V. HAZARDOUS WASTE

Chemical wastes, out-of-date chemicals, and unwanted/unused chemicals all require special handling, storage and, if appropriate, disposal. The EHSO is responsible for coordinating the pick-up and disposal of surplus and waste chemicals from generating departments. In addition, the EHSO has been given the responsibility for determining the status of substances as surplus or wastes. To assure compliance with regulations, safe handling, and efficiency of operations, the EHSO has established the following procedures applicable to the collection, storing, labeling, and packaging of these substances by departments or individuals preparing them for pick-up by EHSO personnel or waste vendors.

A. General Information

1. All departments must follow these procedures for surplus chemicals/ hazardous waste disposal. When departments follow these procedures, the EHSO will coordinate pick-up of the hazardous materials and will manage them for surplus, reuse, recycling, or waste disposal as appropriate. This will be done at no cost to the department with the following exceptions:

    a. waste generated from a spill or incident which is beyond the capability of the EHSO to handle in-house, or

    b. waste generated in a location other than on the OU campus.

    For these exceptional cases, the invoice(s) for disposal will be passed on to the generating department.

2. Departments are responsible for ensuring that their personnel purchase and dispose of chemical and biohazardous materials in a manner that does not generate unknown/unlabeled, abandoned, excessive, or unnecessary waste. This includes making certain that unused chemicals are not abandoned after a researcher's departure or relocation, or major laboratory clean-outs. If such wastes are generated, the invoice(s) for disposal of such items may be forwarded to the department for payment.

3. Department personnel shall not accept any chemical, hazardous substance, or item(s) containing hazardous substances as gifts or donations on the behalf of the University without notifying the EHSO prior to the transfer. This is to assure that no unanticipated future hazardous waste costs result from such a transfer.

4. Department personnel shall not give or sell university property, including hazardous substances, to any person or organization outside the University except through the legally established procedures of the Purchasing Department or, in the instance of hazardous materials, through the EHSO's Hazardous Materials Surplus Program (see Section V.D., “Hazardous Materials Surplus Program.”

5. Under no circumstances is any person to dispose of a hazardous substance down the drain or in the refuse disposal system where the applicable regulations, procedures, and policies regarding its disposal as described in this document prohibit such disposal. Materials currently approved for drain disposal (in moderate quantities) are as follows:

    a. bleach solutions,

    b. hydrogen peroxide,

    c. blood products, and

    d. non-hazardous buffer solutions.

6. For disposal of scintillation fluids, contact the OU Radiation Safety Office.

7. The EHSO will not pick up or handle surplus or waste hazardous substances that have not been properly containerized, labeled, packaged, or manifested according to the procedures described herein.

8. Mineral acids and bases shall be neutralized by the laboratory generating these wastes. Neutralization procedures may be found in Section V.E., "Neutralization of Spent Inorganic Acids and Bases."

B. **Segregation**

1. Unused/unwanted chemicals should remain in their original containers unless the container integrity has been compromised (see Section V.C.2.).

2. Waste chemicals should be collected in separate containers by categories segregated such that spills or leaks would remain isolated from other containers of wastes or chemicals. These categories include but are not necessarily limited to:

    a. waste oil (used oil containing or mixed with hazardous waste),

    b. used oil (oil with no hazardous waste contamination),

    c. PCB wastes,

    d. acids,

    e. caustics,

    f. halogenated solvents,

    g. non-halogenated solvents,

h. mercury wastes,

i. reactive chemicals, and

j. wastes with high heavy metal contamination.

C. **Basic Procedures**

1. Laboratories may accumulate no more than 55 gallons of hazardous waste or one quart of acute (P-listed) hazardous waste. See Appendix G, “Acute Hazardous Wastes” for a listing.

2. Wastes should be collected in the original or other compatible primary container. However, in instances of corrosive wastes or halogenated solvents, the use of metal containers is often unsuitable even if the solvents were originally shipped in metal containers.

   a. Containers must not be leaking or damaged.

   b. Containers should be closed with a properly fitting cap or other closure at all times between filling of the container. Make-shift covers such as tape, parafilm, or a rag stuffed in an opening are unacceptable. Funnels may not be left in the container opening, and should be removed and the container lid placed on the container between fillings.

   c. Plastic bags, where acceptable as containers, should be without punctures or tears and should be tightly sealed. Plastic bags used as primary containers should be packaged in a secondary container such as glass, hard plastic, metal, or cardboard box due to the ease in which damage may result to the bag.

   d. Containers should be inspected weekly for leaks/deterioration.

   e. Glass containers should not exceed one gallon (4 liters) in size and should not be filled into the neck of the fill/pour spout.

   f. Metal or plastic containers should not exceed 5 gallons (20 liters) in size (unless previous arrangements have been made with the EHSO) and should not be filled into the neck of the fill/pour spout. Where containers have flat tops, the liquid level should be at least 1 inch from the fill/pour opening.

3. Containers must be properly labeled as to content.

   a. For single chemical wastes, label the contents with "Waste (chemical name)" or "Hazardous Waste (chemical name)."

   b. For mixtures, a label similar to the table below is required

indicating names of components which comprise at least 1% by volume of the total contents or mixture. Each time material is added to the container, information must be recorded. (These labels are available from the EHSO and the Radiation Safety Office).

AMOUNT	WASTE CHEMICAL NAME Mixtures must indicate % of each	CAS#	HAZARD
500 ml	20% toluene	108-88-3	F

*Hazard Codes:
C: Corrosive E: Explosive F: Flammable
O: Oxidizer I: Irritant R: Reactive
T: Toxic HT/C: Highly Toxic/Carcinogenic

PI NAME: _____

CONTACT PERSON NAME: _____

BLDG/ROOM NUMBER: _____

c. Do not use formulas or chemical equations.

d. In the instances of time-sensitive substances such as ethers, the date of container opening or initial accumulation should be included on the label.

e. For peroxide forming substances, identify the peroxide content and the date tested. See Section IV.H., "Peroxide Forming Chemicals" for more information.

f. Remove or obliterate any other labels or wordings not related to the current substance.

4. When accumulation nears the maximum accumulation amount identified in V.C.1. above, or when accumulation will exceed the available space within the laboratory area, departmental representative should coordinate the pick-up and transfer of the substances with the EHSO.

5. The generator or departmental representative must complete a *Hazardous Materials Pick-up Request* form (see page V-5).

6. Each container must be labeled with the unique identification number developed as a result of the completion of the *Hazardous Materials Pick-*

DEPARTMENT:		
PRINCIPLE INVESTIGATOR:	**CAMPUS ADDRESS:**	**PHONE #:**
INVENTORY SUPERVISOR:	**CAMPUS: ADDRESS:**	**PHONE #:**

UNIVERSITY OF OKLAHOMA

ENVIRONMENTAL HEALTH AND SAFETY OFFICE

HAZARDOUS MATERIALS PICK-UP REQUEST FORM

OUHSC (FAX 271-1606) (Office 271-3000)
OU Norman (FAX 325-7899) (Office 325-2212)

ROOM NUMBER	DEPT CODE*	CONTAINER NUMBER	AMOUNT	SOLID/ LIQUID/ GAS (S/L/G)	PURE/ MIXTURE (P/M)	PRIMARY HAZARD CODE	DESCRIPTION OF CONTENTS 1. Chemical or common name and CAS# of all substances over 1% by volume. 2. Strength (concentration) of individual substance, where applicable. 3. Percentages of mixed chemicals in container (by volume)	EHSO Use Only			
								Surplus Date	Waste Date	Ship Date	Bulk Date
Example: 301	ES	01	4 liters	L	M	F	20% Toluene 108-88-3 80% Xylene 1330-20-7				

Hazard Codes: **C** - **C**orrosive **E** - **E**xplosive **F** - **F**lammable **HT/C** - **H**ighly **T**oxic/**C**arcinogenic
I - **I**rritant **O** - **O**xidizer **R** - **R**eactive

*See reverse for Department Code listing

V–5

This is to certify that the above named materials are properly classified, described, packaged, marked and labeled and are in proper condition for the transportation according to the applicable requirements of the University of Oklahoma Environmental Health and Safety Office.

_____ Signature of Authorized Department Representative

_____ Date

UPON COMPLETION, PLEASE FAX TO: **OUHSC, 271-1606**
OU-NORMAN, 325-7238

EHSO USE

Waste Generated(in lbs.) =

Departmental Codes - Health Sciences Center Campus

(AE) Architectural and Engineering Services
(AF) Animal Resources Facility
(AS) Anatomical Sciences
(AN) Anesthesiology
(AT) Anatomy
(BC) Biochemistry
(BS) Biostatistics
(CD) Clinical Dietetics
(CL) Clinical Laboratory Science
(CN) College of Nursing
(CS) Computing Services
(DC) Dermatology Clinic
(DN) Dentistry
(EH) Occupational and Environmental Health
(EN) Endocrinology
(ES) Environmental Health and Safety Office
(GO) Gynecology and Obstetrics
(LI) Library
(ME) Department of Medicine
(MI) Microbiology and Immunology
(MP) Molecular Pathology
(NE) Neurology
(NP) Nephrology
(NS) Neurosurgery
(NU) Nutritional Sciences
(OP) Ophthalmology
(OR) Otorhinolaryngology
(OS) Orthopedic Surgery
(OT) Occupational Therapy
(PA) Pathology
(PC) Psychiatry
(PE) Pediatrics
(PD) Police Department
(PG) Photographic Services
(PH) College of Pharmacy
(PL) Plastic Surgery
(PM) Pharmacology
(PN) Paint Shop
(PS) Print Shop/Photographic Services
(PT) Physical Therapy
(PY) Physiology
(PU) Pulmonary Medicine
(RS) Radiation Safety
(RT) Radiologic Technology
(SC) Steam and Chilled Water Plant
(SS) Site Support
(SU) Surgery
(TU) Tulsa Campus
(UR) Urology

Departmental Codes - Norman Campus

(AH) Adams Hall
(AFH) Animal Holding Facility
(ARM) Armory
(BH) Buchanan Hall
(BL) Bizzell Memorial Library
(CRAD) Craddock Hall
(CSKF) Ceramics Studio & Kiln Hall
(GLCH) George Lynn Cross Hall
(CEC) Carson Engineering Center
(CHB) Chemistry Building
(CMC) Catlett Music Center
(EL) Engineering Laboratory
(FAC) Fine Arts Center
(FH) Felgar Hall
(FJC) Jones Art Center
(FL) Fears Structural Engineering Lab
(MUD) Mechanical Engineering Mud Lab
(NEL) Nuclear Engineering Laboratory
(OHNH) Sam Noble Oklahoma Museum of Natural History
(PHSC) Physical Sciences Center
(PP) Physical Plant
(RHYN) Rhyne Hall
(SEC) Sarkeys Energy Center
(AD) Athletic Department
(CC) Columns Commissary
(ARS) Archaeological Services
(BS) Biological Station
(LAND) Landscaping
(NH) Nielson Hall
(FR) Fisheries Research
(GH) Gould Hall
(GHC) Goddard Health Center
(LNC) Lloyd Noble Center
(ES) Environmental Services
(ACM) Asbestos Containing Material
(PRTS) Printing Services
(SRTC) Stephenson Research and Technology Center
(PS) Paint Shop
(PRP) Properties
(RH) Richards Hall
(PYS) Psychology
(CPH) Copeland Hall
JAGC Jimmie Austin Golf Course

up Request form. This label identifies the 6-digit date, the 2-letter departmental code, and the sequential numerical numbering starting with the number "01". For example, the label on the container for the example given on the form on page V-5 would read 010197ES01.

7. Contact the EHSO for pick-up.

8. The EHSO will then determine the status of the substances as surplus, for reuse, for recycling, or for waste disposal.

9. In certain instances, the EHSO may request that a department store unwanted chemicals until a chemical pick-up by a hazardous waste management firm is arranged.

10. Departments may be requested to "box up" multiple dry chemical containers for transport. When this is performed, the following safety measures should be followed.

    a. The overpack or container must be capable of carrying the weight of the items to be packed inside.

    b. Where used, cardboard boxes should be reinforced with additional tape on the underside. No more than 4 one gallon glass jars (full) in a cardboard box should be permitted (equates to a weight of 44 pounds).

    c. Chemicals must be compatible. This is to be determined by the departmental representative.

    d. Chemical containers to be packed inside shall be placed upright, side-by-side, one-high only. Each individual container should be labeled with the name of the chemical substance(s) and CAS number.

    e. Packing material should be placed between containers made of glass or where other breakage may occur during handling or transport. This material should be provided by the requesting department.

D. Hazardous Materials Surplus Program

A hazardous materials surplus program has been established to assure maximum use of non-radioactive chemical substances that still useable but deemed of no further use to a particular department. These chemicals are available for redistribution or reuse amongst other OU departments or units. The EHSO has full responsibility for regulating and coordinating this hazardous materials surplus program. Procedures for participating in the program are as follows.

1. Departments identifying a substance as surplus to their needs should

complete a “Hazardous Materials Pick-up Request” Form (see page V-6) and contact the EHSO for pick-up and transfer to the EHSO central storage location.

2. Departments wishing to obtain surplus chemicals may view a current inventory at the EHSO website or may contact the EHSO to request information on a chemical of interest.

3. Surplus chemicals reallocated to University departments will be provided at no charge to the receiving department and there will be no compensation to the department that originally offered the chemical as surplus.

4. Departments have the option of requesting the return of previously surplused chemicals, if still available, at no charge.

5. When a department transfers the surplus chemical to the EHSO, the container must be in good condition and properly labeled with the substance name, hazard(s), and the CAS number.

E. Neutralization of Spent Inorganic Acids and Bases

Spent mineral acids and bases comprise a large portion of the unwanted chemicals being stored in OU campus laboratories. As a part of regular laboratory procedures, OU laboratories should neutralize spent inorganic acids and bases that do not contain metal or organic contaminants. These chemicals will be managed in an "elementary neutralization unit" and, therefore, are not considered a part of the hazardous waste stream for the campus. An "elementary neutralization unit" is any container used for neutralizing corrosive wastes.

Neutralization is a relatively simple procedure that is best done by and in the laboratory that uses inorganic acids and bases on a regular basis. The laboratory that generates spent corrosives usually has the facilities and expertise to neutralize them, and therefore will be responsible for doing so. The following procedures describe the proper technique for neutralization of spent inorganic acids and bases as a part of regular laboratory procedures.

1. Applicability

   a. Tables V-1 and V-2 list corrosives to be managed in-house by OU laboratories. Aqueous corrosive wastes must not contain sulfides, cyanides, metals, or other materials that can give off hazardous gases or vapors upon reaction with the acid or base.

   b. Do **not** use these procedures for:

      (1) inorganic acids that contain metals (e.g., atomic absorption standards),

(2) esters of inorganic acids,

(3) chromic acid,

(4) perchloric acid,

(5) hydrofluoric acid, or

(6) organic acids.

TABLE V-1
INORGANIC ACIDS

Name/Molecular Wt.	Formula	Synonyms
Sulfuric Acid M.W. - 98.08	H_2SO_4	Dipping Acid Oil of Vitrol Sulphuric Acid Vitrol Brown Oil Nordhausen Acid
Boric Acid M.W. - 61.84	BH_3O_3	Boracic Acid Orthoboric Acid
Nitrous Acid M.W. - 47.02	HNO_2	Nitrosyl Hydroxide
Nitric Acid M.W. - 63.02	HNO_3	Aqua Fortis Azotic Acid Hydrogen Nitrate
Hyponitrous Acid	$H_2N_2O_2$	
Hydrochloric Acid M.W. - 36.46	HCl	Chlorohydric Acid Hydrochloride Muriatic Acid Spirits of Salt
Aqua Regia (3:1 mixture)	HCI/HNO_3	Nitrohydrochloric Acid Nitromuriatic Acid
Phosphoric Acid M.W. -98.00	H_3PO_4	Orthophosphoric Acid

TABLE V-2 INORGANIC BASES		
Name/Molecular Wt.	**Formula**	**Synonyms**
Aluminum Hydroxide M.W. - 78.01	$Al(OH)_3$	Alumigel Alumina Hydrate Alumina Trihydrate Aluminum Hydrate Aluminum (III) Hydroxide Aluminum Oxide - $3H_20$ Aluminum Trihydroxide
Calcium Carbonate M.W. - 100.09	$CaCO_3$	Precipitated Chalk Chalk Dolomite Limestone/Marble Portland Stone
Calcium Hydroxide M.W. - 74.10	$Ca(OH)_2$	Slaked Lime Lime Water Hydrated Lime Calcium Hydrate
Calcium Oxide M.W. - 56.08	CaO	Lime Burnt Lime Calcia Calx Lime, Unslaked Quicklime
Magnesium Carbonate M.W. - 84.32	$MgCO_3$	Carbonate Magnesium Magnesia Alba Magnesium Carbonate (precipitated)
Magnesium Hydroxide M.W. - 58.33	$Mg(OH)_2$	Magnesia Magma Magnesium Hydrate Milk of Magnesia
Potassium Hydroxide M.W. - 56.11	KOH	Caustic Potash Lye Potassium Hydrate
Sodium Bicarbonate M.W - 85.01	$NaHCO_3$	Baking Soda Bicarbonate of Soda Sodium Acid Carbonate
Sodium Carbonate M.W. - 105.99	Na_2CO_3	Soda Ash Crystol Carbonate Carbonic Acid - Disodium Salt

TABLE V-2 INORGANIC BASES		
Name/Molecular Wt.	**Formula**	**Synonyms**
Sodium Hydroxide M.W. - 40.00	NaOH	Lye Caustic Soda Soda Lye Sodium Hydrate White Caustic
Miscellaneous Sulfates (**not** Sulfides) Ex: Sodium Sulfate Magnesium Sulfate Calcium Sulfate Aluminum Sulfate	 Na_2SO_4 $MgSO_4$ $CaSO_4$ $Al_2(SO_4)_3$	 Alum

2. Supplies and Equipment Needed

   a. Soda ash or diluted inorganic base for neutralization of an acid, or a diluted inorganic acid for neutralization of a base

   b. Polyethylene bucket (1- or 2-gallon size, as personal preference dictates, but remember that 1 gallon weighs approximately 8 pounds or greater)

   c. Personal protective equipment (See Section V.E.3.)

   d. 500 ml beakers

   e. pH meter, pH indicator strips, or other pH test method

   f. Thermometer

3. Personal Protective Equipment

Read the MSDS for detailed information. Contact the vendor or the EHSO if an MSDS is not available. The minimum recommended personal protection needed when performing the neutralization procedure is as follows.

   a. Ventilation: work in a fume hood.

   b. Gloves: use neoprene, natural rubber, butyl, polyethylene, nitrile butadiene, or polyvinyl chloride depending on the MSDS information.

   c. Clothing: apron (rubber is preferred), laboratory coat (or protective suit or coveralls), and closed-toed shoes.

d. Eye Protection: splash-proof or dust-proof goggles and a face-shield (8 inch minimum).

e. Safety Equipment: an eyewash station and emergency shower should be located in the area. All employees should locate these emergency facilities before starting to work.

4. General Neutralization Procedures

If there are any questions, or if you are hesitant about attempting this procedure with any spent acid or alkali waste, please call the EHSO at 405/271-3000.

a. WARNING: REMEMBER THAT EXTREME HEAT CAN BE PRODUCED BY THIS PROCEDURE UNLESS IT IS DONE VERY SLOWLY. CLOSELY MONITOR THE AMOUNT OF HEAT PRODUCED THROUGH THE USE OF THE THERMOMETER.

b. For concentrated acids and bases, neutralization must be done very slowly and with lots of stirring.

c. The recommended time for the neutralization procedure is when the wastewater flow is at a peak, e.g., 9:00 a.m.

d. Hands should always be washed after working with these chemicals.

5. Acid Neutralization

a. Make a saturated solution of soda ash in a beaker or use an inorganic base diluted in water (1:10 ratio) - set aside.

b. Put tap water into 1- or 2-gallon polyethylene bucket.

c. Dilute acid at least 1:10 (1 part acid to 9 parts of water) by slowly pouring and stirring acid into the water.

d. Slowly add soda ash or other basic solution into diluted acid with stirring, or save diluted acid to neutralize bases as described below.

e. Monitor pH with pH meter, pH indicator strips, or other pH test method.

f. When pH is between 5 and 10.5, dispose in a drain followed with excess water. A pH near 7 is preferred to reduce the possibility of plumbing damage.

HELPFUL HINT: When neutralizing an acid, the pH can be tested quickly by the following method. Make a saturated solution of sodium bicarbonate in water. A

small amount of sodium bicarbonate solution poured into the acid will make a "fizz", which is a release of carbon dioxide. This "fizz" will indicate that the solution is still acidic, and needs more base to be added. Always do a final check of the pH before pouring the neutralized acid down the drain.

6. Base Neutralization

   a. Put tap water into 1 or 2 gallon polyethylene bucket.

   b. Dilute alkali wastes at least 1:10 (1 part alkali to 9 parts water) by slowly pouring and stirring the base into the water.

   c. Neutralize the diluted alkali solution with a previously diluted inorganic acid.

   d. Monitor pH with pH meter, pH indicator strips, or other pH test method.

   e. When pH is between 5 and 10.5, dispose in a drain followed by excess water. A pH near 7 is preferred to reduce the possibility of plumbing damage.

F. Destruction, Decontamination and Disposal of Ethidium Bromide

Ethidium bromide (EtBr) is a powerful mutagen which is widely used in laboratories for visualizing nucleic acids. The compound is an odorless red crystalline solid, is soluble in water, and fluoresces a red-orange under ultraviolet (UV) light. EtBr may be purchased in powder or solution form, and may also be known as:

2,7-diamino-10-ethyl-phenylphenanthridinium bromide
3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide
2,7-diamino-9-phenyl-10-ethylphenanthridinium bromide
2,7-diamino-9-phenylphenanthridine ethobromide
dromilac
homidium bromide
RD 1572
novidium bromide
babidium bromide

Acute exposures are irritating to the eyes, skin, mucous membranes, and upper respiratory tract. Because ethidium bromide is strongly mutagenic, it should be regarded as a reproductive toxin and a possible carcinogen. It may be harmful by inhalation, ingestion, and skin absorption and should be handled only when wearing rubber or nitrile gloves, lab coat, and chemical splash goggles while working in a chemical fume hood.

Work areas where ethidium bromide solutions are prepared or used should be organized and designated with warning signs, which are available from the EHSO. Containers of solid ethidium bromide should be tightly closed and stored in a cool dry well-ventilated area away from strong oxidizing agents.

In case of contact with eyes, immediately flush eyes with copious amounts of water for at least 15-minutes. In case of contact with skin, immediately wash skin with soap and copious amounts of water. Chemical exposures should be immediately reported to the laboratory supervisor and medical attention sought.

1. Principles of Decontamination and Destruction

Several methods have been published for the chemical destruction/ decontamination of EtBr involving treatment with bleach, hypophosphorus acid and sodium nitrate, and potassium nitrate. By-products from these procedures, however, can also be mutagenic. For example, the by-products of bleach decontamination show 20% mutagenic activity of the original EtBr, by-products of the $H_3PO_4/NaNO_2$ procedure show 0.6% mutagenic activity, and by-products of the $KMnO_4$ procedure shows 0.03%. This information clearly demonstrates that the commonly used technique of bleach decontamination is not the best choice. Because potassium nitrate will stain most materials it contacts, the best choice for destruction or decontamination is the sodium nitrate procedure. The reagents (sodium nitrate and a dilute solution of hypophosphorus acid) are inexpensive and relatively nontoxic.

In the laboratory, EtBr might be encountered in a variety of solvents including EtBr dissolved in water, TBE buffer, MOPS buffer, and cesium chloride solution. The method to destroy EtBr in these solvents is based on a deamination procedure. The recommended procedure removes the amino groups from the EtBr and it may cleave the N-containing ring. Sufficient hypophosphorus acid must be supplied to reduce the pH sufficiently (pH<3, approximately) or the reaction may fail. EtBr in solvents which may not be drain disposed (See Section V.A.5.) are considered hazardous waste, and should be collected in accordance with the procedures identified in this Section.

The decontamination should be validated through the use of a fluorescent lamp (while wearing UV protective glasses or goggles). It should be noted that the fluorescent lamp procedure only determines fluorescent compounds (such as EtBr) but that the EtBr can be changed into non-fluorescent but still toxic compounds.

2. Destruction and Decontamination Procedures

The decontamination mixture gives off a small amount of nitrogen dioxide when initially mixed, and should be used in a chemical fume hood. Hypophosphorus acid is corrosive and should be handled carefully. MSDSs for all reagents should be readily accessible to all employees.

a. Destruction of EtBr in Aqueous Solution

(1) Dilute the solution, if necessary, so that the concentration of EtBr does not exceed 0.5 mg/ml.

(2) For each 100 ml of EtBr in H_2O, MOPS buffer (see below),

or 1 g/ml cesium chloride solution, add 20 ml of 5% hypophosphorus acid solution and 12 ml of 0.5 M sodium nitrite solution.

(a) The hypophosphorus acid solution is prepared by adding 10 ml of the commercially available 50% solution to 90 ml of water and stirring briefly.

(b) It is advisable to prepare the hypophosphorus acid solution and the sodium nitrite solution (34.5 g/liter) fresh each day.

(c) Even if less concentrated solutions are to be decontaminated, it is best to add the same volume of hypophosphorus acid and sodium nitrite solutions to ensure the completeness of destruction.

(d) For more concentrated solutions, the volumes should be increased accordingly.

(3) Stir briefly and allow to stand for 20 hours.

(4) Neutralize with sodium bicarbonate ($NaCO_3$), check for completeness of destruction, and discard the solution in the sanitary sewer system.

b. Decontamination of EtBr in Aqueous Solution

(1) Dilute the solution, if necessary, so that the concentration of EtBr does not exceed 0.1 mg/ml.

(2) For each 100 ml of EtBr in solution add approximately 3 g of Amberlite XAD-16 resin or coarse grade activated carbon. Stir for 20 hours, then filter the mixture. Alternatively, commercial systems using similar products are available.

(3) The absorbent, which now contain EtBr, should be packaged and disposed as solid EtBr waste (see Section V.F.3., “Disposal of Ethidium Bromide in Solid Waste”).

(4) Check the liquid for completeness of decontamination (for example, by UV fluorescence) and discard it in the sanitary sewer system.

c. Decontamination of Equipment Contaminated with EtBr

Glass, stainless steel, Formica, floor tile, and the filters of trans-

illuminators have been successfully decontaminated using this technique.

(1) Wash the equipment once with a paper towel soaked in a freshly prepared decontamination solution consisting of 4.2 g of sodium nitrite and 20 ml of hypophosphorus acid (50%) in 300 ml of H_2O.

(2) Wash five times with wet paper towels using a fresh towel each time.

(3) If the decontamination solution (pH 1.8) is felt to be too corrosive for the surface to be decontaminated, then use six H_2O washes.

(4) Soak all the towels in decontamination solution for 1 hour.

(5) Check for completeness of decontamination (for example, by UV fluorescence) and discard the solution in the sanitary sewer system.

d. Decontamination of EtBr in Isopropanol Saturated with Cesium Chloride

(1) Dilute the solution, if necessary, so that the concentration of EtBr in the isopropanol saturated with cesium chloride does not exceed 1 mg/ml.

(2) For each volume of EtBr solution, add 4 volumes of a freshly made decontamination solution consisting of 4.2 g of sodium nitrite and 20 ml of hypophosphorus acid (50%) in 300 ml of H_2O and stir the mixture for 20 hours.

(3) Neutralize with $NaCO_3$, test for completeness of destruction (for example, by UV fluorescence), and discard the solution in the sanitary sewer with copious amounts of water.

e. Decontamination of Ethidium Bromide Spills

Given the wide usage of EtBr in laboratories, spills may be expected to occur, such as on benches, in fume hoods, or on the floor. Another problem that may occur is the build up of EtBr residues on the surfaces of equipment such as centrifuges. Transilluminators are used to view the gels which contain DNA stained with EtBr, and residues of EtBr may build up on the large UV filter which forms the top surface of these units. Any decontamination method should not, of course, damage the optical surface. The decontamination solution described below is less corrosive than potassium permanganate in hydrochloric acid and does not appear to damage surfaces of transilluminator filters and Formica. Another

method using activated charcoal and ethanol may leave activated charcoal on optical surfaces. The method described here does not appear to leave residues on nonporous surfaces.

Although low levels of mutagenic activity were found when high (10 mg/ml) concentrations of EtBr in solution were degraded, the sodium nitrite/hypophosphorus acid method does not produce significantly mutagenic solutions when the EtBr concentration does not exceed 0.5 mg/ml.

(1) De-energize all electrical equipment before decontamination and wear appropriate protective equipment, including rubber gloves, laboratory coat, and chemical goggles. A small amount of nitrogen dioxide is given off when the decontamination solution is mixed, and therefore this procedure is best carried out in a fume hood.

(2) Scrub the contaminated surface or equipment with a paper towel soaked in a freshly prepared decontamination solution (prepared by adding 20 ml of 50% hypophosphorus acid to a solution of 4.2 g of sodium nitrite in 300 ml of water). Scrub another five times with wet paper towels, using a fresh towel each time.

(3) Place all towels in a large container and soak them in fresh decontamination solution for at least 1 hour before disposing of them.

(4) Test the used decontamination solution for fluorescence and/or mutagenicity. Neutralize the used decontamination solution with sodium bicarbonate and discard as aqueous waste in the sanitary sewer system.

(5) Dry off the decontaminated surface or equipment and place it in service again.

3. Disposal of Ethidium Bromide in Solid Waste

Trace amounts of EtBr (less than 0.1%) in electrophoresis gels may be disposed as regular laboratory trash. Higher concentrations, such as when the color of the gel fluoresces dark pink or red under a UV lamp, should not be placed in the laboratory trash or placed with biomedical waste that will be autoclaved, but rather placed in the biomedical waste stream destined for incineration. Gloves, test tubes, paper towels, filters for liquid decontamination, empty containers that once held EtBr, etc. that are contaminated with EtBr should also be disposed in the biomedical waste stream that is destined for incineration.

Sources: Lunn, G. and E.B. Sansone, 1987, "Ethidium Bromide: Destruction and Decontamination of Solutions," *Analytical Biochemistry* 162:453-458

Lunn, G. and E.B. Sansone, 1989, "Decontamination of Ethidium Bromide Spills," *Applied Industrial Hygiene* 4:234-235

G. **Chromic Acid**

Laboratories using cleaning products containing potassium or sodium dichromate in sulfuric acid, including the product *Chromerge*, i.e., chromic acid, for cleaning laboratory glassware should try to abandon the practice as soon as possible. Spent (waste) chromium may not be discharged into the wastewater treatment system. Because the City of Norman has placed limitations on the concentration of chromium in wastewater discharge (0.09 mg/l hexavalent chromium; 1.6 mg/l total chromium), used solutions must be saved, treated, and disposed as a hazardous waste which is expensive and time consuming. The EHSO recommends that laboratories seek alternative glassware cleaning solutions for products to meet their needs.

Glassware cleaning products which do not contain chromium such as *Nochromix* or a simular subtitute, are readily available in the market and are recommended. *Nochromix* mixed with sulfuric acid is as effective as *Chromerge* in removing trace metals and enzyme residues, but it eliminates the need for special disposal since spent solutions of *Nochromix* can be safely disposed of in the sink if neutralized and not contaminated with other metals or toxic substances. CAUTION: the mixing of *Nochromix* and sulfuric acid results in a continual exothermic reaction which requires a vented bottle cap to prevent the container from bursting.

Alternative cleaning solutions are provided in the following sections. For glassware requiring more intensive cleaning, use the "alkaline bath" procedure, followed by the "acid bath" procedure. CAUTION: chromic acid, sulfuric acid, and alternative cleaning solutions are highly corrosive. Take appropriate precautions including the use of personal protective equipment and a fume hood.

In cases where it is imperative that glassware must be cleaned using a cleaning solution containing chromium, the spent cleaning reagent must be processed and packaged by the source laboratory using the guidelines in Section V.C. or V.G.2.

1. Alternative Cleaning Solutions

    a. As an alternative to a sulfuric acid bath, a 95% ethanol/ hydrochloric acid bath or 95% ethanol/potassium hydroxide bath can be used as effectively against organic residues.

    b. An ammonium persulfate/sulfuric acid solution can be utilized by dissolving 36 grams of ammonium peroxydisulfate (a.k.a. ammonium persulfate), $(NH_4)_2S_2O_8$, in a 2.2 liter (one-gallon) bottle of 98% sulfuric acid. More ammonium peroxydisulfate should be added every few weeks as necessary to maintain the oxidizing capability of the solution. This solution is an extremely powerful oxidant that can remove dirt and grease, but can corrode clothing and skin. It should be stored and used in a chemical fume hood.

c. Acid Bath:

464 milliliters hydrochloric acid
3 gallons 95% ethanol

This cleaning solution is recommended for cleaning glassware used in most general laboratory experiments including those involving tissue and other biochemical reactions. Glassware should be washed as thoroughly as possible then soaked in the acid bath for up to, but not over 24 hours. Glassware should then be rinsed several times in deionized water and dried. Note: Soaking glassware over 24 hours in the acid bath may cause etching.

d. Alkaline Bath:

190 grams potassium hydroxide
3 gallons 95% ethanol

This cleaning solution is recommended for glassware used in most general laboratory experiments including those involving organic reactions. Wash glassware thoroughly before soaking in the alkaline bath. Do not allow glassware to soak in the alkaline bath for longer than 24 hours. Rinse with deionized water.

2. Chromic Acid Waste Disposal (for small quantities)

The following should be incorporated as a procedural step in your laboratory research and experiments:

a. In a fume hood, slowly and carefully add the cleaning solution (100 ml) to a container of water (about a 1:1 dilution).

b. Acidify with 3 molar sulfuric acid (35-55 ml) until a pH 1 is reached, as measured by pH indicator strips or other method. To prepare 3 molar sulfuric acid, add 165 ml concentrated (18M) sulfuric acid to 835 ml of water to equal 1 liter. Remember to add the acid to water, not water to the acid.

c. While stirring, slowly add 13.5 g solid sodium thiosulfate until the solution turns cloudy and blue.

d. Neutralize the solution with soda ash. After a few minutes, a blue-gray precipitate is formed.

e. Let the mixture stand for a week or filter immediately through Celite (diatomaceous earth). After a week, much of the supernatant can be decanted.

f. The remaining liquid should be allowed to evaporate or the solid

filtered.

g. The liquid may be washed into the drain with large quantities of water.

h. The solid residue should be washed with hot water to remove the sodium sulfate, then dried, packaged, and labeled as to contents and hazards.

i. Contact the EHSO for pick-up and disposal.

3. Chromic Acid Spillage Disposal

a. Cover the spill with a 1:1:1 mixture by weight of soda ash or calcium carbonate, clay cat litter (bentonite) and sand.

b. Scoop mixture into a container of water in the fume hood. Follow the procedures in Sections V.G.2.

c. The site of the spillage and contaminated clothing should be washed thoroughly with soap and water to remove the oxidant.

Sources: Amour, M.A., et al, *Hazardous Chemicals - Information and Disposal Guide*
Harris, D., *Quantitative Chemical Analysis*, 3rd. Ed.

H. **Lecture Bottles**

Disposal of hazardous lecture bottles is one of the more difficult and expensive waste management operations. Few suppliers are willing to take back their cylinders. Lecture bottles with unknown contents can cost thousands of dollars to dispose. Old bottles can also pose a significant hazard, both to lab personnel and others. Bottles that contained hazardous or poisonous liquid or gas can still be hazardous when "empty", and small leaks in the bottles can damage nearby equipment. Therefore, the following guidelines are offered.

1. Seek an alternative. Don't buy them if you don't need them!

2. If you must use lecture bottles, inquire whether the supplier will take back the cylinder after use. If they will not, try to find another supplier who will.

3. If you cannot find a supplier who will take back the empty/unused portion, plan to purchase only the quantity you need. Plan to use all the bottle contents or develop procedures to destroy any hazardous constituents remaining.

4. When use is finished, return them to the supplier or contact the EHSO for disposition instructions, if needed. DO NOT store unneeded lecture bottles of hazardous gases for some possible future use.

5. Comply with all safety recommendations from the supplier of the lecture bottle and supplier of the regulator or manual control.

6. Ensure that any fixtures attached to the lecture bottle comply with the manufacturer's recommendations.

I. Drug Disposal

Drugs may fall in to three classes: “hazardous” drugs as defined by OSHA which includes cytotoxic/antineoplastic agents (a list may be found in Appendix A of this manual), controlled substances such as barbiturates and morphine, and pharmaceutical drugs. Disposal procedures are as follows:

1. Hazardous Drug Waste Disposal

   a. All hazardous drug waste disposal must be arranged through an outside vendor for incineration.

   b. Special labels indicating “hazardous drug” and “incinerate only” must identify the containers.

   c. If the waste contains animal tissues, carcasses, excreta and other materials contaminated with hazardous drugs including cytotoxic/antineoplastic material, it may be disposed through Animal Resources, but the waste must be identified as such when sent to Animal Resources.

   d. Unused or spent liquid hazardous drugs must be disposed through coordination by the EHSO as hazardous waste, but must not be mixed with other hazardous wastes.

   e. Unused or spent solid hazardous drugs listed by the EPA as hazardous waste [Chlorambucil, Chlornaphazin, Cyclophosphamide, Daunomycin, Diethylstilbesterol, Melphalan, Mitomycin C, Streptozocin (Streptozoticin), and Uracil Mustard] must be disposed as hazardous waste through the EHSO.

   f. Unused or spent solid hazardous drugs not listed by the EPA as hazardous waste and material that has come in contact with any form of hazardous drugs, including empty vials, used containers, syringes, discarded gloves, gowns, goggles and any other disposable material may be disposed through a biomedical waste vendor for incineration.

      (1) All waste material should be placed in sealable plastic biohazard bags of 4 mil thick polyethylene or 2 mil polypropylene, labeled with a “hazardous drug” and “incinerate only” label.

(2) The waste bag should be kept inside a covered waste container indicating "hazardous drug".

(a) If the outer container is also disposable, it should be labeled “incinerate only”.

(b) At least one such receptacle should be located in every area where the drugs are prepared or administered.

(3) Needles, syringes, and breakable items should be placed in a biohazard labeled plastic vial or puncture proof box before they are placed into the bag; needles should not be clipped or capped nor syringes crushed.

(4) The waste should not be moved from one area to another.

(5) The bag should be sealed when filled and the covered waste container taped, or the bag should be placed in an appropriate box or other container supplied by a disposal vendor, then sealed shut.

(6) Precaution should be taken to prevent contamination of the exterior of the outer container.

(a) Personnel disposing of hazardous drug waste should wear gowns and protective gloves when handling waste containers.

(b) A container with a contaminated exterior should be placed in a second container in a manner which eliminates contamination of the second container.

2. Controlled Substances

Controlled substances are habit-forming drugs regulated by the U. S. Drug Enforcement Administration and include amphetamines, depressants, anabolic steroids, and hallucinogens. The substances should be stored in a secure location until the EHSO can arrange for pick-up and disposal of the material. Disposal of such substances is performed as follows.

a. A departmental representative should contact the EHSO who will provide the department with an Oklahoma State Bureau of Investigation (OSBI) *Inventory of Drugs Surrendered* form.

b. The EHSO will contact the OSBI for coordination of appropriate disposal/ destruction. When authorization is provided by OSBI, the EHSO will contact the department so that pick-up and transport may be arranged.

c. After the material has been disposed/destroyed by OSBI, the signed *Inventory of Drugs Surrendered* form will be provided to the EHSO. The EHSO will maintain a copy and provide the original to the generating department. This form should be retained for at least three years by the department in accordance with the Uniform Controlled Dangerous Substance Act.

3. Pharmaceutical Drugs

Pharmaceutical drugs which are not a controlled substance as defined above or listed as a hazardous drug or hazardous waste should be sent back to the supplier/vendor whenever possible. When this is not possible, the drugs may be disposed through a biomedical waste disposal vendor. Place the drugs in a sturdy box (not a biomedical waste disposal box), provide a list of the drug names with the box, mark the box "Incinerate Only", and contact the biomedical waste disposal vendor for pickup and disposal.

J. Other Decontamination/Disposal Procedures

Laboratory solutions containing hazardous chemicals may not be disposed down the drain. They should be collected for disposal as described in this Section unless they are first neutralized or decontaminated according to recognized procedures. The following procedures are available upon request from the EHSO:

1. Disposal of Benzidine and Diaminobenzidine
2. Decontamination of Spills Involving Benzidine and Diaminobenzidine
3. Decontamination of Aqueous Solutions of Benzidine and Diamino-benzidine
4. Disposal of Chloromethylsilane and Dichloromethylsilane
5. Disposal of Cyanides and Cyanogen Bromide
6. Disposal of Dimethyl Sulfate, Diethyl Sulfate, Methyl Methane-sulfonate, Ethyl Methanesulfonate, Diepoxybutane, and 1,3-Propane Sultone
7. Disposal of Ethidium Bromide and Propidium Iodide
8. Decontamination of Equipment Contaminated with Ethidium Bromide
9. Decontamination of Ethidium Bromide in Isopropanol Saturated with Cesium Chloride
10. Decontamination of Ethidium Bromide in Isoamyl Alcohol and 1-Butanol
11. Disposal of Hydrazine

12. Decontamination of Spills of Hydrazine
13. Disposal of Hydrogen Peroxide
14. Reduction of Iodine
15. Disposal of Sodium Azide

Source: *Current Protocols in Molecular Biology*, Supplement 34