



UCCS SAFE OPERATING PROCEDURE

23. SAFE HANDLING AND DISPOSAL OF ETHIDIUM BROMIDE

(For assistance, please contact [Environmental Health & Safety](#))

Ethidium bromide is a nucleic acid stain. It fluoresces under ultraviolet light, especially when bound to double-stranded DNA. It is also a strong mutagen and a possible carcinogen, so must be managed correctly. While it is not specifically regulated as a hazardous waste, the mutagenic properties may present a hazard if it is not managed properly in the laboratory.

Ethidium bromide (EtBr) is a strong mutagen that requires special handling and disposal procedures, as described follows:

- When handling EtBr always wear a lab coat, nitrile gloves, and chemical splash goggles. Proper skin and eye protection are needed when a ultraviolet (UV) light source is used while working with EtBr. Avoid exposing unprotected skin and eyes to intense UV sources. Wear a face shield if UV source is pointing upwards. When working with a UV source for a long time, wrap up lab coat sleeves with tape or other means where the wrist could be exposed
- Unused excess EtBr must be labeled for collection by [EH&S](#).
- Solutions of EtBr containing heavy metals, organics, cyanides or sulfides, or other toxic constituents must be labeled for collection by [EH&S](#).
- pH neutral, aqueous solutions containing less than or equal to 5 ppm (mg/L) EtBr may be disposed via the sanitary sewer so long as the sink is connected to a Publicly Owned Treatment Works (POTW).
- pH neutral, aqueous solutions containing greater than 5 ppm EtBr may be discharged to the sanitary sewer¹ following decontamination or deactivation as listed below³.
- Solvent solutions containing any amount of EtBr must be labeled for collection by [EH&S](#).
- EtBr mixed with a radioactive isotope must be processed for collection by [CU Boulder Health Physics](#).
- Gels that contain ethidium bromide may be double-bagged, labeled "non-hazardous" and placed in the trash if they contain less than 0.1% ethidium bromide. If the gel is pink or red, the ethidium concentration is higher than 0.1%. In this case, the gel is handled as hazardous waste.
- Concentrated solutions (10 mg/ml) of ethidium bromide must be handled as hazardous waste.
- Disposable equipment/consumables (i.e., gloves, pipette tips, etc.) minimally contaminated with EtBr may be bagged and disposed as regular trash. Materials with gross contamination must be bagged in sealed heavyweight plastic bags weighing less than 20 pounds and labeled for collection by EH&S.
- Clean spills of ethidium bromide carefully with soap and water. Collect the soap, water and ethidium bromide mixture onto absorbent material. If concentrated or powdered ethidium bromide was spilled, place it in a sturdy screw top container and dispose of as hazardous waste. All other materials may go in the trash after you double bag it and label it "non-hazardous".
- Other nucleic acid dyes and stains are also mutagens and possible carcinogens. They should be treated like ethidium bromide with the methods above.

Decontamination or Deactivation:²

Charcoal Filtration

Filtering the aqueous EtBr waste solutions (free of other contaminants) through a bed of activated charcoal is a relatively simple and effective method for removal of EtBr. The filtrate may be poured down the drain.

There are two kits available for charcoal filtration.

Funnel Kit:

Commercial filter funnel kits are available that use a packaged charcoal disk that is graduated for easily tracking the amount of aqueous solution that can be run through it. This is particularly useful for labs that generate large amounts of solutions at a time. The kit is available through Schleicher and Schuell or VWR.

- Filter the EtBr solution through the charcoal filter.
- Pour filtrate down the drain.
- Place charcoal filter in a sealed container (mayonnaise jar) and label as a hazardous waste.

GreenBags Kit:

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

- Place the Green Bag into the EtBr solution.
- Allow to sit for the allotted time.
- Pour filtrate down the drain.
- Dispose of the Green Bag in a sealed container (mayonnaise jar) and label as a hazardous waste.

EtBr Chemical Degradation Procedure³

Solutions containing EtBr can be deactivated and rendered nonhazardous by using one of three methods. Deactivation may be confirmed using UV light to detect fluorescence. The three methods are as follows:

➤ **Armour Method:**

This is the simplest method, but is somewhat controversial. One study found traces of mutagenic reaction mixtures using this method. (Lunn, G. and E. Sansone, Analytical Biochemistry, vol. 162, pp. 453-458, 1987)

- Combine equal amounts of EtBr solution and household bleach.
- Stir constantly for four hours or let sit for 2-3 days.
- Adjust pH to 4-9 with sodium hydroxide.
- Pour down drain with copious amounts of water.

➤ **Lunn and Sansone Method:**

For each 100 ml of EtBr solution:

- Add 5% hypophosphorus acid.
- Add 12 ml of 0.5 M sodium nitrite.
- Stir briefly and let stand for 20 hours.
- Adjust pH to 4-9 using sodium hydroxide.
- Pour down drain with copious amounts of water.

➤ **Quillardet and Hoffnung Method:**

This method uses 0.5 M potassium permanganate and 2.5 M hydrochloric acid. Since chlorine gas may be released in significant concentration, EH&S does not recommend using this method.

Contaminated Debris:

Contaminated debris includes gloves, bench paper and other non-labware items. Place contaminated debris into an appropriate containers for hazardous waste disposal. All containers used for collection must

have a top or cover that can be secured. Hazardous waste labels must be used and EH&S contacted when the material is ready for disposal. Bags must not be used unless they are doubled up and kept sealed.

Contaminated Labware:

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

Needles, scalpels, Pasteur pipettes, and other sharps contaminated with EtBr should be disposed of directly into a sharps container. These will be disposed of like other sharps waste.

Volumetric or transfer pipettes, and other disposable glassware incidentally contaminated with EtBr should be disposed of in a sharps container. Grossly contaminated (visibly contaminated) glassware should be washed with bleach before disposal in a sharps container.

Test tubes and centrifuge tubes contaminated with EtBr should first be emptied, with the liquid disposed of according to the procedures given above. Empty incidentally contaminated tubes can be disposed of in the trash. Grossly contaminated (visibly contaminated) tubes should be collected with other laboratory debris, labeled and disposed of as a hazardous waste.

Most other disposable labware (e.g. sample vials, disposable beakers, etc.) incidentally contaminated with EtBr may be disposed of in the normal trash. Grossly contaminated (visibly contaminated) disposable labware should be labeled and managed as a hazardous waste.

Accident Procedures:

Be very careful when using EtBr. Call EH&S at 255-3212 for assistance with spills. Below are procedures to follow in case of contact:

- In the case of eye contact, flush eye(s) with water for at least 15 minutes lifting upper and lower eyelids occasionally.
- If skin is exposed, remove contaminated clothing and wash skin with soap and water immediately.
- If EtBr vapors are inhaled, remove to fresh air.
- If swallowed, get medical attention immediately.

Spill Clean-up:

In case of a small spill, absorb freestanding liquid. Use ultraviolet light to locate the spill. Then prepare decontamination solution by mixing of 4.2 grams of sodium nitrite and 20 mL of hypophosphorous acid (50%) in 300 mL of water. To decontaminate the spill area, wash it with a paper towel soaked in the decontamination solution. Wash the spill area with wet paper towels (soaked in the decontamination solution) an additional 5 times (using fresh paper towel each time). After cleaning up the area put all the used towels in the decontamination solution for 1 hour. Check the completeness of decontamination using the ultraviolet light. If satisfied, put all the decontamination solution in a bottle, label it with a hazardous waste label and call EH&S for waste pick-up.

In case of large spill notify all the people in the laboratory. Evacuate the laboratory immediately. Call EH&S for assistance with a clean up. Post warning signs on all laboratory doors notifying others of the spill. Prevent unnecessary entry into the room until the EH&S personnel arrive. Explain all the details to the EH&S personnel.



Contaminated Equipment:

Laboratory equipment (e.g. transilluminators, laboratory floors and countertops, etc.) contaminated with aqueous solutions of more than 10 mg/L (0.01 %) EtBr should be decontaminated using the spill clean-up procedures listed above.

¹ Again, the sanitary sewer must discharge to the POTW.

² Chemical degradation procedures **MUST** be included in the experiment or research protocols to avoid unauthorized treatment of hazardous waste.

Last reviewed by Cynthia Norton on September 22, 2021