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Ethidium Bromide Disposal Procedure

Ethidium bromide (EtBr) is a powerful mutagen widely used in biochemical research laboratories for visualizing nucleic acids. The compound forms fluorescent complexes by intercalation and these compounds are readily visible under ultraviolet (UV) light. EtBr is generally used in the laboratory dissolved in water or buffer solutions.

Since EtBr is so widely used, proper management of EtBr containing waste and EtBr contaminated materials is important. Below are the procedures to be followed for EtBr containing waste and EtBr contaminated material.

Aqueous Solutions of Ethidium Bromide or Cesium Chloride/Ethidium Bromide

Aqueous solutions of less than 10 mg/L (10 ppm or 0.001%) EtBr may be disposed of in the sanitary sewer.

Aqueous solutions of more than 10 mg/L EtBr must first be treated before they are disposed of in the sanitary sewer. The solution can be filtered through an EtBr filter (below) ensuring that a vacuum flask is used, not an Erlenmeyer flask. While wearing proper protective equipment, the paper carbon filter disk containing EtBr must be removed from the filter unit using a forceps and then processed through the Office of Research waste pickup. The remaining polypropylene filter unit can be reused according to the manufacturer's directions or rinsed with bleach, and disposed of in the trash.

Alternatively, solutions can be deactivated by either the Lunn and Sansone method (below) or the Armour method (below) and then disposed of in the sanitary sewer. The Lunn and Sansone method degrades EtBr by reaction with sodium nitrite and hypophosphorous acid in aqueous solution. The Armour method degrades EtBr with household bleach.

It is strongly recommended to use charcoal filtration over chemical deactivation.

Note: Waste solutions greater than 10 mg/L must not be diluted and then disposed of by sewer under this policy. Dilution as a partial or complete substitute for pretreatment before discharge in a public sewer is prohibited by the Tulsa Publically Owned Treatment and Waterworks Ordinance, Chapter 12, Title 11-C (#19991), Section 1201.

Acrylamide and Agarose Gels Containing Ethidium Bromide

Acrylamide and agarose gels containing less than 10 mg/L (10 ppm or 0.001%) EtBr can be disposed of in the trash. Gels that are trashed should be put in double lined trash bags and labeled as nonhazardous.

Acrylamide and agarose gels containing more than 10 mg/L EtBr must be disposed as hazardous waste through the Research Office, Laboratory Safety Coordinator. Store until announced disposal pickup in double lined plastic bags in a proper sized, leak proof and tightly sealed container.

Alcohol Solutions of Ethidium Bromide

Because alcohols are flammable, all alcohol solutions containing EtBr should be processed as hazardous waste through the Research Office, Laboratory Safety Coordinator.

Lab ware Contaminated with Ethidium Bromide

Contaminated labware includes needles, disposable gloves, pipettes, test tubes, etc. that are contaminated with EtBr. Depending on the type of waste that you generate, follow the procedures below.

1. Needles, scalpels, Pasteur pipettes and other sharps contaminated with EtBr should be disposed of directly into a sharps container.
2. Volumetric or transfer pipettes, and other disposable glassware contaminated with EtBr should be disposed of in a waste container designated for glass disposal. Grossly contaminated (visibly contaminated) glassware should be washed with bleach before disposal in the designated container.
3. Test tubes and centrifuge tubes contaminated with EtBr should first be emptied, with the liquid disposed of according to the procedures given above. Empty tubes can then be disposed of in the trash. Grossly contaminated (visibly contaminated) tubes should be washed with bleach prior to disposal.
4. Laboratory equipment (e.g. transilluminators, laboratory floors and counter tops, etc.) contaminated with aqueous solutions of more than 10 mg/L EtBr should be decontaminated using the Lunn and Sansone method (below) for decontamination of equipment contaminated with EtBr. This is done by scrubbing the contaminated lab equipment with paper towels soaked in a freshly prepared aqueous solution of sodium nitrite and hypophosphorous acid while wearing the proper protective equipment. The towels are then decontaminated in the decontamination solution before disposal in the trash.
5. Most other disposable labware (e.g. sample vials, disposable beakers, etc.) contaminated with EtBr may be disposed of in the normal trash. Grossly contaminated (visibly contaminated) disposable labware should be washed with bleach prior to disposal.

Spills of Ethidium Bromide

Small spills (less than 0.5 L) of aqueous solutions of more than 10 mg/L EtBr and the resulting contaminated lab equipment can be decontaminated using the Lunn and Sansone method (below) for decontamination of equipment contaminated with EtBr. The EtBr is degraded by wiping the surface with paper towels soaked in a freshly prepared decontamination solution (an aqueous solution of sodium nitrite and hypophosphorous acid) while wearing the proper protective equipment.

All spills (more than 100 ml) containing Ethidium Bromide should be reported to the PI and Research Office, Laboratory Coordinator at 586-4607. Leave lab, close doors.

Charcoal Filtration

Filtering the aqueous ethidium bromide waste solutions, free of other contaminants, through a bed of activated charcoal using a vacuum flask, not an Erlenmeyer flask, is a relatively simple and effective method for removal of ethidium bromide. The filtrate may be poured down the drain.

Any kit may be used, however following are two simple kits available for charcoal filtration:

Funnel Kit

Schleicher and Schuell supply a commercial filter funnel kit that uses a packaged charcoal disk that is graduated for easily tracking the amount of aqueous solution calculated for a fixed quantity of ethidium bromide residue. This is particularly useful for labs that generate large amounts of solutions at a time. The kit is available through [VWR](#).



- ▶ Filter the ethidium bromide solution through the charcoal filter using a vacuum (thick walled) flask.
- ▶ Pour filtrate down the drain.
- ▶ Place charcoal filter in a sealed bag (e.g., zip-lock) and place in safe storage for announced hazardous waste pickup. (If the materials contain an infectious agent, it must be disinfected before pickup)

The Green Bag

Another simple charcoal filtration method is the Green Bag, manufactured by BIO 101. The Green Bag® Kit allows rapid and trouble-free concentration of ethidium bromide from large volumes of solutions into a small "tea" bag containing activated carbon which is then conveniently disposed along with other solid hazardous wastes. One kit has the capacity to remove 500 mg of ethidium bromide from solutions (10mg EtBr/bag).



Place the Green Bag into the ethidium bromide solution.
Allow to sit for the allotted time.
Pour filtrate down the drain.
Dispose of the Green bag in safe storage for announced hazardous waste pickup. (If the materials contain an infectious agent, it must be disinfected before pickup).

Green Bags are available through BIO101 ([Bio101 Green Bag.](#))

Chemical Neutralization

Lunn and Sansone Ethidium Bromide Destruction Method (Lunn, George, and Sansone, Eric B. 1994. *Destruction of Hazardous Chemicals in the Laboratory*. John Wiley and Sons, Inc. pp. 185.)

1. Carry out the following steps in a fume hood and follow all laboratory safety precautions, including proper protective clothing.
2. Dilute solutions containing EtBr to concentration $<0.05\%$ w/v (50mg/100mL).
3. For each 100mL of EtBr solution add 20mL of fresh 5% hypophosphorous acid and 12mL of fresh 0.5M sodium nitrite solution. Check that the pH of the solution is <3.0 . Stir briefly.
4. After reacting for at least 20 hours, neutralize with sodium bicarbonate, then rinse the solution down the sanitary sewer with water.

Armour Ethidium Bromide Destruction Method (Armour, Margaret-Ann. 1994. Personal communication.)
(Kaufman, James A. ed. 1990. *Waste Disposal in Academic Institutions*. Lewis Publishers. pp. 127-8)

1. Carry out the following steps in a fume hood and follow all laboratory safety precautions, including proper protective clothing.
2. Dilute solutions containing EtBr to concentration $<0.034\%$ w/v (34mg/100mL).
3. Add 10mL of fresh bleach for every 1mg EtBr.
4. Stir at room temperature for at least 4 hours.
5. The EtBr is converted to the physiologically inactive product 2-carboxybenzophenone, and the solution should then be rinsed down the sanitary sewer with water.

Lunn and Sansone Method for the Decontamination of Equipment Contaminated with Ethidium Bromide (Lunn, George, and Sansone, Eric B. 1994. *Destruction of Hazardous Chemicals in the Laboratory*. John Wiley and Sons, Inc. pp. 186.)

1. Before decontamination, deenergize all electrical equipment and wear the appropriate protective equipment.
2. The decontamination solution is prepared by adding 20mL of 50% hypophosphorous acid to a solution of 2g of sodium nitrite in 300mL of water.
3. Scrub the contaminated surface or equipment with a paper towel soaked in the freshly prepared decontamination solution. Scrub another five times with paper towels soaked in the freshly prepared decontamination solution, using a fresh towel each time.
4. Place all the used towels in a large container and soak them in fresh decontamination solution for at least one hour.
5. Neutralize used decontamination solution and towels with sodium bicarbonate. The towels can then be discarded in the trash, and the solution may be rinsed down the sanitary sewer with water.

Note: A small amount of nitrogen dioxide may be given off when the decontamination solution is initially mixed, so the procedure should be carried out in a chemical fume hood.