20325-40-0
para-Dimethylaminoazobenzene	60-11-7
4-Dimethylaminoazobenzene	60-11-7
trans-2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4- oxadiazole	55738-54-0
7,12-Dimethylbenz(a)anthracene	57-97-6
3,3'-Dimethylbenzidine (ortho-Tolidine)	119-93-7
3,3'-Dimethylbenzidine dihydrochloride	612-82-8
Dimethylcarbamoyl chloride	79-44-7
1,1-Dimethylhydrazine (UDMH)	57-14-7
1,2-Dimethylhydrazine	540-73-8

Dimethyl sulfate	77-78-1
Dimethylvinyl Chloride	513-37-1
1,6-Dinitropyrene	42397-64-8
1,8-Dinitropyrene	42397-65-9
2,4-Dinitrotoluene	121-14-2
2,6-Dinitrotoluene	606-20-2
1,4-Dioxane	123-91-1
Diphenylhydantoin (Phenytoin)	57-41-0
Diphenylhydantoin (Phenytoin), sodium salt	630-93-3
Direct Black 38 (technical grade)	1937-37-7
Direct Blue 6 (technical grade)	2602-46-2
Direct Brown 95 (technical grade)	16071-86-6
Disperse Blue 1	2475-45-8
Epichlorohydrin	106-89-8
Erionite	12510-42-8
Estradiol 17B	50-28-2
Estrone	53-16-7
Ethinylestradiol	57-63-6
Ethyl acrylate	140-88-5
Ethyl methanesulfonate	62-50-0
Ethyl-4,4'-dichlorobenzilate	510-15-6
Ethylene dibromide	106-93-4
Ethylene dichloride (1,2-Dichloroethane)	107-06-2
N-Ethyl-N-nitrosourea	759-73-9
Ethylene oxide	75-21-8
Ethylene thiourea	96-45-7
Ethyleneimine	151-56-4
Folpet	133-07-3
Formaldehyde (gas or aqueous solution)	50-00-0
2-(2-Formylhydrazino)-4-(5-nitro-2-furyl) thiazole	3570-75-0
Furan	110-00-9
Furazolidone	67-45-8
Furmecyclox	60568-05-0
Fusarin C	79748815
Gasoline engine exhaust (condensates/extracts)	n/a
Glasswool fibers (airborne particles of respirable size)	n/a
Glu-P-1 (2-Amino-6-methyldipyrido[1,2- a:3', 2'-d]imidazole)	67730-11-4
Glu-P-2 (2-Aminodipyrido[1,2-a:3',2'-d]imidazole)	67730-10-3
Glycidaldehyde	765-34-4
Glycidol	556-52-5
Griseofulvin	126-07-8
Gyromitrin (Acetaldehyde methylformylhydrazone)	16568-02-8
HC Blue 1	2784-94-3
Heptachlor	76-44-8
Heptachlor epoxide	1024-57-3
Hexachlorobenzene	118-74-1
Hexachlorocyclohexanes (technical grade)	various
Hexachlorodibenzodioxin	34465-46-8

Hexachloroethane	67-72-1
Hexamethylphosphoramide	680-31-9
Hydrazine	302-01-2
Hydrazine sulfate	10034-93-2
Hydrazobenzene (1,2-Diphenylhydrazine)	122-66-7
Indeno [1,2,3-cd]pyrene	193-39-5
IQ (2-Amino-3-methylimidazo[4,5-f]quinoline)	76180-96-6
Iron dextran complex	9004-66-4
Isosafrole	120-58-1
Kepone (Chlordecone)	143-50-0
Lactofen	77501-63-4
Lasiocarpine	303-34-4
Lead acetate	301-04-2
Lead and lead compounds	various
Lead phosphate	7446-27-7
Lindane and other hexachlorocyclohexane isomers	various
Mancozeb	8018-01-7
Maneb	12427-38-2
Me-A-alpha-C (2-Amino-3-methyl-9H-pyrido[2, 3-b]indole)	68006-83-7
Medroxyprogesterone acetate	71-58-9
MeIQ(2-Amino-3,4-dimethylimidazo[4,5-f]quinoline)	7094112
MeIQx(2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline)	7500-04-0
Melphalan	148-82-3
Merphalan	531-76-0
Mestranol	72-33-3
Methoxsalen with ultraviolet A therapy	n/a
8-Methoxypsoralen with ultraviolet A therapy	298-81-7
5-Methoxypsoralen with ultraviolet A therapy	484-20-8
2-Methylaziridine (Propyleneimine)	75-55-8
Methylazoxymethanol	590-96-5
Methylazoxymethanol acetate	592-62-1
3-Methylcholanthrene	56-49-5
5-Methylchrysene	3697-24-3
4,4'-Methylene bis(2-chloroaniline) (MOCA)	101-14-4
4,4'-Methylene bis(N,N-dimethyl)benzenamine	101-61-1
4,4'-Methylene bis(2-methylaniline)	838-88-0
4,4'-Methylenedianiline	101-77-9
4,4'-Methylenedianiline dihydrochloride	13552-44-8
Methylhydrazine and its salts	13552-44-8
Methyl chloromethyl ether	107-30-2
Methyl-CCNU	13909-09-6
Methyl iodide	74-88-4
Methyl methanesulfonate	66-27-3
2-Methyl-1-nitroanthraquinone (of uncertain purity)	129-15-7
N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	70-25-7
N-Methyl-N-nitrosourea	
N-Methylolacrylamide	924-42-5

Methylthiouracil	56-04-2
Metiram	9006-4222
Metronidazole	443-48-1
Michler's ketone	90-94-8
Mineral Oils, untreated and mildly treated	n/a
Mirex	2385-85-5
Mitomycin C	50-07-7
MOPP	
Monocrotaline	135-22-0
5-(Morpholinomethyl)-3-[(5-nitro-fufurylidene)-amino]-2-oxazolidinone	139-91-3
Mustard gas	505-60-2
Nafenopin	3771-19-5
1-Naphthylamine	134-32-7
2-Naphthylamine	91-59-8
3-Naphthylamine	
Nickel and certain nickel compounds	various
Nickel carbonyl	13463-3933
Nickel refinery dust, from the pyrometallurgical process	7440-02-0
Nickel subsulfide	12035-72-2
Niridazole	61-57-4
Nitrilotriacetic acid	139-13-9
Nitrilotriacetic acid, trisodium salt monohydrate	18662-53-8
5-Nitroacenaphthene	602-87-9
5-Nitro-o-anisidine	99-59-2
o-Nitroanisole	91-23-6
4-Nitrobiphenyl	92-93-3
6-Nitrochrysene	7496-02-8
Nitrofen (technical grade)	1836-755
2-Nitrofluorene	607-57-8
Nitrofurazone	59-87-0
1-[(5-Nitrofurfurylidene)amino]-2-imidazollidinone	555-84-0
1-[(5-Nitrofurfurylidene)-N-[4-(5-Nitro-2-furyl)-2 thiazolyl]acetamide	531-82-8
Nitrogen mustard (Mechlorethamine)	51-75-2
Nitrogen mustard hydrochloride (Mechlorethamine hydrochloride)	55-86-7
Nitrogen mustard N-oxide	126-85-2
Nitrogen mustard N-oxide hydrochloride	302-70-5
2-Nitropropane	79-46-9
4-Nitropyrene	57835-92-4
N-Nitrosodi-n-butylamine	924-16-3
N-Nitrosodiethanolamine	1116-54-7
N-Nitrosodiethylamine	55-18-5
N-Nitrosodimethylamine	62-75-9
p-Nitrosodiphenylamine	156-10-5
N-Nitrosodiphenylamine	86-30-6
N-Nitrosodi-n-propylamine	621647
N-Nitroso-N-ethylurea	759-73-9

3-(N-Nitrosomethylamino)propionitrile	60153-49-3
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	64091-91-4
N-Nitrosomethylethylamine	10595-95-6
N-Nitroso-N-methylurea	684-93-5
N-Nitroso-N-methylurethane	615--532
N-Nitrosomethylvinylamine	4549-40-0
N-Nitrosomorpholine	59-89-2
N-Nitrosonornicotine	16543-55-8
N-Nitrosopiperidine	100-75-4
N-Nitrosopyrrolidine	930-55-2
N-Nitrososarcosine	13256-22-9
Norethisterone (Norethindrone)	68-22-4
Ochratoxin A	303-47-9
Oestrogen replacement therapy	n/a
Oestrogen, nonstreoidal	
Oestrogen, steroidal	
Oil Orange SS	2646-17-5
Oral contraceptives, combined	n/a
Oral contraceptives, sequential	n/a
4,4'-Oxydianiline	101-80-4
Oxadiazon	19666-30-9
Oxymetholone	434-07-1
Oxazepam	604-75-1
Panfuran S	794-93-4
Pentachlorophenol	87-86-5
Phenacetin	62-44-2
Phenazopyridine hydrochloride	136-40-3
Phenesterin	3546-10-9
Phenobarbital	50-06-6
Phenoxybenzamine	59961
Phenoxybenzamine hydrochloride	63-92-3
Phenyl glycidyl ether	122-60-1
Phenylhydrazine and its salts	various
o-Phenylphenate, sodium	132-27-4
Phenytoin	57-41-0
PhiP(2-Amino-1-methyl-6-phenylimidazol[4,5-b]pyridine)	105650-23-5
Polybrominated biphenyls	various
Polychlorinated biphenyls	various
Polychlorinated biphenyls (containing 60 or more percent chlorine by molecular weight)	various
Polychlorinated dibenzo-p-dioxins	various
Polychlorinated dibenzofurans	various
Polycyclic aromatic hydrocarbons	various
Polygeenan	53973-98-1
Ponceau MX	3761-53-3
Ponceau 3R	3564-09-8
Potassium bromate	7758-01-2
Procarbazine	671-16-9

Procarbazine hydrochloride	366-70-1
Procymidone	32809-16-8
Progesterone	57-83-0
Progestins	various
1,3-Propane sultone	1120-71-4
Progargite	2312-35-8
beta-Propiolactone	57-57-8
Propylene oxide	75-56-9
Propylthiouracil	51-52-5
Radionuclides	various
Radon	10043-92-2
Reserpine	50-55-5
Residual (heavy) fuel oils	n/a
Saccharin	81-07-2
Saccharin, sodium	128-44-9
Safrole	94-59-7
Selenium sulfide	7446-34-6
Shale-oils	68308-34-9
Silica, crystalline (airborne particles of respirable size)	n/a
Sodium ortho-phenylphenate	
Soots, tars, and mineral oils (untreated and mildly treated oils and used engine oils)	n/a
Sterigmatocystin	10048-13-2
Streptozotocin	18883-66-4
Styrene	100-42-5
Styrene oxide	96-09-3
Sulfallate	95-06-7
Talc containing asbestiform fibers	n/a
Terrazole	2593-15-9
Testosterone and its esters	58-22-0
2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD)	1746-01-6
1,1,2,2-Tetrachloroethane	79-34-5
Tetrachloroethylene (Perchloroethylene)	127-18-4
p-a,a,a-Tetrachlorotoluene	5216-25-1
Tetranitromethane	509-14-8
Thioacetamide	62-55-5
4,4'-Thiodianiline	139-65-1
Thiourea	62-56-6
Thorium dioxide	1314-20-1
Tobacco, oral use of smokeless products	n/a
Tobacco smoke	n/a
Toluene diisocyanate	26471-62-5
ortho-Toluidine	95-53-4
ortho-Toluidine hydrochloride	636-21-5
para-Toluidine	106-49-0
Toxaphene (Polychorinated camphenes)	8001-35-2
Treosulfan (Tresoluphan)	299-75-2
Trichlormethine (Trimustine hydrochloride)	817-09-4
2,4,6-Trichlorophenol	88-06-2

1,2,3-Trichloropropane	96-18-4
Triphenyltin hydroxide	76-87-9
Trichloroethylene	79-01-6
Tris(aziridinyl)-para-benzoquinone (Triaziquone)	68-76-8
Tris(1-aziridinyl)phosphine sulfide (Thiotepa)	52-24-4
Tris(2-chloroethyl) phosphate	115-96-8
Tris(2,3-dibromopropyl)phosphate	126-72-7
Trp-P-1 (Tryptophan-P-1) (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole)	62450-06-0
Trp-P-2 (Tryptophan-P-2) (3-Amino-1-methyl-5H-pyrido[4,3-b]indole)	62450-07-1
Trypan blue (commercial grade)	72-57-1
Unleaded gasoline (wholly vaporized)	n/a
Uracil mustard	66-75-1
Urethane (Ethyl carbamate)	51-79-6
Vinyl bromide	593-60-2
Vinyl chloride	75-01-4
4-Vinyl-1-cyclohexene diepoxide (Vinyl cyclohexene dioxide)	106-87-6
Vinyl trichloride (1,1,2-Trichloroethane)	79-00-5
2,6-Xylidine (2,6-Dimethylaniline)	87-62-7
Zineb	12122-67-7

Developmental Toxicity

Acetohydroxamic acid	546-88-3
Actinomycin D	50-76-0
All-trans retinoic acid	302-79-4
Alprazolam	28981-97-7
Amikacin sulfate	39831-55-5
Aminoglutethimide	125-84-8
Aminoglycosides	various
Aminopterin	54-62-6
Angiotensin converting enzyme (ACE) inhibitors	various
Anisindione	117-37-3
Aspirin	50-78-2
Barbiturates	various
Benomyl	17804-35-2
Benzphetamine hydrochloride	5411-22-3
Benzodiazepines	various
Bischloroethyl nitrosourea (BCNU) (Carmustine)	1540-93-8
Bromoxynil	1689-84-5
Butabarbital sodium	143-81-7
1,4-Butanediol dimethylsulfonate (Busulfan)	55-98-1
Carbon disulfide	75-15-0
Carbon monoxide	630-08-0
Carboplatin	41575-94-4
Chenodiol	474-25-9
Chlorcyclizine hydrochloride	1620-21-9

Chlorambucil	305-03-3
Chlordecone (Kepone)	143-50-0
Chlordiazepoxide	58-25-3
Chlordiazepoxide hydrochloride	438-41-5
1-(2-Chloroethyl)-3-cyclohexyl-l-nitrosourea (CCNU) (Lomustine)	13010-47-4
Clomiphene citrate	50-41-9
Clorazepate dipotassium	57109-90-7
Cocaine	50-36-2
Colchicine	64-86-8
Conjugated estrogens	n/a
Cyanazine	21725-46-2
Cycloheximide	66-81-9
Cyclophosphamide (anhydrous)	50-18-0
Cyclophosphamide (hydrated)	6055-19-2
Cyhexatin	13121-70-5
Cytarabine	147-94-4
Danazol	17230-88-5
Daunorubicin hydrochloride	23541-50-6
Demeclocycline hydrochloride (internal use)	64-73-3
Diazepam	439-14-5
Dicumarol	66-76-2
Diethylstilbestrol (DES)	56-53-1
Dinocap	39300-45-3
Dinoseb	88-85-7
Diphenylhydantoin (Phenytoin)	57-41-0
Doxycycline (internal use)	564-25-0
Doxycycline calcium (internal use)	94088-85-4
Doxycycline hyclate (internal use)	24390-14-5
Doxycycline monohydrate (internal use)	17086-28-1
Ergotamine tartrate	379-79-3
Ethyl alcohol in alcoholic beverages	n/a
Ethylene glycol monoethyl ether	110-80-5
Ethylene glycol monomethyl ether	109-86-4
Ethylene glycol monoethyl ether acetate	111-15-9
Ethylene glycol monomethyl ether acetate	110-49-6
Ethylene thiourea	96-45-7
Etoposide	33419-42-0
Etretinate	54350-48-0
Fluorouracil	51-21-8
Fluoxymesterone	76-43-7
Flurazepam hydrochloride	1172-18-5
Flutamide	13311-84-7
Halazepam	23092-17-3
Hexachlorobenzene	118-74-1
Ifosfamide	3778-73-2
Iodine-131	10043-66-0
Isotretinoin	4759-48-2
Lead	7439-92-1
Lithium carbonate	554-13-2

Lithium citrate	919-16-4
Lorazepam	846-49-1
Lovastatin	75330-75-5
Medroxyprogesterone acetate	71-58-9
Megestrol acetate	595-33-5
Melphalan	148-82-3
Menotropins	9002-68-0
Meprobamate	57-53-4
Mercaptopurine	6112-76-1
Mercury and mercury compounds	various
Methacycline hydrochloride	3963-95-9
Methimazole	60-56-0
Methotrexate	59-05-2
Methotrexate sodium	15475-56-6
Methyl bromide as a structural fumigant	74-83-9
Methyl mercury (dimethyl mercury)	593-74-8
Methyltestosterone	58-18-4
Midazolam hydrochloride	59467-96-8
Minocycline hydrochloride (internal use)	13614-98-7
Misoprostol	59122-46-2
Mitoxantrone hydrochloride	70476-82-3
Nafarelin acetate	86220-42-0
Neomycin sulfate (internal use)	1405-10-3
Netilmicin sulfate	56391-57-2
Nicotine	54-11-5
Nitrogen mustard (Mechlorethamine)	51-75-2
Nitrogen mustard hydrochloride (Mechlorethamine hydrochloride)	55-86-7
Norethisterone (Norethindrone)	68-22-4
Norethisterone acetate (Norethindrone acetate)	51-98-9
Norethisterone (Norethindrone)/Ethinyl estradiol	68-22-4/57-63-6
Norethisterone (Norethindrone)/Mestranol	68-22-4/72-33-3
Norgestrel	6533-00-2
Oxazepam	604-75-1
Oxytetracycline (internal use)	79-57-2
Oxytetracycline hydrochloride(internal use)	2058-46-0
Paramethadione	115-67-3
Penicillamine	52-67-5
Pentobarbital sodium	63-98-9
Phenprocoumon	435-97-2
Pipobroman	54-91-1
Plicamycin	18378-89-7
Polybrominated biphenyls	922-66-0
Polychlorinated biphenyls	various
Procarbazine hydrochloride	366-70-1
Propylthiouracil	51-52-5
Retinol/retinyl esters, when in daily dosages in excess of 10,000 IU, or 3,000 retinol equivalents. (NOTE: Retinol/retinyl esters are required and essential for maintenance of normal reproductive function. The recommended daily level during pregnancy is 8,000 IU.)	
Ribavirin	36791045

Secobarbital sodium	309-43-3
Streptomycin sulfate	3810-74-0
Tamoxifen citrate	54965-24-1
Temazepam	846-50-4
Testosterone cypionate	58-20-8
Testosterone enanthate	315-37-7
2,3,7,8-Tetrachlorodibenzo-para-dioxin (TCDD)	1746-01-6
Tetracyclines (internal use)	various
Tetracycline (internal use)	60-54-8
Tetracycline hydrochloride (internal use)	64-75-5
Thalidomide	50-35-1
Thioguanine	154-42-7
Tobacco smoke (primary)	n/a
Tobramycin sulfate	108-88-3
Triazolam	28911-01-5
Trilostane	13647-35-3
Trimethadione	127-48-0
Uracil mustard	66-75-1
Urethane	51-79-6
Urofollitropin	26995-91-5
Valproate (Valproic acid)	99-66-1
Vinblastine sulfate	143-67-9
Vincristine sulfate	2068-78-2
Warfarin	81-81-2

Female Reproductive Toxicity

Aminopterin	54-62-6
Anabolic steroids	n/a
Aspirin(NOTE: It is especially important not to use aspirin during the last three months of pregnancy, unless specifically directed to do so by a physician because it may cause problems in the unborn child or complications during delivery.)	50-78-2
Carbon disulfide	75-15-0
Cocaine	50-36-2
Cyclophosphamide (anhydrous)	50-18-0
Cyclophosphamide (hydrated)	6055-19-2
Ethylene oxide	75-21-8
Lead	
Tobacco smoke (primary)	n/a
Uracil mustard	66-75-1

Male Reproductive Toxicity

Anabolic steroids	n/a
Benomyl	17804-35-2
Carbon disulfide	75-15-0
Colchicine	64-86-8
Cyclophosphamide (anhydrous)	50-18-0
1,2-Dibromo-3-chloropropane (DBCP)	96-12-8
m-Dinitrobenzene	99-65-0

o-Dinitrobenzene	528-29-0
p-Dinitrobenzene	100-25-4
Dinoseb	88-85-7
Ethylene glycol monoethyl ether	110-80-5
Ethylene glycol monomethyl ether	109-86-4
Ethylene glycol monoethyl ether acetate	111-15-9
Ethylene glycol monomethyl ether acetate	110-49-6
Hexamethylphosphoramide	680-31-9
Lead	7439-92-1
Nitrofurantoin	67-20-9
Tobacco smoke (primary)	n/a
Uracil mustard	66-75-1

Select Biological Toxins

Toxin	CAS
Abrin A	1393-62-0
Botulinum Toxin A	53597-24-3
Botulinum Toxin B	93384-44-2
Conotoxin TxVIA	153700-13-1
Conotoxin SI	115797-06-3
Conotoxin GS	115757-31-8
Diacettoxyscirpenol (DAS)	2270-40-8
Ricin	9009-86-3
Saxotoxin	35523-89-8
Shigatoxin	75757-64-1
T-2 Toxin	21259-20-1
Tetrodotoxin	4368-28-9

APPENDIX D

1. STORAGE AND HANDLING OF PEROXIDE FORMING CHEMICALS

Materials that are susceptible to peroxide formation are ones that typically react with air, moisture or impurities and produce a change in their chemical composition in normal storage. Certain organic solvents are susceptible to peroxide formation and can form potentially explosive peroxides over time. Unless these materials are properly handled they can pose a serious safety hazard to users and a difficult disposal problem to the Environmental Health and Safety Office.

Peroxide forming chemicals are divided into three classes as follows:

Class A: Chemicals that form explosive levels of peroxides without concentration. These are the most hazardous and can form explosive peroxide levels even if not opened.

Divinyl Acetylene	Divinyl Ether	Isopropyl Ether
Sodium or Potassium Amide	Vinylidene Chloride	

Class B: Chemicals that form explosive levels of peroxides when concentrated through distillation evaporation or exposure to air after opening.

Acetal	Cumene	Dioxane
Cyclohexene	Cyclopentene	Dicyclopentadiene
Diethyl Ether	Tetrahydrofuran	Methyl Isobutyl Ketone
Ethyl Vinyl Ether	Methyl Acetylene *	Furan
Diacetylene *	Methyl Cyclopentane	Tetrahydronaphthalene
Glyme (ethylene glycol dimethyl ether)		

Class C: Chemicals which are a hazard due to peroxide initiation of polymerization.

Butadiene	Chlorobutadiene	Chlorotrifluoroethylene *
Acrylonitrile	Vinyl Acetate	Vinyl Acetylene*
Vinyl Chloride*	Vinyl Pyridine	Tetrafluoroethylene*
Styrene	Methyl Methacrylate	

* Gas

2. General Precautions for Storage and Handling of Peroxide Forming Chemicals

a) Minimize the quantity of peroxides or peroxide forming chemicals in the lab.
b) Know the properties and hazards of all chemicals you are using through adequate research and study,including reading the label and MSDS.
c) Label each container with the Date Received, Date Opened and Date Last Tested.
d) Segregate these compounds from incompatible materials. Store away from ignition sources. Protect from flames, static electricity, and sources of heat.
e) Test chemicals for peroxide before any distillation or purification of peroxide forming chemicals.

f) Use extreme caution before concentrating or purifying peroxide forming chemicals as most explosions occur during these processes.
g) Wear proper personal protective equipment, including safety eyewear and face shields, when working with peroxide forming chemicals.
h) Minimize peroxide formation in ethers by storing in tightly sealed containers in a cool place in the absence of light.
i) If solids or crystals are observed in either the liquid or around the cap of peroxide forming chemicals, donot open or move the container but contact EHS for disposal.

3. Storage Limits for Each Class of Peroxide Forming Chemicals

Class A. Storage Unopened: six (6) months maximum. Storage Opened: test upon opening. If the test indicates > 80 ppm peroxides dispose. If < 80 ppm peroxide retest every 3 months and dispose when the test indicates > 80 ppm peroxides, the manufacturer expiration date is reached or one(1) year from the date of receipt which ever occurs first.

Class B. Storage Unopened: one (1) year maximum. Storage Opened: test upon opening. If the test indicates > 80 ppm peroxides dispose. If < 80 ppm peroxide retest every 3 months and dispose when the test indicates > 80 ppm peroxides or the manufacturers expiration date is reached which ever occurs first.

Class C. Storage Unopened: one (1) year maximum. Storage Opened: test upon opening. If the test indicates > 80 ppm peroxides dispose. If < 80 ppm peroxide retest every 3 months and dispose when the test indicates > 80 ppm peroxides or the manufacturers expiration date is reached which ever occurs first.

4. SHOCK SENSITIVE AND POTENTIALLY EXPLOSIVE CHEMICALS

The following chemicals are known to be shock sensitive or potentially explosive and need to be handled with care. The shock sensitivity and explosive potential of solids is increased if they become dry. This list is not all-inclusive, but is limited to those chemicals which may be present at Texas Tech University.

a) Benzoyl Peroxide — White crystalline solid; requires 25-35% water
b) Dinitrophenyl hydrazine + — Red crystalline solid, requires >30% water.
c) Methyl Ethyl Ketone Peroxide — Colorless liquid; strong oxidizer
d) Nitroglycerin — Colorless liquid
e) Nitromethane — Colorless liquid; highly flammable, incompatible with Amines, oxidizers, strong acids or bases.
f) Picramide + (syn. Trinitroaniline) — Yellow crystalline solid; requires >30 % water
g) Picric Acid + (syn. Trinitrophenol) — Yellow crystalline solid; requires >30% water Incompatible with metals, oxidizers or reducers.
h) Picryl Chloride — White crystalline solid; reguires >30% water. Incompatible with oxidizers, inorganic nitrates

i) Picryl Sulfonic Acid + Flammable solid, corrosive; requires >30% water (syn. Trinitrobenzenesulfonic acid)
j) Trinitroanisole (syn. Methyl Picrate)
k) Trinitrobenzene + Yellow crystalline solid; requires >30% water
l) Trinitrobenzoic Acid
m) Trinitrotoluene+
n) Urea Nitrate + Colorless crystalline solid; requires >10% water

+ These items require EHS approval to purchase.

PEROXIDE FORMING CHEMICAL
Date Received _____Date Opened _____
Date/Test Results (Amount Used)_____
Date/Test Results (Amount Used)_____
Date/Test Results (Amount Used)_____
Date/Test Results (Amount Used)_____
Date/Test Results (Amount Used)_____

EXAMPLE
PEROXIDE FORMING CHEMICAL
Date Received _9/16/10___ Date Opened __9/26/10___
Date/Test Results _____9/26/10 10 PPM_____
Date/Test Results _____12/26/10 10 PPM_____
Date/Test Results __3/25/11 30 PPM_____
Date/Test Results __6/25/11 100 PPM_____
Date/Test Results _____

In the above example the chemical was a Class A peroxide forming chemical. It had a manufacturer's expiration date of 10/15/11.
The chemical was opened within the 6 months allowable unopened storage time. The initial test after opening was satisfactory. Subsequent tests every three months were satisfactory until the test on 6/25/06. As this test result is >80 ppm. The chemical must be disposed of as soon as possible.

ADDITIONAL INFORMATION
Peroxide Forming Chemical Labels are available from EHSO
Peroxide Test Strips are available from the following sources.
University of Hawaii at Manoa Chemistry Department Stockroom, Bilger 116
Phone: 956-6021
Hawaii Chemical and Scientific 2363 N. King St. Honolulu, HI, 96819
Phone: 841-4265
Laboratory Safety and Supply (800) 356-0783 Online at www.labsafety.com

APPENDIX E

Emergency Contact Information

PI: Name
Lab Address
Lab Number
Emergency Contact Number

Emergency 9-911
Ambulance, Fire, Police
University Medical Center (UMC)
(806) 775-8200
Texas Tech Police
Non-emergency (806) 742-3931

Building Contact
Title:
Name:
Phone Number:
Lab Safety Coordinator
Name:
Phone Number:

Physical Plant
Maintenance Emergencies (806) 742-3301 or after 5pm (806) 742-3328
Information Technology
(806) 742-HELP or for Server-related issues (806) 742-3649
Texas Department of Public Safety
(806) 472-2700
TTU Emergency Management Coordinator University Council: Ronald Phillips
(806) 742-2121
TTUS Emergency Management Coordinator Executive Director of Public Safety and Emergency Management: Jay Parchman
(806) 742-9000
The Office of Communications and Marketing maintains the **Emergency Communications Center**
212 Administration Building
Lubbock, TX 79409
806.742.2136
806.742.1615 fax
Normal Office Hours: 8 a.m. – 5 p.m. Central, Monday – Friday

APPENDIX F

Mandatory Laboratory Safety Rules:

1. Comply with emergency evacuation procedures.
2. Access to eyewashes, safety showers, and fire extinguishers must be kept clear.
3. Eating and drinking in the laboratories and animal rooms is strictly forbidden at all times.
4. Eye protection must be worn at all times in the laboratories where chemicals are used and where there is the potential for eye injury.
5. Open-toed shoes, sandals, nor shorts, are acceptable in laboratories or animal rooms.
6. Keep the Chemical Hygiene Plan, MSDS's, and emergency phone numbers in a highly visible location in the lab.
7. Immediately report any work related illness or injury to your supervisor and the safety coordinator.
8. Tasks that present unusual hazards must be reviewed with the appropriate supervisor before they are conducted.
9. Labels on containers must not be defaced, and all containers of chemicals must be labeled, listing the contents, hazards, name of owner, date received, and date opened.
10. Dispose of all expired chemicals per annual inventory of the lab. Contact EH&S for all waste chemical disposals at 2-3876.
11. When working with carcinogens, mutagens, or teratogens, comply with all safety procedures.
12. Pipetting by mouth is strictly forbidden.
13. Gas cylinders must be firmly secured with restraints whether in use or stored. Regulators must be removed and caps used when moving cylinders.
14. Do not modify electrical equipment yourself. The electrical shop will modify or fix electrical equipment for your SAFETY.
15. Lab must contain a first aid kit and its location must be known by lab members.
16. Emergency phone numbers should be posted within laboratory and on the outside of the laboratory door (i.e. 9-911, EH&S number 2-3876, Poison Control Center, Principal Investigator's office and home phone numbers, and other employees' or students' home phone numbers.

Exposure Incident:

An exposure incident occurs when potentially infectious material enters the body through a needlestick, contact with mucous membranes (eyes, inside of nose or mouth, genitals) or contact with broken skin (rash, acne, cuts, scrape). If this occurs,

- Immediately wash the infected are with soap and water or disinfectant for 15 minutes.
- If it is an eye exposure, irrigate the eye at an eyewash station for 15 minutes (do not use disinfectant).
- Apply first aid if necessary.
- Notify your supervisor.
- Call the employer's occupational medicine provider to arrange a post-exposure evaluation.
- Take a Bloodborne Pathogen Exposure Incident Report to the provider to be completed at the evaluation.

APPENDIX G

Image: Example of filled out Hazardous Waste label.

INTERIM GUIDANCE FOR HANDLING AND STORING CHEMICAL WASTES AT TTU
December 14, 2011

ALLOWABLE WASTE CONTAINERS:

• Use glass or polyethylene containers that will not corrode.

• Containers must be triple rinsed. (Each rinse must be 1/10 the volume of the container and the rinse also collected for disposal)

• The original container label must be completely defaced or removed.

• Metal cans must be used **only** for solvent waste that is non-corrosive. **If metal cans are used, they must be disposed of within 1 month of accumulation start date or when ••• full, which ever happens first.**

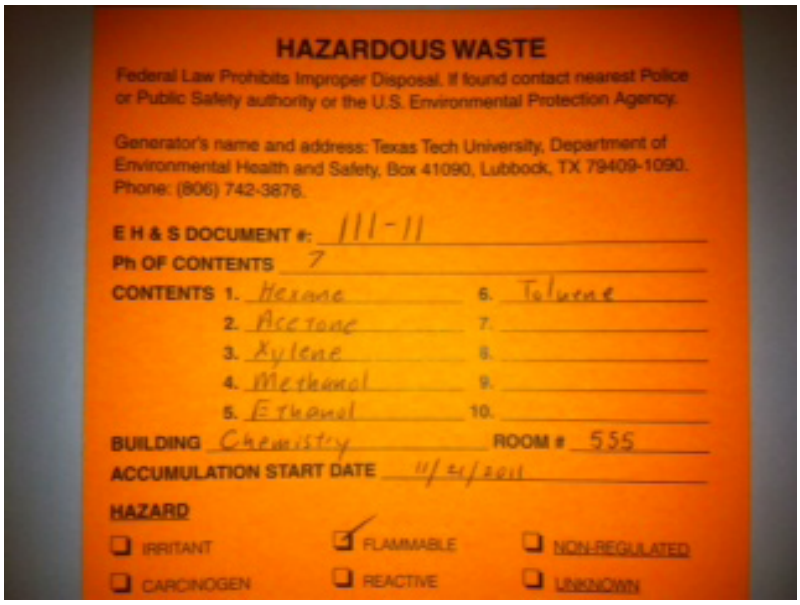
LABEL ALL WASTE CONTAINERS:

• The contents of a container must be shown and displayed prominently with the label facing forward where it can be seen.

• Use labels such as "Xylene To Be Recycled" or "Xylene To Be Reclaimed" to identify chemicals that can be redistilled or put to other uses.

LABEL ALL WASTE CONTAINERS WITH THE WORDS "HAZARDOUS WASTE" AND THE CONTENTS:

• Use the orange "Hazardous Waste" labels provided by Texas Tech Environmental Health and Safety (TTU EH&S -- 742-3876).


HAZARDOUS WASTE

Federal Law Prohibits Improper Disposal. If found contact nearest Police or Public Safety authority or the U.S. Environmental Protection Agency.

Generator's name and address: Texas Tech University, Department of Environmental Health and Safety, Box 41090, Lubbock, TX 79409-1090. Phone: (806) 742-3876.

E H & S DOCUMENT #: 111-11

Ph OF CONTENTS 7

CONTENTS 1. Hexane 6. Toluene
2. Acetone 7.
3. Xylene 8.
4. Methanol 9.
5. Ethanol 10.

BUILDING Chemistry ROOM # 555

ACCUMULATION START DATE 11/14/2011

HAZARD

☐ IRRITANT ☑ FLAMMABLE ☐ NON-REGULATED
☐ CARCINOGEN ☐ REACTIVE ☐ UNKNOWN

• All containers of chemical waste must have an accurate EH&S label that lists
- Accumulation start date
- pH of contents
- Each individual chemical waste (full name)
- Building and room number
- All hazards posed by the waste checked

• Fill in building, room number and accumulation start date before any waste is added. Update other information as waste is added.

WASTE CONTAINERS:
• **All waste containers must be securely capped or capped with an appropriate venting cap.**

• **Do not leave a funnel in the waste container**.

DO NOT ALLOW LABORATORY WASTE TO ACCUMULATE:
• **Store only ONE container of each kind of waste in the laboratory.**

• **When the waste container is ••• full or it has been one month since accumulation start date**, log on to TTU EH&S website to schedule a chemical waste pickup (http://www.depts.ttu.edu/ehs/Web/).

STORAGE OF WASTE CONTAINERS:
• **Store waste only in a fume hood where no reactions are being carried out.** The waste must be segregated, and the sash must be fully closed.

• Store waste away from reactions in progress, equipment and sitting areas inside the laboratory.

• If there is no room in a fume hood to store flammable waste, store it in a flammables cabinet. Do not store flammable waste containers on a bench or floor. To obtain cabinets, contact EH&S (742-3876).

• Segregate waste away from other incompatible waste or chemicals.

• **Do not store waste containers near a sink or floor drain.**

SEPARATING THE WASTE: Incompatible wastes must be stored separately

• **Waste Analysis** - Each person in the laboratory conducting operations must complete a waste analysis. The waste analysis will need to include, but is not limited to what will be generated from the operation, the incompatibilities and how it will be stored. The waste analysis can be either entered in the laboratory notebook that is checked by the PI or in an SOP approved by the PI. If you have any questions about your waste analysis or if you need help contact EH&S at 742-3876.

• **Halogenated and Non-Halogenated waste** - These wastes need to be segregated from one another in separate containers.

• **Store acids and bases in separate cabinets** - Leaking containers or a spill could cause a violent reaction and emit toxic gases.

• **Store acids and organic waste in separate cabinets** - If these chemicals should mix, a fire or explosion can occur.

• **Do not mix incompatible solvents in the same waste container** - For example, nitric acid and ethanol can form an explosive mixture.

• **Practices used for storage of chemicals should also be used for storage of waste** - Store them by storage groups such as:

- Compatible Organic Bases
- Compatible Pyrophoric & Water-Reactive Materials
- Compatible Inorganic Bases
- Compatible Organic Acids
- Compatible Oxidizers including Peroxides
- Compatible Inorganic Acids not including Oxidizers or Combustible
- Not Intrinsically Reactive or Flammable or Combustible
- Poison Compressed Gases
- Compatible Explosive or other highly Unstable Material
- Non-Reactive Flammable and Combustible, including solvents
- **Incompatible with ALL other storage groups**

Link to *Prudent Practices in the Laboratory* over compatible storage groups
http://www.nap.edu/openbook.php?record_id=12654&page=96

ALWAYS STORE THE FOLLOWING TYPES OF WASTES SEPERATELY FROM EACH OTHER:

- Acids and bases.
- Alkali or alkali earth metals, Alkyllithiums and aqueous waste
- Cyanide, sulfide or arsenic compounds and acids
- Mercury or silver and ammonium containing compounds
- Organics and acids.
- Powdered or reactive metals and combustible materials

DO NOT MIX INCOMPATIBLE WASTES:

- **Before mixing wastes, know your chemicals and how they react**. Consult the MSDS for the chemical and a classic reference such as Bretherick, Hazards in the Chemical Laboratory, 7th. Edition, 2007 to identify proper procedures

- For examples of wastes that **must not be combined**, refer to the following links containing incompatibles charts

http://web.princeton.edu/sites/ehs/chemwaste/RCRAChemicalCompatibilityList.pdf and http://web.princeton.edu/sites/ehs/chemwaste/compatability.htm

APPENDIX H

Hazardous Chemicals are denoted in: *Red Italics*
Western Blot Protocol

Required Solutions
10x PBS/Lysis Buffer
80 g NaCl
2 g KCl
14.4 g Na2HPO4
2.4 g KH2PO4 dissolve into 800 ml ddH2O
10ml Triton X-100
Adjust pH to 7.4 With ***HCl***

Add right before use to 50 ml:
10 µl Aprotinin Stock (10mg/ml)
10 µl Leupeptin Stock (10mg/ml)
50 µl ***phenylmethanesulfonylfluoride*** (PMSF) Stock (200mM)

Keep on Ice

When examining phosphorylated proteins, add the following phosphatase inhibitor:
Na-ortho-vanadate to 1 mM (stock 0.1 M)

10x Ponceau S
Ponceau S 2% (w/v) TCA 30% (w/v) Dilute to 1x before use (can be re-used many times).

4x Laemmli Buffer
4.4 ml 0.5 M Tris (pH 6.8) 4.4 ml Glycerol 2.2 ml 20% ***SDS*** 0.5 ml 1% Bromophenol Blue 0.5 ml
Aliquot and store at -20^{o}C. Dilute to 2x before use.

10x Running Buffer
30.3 g (0.25 M) Tris Base 144 g (1.92 M) Glycine 10 g (1%) ***SDS*** or appropriate for concentrated stock qs 1000 ml ddH_2O Dilute 1:10 with ddH_2O. pH will be 8.3

10x Electrotransfer Buffer
30.3 g Tris Base 144 g Glycine qs 1000 ml ddH_2O

Tris Buffered Saline Tween (TBST)
50 mM Tris Base
500 mM NaCl
0.1% Tween 20
pH to 7.6 with ***HCl***

Laemmli Buffer Cell Extracts

Cells can be directly lysed into Laemmli buffer as follows:

1. Harvest cells by trypsination and count cells. Keep everything cold after this step.
2. Spin cells in media for 5 min at 1,000 g at 4°C.
3. Aspirate off media and resuspend pellet in 1 ml cold 1x PBS.
4. Transfer 106 cells to an eppendorf tube and spin in microfuge for 4 min at 4,000 g at 4°C. Aspriate off PBS.
5. Suspend cells in 100 µl hot Laemmli buffer.
6. Heat for 5 min (80- 100°C).
7. Shear DNA through a 28 1/2 gauge insulin syringe 5 times, or sonicate.
8. Heat to 80-100°C for 5 minutes before loading.
9. Load 10 µl (105 cells) per lane.

PBS Lysis Whole Cell Extracts

1. Pellet Cells. Keep everything cold after this step; chill tubes and solutions on ice.
2. Wash once with cold 1x PBS. Cells can be counted in one of these steps.
3. Resuspend cell pellet in 5x the pellet volume of PBS Lysis Buffer (4°C).
4. Incubate on ice for 30 min with occasional mixing (flicking the tube).
5. Spin 15K RPM (microfuge at max setting), 10 min at 4°C.
6. Set some supernatant aside for a Bradford assay and quick-freeze the sample or aliquots on dry ice and store at -80°C.
7. Collect Supernatant and keep on ice.

SDS-PAGE Gel and Western Blot

Run the gel according to the instructions below. Refer to *SDS-PAGE Gels* on page 7 for details.

1. Measure protein concentration in duplicate using Bradford using a BSA standard curve. Run up to 100 µg/lane
2. Add Laemmli buffer and heat to 100°C for 5 min (don't boil too long, proteins get destroyed).
3. Run by SDS-PAGE until the blue front is at the bottom of the gel (refer to *SDS-PAGE Gels* for gel composition).

4. Transfer: Blot onto a PVDF membrane. Pre-wet materials in ***Methanol*** buffer. Stack in the following order:
   - case (clear side)
   - sponge
   - Whatman paper
   - membrane
   - gel
   - Whatman paper
   - sponge
   - case (black side) Place in the transfer apparatus with black side facing black).
   - Run transfer at 500mA for 1.2 hours

5. Stain with 1x Ponceau S for 1 minute and destain in acidified H2O (2.3 l of ddH2O + 4 ml concentrated HCl). Wrap in plastic wrap and Xerox. Rinse in 1x TBST.
6. Block the membrane for 1 hr min in 50 ml TBST + 5% Bovine Sera Albumin + 0.1% Tween 20, in a small Tupperware dish on a shaker.
7. Incubate with primary antibody diluted in 50 ml TBST + 5% BSA + 0.1% Tween 20. Incubate o/n at 4oC or 2 hrs at RT. The primary antibody mix can be re-used (store at 4oC.)
8. Wash 3X for 5-10min in ~50ml PBS +0.1% Tween20 at RT
9. Incubate with secondary antibody for 30 min to 1 hr at RT in 2 ml 1x PBS + 1% BSA + 0.1% Tween 20.
10. Wash 3 x 10 min each in ~50 ml 1x PBS + 0.1% Tween 20 at RT in a small Tupperware on a shaker.
11. Rinse with ddH2O.
12. Detect protein with ECL kit (2 ml/membrane). In a separate tube, mix black and white ECL solutions in a 1:1 ratio. Then aliquot solution onto membranes and

Gel Recipe

Reagents	**5%**	**7%**	**9%**	**12.5%**
	2 minigels	2 minigels	2 minigels	2 minigels
40% Acrylamide mix	1.7 ml	2.4 ml	3.0 ml	4.2 ml
4xLGB	2.5 ml	2.5 ml	2.5 ml	2.5 ml
20% SDS	50 ul	50 ul	50 ul	50 ul
dd Water	5.7 ml	5.0 ml	4.4 ml	3.2 ml
10% APS (0.1g/ml; fresh)	50 ul	50 ul	50 ul	50 ul
TEMED	3.3 ul	3.3 ul	3.3 ul	3.3 ul

Stacking Gel Buffer (SGB, 0.5 M Tris) 12.1 g Tris up to 200 ml of H2O, PH 6.8 (store in 4C)

Lower Gel Buffer (LGB, 1.5 M Tris) 36.3 g Tris, up to 200 ml of H2O, PH 8.8 (store in 4C)

20% Sodium Dodecyl Sulfate (SDS) 20 g SDS, up to 100 ml in H2O

1.5% Ammonium Persulfate (APS) 1.5 g APS, up to 100 ml in H2O (Aliquot and store in -20C)

Hydrochloric Acid (HCl): I have read the MSDS and understand the hazards related to the use of this Hydrochloric Acid as communicated to me by the PI, Dr. Boyd Butler. These include but are not restricted to: Causes Burns if contacted by skin, harmful if inhaled or ingested. I must wear protective equipment when working with the concentrated form of this chemical. These include: gloves, protective eyewear and a laboratory coat.

Phenylmethylsulfonyl fluoride (PMSF): I have read the MSDS and understand the hazards related to the use of Phenylmethylsulfonyl fluoride as communicated to me by the PI, Dr. Boyd Butler. These include but are not restricted to: extreme eye and respiratory irritant therefore I must wear protective equipment when working with the powder form of this chemical. These include: gloves, protective eyewear, protective surgical/particulate mask, and a laboratory coat.

Sodium dodecyl Sulfate (SDS): I have read (MSDS) and understand the hazards related to the use of this Sodium Dodecyl Sulfate as communicated to me by the PI, Dr. Boyd Butler. These include but are not restricted to: extreme eye and respiratory irritant therefore in addition SDS must not be handled in its powder form around open flames due its low flash point. I must wear protective equipment when working with the powder form of this chemical. These include: gloves, protective eyewear, protective surgical/particulate mask, and a laboratory coat.

Methanol: I have read (MSDS) and understand the hazards related to the use of this Methanol as communicated to me by the PI, Dr. Boyd Butler. These include but are not restricted to: eye, skin and respiratory irritant, poison if ingested, and highly flammable. I must wear protective equipment when working with the concentrated form of this chemical. These include: gloves, protective eyewear, protective surgical/particulate mask, and a laboratory coat. In addition I must not work with methanol around open flames.

Temed: I have read (MSDS) and understand the hazards related to the use of this Methanol as communicated to me by the PI, Dr. Boyd Butler. These include but are not restricted to: eye, skin and respiratory irritant, poison if ingested, and highly flammable. I must wear protective equipment when working with the concentrated form of this chemical. These include: gloves, protective eyewear, and a laboratory coat. In addition I must not work with methanol around open flames and chemical should be handled in fume hood.

Student Signatures	Date
Erin Brown	04/05/12
Principal Investigator Signature	**Date**
Boyd Butler	4/5/12

APPENDIX I

Employee Training Record
This Certifies that the Chemical Hygiene Plan, Annual Safety and Lab Specific Hazardous Chemical/Carcinogen/Toxins training has been completed

Printed Name	Signature	EHS Annual Lab Safety Training Date	Lab Specific Hazardous Chemical/Carcinogen /Toxins training Date